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- conferences [Bruand A, Hartmann C and Lesturgez G. 2005];

- thesis [Astiko W, 2013];

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* Please take note of the authors' name. Kindly do not wrongly cite the first and last name. For example, if the author is "Rahimah Ali", the correct citation should be "Ali, R. (2018)" in Reference List AND "Ali (2018)" in-text; BUT NOT "Rahimah, A. (2018)".

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APA REFERENCING STYLE (6TH EDITION)

INTRODUCTION TO THE AMERICAN PSYCHOLOGICAL ASSOCIATION (APA) REFERENCING STYLE

The American Psychological Association referencing style (or APA as it is more commonly known) is used across a variety of disciplines. The sixth and latest edition was published in 2010.

IN-TEXT REFERENCES

- APA uses the 'author-date' style of referencing. That is, in-text references (generally) appear in the following format:
  (Author's Last Name, Year of Publication).
  Example: (Austen, 1813).

- You are also permitted to include the Author's name in a sentence, omitting it from the brackets.
  Example: Austen (1813)

- When directly quoting from a source, you must include page number(s) and enclose the quote in double quotation marks.
  Example: "A woman must have money and a room of her own if she is to write fiction" (Woolf, 1929, p. 6).

  Note: For multiple pages, use the abbreviation 'pp.' Include the full page range, i.e. '64-67'.
  Example: Woolf (1929, pp. 64-67) observes that…

- When paraphrasing or referring to an idea contained in another work, the *Publication manual of the American Psychological Association* advises: "you are encouraged to provide a page or paragraph number, especially when it would help an interested reader locate the relevant passage in a long or complex text" (American Psychological Association [APA], 2010, p. 171). It is recommended you verify this advice with your unit of study coordinator, lecturer or tutor for each subject.

- If you are referring to an entire work, include only the Author's Last Name and Year of Publication in brackets. If you are referring to part of a work, you must include Page Numbers or their equivalent (see specific examples for more information).

- When citing a source you have not read yourself, but which is referred to in a source you have read (also known as 'secondary referencing'), use the following method:
  Moore (as cited in Maxwell, 1999, p. 25) stated that…
  Important: You would cite Maxwell, not Moore, in the Reference List.
  Note: It is always preferable to cite the original source. "Use secondary sources sparingly when the original work is out of print, unavailable through usual sources, or not available in English" (American Psychological Association [APA], 2010, p. 178).

REFERENCE LIST

- The Reference List should appear at the end of your work on a separate page.

- Only include references you have cited in your work.

- All references should have a hanging indent. That is, all lines of a reference subsequent to the first line should be indented (see examples in the tables below).

- In general, references should be listed alphabetically by the last name of the first author of each work.

- Special Reference List cases:
    - In the case of works by different authors with the same family name, list references alphabetically by the authors' initials.
    - In the case of multiple works by the same author in different years, list references chronologically (earliest to latest).
    - In the case of multiple works by the same author in the same year, list references alphabetically by title in the Reference List.

- When referring to Books, Book Chapters, Article Titles or Webpages, capitalise only the first letter of the first word of a title and subtitle, and proper nouns.
  Example: *Aboriginals and the mining industry: Case studies of the Australian experience*

- When referring to Journal Titles, capitalise all major words (do not capitalise words such as 'of', 'and', & 'the' unless they are the first word in the title).
  Example: *Journal of Exercise Science and Fitness*

USEFUL LINKS

REFERENCING AND CITATION STYLES SUBJECT GUIDE: http://libguides.library.usyd.edu.au/citation

ENDNOTE SUBJECT GUIDE: http://libguides.library.usyd.edu.au/endnote

HOW TO REFERENCE TUTORIAL: https://library.sydney.edu.au/help/online-training/referencing/

ACADEMIC LIAISON LIBRARIANS: https://library.sydney.edu.au/contacts/subjectcontacts.html

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University of Canberra Library & Academic Skills Program. (2010). *A guide to referencing with examples in the APA & Harvard styles* (6th ed.). Retrieved from the University of Canberra Library website: http://www.canberra.edu.au/library/attachments/pdf/apa.pdf

Document originally revised by K. Masters, July 2014

Updated by E. Tam and J. Ulyannikova, January 2016

Updated by M. Cassin, March 2017

	IN-TEXT REFERENCE	REFERENCE LIST

BOOKS & BOOK CHAPTERS

One author – in-text reference placement ***Note:*** There are two main ways to use in-text references. Firstly, to focus on the information from your source – 'information prominent'. Secondly, to focus on the author – 'author prominent'.	***'Information prominent' (the author's name is within parentheses):*** The conclusion reached in a recent study (Cochrane, 2007) was that… ***OR*** ***'Author prominent' (the author's name is outside the parentheses):*** Cochrane (2007) concluded that…	Cochrane, A. (2007). *Understanding urban policy: A critical approach.* Malden, MA: Blackwell Publishing.
One author – when fewer than 40 words are quoted Include the material in the paragraph and include specific page number/s. Use **quotation marks** to show the exact words.	An interesting view was expressed that "the connection of high profile developments to their surrounding environment has increasingly been questioned" (Cochrane, 2007, p. 117). ***OR*** An interesting view was expressed by Cochrane (2007) that "the connection of high profile developments to their surrounding environment has increasingly been questioned" (p. 117).	Cochrane, A. (2007). *Understanding urban policy: A critical approach.* Malden, MA: Blackwell Publishing.
One author – when 40 or more words are quoted Begin quoting the material on a new line, indent it 5 spaces (use the indent tool to keep all lines of the quote evenly indented), and include specific page number/s. **Omit** the quotation marks. Use **double spacing** for both your text and the indented quote. Make sure the quote is **exactly** as it was published.	Much has been written about acute care. Finkelman (2006), for example, points out that: There are many changes in acute care services occurring almost daily, and due to the increasing use of outpatient surgery, surgical services have experienced major changes. Hospitals are increasing the size of their outpatient or ambulatory surgery departments and adjusting to the need of moving patients into and out of the surgical service in 1 day or even a few hours. (p. 184). Recently, this trend has been seen in some Australian hospitals and research here…	Finkelman, A. W. (2006). *Leadership and management in nursing.* Upper Saddle River, NJ: Pearson Prentice Hall.

	IN-TEXT REFERENCE	REFERENCE LIST
Two authors	When considering the Howard Government's Indigenous health expenditure, Palmer and Short (2010) maintain that…	Palmer, G. R., & Short, S. D. (2010). *Health care and public policy: An Australian analysis* (4th ed.). Melbourne, Australia: Palgrave Macmillan.
Three to five authors For the first in-text reference, list all the authors' family names, then use the first author's family name followed by 'et al.' for subsequent entries.	A recent study (Seeley, VanPutte, Regan, & Russo, 2011) concluded that… ***Subsequent in-text reference/s:*** (Seeley et al., 2011).	Seeley, R., VanPutte, C., Regan, J., & Russo, A. (2011). *Seeley's anatomy & physiology*. New York, NY: McGraw-Hill.
Six to seven authors For all in-text references, list only the first author's family name followed by 'et al.' All authors are included in the Reference List. ***For books with eight or more authors, please follow the guidelines for journal articles with eight or more authors on page 7.***	The Russian Revolution may never have succeeded if there hadn't already been widespread discontent among the Russian populace (Bulliet et al., 2005).	Bulliet, R. W., Crossley, P. K., Headrick, D. R., Hirsch, S. W., Johnson, L. L., & Northrup, D. (2011). *The earth and its peoples: A global history* (5th ed.). Boston, MA: Wadsworth.
Works by different authors with the same family name For in-text references, include the initials of the authors in question to enable readers to differentiate between them. List references alphabetically by the authors' initials in the Reference List.	These techniques have been shown to improve test scores among primary school aged children (R. Smith, 2010). If funding were enhanced, it is arguable these problems could be ameliorated (C. J. Smith & Laslett, 1993).	Smith, C., & Laslett, R. (1993). *Effective classroom management: A teacher's guide* (2nd ed.). London, United Kingdom: Routledge. Smith, R. (2010). *Rethinking teacher education: Teacher education in the knowledge age*. Sydney, Australia: AACLM Press.

	IN-TEXT REFERENCE	REFERENCE LIST
Several works by the same author in different years When citing references separately, no special rule needs to be observed. When citing references collectively, separate years with a comma and insert years earliest to latest. List references chronologically (earliest to latest) in the Reference List.	These techniques have changed markedly in the last decade (Greenspan, 2000, 2011).	Greenspan, A. (2000). *Orthopedic radiology: A practical approach* (3rd ed.). Philadelphia, PA: Lippincott Williams & Wilkins. Greenspan, A. (2011). *Orthopedic imaging: A practical approach* (5th ed.). Philadelphia, PA: Lippincott Williams & Wilkins.
Several works by the same author in the same year Arrange alphabetically by title in the Reference List. Place lowercase letters (“a”, “b”, “c”, etc.) immediately after the year.	Leadership and change in schools have been major topics of discussion for several years (Fullan, 1996a, 1996b) and this conference… “Educational change” has taken on a new meaning in recent years (Fullan, 1996b) …	Fullan, M. (1996a). Leadership for change. In *International handbook for educational leadership and administration.* New York, NY: Kluwer Academic . Fullan, M. (1996b). *The new meaning of educational change*. London, United Kingdom: Cassell.
Several authors, different years, referred to collectively in your work List sources alphabetically by family name in the in-text reference in the order in which they appear in the Reference List. Separate each reference with a semicolon.	The cyclical process (Carr & Kemmis, 1986; Dick, 2000; Kemmis & McTaggart, 1988; MacIsaac, 1995) suggests…	Carr, W., & Kemmis, S. (1986). *Becoming critical: Education knowledge and action research.* London, United Kingdom: Falmer Press. Dick, B. (2000). *A beginner’s guide to action research.* Retrieved from http://www.scu.edu.au/schools/gcm/ar/arp/guide.html Kemmis, S., & McTaggart, R. (Eds.). (1988). *The action research planner* (3rd ed.). Melbourne, Australia: Deakin University Press.

	IN-TEXT REFERENCE	REFERENCE LIST
eBook – online book - If the URL leads to information about how to obtain the book, use "Available from" instead of "Retrieved from". - If there is a DOI (digital object identifier), include it instead of the 'Retrieved from' statement. A DOI is a unique, permanent identifier assigned to many electronic documents.	We found helpful information about deaf children (Niemann, Greenstein, & David, 2004) that meant we could… ***OR*** Schiraldi (2001) offers solutions to PTSD.	Niemann, S., Greenstein, D., & David, D. (2004). *Helping children who are deaf: Family and community support for children who do not hear well.* Retrieved from http://www.hesperian.org/ publications_download_deaf.php Schiraldi, G. R. (2001). *The post-traumatic stress disorder sourcebook: A guide to healing, recovery, and growth* [Adobe Digital Editions version]. doi:10.1036/0071393722
Chapter in edited book	A discussion about Australia's place in today's world (Richards, 1997) included reference to… ***OR*** Richards (1997) proposed that…	Richards, K. C. (1997). Views on globalization. In H. L. Vivaldi (Ed.), *Australia in a global world* (pp. 29-43). Sydney, Australia: Century.
Brochure – author is also publisher	The security of personal information is addressed in the TransACT brochure (TransACT, n.d.)	TransACT . (n.d.). *Guide to equipment and service* [Brochure]. Canberra, Australia: Author.
Editor	In discussing best practice, Zairi (1999) identified… ***OR*** Best practice indicators in management have been identified (Zairi, 1999) and…	Zairi, M. (Ed.). (1999). *Best practice: Process innovation management.* Oxford, United Kingdom: Butterworth-Heinemann.
Compiler, *or* Reviser, *or* Translator Use the following abbreviations after the person's name in the Reference List: Comp. Rev. Trans.	This novel by Gaarder (1991/1994) provides an appealing approach to… ***OR*** Socrates has been described as "enigmatic" (Gaarder, 1991/1994, p. 50) which provides us with…	Gaarder, J. (1994). *Sophie's world: A novel about the history of philosophy* (P. Møller, Trans.). London, United Kingdom: Phoenix House. (Original work published 1991).

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Corporate author – when the author is also the publisher Spell out the full name of the body each time it is cited in-text, unless it is long and has a familiar/easily understood abbreviation. In the latter case, give the full name with the abbreviation for the first in-text reference. Use the abbreviation only for subsequent references.	A recent study (Australian Institute of Health and Welfare [AIHW], 2009) highlighted … ***Subsequent in-text reference/s:*** The AIHW (2009) found that…	Australian Institute of Health and Welfare. (2009). *Indigenous housing needs 2009: A multi-measure needs model* (AIHW cat. no. HOU 214). Canberra, Australia: Author.
Corporate author – commissioned reports	The report prepared by the South Australian Centre for Economic Studies (2009) was discussed.	South Australian Centre for Economic Studies. (2009). *Local government's current and potential role in water management and conservation: Final report.* Commissioned by the Local Government Association of South Australia. Adelaide, Australia: Author.
No date of publication	Some aspects of forensic science are more challenging than others (Browne, n.d.) and for this reason…	Browne, J. D. (n.d.). *Forensic science as a career*. London, England: Tower.
Second or later edition	Peters (2001, p. 6) argued that "..."	Peters, T. (2001). *The elements of counselling* (2nd ed.). Brisbane, Australia: Macmillan.
Multi-volume work	Inge, Duke and Bryer (1978, p. 27) claim that there is much to learn about these writers which results in… ***OR*** There is so much to learn about our country (Clark, 1978, p. 42) that we kept returning to…	Inge, M. T., Duke, M., & Bryer, J. R. (Eds.). (1978). *Black American writers: Bibliographical essays* (Vols. 1-2). New York, NY: St. Martins. Clark, C. M. H. (1978). *A history of Australia: Vol. 4*. *The earth abideth for ever, 1851-1888.* Australia: Melbourne University Press.

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DICTIONARY / ENCYCLOPAEDIA

Dictionary / Encyclopaedia – print Include information about editions, volume numbers and page numbers in parenthesis following the title in the Reference List.	According to one definition of "bivalence" (VandenBos, 2007, p. 123)…	VandenBos, G. R. (Ed.). (2007). *APA dictionary of psychology.* Washington, DC: American Psychological Association.
Dictionary / Encyclopaedia – online Include information about editions, specific volume numbers or page numbers in parenthesis following the title in the Reference List.	A psychological overview of ADHD (Arcus, 2001)…	Arcus, D. (2001). Attention deficit / hyperactivity disorder (ADHD). In B. Strickland (Ed.), *The Gale encyclopedia of psychology.* Retrieved from http://www.gale.cengage.com/ ***Note:*** If retrieved from a database, do a Web search for the home page of the publisher of the encyclopaedia and use the URL in the reference.

JOURNAL, NEWSPAPER & NEWSLETTER ARTICLES

Journal article with one author – separated paging (paginated by issue) If each issue of a journal begins on page 1, include the issue number in parenthesis immediately after the volume number in the Reference List.	In an earlier article, it was proposed (Jackson, 2007)…	Jackson, A. (2007). New approaches to drug therapy. *Psychology Today and Tomorrow, 27*(1), 54-59.
Journal article with two authors – continuous paging throughout a volume. If the journal volume page numbers run continuously throughout the year, regardless of issue number, do **not** include the issue number in your Reference List entry.	Kramer and Bloggs (2002) stipulated in their latest article… ***OR*** This article on art (Kramer & Bloggs, 2002) stipulated that…	Kramer, E., & Bloggs, T. (2002). On quality in art and art therapy. *American Journal of Art Therapy, 40,* 218-231.

	IN-TEXT REFERENCE	REFERENCE LIST
Journal article with three to five authors For the first in-text reference, list all the authors' family names, then use the first author's family name followed by 'et al.' for subsequent entries.	A recent study to investigate the effects of an organisational stress management program on employees (Elo, Ervasti, Kuosma, & Mattila, 2008) concluded that… ***Subsequent in-text reference/s:*** (Elo et al., 2008)	Elo, A., Ervasti, J., Kuosma, E., & Mattila, P. (2008). Evaluation of an organizational stress management program in a municipal public works organization. *Journal of Occupational Health Psychology*, *13*(1), 10-23. doi: 10.1037/1076-8998.13.1.10
Journal article with six to seven authors For all in-text references, list only the first author's family name followed by 'et al.' All authors are included in the Reference List.	A simple ALMA is described in a recent study (Restouin et al., 2009).	Restouin, A., Aresta, S., Prébet, T., Borg, J., Badache, A., & Collette, Y. (2009). A simplified, 96-well–adapted, ATP luminescence–based motility assay. *BioTechniques, 47*, 871–875. doi: 10.2144/000113250
Journal article with eight or more authors For all in-text references, list only the first author's family name followed by 'et al.' In the Reference List, include the first six authors' names, then insert three ellipsis points (...), and add the last author's name.	Traumatic injury is the leading cause of death and disability worldwide (Steel et al., 2010).	Steel, J., Youssef, M., Pfeifer, R., Ramirez, J. M., Probst, C., Sellei, R., ... Pape, H. C. (2010). Health-related quality of life in patients with multiple injuries and traumatic brain injury 10+ years postinjury. *Journal of Trauma: Injury, Infection, and Critical Care, 69*(3), 523-531. doi: 10.1097/TA.0b013e3181e90c24
Journal or magazine article with no volume or issue number	Wychick and Thompson (2005) foreshadow that scam will still be enticing… ***OR*** An interesting approach to scam (Wychick & Thompson, 2005) suggested that…	Wychick, J., & Thompson, L. (2005, November 24). Fallen for a scam lately? *AustraliaToday,* 54-60.
Journal article retrieved from a database – with a DOI (Digital Object Identifier) A DOI is a unique, permanent identifier assigned to articles in many databases. **Always** include the DOI if one is provided (usually in the article's full-text, abstract or database record). If there is a DOI, no other retrieval information is necessary.	A study examining priming (Johns & Mewhort, 2009) discovered …	Johns, E., & Mewhort, D. (2009). Test sequence priming in recognition memory. *Journal of Experimental Psychology: Learning, Memory and Cognition, 35*, 1162-1174. doi: 10.1037/a0016372

	IN-TEXT REFERENCE	REFERENCE LIST
Journal article – in press	Influence of music in running performance (Lee & Kimmerly, in press) …	Lee, S., & Kimmerly, D. (in press). Influence of music on maximal self-paced running performance and passive post-exercise recovery rate. *The Journal of Sports Medicine and Physical Fitness*.
Journal article – **Cochrane Review with DOI**	Overweight and obesity are increasing throughout the industrialised world (Shaw, O'Rourke, Del Mar, & Kenardy, 2005) …	Shaw, K., O'Rourke, P., Del Mar, C., & Kenardy, J. (2005). Psychological interventions for overweight or obesity. *The Cochrane database of systematic reviews* (2). doi:10.1002/14651858.CD003818.pub2
Journal article retrieved from a database – without a DOI - If there is no DOI, do a Web search to locate the URL of the journal's home page & include it in the Reference List. The journal URL can sometimes be found in the database record or in the full text view of the article. - If the online article is ONLY available from a database (e.g. for discontinued journals where the journal home page doesn't exist), include the entry page URL of the database where it was found. Give the database name if not in the URL.	The effects of climate change on agriculture are studied by Ramalho, Da Silva and Dias (2009)… Primary care is one area marked for improvement (Purtilo, 1995).	***Example using URL of journal home page:*** Ramalho, M., Da Silva, G., & Dias, L. (2009). Genetic plant improvement and climate changes. *Crop Breeding and Applied Biotechnology, 9*(2), 189-195. Retrieved from http://www.sbmp.org.br/cbab ***Example using URL of database (where there is no journal home page):*** Purtilo, R. (1995). Managed care: Ethical issues for the rehabilitation professions. *Trends in Health Care, Law and Ethics, 10*, 105-118. Retrieved from http://www.proquest.com
Book review in a journal	In his review of Thomas Samaras' latest book, Marson (2009) identifies…	Marson, S. M. (2009). How big should we be? A Herculean task accomplished [Review of the book *Human body size and the laws of scaling: Physiological, performance, growth, longevity and ecological ramification,* by T. Samaras]. *Public Health Nutrition, 12*, 1299–1300. doi:10.1017/S1368980009990656
Newspaper article – with an author	The notion of a Bill of Rights may be inappropriate in the Australian context (Waterford, 2007).	Waterford, J. (2007, May 30). Bill of Rights gets it wrong. *The Canberra Times,* p. 11.
Newspaper article – without an author	The redesign of the Internet ("Internet pioneer", 2007) is said to…	Internet pioneer to oversee network redesign. (2007, May 28). *The Canberra Times,* p. 15.

	IN-TEXT REFERENCE	REFERENCE LIST
Newspaper article retrieved from a database Do a Web search to locate the URL of the newspaper's home page & include it in the Reference List.	In an attempt to save the tiger, Darby (2002) provided…	Darby, A. (2002, August 20). Rarest tiger skin a rugged survivor. *Sydney Morning Herald.* Retrieved from http://www.smh.com.au
Article in an online newsletter	Australia's casualty rate was almost 65 per cent - the highest in the British Empire ("Australians and the Western Front", 2009)	Australians and the Western Front. (2009, November). *Ozculture newsletter.* Retrieved from http://www.cultureandrecreation.gov.au/ newsletter/

CONFERENCE / SEMINAR PAPERS

Conference or seminar papers in published proceedings – print If the paper is from a book, use the Book chapter citation format. If it is from regularly published proceedings (e.g. annual), use the Journal article citation format.	In a paper about conservation of photographs (Edge, 1996), the proposition that…	Edge, M. (1996). Lifetime prediction: Fact or fancy? In M. S. Koch, T. Padfield, J. S. Johnsen, & U. B. Kejser (Eds.), *Proceedings of the Conference on Research Techniques in Photographic Conservation* (pp. 97-100). Copenhagen, Denmark: Royal Danish Academy of Fine Arts.
Conference or seminar papers in published proceedings – online	Tester (2008) points to the value of using geothermal sources for power and energy.	Tester, J. W. (2008). The future of geothermal energy as a major global energy supplier. In H. Gurgenci & A. R. Budd (Eds.), *Proceedings of the Sir Mark Oliphant International Frontiers of Science and Technology Australian Geothermal Energy Conference,* Canberra, Australia: Geoscience Australia. Retrieved from http://www.ga.gov.au/image_cache/ GA11825.pdf

	IN-TEXT REFERENCE	REFERENCE LIST

GOVERNMENT PUBLICATIONS

Government department as author Spell out the full name of the body each time it is cited in-text, unless it is long and has a familiar/easily understood abbreviation. In the latter case, give the full name with the abbreviation for the first in-text reference. Use the abbreviation for subsequent references.	The need for guidelines to manage and use multiple channels to deliver e-government services (Department of Finance and Administration [DOFA], 2006) presents Australian Government agencies with… ***Subsequent in-text reference/s:*** DOFA (2006) identified …	Department of Finance and Administration. (2006). *Delivering Australian Government services: Managing multiple channels.* Canberra, Australia: Author.
Government publication – with identifying number Includes report numbers, catalogue numbers, etc.	Recently released statistics from the Australian Bureau of Statistics (ABS) (2007) reveal interesting changes in Australian society. ***Subsequent in-text reference/s:*** The ABS (2007) reported that …	Australian Bureau of Statistics. (2007). *Australian social trends* (Cat. no. 4102.0). Canberra, Australia: ABS.
Government report – online	***First in-text reference:*** A recent government report (Department of the Prime Minister and Cabinet [PM&C], 2008) examines a selection of key topics … ***Subsequent in-text reference/s:*** Families in Australia were highlighted (PM&C, 2008)…	Department of the Prime Minister and Cabinet. (2008). *Families in Australia: 2008.* Retrieved from http://www.dpmc.gov.au/ publications/families/index.cfm#contact
Government approved standards	…and "including data in computer systems, created or received and maintained by an organisation" (Standards Australia, 1996, p. 7) as well as…	Standards Australia. (1996). *Australian Standard AS 4390: Records Management.* Sydney, Australia: Author.

LEGISLATION

Note: For more comprehensive information please consult the following publication: *The bluebook: A uniform system of citation* (19th ed.). (2010). Cambridge, MA: Harvard Law Review Association.

Act – print	According to s. 8.1 of the *Anti-Discrimination Act 1977* (NSW), it is unlawful for an employer to discriminate against a person on the ground of race.	*Anti-Discrimination Act 1977* (NSW) s. 8.1 (Austl.). ***Follow this convention:*** *Short Title of the Act* (in italics) *Year* (in italics) (Jurisdiction abbreviation) Section number Subdivision, if relevant (Country abbreviation).

	IN-TEXT REFERENCE	REFERENCE LIST
Bill – print	The Mental Health Bill 2013 (WA) prohibits…	Mental Health Bill 2013 (WA) (Austl.). ***Follow this convention:*** Bill Name (no italics) Year (Jurisdiction abbreviation) (Country abbreviation).
Act & Bill – online	According to s. 8.1 of the *Anti-Discrimination Act 1977* (NSW), it is unlawful for an employer to discriminate against a person on the ground of race.	*Anti-Discrimination Act 1977* (NSW) s. 8.1 (Austl.). Retrieved from http://www.legislation.nsw.gov.au/maintop/scanact/inforce/NONE/0
Case	According to Ellis v. Wallsend District Hospital (1989)… …in a land right case (*Mabo v. Queensland*, 1988)…	*Ellis v. Wallsend District Hospital* 1989 17 NSWLR 553 (Austl.). *Mabo v. Queensland* 1988 166 CLR 186 (Austl.). ***Follow this convention:*** *Case Name* (in italics) Year Volume number Reporter abbreviation First page number (Country abbreviation).

IMAGES, MUSIC & AUDIOVISUAL MEDIA

CD recording	Lyrics from Paul Kelly's song "From Little Things Big Things Grow" (Kelly, 1997, track 10) were used in recent television advertisements.	Kelly, P. (1997). From little things big things grow. On *Songs from the south: Paul Kelly's greatest hits* [CD]. Melbourne, Australia: Mushroom Records.
DVD / Videorecording	Jane Austen's world came alive in *Sense and sensibility* (Lee, 1995)	Lee, A. (Director). (1995). *Sense and sensibility* [DVD]. Australia: Columbia TriStar Home Video.

	IN-TEXT REFERENCE	REFERENCE LIST
Figure, Table, Graph, Map or Chart Cite each of these as you would for a book. Include, in square brackets, the type of entry immediately after the title: [Figure]. [Table]. [Map]. [Graph]. [Chart].	**Graph** The internal processes were well described (Kaplan & Norton, 2004) which led to… **Map** To locate a property just outside the Australian Capital Territory, use the 1:100 000 map produced by Geoscience Australia (2004) which covers…	**Graph** Kaplan, R. S., & Norton, D. P. (2004). Internal processes deliver value over different time horizons [Graph]. In *Strategy maps: Converting intangible assets into tangible outcomes* (p. 48). Boston, MA: Harvard Business School. **Map** Geoscience Australia [NATMAP] (Cartographer). (2004). *ACT region, New South Wales and Australian Capital Territory* [Map]. Canberra, Australia: Author.
Image – online	The effective use of light in Monet's 'Haystacks' (Monet, 1890)…	Monet, C. (1890). *Haystacks, midday* [Painting]. National Gallery of Australia, Canberra. Retrieved from http://artsearch.nga.gov.au/ Detail-LRG.cfm?IRN=29073&View=LRG
Liner notes	The American jazz trombonist, bandleader and composer Jack Teagarden (Weiner, 1995)…	Weiner, D. J. (1995). [Liner notes]. J. Teagarden (Composer), *Big 'T' jump* [CD]. USA: Jass Records.
Score	Craig Scott is one of Australia's leading bassists (Scott, 2013)	Scott, C. (2013). *C minor waltz: For jazz quintet* [Score]. Sydney, Australia: Craig Scott
Streamed music	An analysis of the jazz piano style of "What's Your Story Morning Glory" (Williams, 1978, track 8) reveals…	Williams, M. L. (1978). What's your story morning glory. On *Mary Lou Williams: Solo recital, Montreux Jazz Festival* [CD]. Fantasy. Retrieved from Naxos Music Library Jazz.
Interview – on radio	In a recent interview with the Prime Minister (Mitchell, 2009)…	Mitchell, N. (Presenter). (2009, October 16). Interview with the Prime Minister, Kevin Rudd. In *Mornings with Neil Mitchell* [Radio broadcast]. Melbourne, Australia: Radio 3AW.
Interview – on television	He demonstrated his professionalism and sensitivity in an interview with Raelene Boyle (Denton, 2006) and…	Denton A. (Producer and Interviewer). (2006, September 25). Interview with Raelene Boyle. In *Enough Rope with Andrew Denton.* [Television broadcast]. Sydney, Australia: Australian Broadcasting Corporation.
Motion picture (movie)	Jackson and Pyke (2003) provide evidence that belief in a world…	Jackson, P. (Director), & Pyke, S. (Producer). (2003). *The lord of the rings: The return of the king* [Motion picture]. New Zealand: Imagine Films. ***Note:*** Give the country where the movie was made – not the city.

	IN-TEXT REFERENCE	REFERENCE LIST
Podcast (audio)	Listening to the news on my MP3 player (Nolan, 2007) was a new experience and I decided…	Nolan, T. (Presenter). (2007, April 28). *AM: News & current affairs* [Audio podcast]. Retrieved from http://abc.net.au/news/ subscribe/amrss.sml
Radio program – broadcast	When discussing how people write about music, Koval (2009)…	Koval, R. (Presenter). (2009, November 19). *The Book Show* [Radio broadcast]. Melbourne, Australia: ABC Radio National.
Radio program – transcript	The views of the internationally renowned author and public speaker, De Bono, prompted me to follow up one of the interviews (Mascall, 2005) which…	Mascall, S. (Reporter). (2005, February 14). Are we hardwired for creativity? In *Innovations* [Radio program] [Transcript]. Melbourne, Australia: ABC Radio Australia. Retrieved from http://www.abc.net.au/ra/innovations/stories/s1302318.htm
Speech – online	In her ANZAC Day speech (Clark, 2007), the Prime Minister of New Zealand referred to…	Clark, H. (2007, April 25). *Prime Minister's 2007 ANZAC Day message* [Transcript]. Retrieved from http://www.anzac.govt.nz
Television advertisement	The problems of teenage anxiety were graphically captured (Beyondblue, 2009)…	Beyondblue (Producer). (2009, November 29). *Beyondblue: Anxiety* [Television advertisement]. Canberra, Australia: WIN TV.
Television program – broadcast	Examining future plans for Canberra's city area (Kimball, 2009)…	Kimball, C. (Presenter). (2009, September 4). *Stateline* [Television broadcast]. Canberra, Australia: ABC TV. ***Note:*** Always check the television station's website and use the transcript, if one is available, for direct quotes.
Television program – transcript	Cyclones often affect Australia, especially in the north (McLaughlin, 2004) and it is worthwhile…	McLaughlin, M. (Presenter). (2004, November 7). Cyclone Tracy. In *Rewind* [Television program] [Transcript]. Sydney, Australia: ABC TV. Retrieved from http://www.abc.net.au/tv/ rewind/txt/s1233697.htm

	IN-TEXT REFERENCE	REFERENCE LIST
THESIS OR DISSERTATION		
Thesis or Dissertation – print	Nurses working in an acute care environment tend to experience a high degree of workplace conflict (Duddle, 2009).	Duddle, M. (2009). *Intraprofessional relations in nursing: A case study* (Unpublished doctoral thesis), University of Sydney, Australia.
Thesis or Dissertation – retrieved from a database	The field of engineering has largely developed around the positivist philosophical position (Hector, 2008).	Hector, D. C. A. (2008). *Towards a new philosophy of engineering: Structuring the complex problems from the sustainability discourse* (Doctoral thesis). Available from Australasian Digital Theses database. (Record No. 185877) ***Note:*** End the reference with the unique number or identifier assigned to the thesis/dissertation.
Thesis or Dissertation – retrieved from the web	Lacey (2011) differentiates between instrumental violence and violence inflicting injury for its own sake.	Lacey, D. (2011). *The role of humiliation in collective political violence* (Masters thesis, University of Sydney, Australia). Retrieved from http://hdl.handle.net/2123/7128

UNIVERSITY PROVIDED STUDY MATERIALS

Lecture / tutorial notes, etc. – online	Septicaemia is one of many infections commonly acquired in hospitals (Maw, 2010) …	Maw, M. (2010). *NURS5082 Developing nursing practice, lecture 2, week 1: Healthcare-associated infections and their prevention* [Lecture PowerPoint slides]. Retrieved from http://learn-on-line.ce.usyd.edu.au/

	IN-TEXT REFERENCE	REFERENCE LIST
SOCIAL MEDIA		
Facebook update List the author's name as it is written (including nicknames).	$52 million will be provided to deploy Australian civilian troops (Rudd, 2009)	Rudd, K. (2009, October 24). Australian civilian corps to help in crises [Facebook update]. Retrieved from http://www. facebook.com/note.php?note_id=200124043571&ref=mf
Blog post - List the author's name as it is used in the posting (including nicknames). - For a blog comment, use 'Blog comment' instead of 'Blog post' and include the exact title (including 'Re:' if used)	The plight of the flapper skate was recently highlighted (Keim, 2009)…	Keim, B. (2009, November 18). ID error leaves fish at edge of extinction [Blog post]. Retrieved from http://www.wired. com/ wiredscience/2009/11/extinction-error/
Video blog post (eg YouTube)	The Prime Minister, speaking about Australia's role in the G20 forum (Rudd, 2009)…	Rudd, K. (2009, September 29). Update on new G20 arrangements [Video file]. Retrieved from http://www.youtube.com/watch?v=i8IdJ-0S5rs
Twitter tweet If the author uses their name as their Twitter 'handle', do not alter its format to follow the convention of 'Family name, Initial(s).'	President Obama announced the launch of the American Graduation Initiative (BarackObama, 2009).	BarackObama. (2009, July 15). Launched American Graduation Initiative to help additional 5 mill. Americans graduate college by 2020: http://bit.ly/gcTX7 [Twitter post]. Retrieved from http://twitter.com/BarackObama/status/2651151366 ***Note:*** This reference would be filed under 'B', not 'O'
Discussion group, list, etc. – online	There are strongly held views about knowledge management (Weidner, 2007) and from personal experience…	Weidner, D. (2007, June 11). KM reducing in popularity [Discussion list message]. Retrieved from http://actkm.org/ mailman/listinfo/actkm_actkm.org
Wiki Include the date retrieved, as the information is likely to change in these sources.	The role of media corporations in the media literacy movement is discussed ("Great debates in media literacy", n.d.)	Great debates in media literacy: Theory and practice of media literacy. (n.d.). In *Wikiversity.* Retrieved October 27, 2009, from http://en.wikiversity.org/wiki/Great_Debates_in_Media_ Literacy

	IN-TEXT REFERENCE	REFERENCE LIST

PERSONAL COMMUNICATION AND EMAIL

Personal communication Includes private letters, memos, email, telephone conversations, personal interviews, etc. These are cited in-text only, not in the Reference List.	J. Francis (personal communication, August 6, 2007) was able to confirm that the floods had not reached their area.	**Not included in Reference List. Cite in-text only.**
Email – NEVER cite addresses without permission of the owner of the address	Ms Coleman (personal communication, July 11, 2007) provided details in an email and we acted on that information.	**Not included in Reference List. Treat as personal communication and cite in-text only.**

WEB RESOURCES

Web document – author or sponsor given, dated ***Note:*** A web document is a file (e.g. a Word or PDF file) found on the Web. Often there are links to Web documents from Web pages. A Web document is not the same as a web page.	An RBA paper (Simon, Smith, & West, 2009) found that participation in a loyalty program and access to an interest-free period…	Simon, J., Smith, K., & West, T. (2009). *Price incentives and consumer payment behaviour.* Retrieved from the Reserve Bank of Australia website: http://www.rba.gov.au/ PublicationsAndResearch/RDP/RDP2009-04.html
Web document – author or sponsor given but not dated	The Commonwealth Scientific and Industrial Research Organisation (CSIRO) is designing several energy-efficient electric machines to reduce greenhouse gas emissions (CSIRO, n.d.).	Commonwealth Scientific and Industrial Research Organisation. (n.d.). *Reducing Australia's greenhouse emissions factsheet.* Retrieved from http://www.csiro.au/ resources/ps282.html

	IN-TEXT REFERENCE	REFERENCE LIST
Web page with no page numbers *Include in in-text references:* - A paragraph number with the abbreviation 'para' (count paragraphs if numbers are not visible) ***OR*** - A section heading and paragraph number (e.g. Introduction, para. 3). A long section heading may be shortened and enclosed in double quotation marks. ***Note:*** Because Web pages can be updated, you must include the date on which you accessed the source.	Usually the author or creator of a work is the copyright owner (University of Sydney, 2010, "Who owns copyright?", para. 1). ***Note:*** The heading of the section was "Who owns copyright?"	University of Sydney. (2010). *Guide to copyright*. Retrieved March 21, 2011, from http://sydney.edu.au/copyright/students/coursework.shtml#who
Web source – no author or sponsor given When there is no author for a source you find on the Web (whether it be a Web document or a Web page), the title moves to the first position of the reference entry. If the title is long, use an abbreviated version of it for in-text citations. Insert double quotation marks around the title ***Note:*** If you were citing the title of a book, periodical, brochure or report, you would use italics rather than double quotation marks.	This vaccine is 6 times more efficient than vaccines previously used to immunise against the condition ("New child vaccine", 2001).	New child vaccine gets funding boost. (2001). Retrieved April 16, 2012, from http://news.ninemsn.com.au/health/story_13178.asp
Website – entire website	The new website of the Department of Education, Employment and Workplace Relations (http://www.deewr.gov.au) includes useful information on current government education policy.	**Not included in Reference list.**

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Revised: Dec 2018

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MANUSCRIPT PREPARATION

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[1]Department of English, Yasouj University, Yasouj, Iran

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A **list** of number of **black and white / colour figures and tables** should also be indicated on this page**.** Figures submitted in color will be printed in colour. See "*5. Figures & Photographs"* for details.

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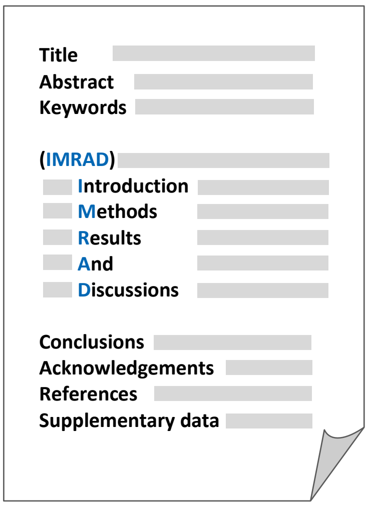

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5	*Indented, italicized, lowercase paragraph heading ending with a period.*

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Table No. (Not italic, align left)

Table Caption (Italic, vice versa with scientific names, align left)

Row and columns, with no horizontal line

Example:

Table 1
PVY infected Nicotiana tabacum *plants optical density in ELISA*

Lines No.	Plant, variety	OD values in ELISA, units		
		7th day	15th day	25th day
10	*N. tabacum,* Samsun	0,008	0,826	1,335
38	*N. tabacum,* Samsun	0,003	1,313	0,767
42	*N. tabacum,* Samsun	0,571	1,211	0,936
43	*N. tabacum,* Samsun	0,497	1,070	0,977
44	*N. tabacum,* Samsun	0,102	0,571	0,232
1000	*N. tabacum,* Samsun	0,180	0,412	0,343
-	Positive	0,865	1,021	0,912
-	Negative	0,019	0,023	0,021

Note. ELISA optical density for the samples № 38, 42, 43 exceeded the commercial positive control on the 15th day of inoculation, which was earlier than expected. It should be noted that OD markedly decreased on the 25th day of inoculation

Figure No. (*Italic*). Figure Caption (Not Italic, align left) Placed at below figure.

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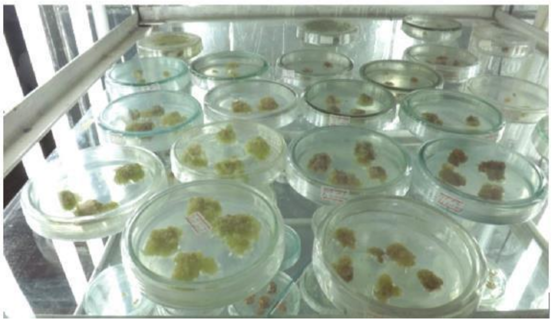

Figure 1. PVY-infected in vitro callus of *Nicotiana tabacum*

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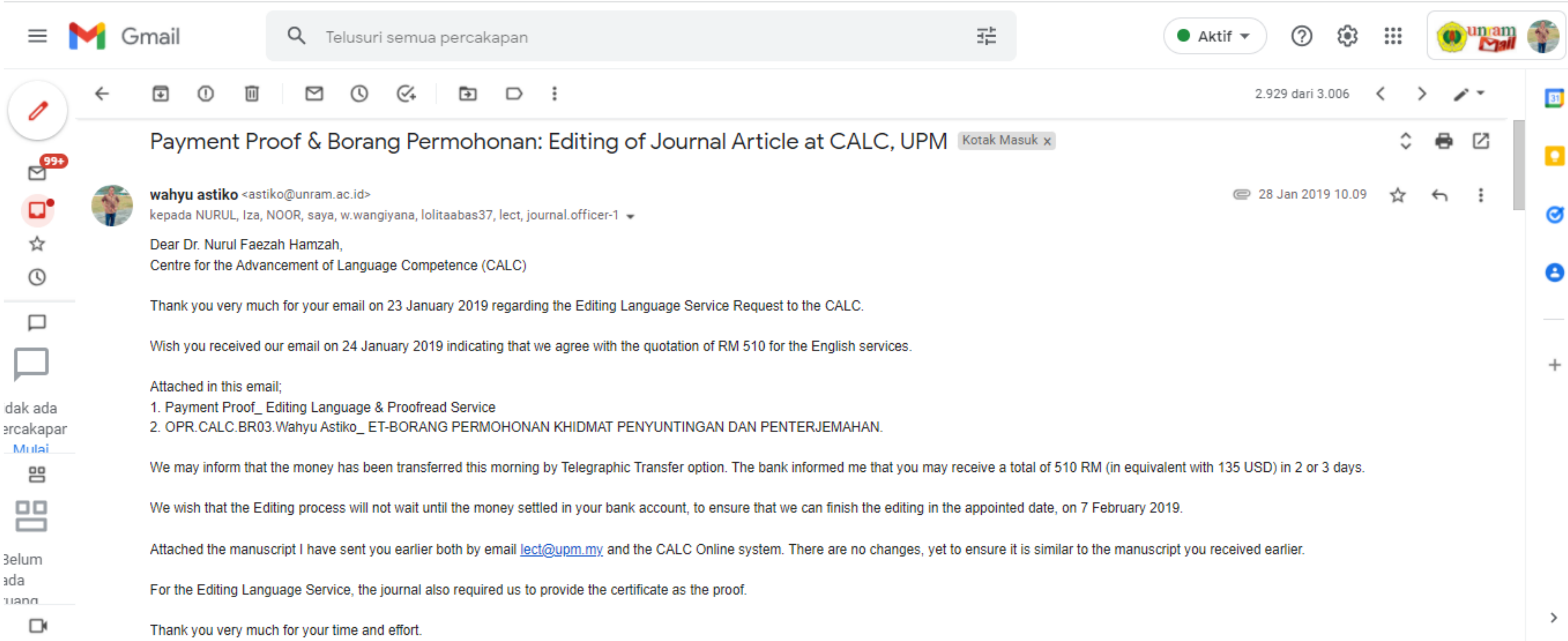

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Mycorrhizal seed-coating on maize-sorghum cropping sequence

Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-uptake and availability on maize-sorghum cropping sequence in Lombok's drylands

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The List of Number of Tables

Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-uptake and availability on maize-sorghum cropping sequence in Lombok's drylands

ABSTRACT

An indigenous arbuscular mycorrhizal fungal (AMF) is expected to improve performance of food crops in sandy and drylands of North Lombok (Indonesia) in dry seasons. To examine the benefits of mycorrhiza to the maize yield at varying doses on plant nutrition (nitrogen and phosphorus), 1 kg of the AMF inoculum was applied to 20 kg maize seeds, in different fertilization packages of cattle manure (15, 12, 9, and 6 ton/ha) and inorganic fertilizers (80, 60, 40 and 20% NPK recommended dose). A 100% NPK recommended dose only was used as the control (200 kg/ha Phonska and 300 kg/ha Urea). Sorghum seeds were planted, subsequently, at the cropping cycle 2 with no additional fertilization and inoculum applied. Results indicated that the AMF applications to the maize-sorghum cropping sequence increased the AMF colonization rate, the N and P nutrition at the soils, N and P uptake, and dry biomass (root, shoot, and grain). The highest corresponding was observed at the crops which utilized a combination of 60% NPK and 12 ton/ha cattle manure, and the performance was higher at the 100 days-after-seeding. When grown simultaneously, mycorrhizal colonization rate on sorghum roots (60-81%) was slightly higher than on maize root (55-75%). This study suggests the AMF inoculation higher the yield of maize, and improves the soil nutrient availability which was very advantageous for the growth of the following crop.

Keywords: Seed coating, arbuscular mycorrhizal fungi (AMF), maize-sorghum, cropping sequence, plant nutrition.

INTRODUCTION

Northern part of North Lombok regency (Indonesia) is dominated by drylands with sandy soils texture. With a very short and low number of rainy days per wet month or no rain during the long dry seasons, consequently, no food crops can normally be cultivated especially in the areas having no deep wells. Moreover, an inadequate phosphorus (P) availability is also one of the factors limiting the productivity of maize and other food crops in the dryland of North Lombok. The requirement of maize crop for P is very high, i.e. for the production of 10 ton/ha, maize crops need 102 kg/ha of P_2O_5 and 76% of it is transported into the seeds (Calderón-Vázquez et al., 2009). In contrast, plant roots can take up P only about 8-13% of the amount of P fertilizer applied (Supardi, 1996). Thus, to cope the unavailability of soil water and P and other essential nutrients in drylands of North Lombok, arbuscular mycorrhizal fungal (AMF) symbiosis is expected to improve performance of food crops especially during the dry seasons. Many have reported that the soil fungi, in symbiosis with their host plants, can help the plants to improve water relation and makes their host plants more tolerant to drought (Augé, 2004). The AMF colonization also increases nutrient uptake from soils and enhance growth and yield of the host plants, although it depends on the species of AMF colonizing the host plants (Marulanda et al., 2003).

The most common findings show that the external hyphae of AMF improves the P uptake and transport the nutrient to root cortex of their host (Azcón et al., 2003; Feng et al., 2003; George et al., 1995; Koide & Kabir, 2000; Tarafdar & Marschner, 1994; Tawaraya et al., 2006). Although there is no growth improvement of the host plants due to the AMF symbiosis, the amount of P uptake through AMF (mycorrhizal pathway) can be higher than the movement through the host roots or the direct pathway (Smith & Read, 2010). AMF can also help their hosts in increasing the uptake of other essential nutrients such as N, K, Ca, Mg, S, Cu, Zn, Mn, Fe, and B, when compared with non-mycorrhizal plants (Dhillion & Ampornpan, 1992; Hawkins et al., 2000; Leigh et al., 2009; Menge, 1983). Most of these capabilities of the AM plants are due to the extensive growth of AMF external hyphae beyond the host roots (Drew et al., 2003; Zhu et al., 2001). The length ratio of AMF hyphae to roots in soil can be 100:1 or higher (George et al., 1995), and with the external hyphae, the mycorrhizal roots can explore further, 10-100 times more volume of soils compare the non-mycorrhizal roots (Sieverding et al., 1991). By applying the Plenchette's mycorrhizal dependency formula to the shoot dry weight of maize and sorghum (Plenchette et al., 1983), the results showed a high mycorrhizal dependency, both for maize (up to 70.12%) and for sorghum (up to 79.42%) (Guo et al., 2013; Tawaraya, 2003). The study indicates that the higher the dependent rate on AMF symbiosis, the more the dry matter produced of the crops. With the high porosity and low water retention capacity on dryland and sandy areas (Bruand et al., 2005) in most areas of North Lombok, the levels of crop dependence on symbiosis with AMF could be higher. Moreover, the symbiosis of crops with AMF could significantly reduce nutrient loss from soil through leaching (Cavagnaro et al., 2015).

Establishment of AM symbiosis can be done through inoculation with AMF propagules. Our previous study orchestrates that the indigenous AMF inoculation in maize plants in sandy soils presents positive implications for the improvement of soil properties by increasing the rates of nutrient uptake by maize crop from the soil and improving its grain yield (Astiko et al., 2013a). Our research group have also shown the benefit of this local inoculation in increasing the soybean crops grown and yield by improving the P uptake from the soils, compared with those without AMF inoculation (Astiko et al., 2013b). However, as found from other studies, the development of AMF in the soil and its contribution to growth, yield and nutrient uptake of host plants could be influenced by the order of plant species cultivated in sequence in the cropping system (Johnson et al., 1992; Vivekanandan & Fixen, 1991). Those two studies reported that the P uptake and the AMF colonization were higher on maize crops grown following soybean compared with those grown following maize or barley.

From the previous study on maize-soybean cropping patterns, it was found that the AMF inoculated in maize crops grown at the first cycle increased root colonization and AMF sporulation which was very advantageous for the growth of the following crops in the cropping sequence (Astiko, 2013). Different AMF populations (colonization and spore counts) were also found between cropping seasons or between crop species in the same cropping season in Central Lombok, Indonesia (Wangiyana et al., 2006). This present study examines the effects of several combinations of fertilizer packages consisting of indigenous AMF bio-fertilizer and varying doses of organic and NPK fertilizers applied to maize crops on N and P status in soil and uptake by maize and by the subsequent sorghum crops, as well as growth and yield components of the maize and sorghum crops in a maize-sorghum cropping sequence on drylands in North Lombok, Indonesia.

MATERIALS AND METHODS

Design of the experiment

The field experiment of maize-sorghum cropping sequence in this study was established in the Akar-Akar village located in North Lombok regency, Indonesia, from January to August 2016, which was arranged according to the Randomized Complete Block Design (RCBD) with four replications (blocks). The treatments were five fertilizer packages consisting of different combinations of organic, inorganic and indigenous AMF bio-fertilizer, which were applied only to the maize crop in the first cropping cycle of the maize-sorghum cropping sequence. The details of the treatments or fertilization packages are listed in Table 1.

Table 1. ***The mycorrhizal-based fertilization packages tested, and applied to maize only in the maize-sorghum cropping sequence***

Fertilization packages	Doses of the packages applied to maize plants only (in the cropping cycle 1)	Sorghum (cropping cycle 2)
D_0	NPK only at 100% the recommended doses (RD), i.e. 200 kg/ha Phonska (NPK 15:15:15) and 300 kg/ha Urea	No fertilizer applied
D_1	NPK at 80% RD + 15 ton/ha cattle manure + AMF	No fertilizer applied
D_2	NPK at 60% RD + 12 ton/ha cattle manure + AMF	No fertilizer applied
D_3	NPK at 40% RD + 9 ton/ha cattle manure + AMF	No fertilizer applied
D_4	NPK at 20% RD + 6 ton/ha cattle manure + AMF	No fertilizer applied

A piece of land used in this study was a sandy soil type (69% sand, 29% silt, and 2% clay, containing an average of 421 AMF spores per 100 g soil), typical of North Lombok area. The land is located at the geographic position of -8.221650, 116.350283. After being cultivated using minimum tillage and cleaned from weeds, the land was split into 20 treatment plots of 7m x 5m based on the RCBD plotting layout. The different fertilization packages are consisting of the AMF inoculum, organic fertilizer (cattle manure) and inorganic fertilizer (NPK and Urea), were applied only to the maize crop in the first cropping cycle.

An indigenous AMF inoculum, i.e. the M_{AA01} mycorrhizal isolate, which was originally isolated from dryland area in Akar Akar village of North Lombok, was applied through seed-coating of maize seeds prior to seeding. Before application, 1 kg of the AMF inoculum was mixed with charcoal powder and tapioca flour as the carrier materials, then this inoculum mixture was mixed with 20 kg maize seeds so that the seeds were coated by the inoculum mixture. For the cropping cycle 1, the uncoated or AMF coated maize seeds (“Bisma” variety) were planted by dibbling 2 seeds per planting hole at 70 cm x 20 cm plant spacing. The entire dose of cattle manure (organic fertilizer) at different doses depending on the treatments (Table 1) was applied at the time of planting the maize seeds by burying the whole dose in the planting hole in the position below the seeds. The inorganic (NPK) fertilizers with the recommended doses of 200 kg/ha Phonska (NPK 15:15:15) and 300 kg/ha Urea (46% N) were applied twice, i.e. at 7 DAS (days after seeding) and at 21 DAS, with the amount applied depending on the treatments (Table 1). At 7 DAS, the maize plants were fertilized with a mixture of the entire Phonska and one third of the Urea treatment doses, followed by application of the remaining Urea treatment doses at 21 DAS. The NPK fertilizers were applied in a furrow of 5 cm alongside the maize plant row at 5-7 cm depth and then were covered with soil.

For the cropping cycle 2, the plots were cleaned from the maize crop debris and weeds; then seeds of sorghum ("Numbu" variety) were direct seeded using dibbling 2 seeds per planting holes made around the maize stubbles. The sorghum plants were not fertilized nor inoculated with AMF inoculum. For both crops, the young maize and sorghum plants were tinned at 7 DAS by leaving one maize or sorghum plant per planting hole. Weeding and soil piling of the maize and sorghum plant base were done at 15 and 30 DAS. Plant protection was done by spraying "OrgaNeem" (an organic pesticide of plant origin containing Azadirachtin extracted from neem leaves) at a concentration of 5 ml OrgaNeem per liter of water. This OrgaNeem solution was applied to both crops (maize or sorghum) from 10 to 60 DAS at 3 days intervals. Harvesting of maize or sorghum crops was done at 100 DAS.

Measurement and data analysis

The variables measured were AMF development, N and P nutrition, and growth and yield of maize and sorghum. The AMF development includes spore counts at 60 and 100 DAS, and root colonization levels at 60 DAS. The N and P nutrition include concentration of total N and available P in the rhizosphere of maize and sorghum at 60 and 100 DAS. The crop variables include dry weight (shoots and roots) and yield components (grain).

Determination of N was by Kjeldhal, and P by spectrometer. AMF spore extraction from soil (100 g soil sample) was done using wet sieving and decanting technique (Brundrett et al., 1996). The spores collected from the 38 μm sieve after the final centrifugation were captured in a filter paper, which were then observed on a Petri dish using a stereo microscope at 40 x magnification, and counted as spore number per 100 g of soil. The percentage of root colonization was determined using the Gridline Intersect technique (Giovannetti & Mosse, 1980) under a stereo microscope after the root pieces were stained using the clearing and staining method of Brundrett et al. (1996).

Data were analyzed using analysis of variance (ANOVA) and the Tukey's HSD (Honestly Significant Difference) means tested at 5% level of significance.

RESULTS AND DISCUSSION

AMF development

In terms of root colonization rates by AMF, there were slight differences between maize and sorghum between treatments of fertilizer packages. This can be seen from Table 2 orchestrating the levels of root colonization by the indigenous AMF are slightly higher on sorghum roots than on maize roots in each treatment, especially in the D3 treatment, it was 77% on sorghum and 65% on maize roots. When grown simultaneously, Carrenho et al. (2007) found higher colonization rates on maize than on sorghum (Carrenho et al., 2007). In this study, however, maize and sorghum were grown in sequence, in which sorghum was direct seeded without treatments and without tillage following harvest of maize plants. All treatments, including AMF inoculation, were applied only to maize plants in the first cropping cycle. Therefore, it means that sorghum in the cropping cycle 2 was grown after AMF propagules have built up in the soil during the growth of the maize plants in the cropping cycle 1. This is in line with the results reported in previous study revealing that AMF root colonization and shoot dry weight of maize at 58 DAS were affected by previous crops, whether they were host or non-host of AMF (Arihara & Karasawa, 2000), however the P fertilization did not affect root colonization, especially on maize following AMF host plants.

In more details, the degree of root colonization by AMF may higher in the roots of maize fertilized with highly soluble mineral fertilizers (NPK, diammonium phosphate, ammonium sulfate and Urea), in the other hand, the length of extra-radical mycelium and number of AMF spores were significantly higher in maize fertilized with organic fertilizer (Bokashi manure) (Bautista-Cruz et al., 2014). This indicates that organic fertilization is more favorable for spore production compared with conventional fertilization. It can also be seen from Table 2 that AMF spore numbers were higher at harvest of the maize crop (100 DAS) compared with those at 60 DAS, especially on the D_2 treatment, indicating a high build up of AMF propagules for the subsequent sorghum crop. This condition seems to favor higher AMF colonization levels in roots of sorghum in the second cropping cycle compared with that of maize in the first cropping cycle. With no tillage treatment, the AMF colonization levels in sorghum may higher than in maize or bean roots (Alguacil et al., 2008). AMF colonization rates may also higher in roots of sorghum than maize, either inoculated with *Glomus mosseae* or *G. versiforme*, indicating that sorghum is more favorable for AMF development than maize plants (Guo et al., 2013).

There are many factors influencing the degrees of AMF colonization of crop roots as reported by Carrenho et al. (2007). In examining the effects of different phosphorus, liming, organic matter, and soil texture on root colonization of peanuts, sorghum and maize in a factorial experiment, each factor significantly affected root colonization level, alone or in interaction with other factors (Carrenho et al., 2007). The most surprising results were the significant interaction effects of plant, phosphorous and organic matter although there were no significant effects of phosphorous (simple superphosphate of 166.6 mg/L (w/v) soil) nor organic matter (22 mg/L (w/v) soil. In this interaction effect, for maize, there was no significant effect of phosphorous and organic matter on AMF colonization of maize roots. On the other hand, the application of both phosphorous and organic matter significantly reduced AMF colonization on sorghum roots (Carrenho et al., 2007).

Table 2. ***Mean spore number (spores per 100 g soil) at 60 and 100 DAS, and colonization rates (%-colonization) on maize and sorghum in a maize-sorghum cropping sequence***

Fertilization packages	AMF on maize (1st crop)			AMF sorghum (2nd crop)		
	Spore per 100 g soil		% colonization	Spore per 100 g soil		% colonization
	60 DAS	100 DAS	60 DAS	60 DAS	100 DAS	60 DAS
D_0	764^e	3464^d	30^d	1231^e	3761^d	35^e
D_1	1059^d	3672^c	55^c	1343^d	4942^b	60^d
D_2	2119^a	4327^a	75^a	2981^a	5165^a	81^a
D_3	1690^b	3894^b	65^b	1881^b	4831^c	77^b
D_4	1294^c	3881^b	63^b	1769^c	4819^c	68^c
HSD 5%	231	13	2.0	109	12	6.5

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages.

In terms of fertilization effects, AMF colonization rate was reported higher on crop roots in unfertilized soils, followed by organic and biodynamic, and the least in exclusively mineral fertilized and conventional farming systems; and they concluded that the amount of soluble P in the soil was the most limiting factor for AMF colonization in roots of those crops (winter wheat, vetch-rye and grass-clover) (Mäder et al., 2000). Moreover, it was reported that the increasing P level in the growing media from 0.1 to 0.5 mM P significantly reduced AMF colonization in roots of lettuce plants especially with increasing levels of N contents (1, 5 or 9 mM N) (Azcón et al., 2003).

A negative impact of soil condition will start to occur when the accumulation of soil P has increased beyond requirement of the crops cultivated (Grant et al., 2005). In the study we report here, it seems that the amount of P input from the NPK fertilizer as applied in the D_2 treatment is most favorable for AMF development in maize crops. In D_2 treatment, the NPK fertilizer was reduced to 120 kg/ha Phonska (60% of the recommended NPK dose of 200 kg/ha Phonska in the D_0 treatment) combined with an application of 12 ton/ha cattle manure and inoculation with AMF. Among the treatments with AMF in combination with cattle manure, the D_1 treatment had the highest NPK fertilizer dose (Table 1), and this level of NPK fertilization seems to limit the AMF infection and development in maize roots compared with those in the D_2 treatment, resulting in a higher AMF colonization rates on D_2 than on D_1 treatment. Therefore, AMF colonization and spore numbers in maize were highest in the D_2 treatment, especially when compared with the D_1 or D_0 treatment. Using the same indigenous AMF applied to a pot experiment in which the soil for the growing media was taken from the same field in North Lombok, it was found that the highest level of AMF colonization of maize roots was in the treatment with AMF combined with cattle manure compared with AMF combined with NPK fertilizer or phosphate rock (Astiko et al., 2013a). The previous study also reported the significantly higher AMF spore number in plots fertilized with cattle manure or cattle manure combined with mineral fertilizers compared with those fertilized with mineral fertilizers only (Gryndler et al., 2006).

Soil nutrient status and nutrient sorption by maize and sorghum

There were significant effects of the different fertilizer packages on soil nutrient status (N and P contents of the soil) of the rhizosphere of both maize and sorghum crops at 60 and 100 DAS (Table 3). In general, there is a tendency that the highest values of the soil nutrient status, measured either at 60 or 100 DAS, are highest under the D_2 (60% NPK + 12 t/ha manure + AMF) or D_1 (80% NPK + 15 t/ha manure + AMF) treatment. The values of the soil nutrient status in these treatments are higher than those in D_0 (100% NPK only) treatment, although the dose of NPK fertilizers was reduced in the D_1 and D_2 treatments (Table 3).

Table 3. ***Mean concentration of total N and available P of soil in the rhizospheres of maize (1st crop) and sorghum (2nd crop) for each treatment of fertilization packages, measured at 60 and 100 DAS***

Fertilization packages	Total N ($g.kg^{-1}$) at 60 DAS		Available P ($mg.kg^{-1}$) at 60 DAS		Total N ($g.kg^{-1}$) at 100 DAS		Available P ($mg.kg^{-1}$) at 100 DAS	
	maize	sorghum	maize	sorghum	maize	sorghum	maize	sorghum
D_0	1.24^{c}	1.10^{c}	14.97^{d}	11.41^{d}	1.33^{d}	1.23^{d}	17.43^{d}	10.15^{d}
D_1	1.45^{a}	1.29^{a}	28.44^{b}	16.25^{b}	1.69^{b}	1.38^{b}	29.51^{b}	21.58^{b}
D_2	1.47^{a}	1.25^{ab}	35.02^{a}	28.49^{a}	1.86^{a}	1.48^{a}	36.56^{a}	29.99^{a}
D_3	1.39^{b}	1.22^{ab}	17.52^{c}	14.59^{bc}	1.47^{c}	1.31^{c}	19.37^{c}	18.59^{c}
D_4	1.33^{b}	1.20^{b}	16.29^{c}	14.25^{c}	1.42^{c}	1.31^{c}	18.53^{c}	17.32^{c}
HSD 5%	0.08	0.07	1.31	1.99	0.08	0.06	1.0	2.98
Before planting [1)]	1.20	-	12.28	-	-	-	-	-

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages.

Since the NPK fertilizers applied, i.e. Phonska and Urea at 7 DAS and Urea at 21 DAS, are all easy to get dissolved in water, significant amount of its nutrients could have been loss through infiltration during rainy season. Previous study shows the sand content of the cultivated land has a positive correlation with infiltration rate, with an r-value of +0.75 (Patle et al., 2018). Since cattle manure is slow in releasing nutrients, it is possible that, at the time of measurement (60 and 100 DAS), the loss of released nutrients from the NPK fertilizers through leaching has been much higher in the D_0 than in the other treatments of fertilization packages due to dissolution by the rain water during that rainy season. However, there were small increases in nutrient status of the soil from 60 to 100 DAS around the rhizospheres of maize crop, especially in those treated with manure and AMF application, indicating some slow release of N and P nutrients from manure after application to the maize plants in the first cropping cycle, which could be due to AMF inoculation. Many have reported that AMF can mobilize and take N and P from organic matter for their hosts (Feng et al., 2003; Hawkins et al., 2000; Koide & Kabir, 2000; Leigh et al., 2009; Tarafdar & Marschner, 1994), and it can be seen from Table 2 that the highest average of AMF colonization levels in maize roots was in the D_2 treatment. Therefore, N and P uptake in shoots of maize and sorghum are highest in the D_2 treatment (Table 4).

In addition, soil contents of those nutrients were also in a good corresponding with the levels of N and P uptake of the crops, i.e. all showing positive correlation coefficients, although it is not significant for P uptake, but the highest values of N and P uptake are also in the D_2 treatment, both for maize and sorghum (Table 4). Based on correlation analysis of the mean values obtained at 60 DAS, there is a significant correlation between soil N and N sorption in the shoots, with a value of r = + 0.970 (R-square = 94.1%, p = 0.006) for maize plants, and r = + 0.904 (R-square = 81.7%, p = 0.035) for sorghum plants. These indicate some contributions of those fertilizers in the packages to nutrient contents of the crops, which are significantly different between treatments of fertilization packages (Table 4).

Table 4. ***Mean N and P sorption (mg.g^{-1} plant dry weight) by each crop at 60 DAS for each treatment of fertilization packages***

Fertilization packages	N and P uptake (mg.g^{-1} plant dry weight) by each crop at 60 DAS			
	Maize (1st cropping cycle)		Sorghum (2nd cropping cycle)	
	N	P	N	P
D_0	18.62^{e}	1.71^{d}	12.74^{d}	0.65^{d}
D_1	27.58^{b}	2.60^{b}	18.92^{b}	0.85^{c}
D_2	28.00^{a}	2.72^{a}	20.86^{a}	1.48^{a}
D_3	23.10^{c}	2.41^{c}	17.29^{c}	1.36^{b}
D_4	20.44^{d}	2.41^{c}	17.22^{c}	1.32^{b}
HSD 5%	0.41	0.11	0.07	0.04

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages.

However, P status of the soil did not show a significant correlation with P uptake either by maize or sorghum crop. In spite of that, there is a significant positive correlation between the degree of AMF colonization in the roots and P sorption in the shoot of maize plants, with an r = + 0.909 (R-square = 82.6%, p = 0.032), and that of sorghum plants, with an r = + 0.940 (R-square = 88.4%, p = 0.017). These mean that there were significant contributions of AMF colonization in the roots to the amount of P taken up by the plants, both maize and sorghum. If soil soluble P has not exceeded crop requirements, AMF association will still result in significantly positive effects on P uptake and biomass production, because of P requirements of the crops; and for optimum growth and yields, crops require P since the early stage of their growth, either from soil, fertilizer application or from AMF associations (Grant et al., 2005).

Although sorghum crop in the second cropping cycle was not fertilized with manure nor NPK fertilizers, direct seeding of sorghum after harvesting the maize plants without tillage seems to be a favorable condition in establishing AMF association in the sorghum crop, which resulted in higher AMF colonization rates in sorghum than in maize roots for each fertilizer package (Table 2). These AMF associations seem to enable the sorghum crops to absorb adequate P and other nutrients from soil and residues of the manure applied in the first cropping cycle even though sorghum crops were not fertilized. This could occur because of the potential of AMF external hyphae to help the host plants in absorbing and dissolving nutrients from soil and manures and other nutrient pools, as well as absorbing other nutrients unavailable for uptake by plant roots (Menge, 1983; Smith & Read, 2010; Tawaraya et al., 2006). Guo et al. (2013) also reported significant effects of AMF inoculation on C, N, P and K contents (mg/pot) in shoot and roots of maize and sorghum grown in pots filled with mixtures of mine tailing and topsoil, and between the AMF species used, *G. versiforme* showed higher colonization rates resulted in higher nutrient sorption and biomass of maize and sorghum compared with *G. mosseae* (Guo et al., 2013).

Biomass and yield components of maize and sorghum

In terms of biomass production and yield components of maize and sorghum, there were also significant effects of the fertilization packages which D_2 treatment presents the highest values of biomass production (Table 5) and yield components (Table 6). This means that nutrient status of the soils was in a good corresponding with biomass weight of the crops, indicated by the positive correlation coefficients, although only some of them are significant. The AMF applications to maize crop, observed at the 60 DAS, orchestrate a significant correlation to dry shoot weight, N and P status of the soil, and the N and P sorption. In contrast, AMF colonization levels of maize roots only show significant correlation with root dry weight at 60 DAS, with an r = + 0.912 (R^2 = 83.2%, p= 0.031), and shoot dry weight at maturity (100 DAS), with an r = + 0.892 (R^2 = 79.6%, p= 0.042). This could mean that during the vegetative growth, AMF colonization maybe focused to improve root growth in order to increase nutrient sorption during the vegetative growth of maize crop.

Table 5. ***Mean dry biomass weight (g/plant) of maize and sorghum for each treatment of fertilization packages***

Fertilization packages	Dry biomass weights (g/plant) of maize (1st crop) and sorghum (2nd crop)							
	Maize root		Maize shoot		Sorghum root		Sorghum shoot	
	60 DAS	100 DAS	60 DAS	100 DAS	60 DAS	100 DAS	60 DAS	100 DAS
D_0	8.13^d	10.43^e	34.15^d	62.29^e	0.82^d	12.30^d	8.31^d	47.74^e
D_1	15.13^b	22.39^b	61.92^b	109.03^b	2.22^b	22.34^b	16.89^b	95.01^b
D_2	17.12^a	34.56^a	72.86^a	111.25^a	3.37^a	24.44^a	23.53^a	105.14^a
D_3	13.65^c	18.48^c	52.26^c	101.05^c	1.68^c	14.52^c	12.01^c	85.46^c
D_4	13.34^c	15.43^d	51.29^c	95.87^d	1.38^c	14.47^c	11.81^c	66.06^d
HSD 5%	0.31	3.04	0.97	2.21	0.30	0.05	0.20	9.54

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages.

In relation to nutrient uptake, although AMF colonization levels did not show significant correlation with N uptake in shoots of maize or sorghum, they showed positive significant correlation with P uptake both in shoots of maize and sorghum, with an r = + 0.909 (R^2 = 82.6%, p= 0.032) for maize, and r = + 0.940 (R^2 = 88.4%, p= 0.017) for sorghum. These could be due to the ability of AMF extra-radical hyphae in mobilizing and absorbing P from organic matter for their hosts (Feng et al., 2003; Koide & Kabir, 2000; Tarafdar & Marschner, 1994). Based on the strength of the relationships between AMF colonization levels and P uptake, the value of R^2 is higher in sorghum (R^2 = 88.4%) than in maize (R^2 = 82.6%), which means that contribution of AMF colonization in roots to P uptake in shoots was higher in sorghum crop at the second cropping cycle than in maize in the first cropping cycle. Since sorghum did not receive any fertilizers, and cattle manure is a nutrient slow-releasing organic matter, it could mean that most P nutrient contained in the shoots, especially in shoots of sorghum in the second cropping cycle, was mostly taken up from the manure under contribution of AMF colonization in the roots, although this still needs to be confirmed with further research. This view is supported by the conditions of the study area, which is dominated by sand, and if leaching happened during rainy season, the loss of the dissolved nutrients from the NPK fertilizers applied in the first cropping cycle could be high. Therefore, the main source of nutrients, especially for sorghum crop in the second cropping cycle is cattle manure applied to the maize crop in the first cropping cycle.

Table 6. ***Mean weights of total dry grains (kg/plot) and 100 dry grains for each crop and each treatment of fertilization packages***

Fertilization packages	Mean dry grain yield (kg/plot) and weight of 100 dry grains (g)			
	Maize in the 1st cropping cycle		Sorghum in the 2nd cropping cycle	
	Grain yield	100 grains	Grain yield	100 grains
D_0	9.60^e	22.48^d	3.57^d	2.73^d
D_1	17.40^b	26.94^b	5.05^b	3.01^b
D_2	22.80^a	28.12^a	6.65^a	3.61^a
D_3	15.60^c	25.98^c	4.43^c	2.90^c
D_4	10.20^d	24.61^c	4.17^c	2.81^{cd}
HSD 5%	0.59	1.37	0.26	0.09

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages.

In relation to nitrogen nutrient, there was a very low and non-significant correlation between AMF colonization levels and N uptake in maize shoots with an R^2 of only 41.2%, however, the N uptake of maize at 60 DAS was highly significantly correlated with the N status of the soil with an R^2 = 94.1% (p= 0.006). Moreover, the N soil availability also showed a significant correlation with biomass and grain yield of maize, i.e. with an R^2 = 84.1% (p= 0.029) with grain yield at 60 DAS, R^2 = 92.2% (p= 0.009) with shoot dry weight at 60 DAS, and R^2 = 90.6% (p= 0.012) with shoot dry weight at 100 DAS.

In contrast, for sorghum in the second cropping cycle, soil N status at 60 DAS showed a significant correlation only with shoot dry weight at 100 DAS. Unlike in maize crop where

AMF colonization levels showed a significant positive correlation with shoot dry weight at maturity (100 DAS), AMF colonization levels in sorghum roots did not show any significant correlation with biomass weight nor yield components of sorghum. However, AMF colonization rates in sorghum roots showed a sufficiently high correlation with N uptake in sorghum shoots, with an R^2 = 71.9% (p= 0.069), and N uptake in sorghum shoots showed positive significant correlation with biomass and grain yield of sorghum, i.e. with an R^2 = 80.5% (p= 0.039) with grain yield, R^2 = 83.0% (p= 0.031) with shoot dry weight at 60 DAS, and R^2 = 89.9% (p= 0.014) with shoot dry weight at 100 DAS. These could mean that for maize crop, most nutrients derived from NPK fertilizers while for sorghum, N and P nutrients were derived from the residues of manure with contribution from AMF colonization in sorghum roots. Many researchers have also showed the ability of AMF to utilize organic sources to supply N to their host (e.g. Hawkins et al., 2000; Leigh et al., 2009), although it was also found that AMF colonization did not necessarily resulted in significantly higher N status of the mycorrhizal than non-mycorrhizal hosts (Hawkins et al., 2000).

However, when correlation analysis was done between averages of colonization levels, biomass and grain yield between maize in the first and sorghum in the second cropping cycle, the results mostly show significant positive coefficients of correlation. For example, correlation of AMF colonization levels between roots of maize and sorghum is highly significant, with an r = + 0.988 (R^2 = 97.6%, p= 0.002), which means that the pattern of differences in AMF colonization levels between roots of maize and sorghum is highly similar. In other words, AMF colonization levels in roots of maize in the first cropping cycle were carried over into the second cropping cycle where sorghum was grown as the rotation crop. This is because both maize and sorghum are hosts of AMF, and both crops have a high mycorrhizal dependency (Guo et al., 2013).

In addition, when a t-test of “Paired Two Sample for Means” was run on the averages of AMF colonization levels and spore number in soil at maturity of the crops between maize and sorghum in Table 2, there were higher significant values (p<0.01) on sorghum than on maize. This could be due to some build up of AMF in the soil after harvest of the maize crop in the first copping cycle before sorghum was direct seeded without tillage. Even both crops were grown simultaneously, it was also found that AMF colonization level was higher on sorghum than on maize (Guo et al., 2013). This means that sorghum can be used to improve mycorrhizal conditions of the soil under a maize-sorghum cropping sequence.

CONCLUSION

Among the mycorrhiza-based fertilization packages tested on the maize-sorghum cropping sequence system at the drylands in North Lombok of Indonesia, the D_2 package, consisting of 60% NPK recommended dose, 12 t/ha cattle manure, and the indigenous AMF inoculation, was found to be the best fertilization package for improving the crop yield and soil nutrient availability. This study noted that the AMF development was higher in the sorghum at the second cropping cycle compared to the growth in the maize at the first cropping cycle. This condition led to the higher NP-uptake and soil nutrient availability in the following crops, at the sandy and dry land, with no additional fertilization and mycorrhizal propagules applied.

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Mycorrhizal seed-coating on maize-sorghum cropping sequence

Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-uptake and availability on maize-sorghum cropping sequence in Lombok's drylands

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The List of Number of Tables

Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-uptake and availability on maize-sorghum cropping sequence in Lombok's drylands

ABSTRACT

An indigenous arbuscular mycorrhizal fungal (AMF) is expected to improve performance of food crops in sandy and drylands of North Lombok (Indonesia) in dry seasons. To examine the benefits of mycorrhiza to maize yield at varying doses on plant nutrition (nitrogen and phosphorus), 1 kg of the AMF inoculum was applied to 20 kg maize seeds in different fertilization packages of cattle manure (15, 12, 9, and 6 ton/ha) and inorganic fertilizers (80, 60, 40 and 20% NPK recommended dose). A 100% NPK recommended dose was used as the control (200 kg/ha Phonska and 300 kg/ha Urea). Sorghum seeds were then planted in cropping cycle 2 with no additional fertilization and inoculum applied. Results indicated that the AMF applications to the maize-sorghum cropping sequence increased the AMF colonization rate, the N and P nutrition at the soils, N and P uptake, and dry biomass (root, shoot, and grain). The highest correspondence was observed in the crops which utilized a combination of 60% NPK and 12 ton/ha cattle manure, and the performance was higher at day 100 after seeding. When grown simultaneously, mycorrhizal colonization rate on sorghum roots (60-81%) was slightly higher than the rate seen on maize root (55-75%). This study suggests that AMF inoculation maize yield and improves soil nutrient availability which is very advantageous for the growth of the subsequent crop.

Keywords: Seed coating, arbuscular mycorrhizal fungi (AMF), maize-sorghum, cropping sequence, plant nutrition

INTRODUCTION

The northern part of North Lombok regency (Indonesia) is dominated by drylands with sandy soils texture. With a very short and low number of rainy days per wet month or no rain during the long dry seasons, no food crops can be cultivated normally especially in the areas without deep wells. Moreover, inadequate phosphorus (P) availability is also one of the factors limiting the productivity of maize and other food crops in the dryland of North Lombok. Maize crop requires very high P; for example, for the production of 10 ton/ha, maize crops need 102 kg/ha of P_2O_5 and 76% of it is transported into the seeds (Calderón-Vázquez et al., 2009). In contrast, plant roots can take up P at only about 8-13% of the amount of P fertilizer applied (Supardi, 1996). Thus, to cope with the unavailability of soil water and P and other essential nutrients in drylands of North Lombok, arbuscular mycorrhizal fungal (AMF) symbiosis has been used as it is expected to improve the performance of food crops especially during the dry seasons. Many have reported that the soil fungi, in symbiosis with their host plants, can help plants to improve water retention and make their host plants more tolerant to drought (Augé, 2004). AMF colonization also increases nutrient uptake from soils and enhances growth and yield of the host plants although it depends on the species of AMF colonizing the host plants (Marulanda et al., 2003).

The most common findings show that the external hyphae of AMF improve P uptake and transport the nutrient to root cortex of their host (Azcón et al., 2003; Feng et al., 2003; George et al., 1995; Koide & Kabir, 2000; Tarafdar & Marschner, 1994; Tawaraya et al., 2006). Although there is no growth improvement of the host plants due to the AMF symbiosis, the amount of P uptake through AMF (mycorrhizal pathway) can be higher than through the host roots or the direct pathway (Smith & Read, 2010). AMF can also help their hosts to increase the uptake of other essential nutrients such as N, K, Ca, Mg, S, Cu, Zn, Mn, Fe, and B when compared with non-mycorrhizal plants (Dhillion & Ampornpan, 1992; Hawkins et al., 2000; Leigh et al., 2009; Menge, 1983). Most of these capabilities of the AM plants are due to the extensive growth of AMF external hyphae beyond the host roots (Drew et al., 2003; Zhu et al., 2001). The length ratio of AMF hyphae to roots in soil can be 100:1 or higher (George et al., 1995), and with the external hyphae, the mycorrhizal roots can explore further, 10-100 times more volume of soils compared to the non-mycorrhizal roots (Sieverding et al., 1991). By applying the Plenchette's mycorrhizal dependency formula to the shoot dry weight of maize and sorghum (Plenchette et al., 1983), the results showed ~~a~~ high mycorrhizal dependency, both for maize (up to 70.12%) and for sorghum (up to 79.42%) (Guo et al., 2013; Tawaraya, 2003).

The findings of the study indicate that the higher the dependent rate is on AMF symbiosis, the more the dry matter is produced of the crops. With the high porosity and low water retention capacity on dryland and sandy areas (Bruand et al., 2005) in most areas of North Lombok, the levels of crop dependence on symbiosis with AMF could be higher. Moreover, the symbiosis of crops with AMF could significantly reduce nutrient loss from soil through leaching (Cavagnaro et al., 2015).

The establishment of AM symbiosis can be done through inoculation with AMF propagules. Our previous study (Astiko et al., 2013a) showed that the indigenous AMF inoculation in maize plants in sandy soils had positive implications for the improvement of soil properties by increasing the rates of nutrient uptake by maize crop from the soil and improving its grain yield. Our research group has also shown the benefit of this local inoculation in increasing the growth of soybean and its yield by improving P uptake from the soils, compared to those without AMF inoculation (Astiko et al., 2013b). However, as found from other studies, the development of AMF in the soil and its contribution to growth, yield and nutrient uptake of host plants could be influenced by the order of plant species cultivated in sequence in the cropping system (Johnson et al., 1992; Vivekanandan & Fixen, 1991). The studies by Johnson et al. (1992) an Vivekanandan and Fixen (1991) reported that the P uptake and the AMF colonization were higher on maize crops grown following soybean compared to when maize crops were grown following maize or barley.

From the previous study on maize-soybean cropping patterns, it was found that the AMF inoculated in maize crops grown in the first cycle increased root colonization and AMF sporulation which was very advantageous for the growth of the following crop in the cropping sequence (Astiko, 2013). Different AMF populations (colonization and spore counts) were also found between cropping seasons or between crop species in the same cropping season in Central Lombok, Indonesia (Wangiyana et al., 2006). The present study examined the effects of several combinations of fertilizer packages consisting of indigenous AMF bio-fertilizer and varying doses of organic and NPK fertilizers applied to maize crops on N and P status in soil, and uptake by maize and subsequent sorghum crops as well as the growth and yield components of the maize and sorghum crops in a maize-sorghum cropping sequence on drylands in North Lombok, Indonesia.

MATERIALS AND METHODS

Design of the experiment

The field experiment of maize-sorghum cropping sequence in this study was arranged according to the Randomized Complete Block Design (RCBD) with four replications (blocks). The study was carried out in the Akar-Akar village located in North Lombok regency, Indonesia, from January to August 2016. Treatments involving the use of five fertilizer packages consisting of different combinations of organic, inorganic and indigenous AMF bio-fertilizer were applied only to the maize crop in the first cropping cycle of the maize-sorghum cropping sequence. The details of the treatments or fertilization packages are listed in Table 1.

Table 1. ***The mycorrhizal-based fertilization packages tested and applied to maize only in the maize-sorghum cropping sequence***

Fertilization packages	Doses of the packages applied to maize plants only (in the cropping cycle 1)	Sorghum (cropping cycle 2)
D_0	NPK only at 100% the recommended doses (RD), i.e. 200 kg/ha Phonska (NPK 15:15:15) and 300 kg/ha Urea	No fertilizer applied
D_1	NPK at 80% RD + 15 ton/ha cattle manure + AMF	No fertilizer applied
D_2	NPK at 60% RD + 12 ton/ha cattle manure + AMF	No fertilizer applied
D_3	NPK at 40% RD + 9 ton/ha cattle manure + AMF	No fertilizer applied
D_4	NPK at 20% RD + 6 ton/ha cattle manure + AMF	No fertilizer applied

A piece of land used in this study was of a sandy soil type (69% sand, 29% silt, and 2% clay, containing an average of 421 AMF spores per 100 g soil), typical of North Lombok area. The land is located at a geographic position of -8.221650, 116.350283. After being cultivated using minimum tillage and cleared of weeds, the land was split into 20 treatment plots of 7m x 5m based on the RCBD plotting layout. The different fertilization packages consisted of AMF inoculum, organic fertilizer (cattle manure), and inorganic fertilizer (NPK and urea), were applied only to the maize crop in the first cropping cycle.

An indigenous AMF inoculum, i.e. the M_{AA01} mycorrhizal isolate, which was originally isolated from dryland area in Akar-Akar village of North Lombok, was applied through seed-coating of maize seeds prior to seeding. Before application, 1 kg of the AMF inoculum was mixed with charcoal powder and tapioca flour as the carrier materials. Then this inoculum mixture was mixed with 20 kg maize seeds so that the seeds were coated by the inoculum mixture. For cropping cycle 1, the uncoated or AMF coated maize seeds ("Bisma" variety) were planted by dibbling 2 seeds per planting hole at 70 cm x 20 cm plant spacing. The entire dose of cattle manure (organic fertilizer) at different doses depending on the treatments (Table 1) was applied at the time of planting the maize seeds by burying the whole dose in the planting hole in the position below the seeds. The inorganic (NPK) fertilizers with the recommended doses of 200 kg/ha Phonska (NPK 15:15:15) and 300 kg/ha Urea (46% N) were applied twice, i.e. at 7 DAS (days after seeding) and at 21 DAS, with the amount applied depending on the treatments (Table 1). At 7 DAS, the maize plants were fertilized with a mixture of the entire Phonska and one third of the Urea treatment doses, followed by application of the remaining Urea treatment doses at 21 DAS. The NPK fertilizers were applied in a furrow of 5 cm alongside the maize plant row at 5-7 cm depth before covered with soil.

In cropping cycle 2, the plots were cleared from maize crop debris and weeds before seeds of sorghum ("Numbu" variety) were directly seeded by dibbling 2 seeds per planting holes made around the maize stubbles. These sorghum plants were not fertilized nor inoculated with AMF inoculum. For both crops, the young maize and sorghum plants were tinned at 7 DAS by leaving one maize or sorghum plant per planting hole. Weeding and soil piling of the maize and sorghum plant base were done at 15 and 30 DAS. Plant protection was done by spraying "OrgaNeem" (an organic pesticide of plant origin containing Azadirachtin extracted from neem leaves) at a concentration of 5 ml OrgaNeem per liter of water. The OrgaNeem solution was applied to both crops (maize or sorghum) from 10 to 60 DAS at a 3-day interval. Harvesting of maize or sorghum crops was done at 100 DAS.

Measurement and data analysis

The variables measured were AMF development, N and P nutrition, and growth and yield of maize and sorghums. The AMF development includes spore counts at 60 and 100 DAS, and root colonization levels at 60 DAS. The N and P nutrition includes concentration of total N and available P in the rhizosphere of maize and sorghum at 60 and 100 DAS. The crop variables include dry weight (shoots and roots) and yield components (grain).

Determination of N was done using the Kjeldhal method and P by using a spectrometer. AMF spore extraction from soil (100 g soil sample) was done using the wet sieving and decanting technique (Brundrett et al., 1996). The spores collected from the 38 μm sieve after the final centrifugation were captured in a filter paper, which were then observed on a Petri dish using a stereo microscope at 40 x magnification, and counted as spore number per 100 g of soil. The percentage of root colonization was determined using the Gridline Intersect technique (Giovannetti & Mosse, 1980) under a stereo microscope after the root pieces were stained using the clearing and staining method of Brundrett et al. (1996).

Data were analyzed using analysis of variance (ANOVA) and the Tukey's HSD (Honestly Significant Difference) means tested at 5% level of significance.

RESULTS AND DISCUSSION

AMF development

In terms of root colonization rates by AMF, there were slight differences between maize and sorghum between treatments fertilizer packages. This can be seen from Table 2 which displays the levels of root colonization by the indigenous AMF which are slightly higher on sorghum roots than on maize roots in each treatment, especially in the D3 treatment where it was 77% on sorghum and 65% on maize roots. When grown simultaneously, Carrenho et al. (2007) found higher colonization rates on maize than on sorghum (Carrenho et al., 2007). In this study, however, maize and sorghum were grown in sequence, in which sorghum was directly seeded without treatments and without tillage following the harvest of maize plants. All treatments, including AMF inoculation, were applied only to maize plants in the first cropping cycle. Therefore, it means that sorghum in cropping cycle 2 was grown after AMF propagules have built up in the soil during the growth of the maize plants in cropping cycle 1. This is in line with the results reported in the previous study (Arihara & Karasawa, 2000) which revealed that AMF root colonization and shoot dry weight of maize at 58 DAS were affected by the previous crop grown, whether they were host or non-host of AMF. However, the P fertilization did not affect root colonization, especially on maize following AMF host plants.

Specifically, the degree of root colonization by AMF may be higher in the roots of maize fertilized with highly soluble mineral fertilizers (NPK, diammonium phosphate, ammonium sulfate and Urea). On the other hand, the length of extra-radical mycelium and number of AMF spores were significantly higher in maize fertilized with organic fertilizers (Bokashi manure) (Bautista-Cruz et al., 2014). This indicates that organic fertilization is more favorable for spore production compared with conventional fertilization. It can also be seen from Table 2 that AMF spore numbers are higher at harvest of the maize crop (100 DAS) compared with those at 60 DAS, especially on the D_2 treatment. This indicates a high buildup of AMF propagules for the subsequent sorghum crops. This condition seems to favor higher AMF colonization levels in roots of sorghum in the second cropping cycle compared with that of maize in the first cropping cycle. With no tillage treatment, the AMF colonization levels in sorghum may be higher than the levels in maize or bean roots (Alguacil et al., 2008). In addition, AMF colonization rates may be higher in roots of sorghum than in the roots of maize, either inoculated with *Glomus* *mosseae* or *G. versiforme*, indicating that sorghum is more favorable for AMF development than maize plants (Guo et al., 2013).

There are many factors influencing the degrees of AMF colonization of crop roots as reported by Carrenho et al. (2007). In examining the effects of different phosphorus, liming, organic matter, and soil texture on root colonization of peanuts, sorghum, and maize in a factorial experiment, each factor significantly affected root colonization level, alone or in interaction with other factors (Carrenho et al., 2007). The most surprising results were the significant interaction effects of plant, phosphorous, and organic matter although there were no significant effects of phosphorous (simple superphosphate of 166.6 mg/L (w/v) soil) nor organic matter (22 mg/L (w/v) soil). As for the interaction effect involving maize, there was no significant effect of phosphorous and organic matter on AMF colonization of maize roots. On the other hand, the application of both phosphorous and organic matter significantly reduced AMF colonization on sorghum roots (Carrenho et al., 2007).

Table 2. ***Mean spore number (spores per 100 g soil) at 60 and 100 DAS, and colonization rates (%-colonization) on maize and sorghum in a maize-sorghum cropping sequence***

Fertilization packages	AMF on maize (1st crop)			AMF sorghum (2nd crop)		
	Spore per 100 g soil		% colonization	Spore per 100 g soil		% colonization
	60 DAS	100 DAS	60 DAS	60 DAS	100 DAS	60 DAS
D_0	764[e]	3464[d]	30[d]	1231[e]	3761[d]	35[e]
D_1	1059[d]	3672[c]	55[c]	1343[d]	4942[b]	60[d]
D_2	2119[a]	4327[a]	75[a]	2981[a]	5165[a]	81[a]
D_3	1690[b]	3894[b]	65[b]	1881[b]	4831[c]	77[b]
D_4	1294[c]	3881[b]	63[b]	1769[c]	4819[c]	68[c]
HSD 5%	231	13	2.0	109	12	6.5

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for descriptions of the packages.

In terms of fertilization effects, AMF colonization rate was reported to be higher on crop roots in unfertilized soils, followed by organic and biodynamic, and the least was seen in exclusively mineral fertilized and conventional farming systems. Thus, it was concluded that the amount of soluble P in the soil was the most limiting factor for AMF colonization in roots of those crops (winter wheat, vetch-rye, and grass-clover) (Mäder et al., 2000). Moreover, it was reported that the increasing P level in the growing media from 0.1 to 0.5 mM P significantly reduced AMF colonization in roots of lettuce plants especially with increasing levels of N contents (1, 5, or 9 mM N) (Azcón et al., 2003).

A negative impact on soil condition occur when the accumulation of soil P has increased beyond the requirement of the crops cultivated (Grant et al., 2005). In the study, it seemed that the amount of P input from the NPK fertilizer as applied in the D_2 treatment was most favorable for AMF development in maize crops. In the D_2 treatment, the NPK fertilizer was reduced to 120 kg/ha Phonska (60% of the recommended NPK dose of 200 kg/ha Phonska in the D_0 treatment) combined with an application of 12 ton/ha cattle manure and inoculation with AMF. Among the treatments with AMF in combination with cattle manure, the D_1 treatment had the highest NPK fertilizer dose (Table 1), and this level of NPK fertilization seems to limit the AMF infection and development in maize roots compared with those in the D_2 treatment, resulting in a higher AMF colonization rate on D_2 than on D_1 treatment. Therefore, AMF colonization and spore numbers in maize were highest in the D_2 treatment, especially when compared with the D_1 or D_0 treatment. Using the same indigenous AMF applied to a pot experiment in which the soil for the growing media was taken from the same field in North Lombok, it was found that the highest level of AMF colonization of maize roots was in the treatment with AMF combined with cattle manure compared with AMF combined with NPK fertilizer or phosphate rock (Astiko et al., 2013a). The previous study also reported a significantly higher AMF spore number in plots fertilized with cattle manure or cattle manure combined with mineral fertilizers compared with those fertilized with mineral fertilizers only (Gryndler et al., 2006).

Soil nutrient status and nutrient sorption by maize and sorghum

There were significant effects of the different fertilizer packages on soil nutrient status (N and P contents of the soil) of the rhizosphere of both maize and sorghum crops at 60 and 100 DAS (Table 3). In general, there is a tendency that the highest values of the soil nutrient status, measured either at 60 or 100 DAS, are highest under the D_2 (60% NPK + 12 t/ha manure + AMF) or D_1 (80% NPK + 15 t/ha manure + AMF) treatment. The values of the soil nutrient status in these treatments were higher than those in D_0 (100% NPK only) treatment, although the dose of NPK fertilizers was reduced in the D_1 and D_2 treatments (Table 3).

Table 3. ***Mean concentration of total N and available P of soil in the rhizospheres of maize (1st crop) and sorghum (2nd crop) for each treatment of fertilization packages, measured at 60 and 100 DAS***

Fertilization packages	Total N ($g.kg^{-1}$) at 60 DAS		Available P ($mg.kg^{-1}$) at 60 DAS		Total N ($g.kg^{-1}$) at 100 DAS		Available P ($mg.kg^{-1}$) at 100 DAS	
	maize	sorghum	maize	sorghum	maize	sorghum	maize	sorghum
D_0	1.24^{c}	1.10^{c}	14.97^{d}	11.41^{d}	1.33^{d}	1.23^{d}	17.43^{d}	10.15^{d}
D_1	1.45^{a}	1.29^{a}	28.44^{b}	16.25^{b}	1.69^{b}	1.38^{b}	29.51^{b}	21.58^{b}
D_2	1.47^{a}	1.25^{ab}	35.02^{a}	28.49^{a}	1.86^{a}	1.48^{a}	36.56^{a}	29.99^{a}
D_3	1.39^{b}	1.22^{ab}	17.52^{c}	14.59^{bc}	1.47^{c}	1.31^{c}	19.37^{c}	18.59^{c}
D_4	1.33^{b}	1.20^{b}	16.29^{c}	14.25^{c}	1.42^{c}	1.31^{c}	18.53^{c}	17.32^{c}
HSD 5%	0.08	0.07	1.31	1.99	0.08	0.06	1.0	2.98
Before planting [1)]	1.20	-	12.28	-	-	-	-	-

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages.

Since the NPK fertilizers applied, i.e. Phonska and Urea at 7 DAS and Urea at 21 DAS, are easily dissolved in water, significant amount of the nutrients could have been loss through infiltration during the rainy season. Previous study shows that the sand content of the cultivated land had a positive correlation with infiltration rate, with an r-value of +0.75 (Patle et al., 2018). Since cattle manure is slow in releasing nutrients, it is possible that at the time of measurement (60 and 100 DAS), the loss of released nutrients from the NPK fertilizers through leaching was much higher in the D_0 than in the other treatments of fertilization packages due to dissolution by rain water during the rainy season. However, there were small increases in nutrient status of the soil from 60 to 100 DAS around the rhizospheres of maize crop, especially in those treated with manure and AMF application. This indicated a slow release of N and P nutrients from manure after application to the maize plants in the first cropping cycle, which could be due to AMF inoculation. Many studies have reported that AMF can mobilize and take N and P from organic matters for their hosts (Feng et al., 2003; Hawkins et al., 2000; Koide & Kabir, 2000; Leigh et al., 2009; Tarafdar & Marschner, 1994), and it can be seen from Table 2 that the highest average of AMF colonization levels in maize roots was in the D_2 treatment. Therefore, N and P uptake in shoots of maize and sorghum was highest in the D_2 treatment (Table 4).

In addition, soil contents of those nutrients corresponded well with the levels of N and P uptake of the crops, i.e. all showing positive correlation coefficients. Although it is not significant for P uptake, the highest values of N and P uptake were also seen in the D_2 treatment for both maize and sorghum (Table 4). The correlation analysis of the mean values obtained at 60 DAS showed a significant correlation between soil N and N sorption in the shoots, with a value of r = + 0.970 (R-square = 94.1%, p = 0.006) for maize plants, and r = + 0.904 (R-square = 81.7%, p = 0.035) for sorghum plants. These indicate those fertilizers in the packages contribute to nutrient contents of the crops, which are significantly different between treatments of fertilization packages (Table 4).

Table 4. ***Mean N and P sorption (mg.g^{-1} plant dry weight) by each crop at 60 DAS for each treatment of fertilization packages***

Fertilization packages	N and P uptake (mg.g^{-1} plant dry weight) by each crop at 60 DAS			
	Maize (1st cropping cycle)		Sorghum (2nd cropping cycle)	
	N	P	N	P
D_0	18.62^{e}	1.71^{d}	12.74^{d}	0.65^{d}
D_1	27.58^{b}	2.60^{b}	18.92^{b}	0.85^{c}
D_2	28.00^{a}	2.72^{a}	20.86^{a}	1.48^{a}
D_3	23.10^{c}	2.41^{c}	17.29^{c}	1.36^{b}
D_4	20.44^{d}	2.41^{c}	17.22^{c}	1.32^{b}
HSD 5%	0.41	0.11	0.07	0.04

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages.

However, P status of the soil did not show a significant correlation with P uptake either by maize or sorghum crop. In spite of that, there is a significant positive correlation between the degree of AMF colonization in the roots and P sorption in the shoot of maize plants, with an r = + 0.909 (R-square = 82.6%, p = 0.032), and that of sorghum plants, with an r = + 0.940 (R-square = 88.4%, p = 0.017). This shows that there were significant contributions of AMF colonization in the roots to the amount of P taken up by the plants, both maize and sorghum. If soil soluble P has not exceeded crop requirements, AMF association will still result in significantly positive effects on P uptake and biomass production because of P requirements of the crops; for optimum growth and yields, crops require P since the early stage of their growth, either from soil, fertilizer application, or from AMF associations (Grant et al., 2005).

Although sorghum crop in the second cropping cycle was not fertilized with manure nor NPK fertilizers, direct seeding of sorghum after harvesting the maize plants without tillage seemed to be a favorable condition in establishing AMF association in the sorghum crop, which resulted in higher AMF colonization rates in sorghum than in maize roots for each fertilizer package (Table 2). The AMF associations seemed to enable the sorghum crops to absorb adequate P and other nutrients from soil and residues of the manure applied in the first cropping cycle even though these sorghum crops were not fertilized. This could occur because of the potential of AMF external hyphae that help the host plants in absorbing and dissolving nutrients from soil and manures and other nutrient pools, as well as other nutrients unavailable for uptake by plant roots (Menge, 1983; Smith & Read, 2010; Tawaraya et al., 2006). Guo et al. (2013)

also reported significant effects of AMF inoculation on C, N, P, and K contents (mg/pot) in shoot and roots of maize and sorghum grown in pots filled with mixtures of mine tailing and topsoil, and between the AMF species used, *G. versiforme* showed higher colonization rates which resulted in higher nutrient sorption and biomass of maize and sorghum compared with *G. mosseae* (Guo et al., 2013).

Biomass and yield components of maize and sorghum

In terms of biomass production and yield components of maize and sorghum, there were also significant effects of the fertilization packages in which D_2 treatment presents the highest values of biomass production (Table 5) and yield components (Table 6). This means that the nutrient status of the soils corresponded well with the biomass weight of the crops, indicated by the positive correlation coefficients, although only some of them are significant. The AMF applications to maize crops, observed at 60 DAS, showed a significant correlation to dry shoot weight, N and P status of the soil, and the N and P sorption. In contrast, AMF colonization levels of maize roots only showed significant correlation with root dry weight at 60 DAS, with an r = + 0.912 (R^2 = 83.2%, p= 0.031) and shoot dry weight at maturity (100 DAS), with an r = + 0.892 (R^2 = 79.6%, p= 0.042). This could mean that during the vegetative growth, AMF colonization have focused on improving root growth to increase nutrient sorption during the vegetative growth of maize crops.

Table 5. ***Mean dry biomass weight (g/plant) of maize and sorghum for each treatment of fertilization packages***

Fertilization packages	Dry biomass weights (g/plant) of maize (1st crop) and sorghum (2nd crop)							
	Maize root		Maize shoot		Sorghum root		Sorghum shoot	
	60 DAS	100 DAS	60 DAS	100 DAS	60 DAS	100 DAS	60 DAS	100 DAS
D_0	8.13^d	10.43^e	34.15^d	62.29^e	0.82^d	12.30^d	8.31^d	47.74^e
D_1	15.13^b	22.39^b	61.92^b	109.03^b	2.22^b	22.34^b	16.89^b	95.01^b
D_2	17.12^a	34.56^a	72.86^a	111.25^a	3.37^a	24.44^a	23.53^a	105.14^a
D_3	13.65^c	18.48^c	52.26^c	101.05^c	1.68^c	14.52^c	12.01^c	85.46^c
D_4	13.34^c	15.43^d	51.29^c	95.87^d	1.38^c	14.47^c	11.81^c	66.06^d
HSD 5%	0.31	3.04	0.97	2.21	0.30	0.05	0.20	9.54

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages.

In relation to nutrient uptake, although AMF colonization levels did not show significant correlation with N uptake in shoots of maize or sorghum, they did show a positive significant correlation with P uptake both in shoots of maize and sorghum, with an r = + 0.909 (R^2 = 82.6%, p= 0.032) for maize, and r = + 0.940 (R^2 = 88.4%, p= 0.017) for sorghum. These could be due to the ability of AMF extra-radical hyphae in mobilizing and absorbing P from organic matters for their hosts (Feng et al., 2003; Koide & Kabir, 2000; Tarafdar & Marschner, 1994). Based on the strength of the relationships between AMF colonization levels and P uptake, the value of R^2 is higher in sorghum (R^2 = 88.4%) than in maize (R^2 = 82.6%). This means that the contribution of AMF colonization in roots to P uptake in shoots was higher in sorghum crop at the second cropping cycle than in maize in the first cropping cycle. Since sorghum did not receive any fertilizers, and cattle manure is a slow-release organic fertilizer, it could mean that most P nutrient contained in the shoots, especially in shoots of sorghum in the second cropping cycle, was mostly taken up from the manure under the contribution of AMF colonization in the roots. However, this still needs to be confirmed by further research. This view is supported by the conditions of the study area, which was dominated by sand, and if leaching happened during the rainy season, the loss of dissolved nutrients from the NPK fertilizers applied in the first cropping cycle could be high. Therefore, the main source of nutrients, especially for sorghum crop in the second cropping cycle was the cattle manure applied to the maize crop in the first cropping cycle.

Table 6. ***Mean weights of total dry grains (kg/plot) and 100 dry grains for each crop and each treatment of fertilization packages***

Fertilization packages	Mean dry grain yield (kg/plot) and weight of 100 dry grains (g)			
	Maize in the 1st cropping cycle		Sorghum in the 2nd cropping cycle	
	Grain yield	100 grains	Grain yield	100 grains
D_0	9.60^e	22.48^d	3.57^d	2.73^d
D_1	17.40^b	26.94^b	5.05^b	3.01^b
D_2	22.80^a	28.12^a	6.65^a	3.61^a
D_3	15.60^c	25.98^c	4.43^c	2.90^c
D_4	10.20^d	24.61^c	4.17^c	2.81^{cd}
HSD 5%	0.59	1.37	0.26	0.09

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages.

In relation to nitrogen nutrient, there was a very low and non-significant correlation between AMF colonization levels and N uptake in maize shoots with an R^2 of only 41.2%. However, the N uptake of maize at 60 DAS was highly significantly correlated with the N status of the soil with an R^2 = 94.1% (p= 0.006). Moreover, the N soil availability also showed a significant correlation with biomass and grain yield of maize, i.e. with an R^2 = 84.1% (p= 0.029) with grain yield at 60 DAS, R^2 = 92.2% (p= 0.009) with shoot dry weight at 60 DAS, and R^2 = 90.6% (p= 0.012) with shoot dry weight at 100 DAS.

In contrast, for sorghum in the second cropping cycle, the soil N status at 60 DAS showed a significant correlation only with shoot dry weight at 100 DAS. Unlike in maize crop where AMF colonization levels showed a significant and positive correlation with shoot dry weight at maturity (100 DAS), AMF colonization levels in sorghum roots did not show any significant correlation with biomass weight or yield components of sorghum. However, AMF colonization rates in sorghum roots showed a sufficiently high correlation with N uptake in sorghum shoots, with an R^2 = 71.9% (p= 0.069), and N uptake in sorghum shoots showed positive significant correlation with biomass and grain yield of sorghum, i.e. with an R^2 = 80.5% (p= 0.039) with grain yield, R^2 = 83.0% (p= 0.031) with shoot dry weight at 60 DAS, and R^2 = 89.9% (p= 0.014) with shoot dry weight at 100 DAS. These could mean that for maize crop, most nutrients were derived from NPK fertilizers while for sorghum, N and P nutrients were derived from the residues of manure contributed from AMF colonization in sorghum roots. Many researchers have also showed the ability of AMF to utilize organic sources to supply N to their host (e.g. Hawkins et al., 2000; Leigh et al., 2009) although it was also found that AMF colonization did not necessarily result in significantly higher N status of the mycorrhizal than non-mycorrhizal hosts (Hawkins et al., 2000).

However, when the correlation analysis was done between averages of colonization levels, biomass and grain yield between maize in the first and sorghum in the second cropping cycle, in general the results show significant and positive coefficients of correlation. For example, correlation of AMF colonization levels between roots of maize and sorghum are highly significant, with an r = + 0.988 (R^2 = 97.6%, p= 0.002). This means that the pattern of differences in AMF colonization levels between roots of maize and sorghum is highly similar. In other words, AMF colonization levels in roots of maize in the first cropping cycle were carried over into the second cropping cycle where sorghum was grown as the rotation crop. This is because both maize and sorghum are hosts of AMF, and both crops have high mycorrhizal dependency (Guo et al., 2013).

In addition, when a t-test of ‘Paired Two Sample for Means’ was run on the averages of AMF colonization levels and spore number in soil at maturity of the crops between maize and sorghum in Table 2, higher significant values (p<0.01) were seen on sorghum than on maize. This could be due to the buildup of AMF in the soil after maize crop in the first copping cycle was harvested before sorghum was directly seeded without tillage. Even though both crops were grown simultaneously, it was also found that AMF colonization levels were higher on sorghum than on maize (Guo et al., 2013). This means that sorghum can be used to improve mycorrhizal conditions of the soil under a maize-sorghum cropping sequence.

CONCLUSION

Among the mycorrhiza-based fertilization packages tested on the maize-sorghum cropping sequence system at the drylands in North Lombok of Indonesia, the D_2 package, consisting of 60% NPK recommended dose, 12 t/ha cattle manure, and the indigenous AMF inoculation, was found to be the best fertilization package to improve crop yield and soil nutrient availability. This study noted that the AMF development was higher in the sorghum at the second cropping cycle compared to the growth in the maize at the first cropping cycle. This condition led to the higher NP-uptake and soil nutrient availability in subsequent crops, both at the sandy and dry land, with no additional fertilization and mycorrhizal propagules applied.

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Dear Author(s),

Sorry for the late reply. Your revised manuscript JTAS-1651-2018.R1 as well as the English editing certificate had been received. The checklist had been done as well.

However, sorry to say that there are some citation mistakes for the references. Kindly check well the unsubmitted e-mail and make the corrections accordingly.

Please do not hesitate to contact me if you have any questions. Thank you.

Regards,

(Ms. Syin Ying, Tee on behalf of CEE)

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Recently, you received a decision on Manuscript ID JTAS-1651-2018, entitled "Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-uptake and availability on maize-sorghum cropping sequence in Lombok's drylands." The manuscript and decision letter are located in your Author Center at https://mc.manuscriptcentral.com/upm-jtas.

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Dear Assoc. Prof. Astiko:

Your manuscript entitled "Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-uptake and availability on maize-sorghum cropping sequence in Lombok's drylands" has been successfully submitted online and is presently being given full consideration for publication in the Journal of Tropical Agricultural Science.

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Dear Assoc. Prof. Astiko,

Your manuscript, JTAS-1651-2018.R1, entitled "Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-uptake and availability on maize-sorghum cropping sequence in Lombok's drylands" has been unsubmitted from the Journal of Tropical Agricultural Science. The reason of "unsubmission" is due to the following issue(s):

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Document originally revised by K. Masters, July 2014

Updated by E. Tam and J. Ulyannikova, January 2016

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One author – in-text reference placement ***Note:*** There are two main ways to use in-text references. Firstly, to focus on the information from your source – 'information prominent'. Secondly, to focus on the author – 'author prominent'.	***'Information prominent' (the author's name is within parentheses):*** The conclusion reached in a recent study (Cochrane, 2007) was that… ***OR*** ***'Author prominent' (the author's name is outside the parentheses):*** Cochrane (2007) concluded that…	Cochrane, A. (2007). *Understanding urban policy: A critical approach.* Malden, MA: Blackwell Publishing.
One author – when fewer than 40 words are quoted Include the material in the paragraph and include specific page number/s. Use **quotation marks** to show the exact words.	An interesting view was expressed that "the connection of high profile developments to their surrounding environment has increasingly been questioned" (Cochrane, 2007, p. 117). ***OR*** An interesting view was expressed by Cochrane (2007) that "the connection of high profile developments to their surrounding environment has increasingly been questioned" (p. 117).	Cochrane, A. (2007). *Understanding urban policy: A critical approach.* Malden, MA: Blackwell Publishing.
One author – when 40 or more words are quoted Begin quoting the material on a new line, indent it 5 spaces (use the indent tool to keep all lines of the quote evenly indented), and include specific page number/s. **Omit** the quotation marks. Use **double spacing** for both your text and the indented quote. Make sure the quote is **exactly** as it was published.	Much has been written about acute care. Finkelman (2006), for example, points out that: There are many changes in acute care services occurring almost daily, and due to the increasing use of outpatient surgery, surgical services have experienced major changes. Hospitals are increasing the size of their outpatient or ambulatory surgery departments and adjusting to the need of moving patients into and out of the surgical service in 1 day or even a few hours. (p. 184). Recently, this trend has been seen in some Australian hospitals and research here…	Finkelman, A. W. (2006). *Leadership and management in nursing.* Upper Saddle River, NJ: Pearson Prentice Hall.

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Two authors	When considering the Howard Government's Indigenous health expenditure, Palmer and Short (2010) maintain that…	Palmer, G. R., & Short, S. D. (2010). *Health care and public policy: An Australian analysis* (4th ed.). Melbourne, Australia: Palgrave Macmillan.
Three to five authors For the first in-text reference, list all the authors' family names, then use the first author's family name followed by 'et al.' for subsequent entries.	A recent study (Seeley, VanPutte, Regan, & Russo, 2011) concluded that… ***Subsequent in-text reference/s:*** (Seeley et al., 2011).	Seeley, R., VanPutte, C., Regan, J., & Russo, A. (2011). *Seeley's anatomy & physiology*. New York, NY: McGraw-Hill.
Six to seven authors For all in-text references, list only the first author's family name followed by 'et al.' All authors are included in the Reference List. ***For books with eight or more authors, please follow the guidelines for journal articles with eight or more authors on page 7.***	The Russian Revolution may never have succeeded if there hadn't already been widespread discontent among the Russian populace (Bulliet et al., 2005).	Bulliet, R. W., Crossley, P. K., Headrick, D. R., Hirsch, S. W., Johnson, L. L., & Northrup, D. (2011). *The earth and its peoples: A global history* (5th ed.). Boston, MA: Wadsworth.
Works by different authors with the same family name For in-text references, include the initials of the authors in question to enable readers to differentiate between them. List references alphabetically by the authors' initials in the Reference List.	These techniques have been shown to improve test scores among primary school aged children (R. Smith, 2010). If funding were enhanced, it is arguable these problems could be ameliorated (C. J. Smith & Laslett, 1993).	Smith, C., & Laslett, R. (1993). *Effective classroom management: A teacher's guide* (2nd ed.). London, United Kingdom: Routledge. Smith, R. (2010). *Rethinking teacher education: Teacher education in the knowledge age*. Sydney, Australia: AACLM Press.

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Several works by the same author in different years When citing references separately, no special rule needs to be observed. When citing references collectively, separate years with a comma and insert years earliest to latest. List references chronologically (earliest to latest) in the Reference List.	These techniques have changed markedly in the last decade (Greenspan, 2000, 2011).	Greenspan, A. (2000). *Orthopedic radiology: A practical approach* (3rd ed.). Philadelphia, PA: Lippincott Williams & Wilkins. Greenspan, A. (2011). *Orthopedic imaging: A practical approach* (5th ed.). Philadelphia, PA: Lippincott Williams & Wilkins.
Several works by the same author in the same year Arrange alphabetically by title in the Reference List. Place lowercase letters (“a”, “b”, “c”, etc.) immediately after the year.	Leadership and change in schools have been major topics of discussion for several years (Fullan, 1996a, 1996b) and this conference… “Educational change” has taken on a new meaning in recent years (Fullan, 1996b) …	Fullan, M. (1996a). Leadership for change. In *International handbook for educational leadership and administration.* New York, NY: Kluwer Academic . Fullan, M. (1996b). *The new meaning of educational change*. London, United Kingdom: Cassell.
Several authors, different years, referred to collectively in your work List sources alphabetically by family name in the in-text reference in the order in which they appear in the Reference List. Separate each reference with a semicolon.	The cyclical process (Carr & Kemmis, 1986; Dick, 2000; Kemmis & McTaggart, 1988; MacIsaac, 1995) suggests…	Carr, W., & Kemmis, S. (1986). *Becoming critical: Education knowledge and action research.* London, United Kingdom: Falmer Press. Dick, B. (2000). *A beginner’s guide to action research.* Retrieved from http://www.scu.edu.au/schools/gcm/ar/arp/guide.html Kemmis, S., & McTaggart, R. (Eds.). (1988). *The action research planner* (3rd ed.). Melbourne, Australia: Deakin University Press.

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eBook – online book - If the URL leads to information about how to obtain the book, use "Available from" instead of "Retrieved from". - If there is a DOI (digital object identifier), include it instead of the 'Retrieved from' statement. A DOI is a unique, permanent identifier assigned to many electronic documents.	We found helpful information about deaf children (Niemann, Greenstein, & David, 2004) that meant we could… ***OR*** Schiraldi (2001) offers solutions to PTSD.	Niemann, S., Greenstein, D., & David, D. (2004). *Helping children who are deaf: Family and community support for children who do not hear well.* Retrieved from http://www.hesperian.org/ publications_download_deaf.php Schiraldi, G. R. (2001). *The post-traumatic stress disorder sourcebook: A guide to healing, recovery, and growth* [Adobe Digital Editions version]. doi:10.1036/0071393722
Chapter in edited book	A discussion about Australia's place in today's world (Richards, 1997) included reference to… ***OR*** Richards (1997) proposed that…	Richards, K. C. (1997). Views on globalization. In H. L. Vivaldi (Ed.), *Australia in a global world* (pp. 29-43). Sydney, Australia: Century.
Brochure – author is also publisher	The security of personal information is addressed in the TransACT brochure (TransACT, n.d.)	TransACT . (n.d.). *Guide to equipment and service* [Brochure]. Canberra, Australia: Author.
Editor	In discussing best practice, Zairi (1999) identified… ***OR*** Best practice indicators in management have been identified (Zairi, 1999) and…	Zairi, M. (Ed.). (1999). *Best practice: Process innovation management.* Oxford, United Kingdom: Butterworth-Heinemann.
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Corporate author – commissioned reports	The report prepared by the South Australian Centre for Economic Studies (2009) was discussed.	South Australian Centre for Economic Studies. (2009). *Local government's current and potential role in water management and conservation: Final report.* Commissioned by the Local Government Association of South Australia. Adelaide, Australia: Author.
No date of publication	Some aspects of forensic science are more challenging than others (Browne, n.d.) and for this reason…	Browne, J. D. (n.d.). *Forensic science as a career*. London, England: Tower.
Second or later edition	Peters (2001, p. 6) argued that "..."	Peters, T. (2001). *The elements of counselling* (2nd ed.). Brisbane, Australia: Macmillan.
Multi-volume work	Inge, Duke and Bryer (1978, p. 27) claim that there is much to learn about these writers which results in… ***OR*** There is so much to learn about our country (Clark, 1978, p. 42) that we kept returning to…	Inge, M. T., Duke, M., & Bryer, J. R. (Eds.). (1978). *Black American writers: Bibliographical essays* (Vols. 1-2). New York, NY: St. Martins. Clark, C. M. H. (1978). *A history of Australia: Vol. 4*. *The earth abideth for ever, 1851-1888.* Australia: Melbourne University Press.

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Dictionary / Encyclopaedia – print Include information about editions, volume numbers and page numbers in parenthesis following the title in the Reference List.	According to one definition of “bivalence” (VandenBos, 2007, p. 123)…	VandenBos, G. R. (Ed.). (2007). *APA dictionary of psychology.* Washington, DC: American Psychological Association.
Dictionary / Encyclopaedia – online Include information about editions, specific volume numbers or page numbers in parenthesis following the title in the Reference List.	A psychological overview of ADHD (Arcus, 2001)…	Arcus, D. (2001). Attention deficit / hyperactivity disorder (ADHD). In B. Strickland (Ed.), *The Gale encyclopedia of psychology.* Retrieved from http://www.gale.cengage.com/ ***Note:*** If retrieved from a database, do a Web search for the home page of the publisher of the encyclopaedia and use the URL in the reference.

JOURNAL, NEWSPAPER & NEWSLETTER ARTICLES

Journal article with one author – separated paging (paginated by issue) If each issue of a journal begins on page 1, include the issue number in parenthesis immediately after the volume number in the Reference List.	In an earlier article, it was proposed (Jackson, 2007)…	Jackson, A. (2007). New approaches to drug therapy. *Psychology Today and Tomorrow, 27*(1), 54-59.
Journal article with two authors – continuous paging throughout a volume. If the journal volume page numbers run continuously throughout the year, regardless of issue number, do **not** include the issue number in your Reference List entry.	Kramer and Bloggs (2002) stipulated in their latest article… ***OR*** This article on art (Kramer & Bloggs, 2002) stipulated that…	Kramer, E., & Bloggs, T. (2002). On quality in art and art therapy. *American Journal of Art Therapy, 40,* 218-231.

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Journal article with three to five authors For the first in-text reference, list all the authors' family names, then use the first author's family name followed by 'et al.' for subsequent entries.	A recent study to investigate the effects of an organisational stress management program on employees (Elo, Ervasti, Kuosma, & Mattila, 2008) concluded that… ***Subsequent in-text reference/s:*** (Elo et al., 2008)	Elo, A., Ervasti, J., Kuosma, E., & Mattila, P. (2008). Evaluation of an organizational stress management program in a municipal public works organization. *Journal of Occupational Health Psychology*, *13*(1), 10-23. doi: 10.1037/1076-8998.13.1.10
Journal article with six to seven authors For all in-text references, list only the first author's family name followed by 'et al.' All authors are included in the Reference List.	A simple ALMA is described in a recent study (Restouin et al., 2009).	Restouin, A., Aresta, S., Prébet, T., Borg, J., Badache, A., & Collette, Y. (2009). A simplified, 96-well–adapted, ATP luminescence–based motility assay. *BioTechniques, 47*, 871–875. doi: 10.2144/000113250
Journal article with eight or more authors For all in-text references, list only the first author's family name followed by 'et al.' In the Reference List, include the first six authors' names, then insert three ellipsis points (...), and add the last author's name.	Traumatic injury is the leading cause of death and disability worldwide (Steel et al., 2010).	Steel, J., Youssef, M., Pfeifer, R., Ramirez, J. M., Probst, C., Sellei, R., ... Pape, H. C. (2010). Health-related quality of life in patients with multiple injuries and traumatic brain injury 10+ years postinjury. *Journal of Trauma: Injury, Infection, and Critical Care, 69*(3), 523-531. doi: 10.1097/TA.0b013e3181e90c24
Journal or magazine article with no volume or issue number	Wychick and Thompson (2005) foreshadow that scam will still be enticing… ***OR*** An interesting approach to scam (Wychick & Thompson, 2005) suggested that…	Wychick, J., & Thompson, L. (2005, November 24). Fallen for a scam lately? *AustraliaToday,* 54-60.
Journal article retrieved from a database – with a DOI (Digital Object Identifier) A DOI is a unique, permanent identifier assigned to articles in many databases. **Always** include the DOI if one is provided (usually in the article's full-text, abstract or database record). If there is a DOI, no other retrieval information is necessary.	A study examining priming (Johns & Mewhort, 2009) discovered …	Johns, E., & Mewhort, D. (2009). Test sequence priming in recognition memory. *Journal of Experimental Psychology: Learning, Memory and Cognition, 35*, 1162-1174. doi: 10.1037/a0016372

	IN-TEXT REFERENCE	REFERENCE LIST
Journal article – in press	Influence of music in running performance (Lee & Kimmerly, in press) …	Lee, S., & Kimmerly, D. (in press). Influence of music on maximal self-paced running performance and passive post-exercise recovery rate. *The Journal of Sports Medicine and Physical Fitness*.
Journal article – Cochrane Review with DOI	Overweight and obesity are increasing throughout the industrialised world (Shaw, O'Rourke, Del Mar, & Kenardy, 2005) …	Shaw, K., O'Rourke, P., Del Mar, C., & Kenardy, J. (2005). Psychological interventions for overweight or obesity. *The Cochrane database of systematic reviews* (2). doi:10.1002/14651858.CD003818.pub2
Journal article retrieved from a database – without a DOI - If there is no DOI, do a Web search to locate the URL of the journal's home page & include it in the Reference List. The journal URL can sometimes be found in the database record or in the full text view of the article. - If the online article is ONLY available from a database (e.g. for discontinued journals where the journal home page doesn't exist), include the entry page URL of the database where it was found. Give the database name if not in the URL.	The effects of climate change on agriculture are studied by Ramalho, Da Silva and Dias (2009)… Primary care is one area marked for improvement (Purtilo, 1995).	***Example using URL of journal home page:*** Ramalho, M., Da Silva, G., & Dias, L. (2009). Genetic plant improvement and climate changes. *Crop Breeding and Applied Biotechnology, 9*(2), 189-195. Retrieved from http://www.sbmp.org.br/cbab ***Example using URL of database (where there is no journal home page):*** Purtilo, R. (1995). Managed care: Ethical issues for the rehabilitation professions. *Trends in Health Care, Law and Ethics, 10*, 105-118. Retrieved from http://www.proquest.com
Book review in a journal	In his review of Thomas Samaras' latest book, Marson (2009) identifies…	Marson, S. M. (2009). How big should we be? A Herculean task accomplished [Review of the book *Human body size and the laws of scaling: Physiological, performance, growth, longevity and ecological ramification,* by T. Samaras]. *Public Health Nutrition, 12*, 1299–1300. doi:10.1017/S1368980009990656
Newspaper article – with an author	The notion of a Bill of Rights may be inappropriate in the Australian context (Waterford, 2007).	Waterford, J. (2007, May 30). Bill of Rights gets it wrong. *The Canberra Times,* p. 11.
Newspaper article – without an author	The redesign of the Internet ("Internet pioneer", 2007) is said to…	Internet pioneer to oversee network redesign. (2007, May 28). *The Canberra Times,* p. 15.

	IN-TEXT REFERENCE	REFERENCE LIST
Newspaper article retrieved from a database Do a Web search to locate the URL of the newspaper's home page & include it in the Reference List.	In an attempt to save the tiger, Darby (2002) provided…	Darby, A. (2002, August 20). Rarest tiger skin a rugged survivor. *Sydney Morning Herald.* Retrieved from http://www.smh.com.au
Article in an online newsletter	Australia's casualty rate was almost 65 per cent - the highest in the British Empire ("Australians and the Western Front", 2009)	Australians and the Western Front. (2009, November). *Ozculture newsletter.* Retrieved from http://www.cultureandrecreation.gov.au/ newsletter/

CONFERENCE / SEMINAR PAPERS

Conference or seminar papers in published proceedings – print If the paper is from a book, use the Book chapter citation format. If it is from regularly published proceedings (e.g. annual), use the Journal article citation format.	In a paper about conservation of photographs (Edge, 1996), the proposition that…	Edge, M. (1996). Lifetime prediction: Fact or fancy? In M. S. Koch, T. Padfield, J. S. Johnsen, & U. B. Kejser (Eds.), *Proceedings of the Conference on Research Techniques in Photographic Conservation* (pp. 97-100). Copenhagen, Denmark: Royal Danish Academy of Fine Arts.
Conference or seminar papers in published proceedings – online	Tester (2008) points to the value of using geothermal sources for power and energy.	Tester, J. W. (2008). The future of geothermal energy as a major global energy supplier. In H. Gurgenci & A. R. Budd (Eds.), *Proceedings of the Sir Mark Oliphant International Frontiers of Science and Technology Australian Geothermal Energy Conference,* Canberra, Australia: Geoscience Australia. Retrieved from http://www.ga.gov.au/image_cache/ GA11825.pdf

	IN-TEXT REFERENCE	REFERENCE LIST

GOVERNMENT PUBLICATIONS

Government department as author Spell out the full name of the body each time it is cited in-text, unless it is long and has a familiar/easily understood abbreviation. In the latter case, give the full name with the abbreviation for the first in-text reference. Use the abbreviation for subsequent references.	The need for guidelines to manage and use multiple channels to deliver e-government services (Department of Finance and Administration [DOFA], 2006) presents Australian Government agencies with… ***Subsequent in-text reference/s:*** DOFA (2006) identified …	Department of Finance and Administration. (2006). *Delivering Australian Government services: Managing multiple channels.* Canberra, Australia: Author.
Government publication – with identifying number Includes report numbers, catalogue numbers, etc.	Recently released statistics from the Australian Bureau of Statistics (ABS) (2007) reveal interesting changes in Australian society. ***Subsequent in-text reference/s:*** The ABS (2007) reported that …	Australian Bureau of Statistics. (2007). *Australian social trends* (Cat. no. 4102.0). Canberra, Australia: ABS.
Government report – online	***First in-text reference:*** A recent government report (Department of the Prime Minister and Cabinet [PM&C], 2008) examines a selection of key topics … ***Subsequent in-text reference/s:*** Families in Australia were highlighted (PM&C, 2008)…	Department of the Prime Minister and Cabinet. (2008). *Families in Australia: 2008.* Retrieved from http://www.dpmc.gov.au/ publications/families/index.cfm#contact
Government approved standards	…and "including data in computer systems, created or received and maintained by an organisation" (Standards Australia, 1996, p. 7) as well as…	Standards Australia. (1996). *Australian Standard AS 4390: Records Management.* Sydney, Australia: Author.

LEGISLATION

Note: For more comprehensive information please consult the following publication: *The bluebook: A uniform system of citation* (19th ed.). (2010). Cambridge, MA: Harvard Law Review Association.

Act – print	According to s. 8.1 of the *Anti-Discrimination Act 1977* (NSW), it is unlawful for an employer to discriminate against a person on the ground of race.	*Anti-Discrimination Act 1977* (NSW) s. 8.1 (Austl.). ***Follow this convention:*** *Short Title of the Act* (in italics) *Year* (in italics) (Jurisdiction abbreviation) Section number Subdivision, if relevant (Country abbreviation).

	IN-TEXT REFERENCE	REFERENCE LIST
Bill – print	The Mental Health Bill 2013 (WA) prohibits…	Mental Health Bill 2013 (WA) (Austl.). ***Follow this convention:*** Bill Name (no italics) Year (Jurisdiction abbreviation) (Country abbreviation).
Act & Bill – online	According to s. 8.1 of the *Anti-Discrimination Act 1977* (NSW), it is unlawful for an employer to discriminate against a person on the ground of race.	*Anti-Discrimination Act 1977* (NSW) s. 8.1 (Austl.). Retrieved from http://www.legislation.nsw.gov.au/maintop/scanact/inforce/NONE/0
Case	According to Ellis v. Wallsend District Hospital (1989)… …in a land right case (*Mabo v. Queensland*, 1988)…	*Ellis v. Wallsend District Hospital* 1989 17 NSWLR 553 (Austl.). *Mabo v. Queensland* 1988 166 CLR 186 (Austl.). ***Follow this convention:*** *Case Name* (in italics) Year Volume number Reporter abbreviation First page number (Country abbreviation).

IMAGES, MUSIC & AUDIOVISUAL MEDIA

CD recording	Lyrics from Paul Kelly's song "From Little Things Big Things Grow" (Kelly, 1997, track 10) were used in recent television advertisements.	Kelly, P. (1997). From little things big things grow. On *Songs from the south: Paul Kelly's greatest hits* [CD]. Melbourne, Australia: Mushroom Records.
DVD / Videorecording	Jane Austen's world came alive in *Sense and sensibility* (Lee, 1995)	Lee, A. (Director). (1995). *Sense and sensibility* [DVD]. Australia: Columbia TriStar Home Video.

	IN-TEXT REFERENCE	REFERENCE LIST
Figure, Table, Graph, Map or Chart Cite each of these as you would for a book. Include, in square brackets, the type of entry immediately after the title: [Figure]. [Table]. [Map]. [Graph]. [Chart].	**Graph** The internal processes were well described (Kaplan & Norton, 2004) which led to… **Map** To locate a property just outside the Australian Capital Territory, use the 1:100 000 map produced by Geoscience Australia (2004) which covers…	**Graph** Kaplan, R. S., & Norton, D. P. (2004). Internal processes deliver value over different time horizons [Graph]. In *Strategy maps: Converting intangible assets into tangible outcomes* (p. 48). Boston, MA: Harvard Business School. **Map** Geoscience Australia [NATMAP] (Cartographer). (2004). *ACT region, New South Wales and Australian Capital Territory* [Map]. Canberra, Australia: Author.
Image – online	The effective use of light in Monet's 'Haystacks' (Monet, 1890)…	Monet, C. (1890). *Haystacks, midday* [Painting]. National Gallery of Australia, Canberra. Retrieved from http://artsearch.nga.gov.au/ Detail-LRG.cfm?IRN=29073&View=LRG
Liner notes	The American jazz trombonist, bandleader and composer Jack Teagarden (Weiner, 1995)…	Weiner, D. J. (1995). [Liner notes]. J. Teagarden (Composer), *Big 'T' jump* [CD]. USA: Jass Records.
Score	Craig Scott is one of Australia's leading bassists (Scott, 2013)	Scott, C. (2013). *C minor waltz: For jazz quintet* [Score]. Sydney, Australia: Craig Scott
Streamed music	An analysis of the jazz piano style of "What's Your Story Morning Glory" (Williams, 1978, track 8) reveals…	Williams, M. L. (1978). What's your story morning glory. On *Mary Lou Williams: Solo recital, Montreux Jazz Festival* [CD]. Fantasy. Retrieved from Naxos Music Library Jazz.
Interview – on radio	In a recent interview with the Prime Minister (Mitchell, 2009)…	Mitchell, N. (Presenter). (2009, October 16). Interview with the Prime Minister, Kevin Rudd. In *Mornings with Neil Mitchell* [Radio broadcast]. Melbourne, Australia: Radio 3AW.
Interview – on television	He demonstrated his professionalism and sensitivity in an interview with Raelene Boyle (Denton, 2006) and…	Denton A. (Producer and Interviewer). (2006, September 25). Interview with Raelene Boyle. In *Enough Rope with Andrew Denton.* [Television broadcast]. Sydney, Australia: Australian Broadcasting Corporation.
Motion picture (movie)	Jackson and Pyke (2003) provide evidence that belief in a world…	Jackson, P. (Director), & Pyke, S. (Producer). (2003). *The lord of the rings: The return of the king* [Motion picture]. New Zealand: Imagine Films. ***Note:*** Give the country where the movie was made – not the city.

	IN-TEXT REFERENCE	REFERENCE LIST
Podcast (audio)	Listening to the news on my MP3 player (Nolan, 2007) was a new experience and I decided…	Nolan, T. (Presenter). (2007, April 28). *AM: News & current affairs* [Audio podcast]. Retrieved from http://abc.net.au/news/ subscribe/amrss.sml
Radio program – broadcast	When discussing how people write about music, Koval (2009)…	Koval, R. (Presenter). (2009, November 19). *The Book Show* [Radio broadcast]. Melbourne, Australia: ABC Radio National.
Radio program – transcript	The views of the internationally renowned author and public speaker, De Bono, prompted me to follow up one of the interviews (Mascall, 2005) which…	Mascall, S. (Reporter). (2005, February 14). Are we hardwired for creativity? In *Innovations* [Radio program] [Transcript]. Melbourne, Australia: ABC Radio Australia. Retrieved from http://www.abc.net.au/ra/innovations/stories/s1302318.htm
Speech – online	In her ANZAC Day speech (Clark, 2007), the Prime Minister of New Zealand referred to…	Clark, H. (2007, April 25). *Prime Minister's 2007 ANZAC Day message* [Transcript]. Retrieved from http://www.anzac.govt.nz
Television advertisement	The problems of teenage anxiety were graphically captured (Beyondblue, 2009)…	Beyondblue (Producer). (2009, November 29). *Beyondblue: Anxiety* [Television advertisement]. Canberra, Australia: WIN TV.
Television program – broadcast	Examining future plans for Canberra's city area (Kimball, 2009)…	Kimball, C. (Presenter). (2009, September 4). *Stateline* [Television broadcast]. Canberra, Australia: ABC TV. ***Note:*** Always check the television station's website and use the transcript, if one is available, for direct quotes.
Television program – transcript	Cyclones often affect Australia, especially in the north (McLaughlin, 2004) and it is worthwhile…	McLaughlin, M. (Presenter). (2004, November 7). Cyclone Tracy. In *Rewind* [Television program] [Transcript]. Sydney, Australia: ABC TV. Retrieved from http://www.abc.net.au/tv/ rewind/txt/s1233697.htm

	IN-TEXT REFERENCE	REFERENCE LIST

THESIS OR DISSERTATION

Thesis or Dissertation – print	Nurses working in an acute care environment tend to experience a high degree of workplace conflict (Duddle, 2009).	Duddle, M. (2009). *Intraprofessional relations in nursing: A case study* (Unpublished doctoral thesis), University of Sydney, Australia.
Thesis or Dissertation – retrieved from a database	The field of engineering has largely developed around the positivist philosophical position (Hector, 2008).	Hector, D. C. A. (2008). *Towards a new philosophy of engineering: Structuring the complex problems from the sustainability discourse* (Doctoral thesis). Available from Australasian Digital Theses database. (Record No. 185877) ***Note:*** End the reference with the unique number or identifier assigned to the thesis/dissertation.
Thesis or Dissertation – retrieved from the web	Lacey (2011) differentiates between instrumental violence and violence inflicting injury for its own sake.	Lacey, D. (2011). *The role of humiliation in collective political violence* (Masters thesis, University of Sydney, Australia). Retrieved from http://hdl.handle.net/2123/7128

UNIVERSITY PROVIDED STUDY MATERIALS

Lecture / tutorial notes, etc. – online	Septicaemia is one of many infections commonly acquired in hospitals (Maw, 2010) …	Maw, M. (2010). *NURS5082 Developing nursing practice, lecture 2, week 1: Healthcare-associated infections and their prevention* [Lecture PowerPoint slides]. Retrieved from http://learn-on-line.ce.usyd.edu.au/

	IN-TEXT REFERENCE	REFERENCE LIST
SOCIAL MEDIA		
Facebook update List the author's name as it is written (including nicknames).	$52 million will be provided to deploy Australian civilian troops (Rudd, 2009)	Rudd, K. (2009, October 24). Australian civilian corps to help in crises [Facebook update]. Retrieved from http://www.facebook.com/note.php?note_id=200124043571&ref=mf
Blog post - List the author's name as it is used in the posting (including nicknames). - For a blog comment, use 'Blog comment' instead of 'Blog post' and include the exact title (including 'Re:' if used)	The plight of the flapper skate was recently highlighted (Keim, 2009)…	Keim, B. (2009, November 18). ID error leaves fish at edge of extinction [Blog post]. Retrieved from http://www.wired. com/wiredscience/2009/11/extinction-error/
Video blog post (eg YouTube)	The Prime Minister, speaking about Australia's role in the G20 forum (Rudd, 2009)…	Rudd, K. (2009, September 29). Update on new G20 arrangements [Video file]. Retrieved from http://www.youtube.com/watch?v=i8IdJ-0S5rs
Twitter tweet If the author uses their name as their Twitter 'handle', do not alter its format to follow the convention of 'Family name, Initial(s).'	President Obama announced the launch of the American Graduation Initiative (BarackObama, 2009).	BarackObama. (2009, July 15). Launched American Graduation Initiative to help additional 5 mill. Americans graduate college by 2020: http://bit.ly/gcTX7 [Twitter post]. Retrieved from http://twitter.com/BarackObama/status/2651151366 ***Note:*** This reference would be filed under 'B', not 'O'
Discussion group, list, etc. – online	There are strongly held views about knowledge management (Weidner, 2007) and from personal experience…	Weidner, D. (2007, June 11). KM reducing in popularity [Discussion list message]. Retrieved from http://actkm.org/mailman/listinfo/actkm_actkm.org
Wiki Include the date retrieved, as the information is likely to change in these sources.	The role of media corporations in the media literacy movement is discussed ("Great debates in media literacy", n.d.)	Great debates in media literacy: Theory and practice of media literacy. (n.d.). In *Wikiversity.* Retrieved October 27, 2009, from http://en.wikiversity.org/wiki/Great_Debates_in_Media_Literacy

	IN-TEXT REFERENCE	REFERENCE LIST
PERSONAL COMMUNICATION AND EMAIL		
Personal communication Includes private letters, memos, email, telephone conversations, personal interviews, etc. These are cited in-text only, not in the Reference List.	J. Francis (personal communication, August 6, 2007) was able to confirm that the floods had not reached their area.	**Not included in Reference List. Cite in-text only.**
Email – NEVER cite addresses without permission of the owner of the address	Ms Coleman (personal communication, July 11, 2007) provided details in an email and we acted on that information.	**Not included in Reference List. Treat as personal communication and cite in-text only.**
WEB RESOURCES		
Web document – author or sponsor given, dated ***Note:*** A web document is a file (e.g. a Word or PDF file) found on the Web. Often there are links to Web documents from Web pages. A Web document is not the same as a web page.	An RBA paper (Simon, Smith, & West, 2009) found that participation in a loyalty program and access to an interest-free period…	Simon, J., Smith, K., & West, T. (2009). *Price incentives and consumer payment behaviour.* Retrieved from the Reserve Bank of Australia website: http://www.rba.gov.au/ PublicationsAndResearch/RDP/RDP2009-04.html
Web document – author or sponsor given but not dated	The Commonwealth Scientific and Industrial Research Organisation (CSIRO) is designing several energy-efficient electric machines to reduce greenhouse gas emissions (CSIRO, n.d.).	Commonwealth Scientific and Industrial Research Organisation. (n.d.). *Reducing Australia's greenhouse emissions factsheet.* Retrieved from http://www.csiro.au/ resources/ps282.html

	IN-TEXT REFERENCE	REFERENCE LIST
Web page with no page numbers *Include in in-text references:* - A paragraph number with the abbreviation 'para' (count paragraphs if numbers are not visible) ***OR*** - A section heading and paragraph number (e.g. Introduction, para. 3). A long section heading may be shortened and enclosed in double quotation marks. ***Note:*** Because Web pages can be updated, you must include the date on which you accessed the source.	Usually the author or creator of a work is the copyright owner (University of Sydney, 2010, "Who owns copyright?", para. 1). ***Note:*** The heading of the section was "Who owns copyright?"	University of Sydney. (2010). *Guide to copyright*. Retrieved March 21, 2011, from http://sydney.edu.au/copyright/students/coursework.shtml#who
Web source – no author or sponsor given When there is no author for a source you find on the Web (whether it be a Web document or a Web page), the title moves to the first position of the reference entry. If the title is long, use an abbreviated version of it for in-text citations. Insert double quotation marks around the title ***Note:*** If you were citing the title of a book, periodical, brochure or report, you would use italics rather than double quotation marks.	This vaccine is 6 times more efficient than vaccines previously used to immunise against the condition ("New child vaccine", 2001).	New child vaccine gets funding boost. (2001). Retrieved April 16, 2012, from http://news.ninemsn.com.au/health/story_13178.asp
Website – entire website	The new website of the Department of Education, Employment and Workplace Relations (http://www.deewr.gov.au) includes useful information on current government education policy.	**Not included in Reference list.**

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SPECIALISATION*(e.g. agriculture)*	OCCUPATION *(e.g. R&D, student)*	JOB TITLE *(e.g. professor, etc)*
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[1]Department of English, Yasouj University, Yasouj, Iran

[2]Department of Language and Humanities Education, Faculty of Language Studies, Universiti Putra Malaysia, UPM, 43400 Serdang, Selangor, Malaysia

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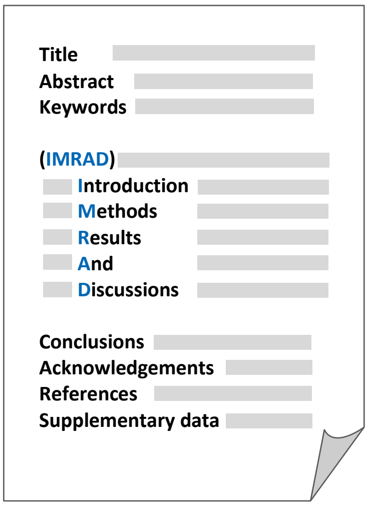

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The Introduction explains the scope and objective of the study in the light of current knowledge on the subject; the Materials and Methods describes how the study was conducted; the Results section reports what was found in the study; and the Discussion section explains meaning and significance of the results and provides suggestions for future directions of research. The manuscript must be prepared according to the Journal's instructions to authors.

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1	**LEFT, BOLDFACE, UPPERCASE**
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3	**Indented, boldface, lowercase paragraph heading ending with a period.**
4	***Indented, boldface, italicized, lowercase paragraph heading ending with a period.***
5	*Indented, italicized, lowercase paragraph heading ending with a period.*

Examples:

METHOD *(1st level of heading)*

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Sample and Participant Selection. Sample and Participant Selection. Sample and Participant Selection. Sample and Participant Selection. Sample and Participant Selection. Sample and Participant Selection. Sample and Participant Selection. Sample and Participant Selection. Sample and Participant Selection.

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Assessments and Measures *(2nd level of heading)*

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TABLES AND FIGURES

Table No. (Not italic, align left)

Table Caption (Italic, vice versa with scientific names, align left)

Row and columns, with no horizontal line

Example:

Table 1
PVY infected Nicotiana tabacum *plants optical density in ELISA*

Lines No.	Plant, variety	OD values in ELISA, units		
		7th day	15th day	25th day
10	*N. tabacum,* Samsun	0,008	0,826	1,335
38	*N. tabacum,* Samsun	0,003	1,313	0,767
42	*N. tabacum,* Samsun	0,571	1,211	0,936
43	*N. tabacum,* Samsun	0,497	1,070	0,977
44	*N. tabacum,* Samsun	0,102	0,571	0,232
1000	*N. tabacum,* Samsun	0,180	0,412	0,343
-	Positive	0,865	1,021	0,912
-	Negative	0,019	0,023	0,021

Note. ELISA optical density for the samples № 38, 42, 43 exceeded the commercial positive control on the 15th day of inoculation, which was earlier than expected. It should be noted that OD markedly decreased on the 25th day of inoculation

Figure No. (*Italic*). Figure Caption (Not Italic, align left) Placed at below figure.

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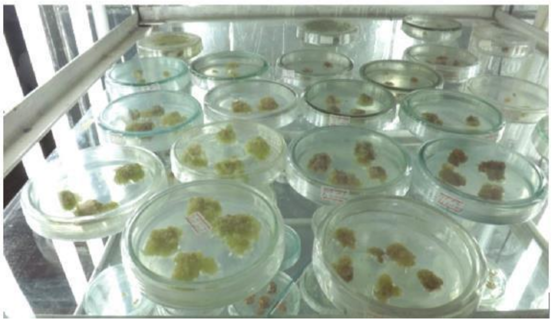

Figure 1. PVY-infected in vitro callus of *Nicotiana tabacum*

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wahyu astiko <astiko@unram.ac.id>
kepada Tee

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Thank you very much for your kind help.

Bests,

Assoc. Prof. Dr. Wahyu Astiko
Agroecotechnology
Faculty of Agriculture, University of Mataram

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Comments to the Corresponding Author
this paper is a good paper, but can be improved with additional characterisation data and details of methods etc to improve the understanding of the work and the results.

Reviewer: 2

Comments to the Corresponding Author
Since cattle manure is part of the fertilization packages, it will be good to include the nutrient content of the manure in the in the mauscript.

Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-uptake and availability on maize-sorghum cropping sequence in Lombok's drylands

Journal:	*Journal of Tropical Agricultural Science*
Manuscript ID	JTAS-1651-2018.R1
Manuscript Type:	Regular Article
Scope of the Journal:	Crop nutrition < Crop and pasture production < AGRICULTURAL SCIENCES, Soil fertility < Crop and pasture production < AGRICULTURAL SCIENCES, Physicochemical assimilation < Plant physiology < AGRICULTURAL SCIENCES, Plant nutrition < Soil and water sciences < AGRICULTURAL SCIENCES, Soil biology < Soil and water sciences < AGRICULTURAL SCIENCES, Micropropagation techniques < Biotechnology < BIOLOGICAL SCIENCES, Microbiology < BIOLOGICAL SCIENCES
Keywords:	Seed coating, Arbuscular Mycorrhizal Fungi, AMF, maize-sorghum, cropping sequence, plant nutrition
Abstract:	ABSTRACT An indigenous arbuscular mycorrhizal fungal (AMF) is expected to improve performance of food crops in sandy and drylands of North Lombok (Indonesia) in dry seasons. To examine the benefits of mycorrhiza to maize yield at varying doses on plant nutrition (nitrogen and phosphorus), 1 kg of the AMF inoculum was applied to 20 kg maize seeds in different fertilization packages of cattle manure (15, 12, 9, and 6 ton/ha) and inorganic fertilizers (80, 60, 40 and 20% NPK recommended dose). A 100% NPK recommended dose was used as the control (200 kg/ha Phonska and 300 kg/ha Urea). Sorghum seeds were then planted in cropping cycle 2 with no additional fertilization and inoculum applied. Results indicated that the AMF applications to the maize-sorghum cropping sequence increased the AMF colonization rate, the N and P nutrition at the soils, N and P uptake, and dry biomass (root, shoot, and grain). The highest correspondence was observed in the crops which utilized a combination of 60% NPK and 12 ton/ha cattle manure, and the performance was higher at day 100 after seeding. When grown simultaneously, mycorrhizal colonization rate on sorghum roots (60-81%) was slightly higher than the rate seen on maize root (55-75%). This study suggests that AMF inoculation maize yield and improves soil nutrient availability which is very advantageous for the growth of the subsequent crop. Keywords: Seed coating, arbuscular mycorrhizal fungi (AMF), maize-sorghum, cropping sequence, plant nutrition

Running Title:

Mycorrhizal seed-coating on maize-sorghum cropping sequence

Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-uptake and availability on maize-sorghum cropping sequence in Lombok's drylands

The List of Number of Tables

Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-uptake and availability on maize-sorghum cropping sequence in Lombok's drylands

ABSTRACT

An indigenous arbuscular mycorrhizal fungal (AMF) is expected to improve performance of food crops in sandy and drylands of North Lombok (Indonesia) in dry seasons. To examine the benefits of mycorrhiza to maize yield at varying doses on plant nutrition (nitrogen and phosphorus), 1 kg of the AMF inoculum was applied to 20 kg maize seeds in different fertilization packages of cattle manure (15, 12, 9, and 6 ton/ha) and inorganic fertilizers (80, 60, 40 and 20% NPK recommended dose). A 100% NPK recommended dose was used as the control (200 kg/ha Phonska and 300 kg/ha Urea). Sorghum seeds were then planted in cropping cycle 2 with no additional fertilization and inoculum applied. Results indicated that the AMF applications to the maize-sorghum cropping sequence increased the AMF colonization rate, the N and P nutrition at the soils, N and P uptake, and dry biomass (root, shoot, and grain). The highest correspondence was observed in the crops which utilized a combination of 60% NPK and 12 ton/ha cattle manure, and the performance was higher at day 100 after seeding. When grown simultaneously, mycorrhizal colonization rate on sorghum roots (60-81%) was slightly higher than the rate seen on maize root (55-75%). This study suggests that AMF inoculation maize yield and improves soil nutrient availability which is very advantageous for the growth of the subsequent crop.

Keywords: Seed coating, arbuscular mycorrhizal fungi (AMF), maize-sorghum, cropping sequence, plant nutrition

INTRODUCTION

The northern part of North Lombok regency (Indonesia) is dominated by drylands with sandy soils texture. With a very short and low number of rainy days per wet month or no rain during the long dry seasons, no food crops can be cultivated normally especially in the areas without deep wells. Moreover, inadequate phosphorus (P) availability is also one of the factors limiting the productivity of maize and other food crops in the dryland of North Lombok. Maize crop requires very high P; for example, for the production of 10 ton/ha, maize crops need 102 kg/ha of P_2O_5 and 76% of it is transported into the seeds (Calderón-Vázquez et al., 2009). In contrast, plant roots can take up P at only about 8-13% of the amount of P fertilizer applied (Supardi, 1996). Thus, to cope with the unavailability of soil water and P and other essential nutrients in drylands of North Lombok, arbuscular mycorrhizal fungal (AMF) symbiosis has been used as it is expected to improve the performance of food crops especially during the dry seasons. Many have reported that the soil fungi, in symbiosis with their host plants, can help plants to improve water retention and make their host plants more tolerant to drought (Augé, 2004). AMF colonization also increases nutrient uptake from soils and enhances growth and yield of the host plants although it depends on the species of AMF colonizing the host plants (Marulanda et al., 2003).

The most common findings show that the external hyphae of AMF improve P uptake and transport the nutrient to root cortex of their host (Azcón et al., 2003; Feng et al., 2003; George et al., 1995; Koide & Kabir, 2000; Tarafdar & Marschner, 1994; Tawaraya et al., 2006). Although there is no growth improvement of the host plants due to the AMF symbiosis, the amount of P uptake through AMF (mycorrhizal pathway) can be higher than through the host roots or the direct pathway (Smith & Read, 2010). AMF can also help their hosts to increase the uptake of other essential nutrients such as N, K, Ca, Mg, S, Cu, Zn, Mn, Fe, and B when compared with non-mycorrhizal plants (Dhillion & Ampornpan, 1992; Hawkins et al., 2000; Leigh et al., 2009; Menge, 1983). Most of these capabilities of the AM plants are due to the extensive growth of AMF external hyphae beyond the host roots (Drew et al., 2003; Zhu et al., 2001). The length ratio of AMF hyphae to roots in soil can be 100:1 or higher and with the external hyphae, the mycorrhizal roots can explore further (George et al., 1995). By applying the Plenchette's mycorrhizal dependency formula to the shoot dry weight of maize and sorghum (Plenchette et al., 1983), the results showed ~~a~~ high mycorrhizal dependency, both for maize (up to 70.12%) and for sorghum (up to 79.42%) (Guo et al., 2013; Tawaraya, 2003). The findings of the study indicate that the higher the dependent rate is on AMF symbiosis, the more the dry

matter is produced of the crops. With the high porosity and low water retention capacity on dryland and sandy areas (Bruand et al., 2005) in most areas of North Lombok, the levels of crop dependence on symbiosis with AMF could be higher. Moreover, the symbiosis of crops with AMF could significantly reduce nutrient loss from soil through leaching (Cavagnaro et al., 2015).

The establishment of AM symbiosis can be done through inoculation with AMF propagules. Our previous study (Astiko et al., 2013a) showed that the indigenous AMF inoculation in maize plants in sandy soils had positive implications for the improvement of soil properties by increasing the rates of nutrient uptake by maize crop from the soil and improving its grain yield. Our research group has also shown the benefit of this local inoculation in increasing the growth of soybean and its yield by improving P uptake from the soils, compared to those without AMF inoculation (Astiko et al., 2013b). However, as found from other studies, the development of AMF in the soil and its contribution to growth, yield and nutrient uptake of host plants could be influenced by the order of plant species cultivated in sequence in the cropping system (Johnson et al., 1992; Vivekanandan & Fixen, 1991). The studies by Johnson et al. (1992) an Vivekanandan and Fixen (1991) reported that the P uptake and the AMF colonization were higher on maize crops grown following soybean compared to when maize crops were grown following maize or barley.

From the previous study on maize-soybean cropping patterns, it was found that the AMF inoculated in maize crops grown in the first cycle increased root colonization and AMF sporulation which was very advantageous for the growth of the following crop in the cropping sequence (Astiko, 2013). Different AMF populations (colonization and spore counts) were also found between cropping seasons or between crop species in the same cropping season in Central Lombok, Indonesia (Wangiyana et al., 2006). The present study examined the effects of several combinations of fertilizer packages consisting of indigenous AMF bio-fertilizer and varying doses of organic and NPK fertilizers applied to maize crops on N and P status in soil, and uptake by maize and subsequent sorghum crops as well as the growth and yield components of the maize and sorghum crops in a maize-sorghum cropping sequence on drylands in North Lombok, Indonesia.

MATERIALS AND METHODS

Design of the experiment

The field experiment of maize-sorghum cropping sequence in this study was arranged according to the Randomized Complete Block Design (RCBD) with four replications (blocks). The study was carried out in the Akar-Akar village located in North Lombok regency, Indonesia, from January to August 2016. Treatments involving the use of five fertilizer packages consisting of different combinations of organic, inorganic and indigenous AMF bio-fertilizer were applied only to the maize crop in the first cropping cycle of the maize-sorghum cropping sequence. The details of the treatments or fertilization packages are listed in Table 1.

Table 1. ***The mycorrhizal-based fertilization packages tested and applied to maize only in the maize-sorghum cropping sequence***

Fertilization packages	Doses of the packages applied to maize plants only (in the cropping cycle 1)	Sorghum (cropping cycle 2)
D_0	NPK only at 100% the recommended doses (RD), i.e. 200 kg/ha Phonska (NPK 15:15:15) and 300 kg/ha Urea	No fertilizer applied
D_1	NPK at 80% RD + 15 ton/ha cattle manure + AMF	No fertilizer applied
D_2	NPK at 60% RD + 12 ton/ha cattle manure + AMF	No fertilizer applied
D_3	NPK at 40% RD + 9 ton/ha cattle manure + AMF	No fertilizer applied
D_4	NPK at 20% RD + 6 ton/ha cattle manure + AMF	No fertilizer applied

A piece of land used in this study was of a sandy soil type (69% sand, 29% silt, and 2% clay, containing an average of 421 AMF spores per 100 g soil), typical of North Lombok area. The land is located at a geographic position of -8.221650, 116.350283. After being cultivated using minimum tillage and cleared of weeds, the land was split into 20 treatment plots of 7m x 5m based on the RCBD plotting layout. The different fertilization packages consisted of AMF inoculum, organic fertilizer (cattle manure), and inorganic fertilizer (NPK and urea), were applied only to the maize crop in the first cropping cycle.

An indigenous AMF inoculum, i.e. the M_{AA01} mycorrhizal isolate, which was originally isolated from dryland area in Akar-Akar village of North Lombok, was applied through seed-coating of maize seeds prior to seeding. Before application, 1 kg of the AMF inoculum was mixed with charcoal powder and tapioca flour as the carrier materials. Then this inoculum mixture was mixed with 20 kg maize seeds so that the seeds were coated by the inoculum mixture. For cropping cycle 1, the uncoated or AMF coated maize seeds ("Bisma" variety) were planted by dibbling 2 seeds per planting hole at 70 cm x 20 cm plant spacing. The entire dose of cattle manure (organic fertilizer) at different doses depending on the treatments (Table 1) was applied at the time of planting the maize seeds by burying the whole dose in the planting hole in the position below the seeds. The inorganic (NPK) fertilizers with the recommended doses of 200 kg/ha Phonska (NPK 15:15:15) and 300 kg/ha Urea (46% N) were applied twice, i.e. at 7 DAS (days after seeding) and at 21 DAS, with the amount applied depending on the treatments (Table 1). At 7 DAS, the maize plants were fertilized with a mixture of the entire Phonska and one third of the Urea treatment doses, followed by application of the remaining Urea treatment doses at 21 DAS. The NPK fertilizers were applied in a furrow of 5 cm alongside the maize plant row at 5-7 cm depth before covered with soil.

In cropping cycle 2, the plots were cleared from maize crop debris and weeds before seeds of sorghum ("Numbu" variety) were directly seeded by dibbling 2 seeds per planting holes made around the maize stubbles. These sorghum plants were not fertilized nor inoculated with AMF inoculum. For both crops, the young maize and sorghum plants were tinned at 7 DAS by leaving one maize or sorghum plant per planting hole. Weeding and soil piling of the maize and sorghum plant base were done at 15 and 30 DAS. Plant protection was done by spraying "OrgaNeem" (an organic pesticide of plant origin containing Azadirachtin extracted from neem leaves) at a concentration of 5 ml OrgaNeem per liter of water. The OrgaNeem solution was applied to both crops (maize or sorghum) from 10 to 60 DAS at a 3-day interval. Harvesting of maize or sorghum crops was done at 100 DAS.

Measurement and data analysis

The variables measured were AMF development, N and P nutrition, and growth and yield of maize and sorghums. The AMF development includes spore counts at 60 and 100 DAS, and root colonization levels at 60 DAS. The N and P nutrition includes concentration of total N and available P in the rhizosphere of maize and sorghum at 60 and 100 DAS. The crop variables include dry weight (shoots and roots) and yield components (grain).

Determination of N was done using the Kjeldhal method and P by using a spectrometer. AMF spore extraction from soil (100 g soil sample) was done using the wet sieving and decanting technique (Brundrett et al., 1996). The spores collected from the 38 µm sieve after the final centrifugation were captured in a filter paper, which were then observed on a Petri dish using a stereo microscope at 40 x magnification, and counted as spore number per 100 g of soil. The perceantage of root colonization was determined using the Gridline Intersect technique (Giovannetti & Mosse, 1980) under a stereo microscope after the root pieces were stained using the clearing and staining method of Brundrett et al. (1996).

Data were analyzed using analysis of variance (ANOVA) and the Tukey's HSD (Honestly Significant Difference) means tested at 5% level of significance.

RESULTS AND DISCUSSION

AMF development

In terms of root colonization rates by AMF, there were slight differences between maize and sorghum between treatments fertilizer packages. This can be seen from Table 2 which displays the levels of root colonization by the indigenous AMF which are slightly higher on sorghum roots than on maize roots in each treatment, especially in the D3 treatment where it was 77% on sorghum and 65% on maize roots. When grown simultaneously, Carrenho et al. (2007) found higher colonization rates on maize than on sorghum (Carrenho et al., 2007). In this study, however, maize and sorghum were grown in sequence, in which sorghum was directly seeded without treatments and without tillage following the harvest of maize plants. All treatments, including AMF inoculation, were applied only to maize plants in the first cropping cycle. Therefore, it means that sorghum in cropping cycle 2 was grown after AMF propagules have built up in the soil during the growth of the maize plants in cropping cycle 1. This is in line with the results reported in the previous study (Arihara & Karasawa, 2000) which revealed that AMF root colonization and shoot dry weight of maize at 58 DAS were affected by the previous crop grown, whether they were host or non-host of AMF. However, the P fertilization did not affect root colonization, especially on maize following AMF host plants.

Specifically, the degree of root colonization by AMF may be higher in the roots of maize fertilized with highly soluble mineral fertilizers (NPK, diammonium phosphate, ammonium sulfate and Urea). On the other hand, the length of extra-radical mycelium and number of AMF spores were significantly higher in maize fertilized with organic fertilizers (Bokashi manure) (Bautista-Cruz et al., 2014). This indicates that organic fertilization is more favorable for spore production compared with conventional fertilization. It can also be seen from Table 2 that AMF spore numbers are higher at harvest of the maize crop (100 DAS) compared with those at 60 DAS, especially on the D_2 treatment. This indicates a high buildup of AMF propagules for the subsequent sorghum crops. This condition seems to favor higher AMF colonization levels in roots of sorghum in the second cropping cycle compared with that of maize in the first cropping cycle. With no tillage treatment, the AMF colonization levels in sorghum may be higher than the levels in maize or bean roots (Alguacil et al., 2008). In addition, AMF colonization rates may be higher in roots of sorghum than in the roots of maize, either inoculated with *Glomus mosseae* or *G. versiforme*, indicating that sorghum is more favorable for AMF development than maize plants (Guo et al., 2013).

There are many factors influencing the degrees of AMF colonization of crop roots as reported by Carrenho et al. (2007). In examining the effects of different phosphorus, liming, organic matter, and soil texture on root colonization of peanuts, sorghum, and maize in a factorial experiment, each factor significantly affected root colonization level, alone or in interaction with other factors (Carrenho et al., 2007). The most surprising results were the significant interaction effects of plant, phosphorous, and organic matter although there were no significant effects of phosphorous (simple superphosphate of 166.6 mg/L (w/v) soil) nor organic matter (22 mg/L (w/v) soil). As for the interaction effect involving maize, there was no significant effect of phosphorous and organic matter on AMF colonization of maize roots. On the other hand, the application of both phosphorous and organic matter significantly reduced AMF colonization on sorghum roots (Carrenho et al., 2007).

Table 2. ***Mean spore number (spores per 100 g soil) at 60 and 100 DAS, and colonization rates (%-colonization) on maize and sorghum in a maize-sorghum cropping sequence***

Fertilization packages	AMF on maize (1st crop)			AMF sorghum (2nd crop)		
	Spore per 100 g soil		% colonization	Spore per 100 g soil		% colonization
	60 DAS	100 DAS	60 DAS	60 DAS	100 DAS	60 DAS
D_0	764[e]	3464[d]	30[d]	1231[e]	3761[d]	35[e]
D_1	1059[d]	3672[c]	55[c]	1343[d]	4942[b]	60[d]
D_2	2119[a]	4327[a]	75[a]	2981[a]	5165[a]	81[a]
D_3	1690[b]	3894[b]	65[b]	1881[b]	4831[c]	77[b]
D_4	1294[c]	3881[b]	63[b]	1769[c]	4819[c]	68[c]
HSD 5%	231	13	2.0	109	12	6.5

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for descriptions of the packages.

In terms of fertilization effects, AMF colonization rate was reported to be higher on crop roots in unfertilized soils, followed by organic and biodynamic, and the least was seen in exclusively mineral fertilized and conventional farming systems. Thus, it was concluded that the amount of soluble P in the soil was the most limiting factor for AMF colonization in roots of those crops (winter wheat, vetch-rye, and grass-clover) (Mäder et al., 2000). Moreover, it was reported that the increasing P level in the growing media from 0.1 to 0.5 mM P significantly reduced AMF colonization in roots of lettuce plants especially with increasing levels of N contents (1, 5, or 9 mM N) (Azcón et al., 2003).

A negative impact on soil condition occur when the accumulation of soil P has increased beyond the requirement of the crops cultivated (Grant et al., 2005). In the study, it seemed that the amount of P input from the NPK fertilizer as applied in the D_2 treatment was most favorable for AMF development in maize crops. In the D_2 treatment, the NPK fertilizer was reduced to 120 kg/ha Phonska (60% of the recommended NPK dose of 200 kg/ha Phonska in the D_0

treatment) combined with an application of 12 ton/ha cattle manure and inoculation with AMF. Among the treatments with AMF in combination with cattle manure, the D_1 treatment had the highest NPK fertilizer dose (Table 1), and this level of NPK fertilization seems to limit the AMF infection and development in maize roots compared with those in the D_2 treatment, resulting in a higher AMF colonization rate on D_2 than on D_1 treatment. Therefore, AMF colonization and spore numbers in maize were highest in the D_2 treatment, especially when compared with the D_1 or D_0 treatment. Using the same indigenous AMF applied to a pot experiment in which the soil for the growing media was taken from the same field in North Lombok, it was found that the highest level of AMF colonization of maize roots was in the treatment with AMF combined with cattle manure compared with AMF combined with NPK fertilizer or phosphate rock (Astiko et al., 2013a). The previous study also reported a significantly higher AMF spore number in plots fertilized with cattle manure or cattle manure combined with mineral fertilizers compared with those fertilized with mineral fertilizers only (Gryndler et al., 2006).

Soil nutrient status and nutrient sorption by maize and sorghum

There were significant effects of the different fertilizer packages on soil nutrient status (N and P contents of the soil) of the rhizosphere of both maize and sorghum crops at 60 and 100 DAS (Table 3). In general, there is a tendency that the highest values of the soil nutrient status, measured either at 60 or 100 DAS, are highest under the D_2 (60% NPK + 12 t/ha manure + AMF) or D_1 (80% NPK + 15 t/ha manure + AMF) treatment. The values of the soil nutrient status in these treatments were higher than those in D_0 (100% NPK only) treatment, although the dose of NPK fertilizers was reduced in the D_1 and D_2 treatments (Table 3).

Table 3. ***Mean concentration of total N and available P of soil in the rhizospheres of maize (1st crop) and sorghum (2nd crop) for each treatment of fertilization packages, measured at 60 and 100 DAS***

Fertilization packages	Total N (g.kg^{-1}) at 60 DAS		Available P (mg.kg^{-1}) at 60 DAS		Total N (g.kg^{-1}) at 100 DAS		Available P (mg.kg^{-1}) at 100 DAS	
	maize	sorghum	maize	sorghum	maize	sorghum	maize	sorghum
D_0	1.24^{c}	1.10^{c}	14.97^{d}	11.41^{d}	1.33^{d}	1.23^{d}	17.43^{d}	10.15^{d}
D_1	1.45^{a}	1.29^{a}	28.44^{b}	16.25^{b}	1.69^{b}	1.38^{b}	29.51^{b}	21.58^{b}
D_2	1.47^{a}	1.25^{ab}	35.02^{a}	28.49^{a}	1.86^{a}	1.48^{a}	36.56^{a}	29.99^{a}
D_3	1.39^{b}	1.22^{ab}	17.52^{c}	14.59^{bc}	1.47^{c}	1.31^{c}	19.37^{c}	18.59^{c}
D_4	1.33^{b}	1.20^{b}	16.29^{c}	14.25^{c}	1.42^{c}	1.31^{c}	18.53^{c}	17.32^{c}
HSD 5%	0.08	0.07	1.31	1.99	0.08	0.06	1.0	2.98
Before planting [1)]	1.20	-	12.28	-	-	-	-	-

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages.

Since the NPK fertilizers applied, i.e. Phonska and Urea at 7 DAS and Urea at 21 DAS, are easily dissolved in water, significant amount of the nutrients could have been loss through infiltration during the rainy season. Previous study shows that the sand content of the cultivated land had a positive correlation with infiltration rate, with an r-value of +0.75 (Patle et al., 2018). Since cattle manure is slow in releasing nutrients, it is possible that at the time of measurement (60 and 100 DAS), the loss of released nutrients from the NPK fertilizers through leaching was much higher in the D_0 than in the other treatments of fertilization packages due to dissolution by rain water during the rainy season. However, there were small increases in nutrient status of the soil from 60 to 100 DAS around the rhizospheres of maize crop, especially in those treated with manure and AMF application. This indicated a slow release of N and P nutrients from manure after application to the maize plants in the first cropping cycle, which could be due to AMF inoculation. Many studies have reported that AMF can mobilize and take N and P from organic matters for their hosts (Feng et al., 2003; Hawkins et al., 2000; Koide & Kabir, 2000; Leigh et al., 2009; Tarafdar & Marschner, 1994), and it can be seen from Table 2 that the highest average of AMF colonization levels in maize roots was in the D_2 treatment. Therefore, N and P uptake in shoots of maize and sorghum was highest in the D_2 treatment (Table 4).

In addition, soil contents of those nutrients corresponded well with the levels of N and P uptake of the crops, i.e. all showing positive correlation coefficients. Although it is not significant for P uptake, the highest values of N and P uptake were also seen in the D_2 treatment for both maize and sorghum (Table 4). The correlation analysis of the mean values obtained at 60 DAS showed a significant correlation between soil N and N sorption in the shoots, with a value of r = + 0.970 (R-square = 94.1%, p = 0.006) for maize plants, and r = + 0.904 (R-square = 81.7%, p = 0.035) for sorghum plants. These indicate those fertilizers in the packages contribute to nutrient contents of the crops, which are significantly different between treatments of fertilization packages (Table 4).

Table 4. ***Mean N and P sorption (mg.g^{-1} plant dry weight) by each crop at 60 DAS for each treatment of fertilization packages***

Fertilization packages	N and P uptake (mg.g^{-1} plant dry weight) by each crop at 60 DAS			
	Maize (1st cropping cycle)		Sorghum (2nd cropping cycle)	
	N	P	N	P
D_0	18.62^e	1.71^d	12.74^d	0.65^d
D_1	27.58^b	2.60^b	18.92^b	0.85^c
D_2	28.00^a	2.72^a	20.86^a	1.48^a
D_3	23.10^c	2.41^c	17.29^c	1.36^b
D_4	20.44^d	2.41^c	17.22^c	1.32^b
HSD 5%	0.41	0.11	0.07	0.04

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages.

However, P status of the soil did not show a significant correlation with P uptake either by maize or sorghum crop. In spite of that, there is a significant positive correlation between the degree of AMF colonization in the roots and P sorption in the shoot of maize plants, with an r = + 0.909 (R-square = 82.6%, p = 0.032), and that of sorghum plants, with an r = + 0.940 (R-square = 88.4%, p = 0.017). This shows that there were significant contributions of AMF colonization in the roots to the amount of P taken up by the plants, both maize and sorghum. If soil soluble P has not exceeded crop requirements, AMF association will still result in significantly positive effects on P uptake and biomass production because of P requirements of the crops; for optimum growth and yields, crops require P since the early stage of their growth, either from soil, fertilizer application, or from AMF associations (Grant et al., 2005).

Although sorghum crop in the second cropping cycle was not fertilized with manure nor NPK fertilizers, direct seeding of sorghum after harvesting the maize plants without tillage seemed to be a favorable condition in establishing AMF association in the sorghum crop, which resulted in higher AMF colonization rates in sorghum than in maize roots for each fertilizer package (Table 2). The AMF associations seemed to enable the sorghum crops to absorb adequate P and other nutrients from soil and residues of the manure applied in the first cropping cycle even though these sorghum crops were not fertilized. This could occur because of the potential of AMF external hyphae that help the host plants in absorbing and dissolving nutrients from soil and manures and other nutrient pools, as well as other nutrients unavailable for uptake by plant roots (Menge, 1983; Smith & Read, 2010; Tawaraya et al., 2006). Guo et al. (2013)

also reported significant effects of AMF inoculation on C, N, P, and K contents (mg/pot) in shoot and roots of maize and sorghum grown in pots filled with mixtures of mine tailing and topsoil, and between the AMF species used, *G. versiforme* showed higher colonization rates which resulted in higher nutrient sorption and biomass of maize and sorghum compared with *G. mosseae* (Guo et al., 2013).

Biomass and yield components of maize and sorghum

In terms of biomass production and yield components of maize and sorghum, there were also significant effects of the fertilization packages in which D_2 treatment presents the highest values of biomass production (Table 5) and yield components (Table 6). This means that the nutrient status of the soils corresponded well with the biomass weight of the crops, indicated by the positive correlation coefficients, although only some of them are significant. The AMF applications to maize crops, observed at 60 DAS, showed a significant correlation to dry shoot weight, N and P status of the soil, and the N and P sorption. In contrast, AMF colonization levels of maize roots only showed significant correlation with root dry weight at 60 DAS, with an r = + 0.912 (R^2 = 83.2%, p= 0.031) and shoot dry weight at maturity (100 DAS), with an r = + 0.892 (R^2 = 79.6%, p= 0.042). This could mean that during the vegetative growth, AMF colonization have focused on improving root growth to increase nutrient sorption during the vegetative growth of maize crops.

Table 5. ***Mean dry biomass weight (g/plant) of maize and sorghum for each treatment of fertilization packages***

Fertilization packages	Dry biomass weights (g/plant) of maize (1st crop) and sorghum (2nd crop)							
	Maize root		Maize shoot		Sorghum root		Sorghum shoot	
	60 DAS	100 DAS	60 DAS	100 DAS	60 DAS	100 DAS	60 DAS	100 DAS
D_0	8.13^d	10.43^e	34.15^d	62.29^e	0.82^d	12.30^d	8.31^d	47.74^e
D_1	15.13^b	22.39^b	61.92^b	109.03^b	2.22^b	22.34^b	16.89^b	95.01^b
D_2	17.12^a	34.56^a	72.86^a	111.25^a	3.37^a	24.44^a	23.53^a	105.14^a
D_3	13.65^c	18.48^c	52.26^c	101.05^c	1.68^c	14.52^c	12.01^c	85.46^c
D_4	13.34^c	15.43^d	51.29^c	95.87^d	1.38^c	14.47^c	11.81^c	66.06^d
HSD 5%	0.31	3.04	0.97	2.21	0.30	0.05	0.20	9.54

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages.

In relation to nutrient uptake, although AMF colonization levels did not show significant correlation with N uptake in shoots of maize or sorghum, they did show a positive significant correlation with P uptake both in shoots of maize and sorghum, with an r = + 0.909 (R^2 = 82.6%, p= 0.032) for maize, and r = + 0.940 (R^2 = 88.4%, p= 0.017) for sorghum. These could be due to the ability of AMF extra-radical hyphae in mobilizing and absorbing P from organic matters for their hosts (Feng et al., 2003; Koide & Kabir, 2000; Tarafdar & Marschner, 1994). Based on the strength of the relationships between AMF colonization levels and P uptake, the value of R^2 is higher in sorghum (R^2 = 88.4%) than in maize (R^2 = 82.6%). This means that the contribution of AMF colonization in roots to P uptake in shoots was higher in sorghum crop at the second cropping cycle than in maize in the first cropping cycle. Since sorghum did not receive any fertilizers, and cattle manure is a slow-release organic fertilizer, it could mean that most P nutrient contained in the shoots, especially in shoots of sorghum in the second cropping cycle, was mostly taken up from the manure under the contribution of AMF colonization in the roots. However, this still needs to be confirmed by further research. This view is supported by the conditions of the study area, which was dominated by sand, and if leaching happened during the rainy season, the loss of dissolved nutrients from the NPK fertilizers applied in the first cropping cycle could be high. Therefore, the main source of nutrients, especially for sorghum crop in the second cropping cycle was the cattle manure applied to the maize crop in the first cropping cycle.

Table 6. ***Mean weights of total dry grains (kg/plot) and 100 dry grains for each crop and each treatment of fertilization packages***

Fertilization packages	Mean dry grain yield (kg/plot) and weight of 100 dry grains (g)			
	Maize in the 1st cropping cycle		Sorghum in the 2nd cropping cycle	
	Grain yield	100 grains	Grain yield	100 grains
D_0	9.60^e	22.48^d	3.57^d	2.73^d
D_1	17.40^b	26.94^b	5.05^b	3.01^b
D_2	22.80^a	28.12^a	6.65^a	3.61^a
D_3	15.60^c	25.98^c	4.43^c	2.90^c
D_4	10.20^d	24.61^c	4.17^c	2.81^{cd}
HSD 5%	0.59	1.37	0.26	0.09

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages.

In relation to nitrogen nutrient, there was a very low and non-significant correlation between AMF colonization levels and N uptake in maize shoots with an R^2 of only 41.2%. However, the N uptake of maize at 60 DAS was highly significantly correlated with the N status of the soil with an R^2 = 94.1% (p= 0.006). Moreover, the N soil availability also showed a significant correlation with biomass and grain yield of maize, i.e. with an R^2 = 84.1% (p= 0.029) with grain yield at 60 DAS, R^2 = 92.2% (p= 0.009) with shoot dry weight at 60 DAS, and R^2 = 90.6% (p= 0.012) with shoot dry weight at 100 DAS.

In contrast, for sorghum in the second cropping cycle, the soil N status at 60 DAS showed a significant correlation only with shoot dry weight at 100 DAS. Unlike in maize crop where AMF colonization levels showed a significant and positive correlation with shoot dry weight at maturity (100 DAS), AMF colonization levels in sorghum roots did not show any significant correlation with biomass weight or yield components of sorghum. However, AMF colonization rates in sorghum roots showed a sufficiently high correlation with N uptake in sorghum shoots, with an R^2 = 71.9% (p= 0.069), and N uptake in sorghum shoots showed positive significant correlation with biomass and grain yield of sorghum, i.e. with an R^2 = 80.5% (p= 0.039) with grain yield, R^2 = 83.0% (p= 0.031) with shoot dry weight at 60 DAS, and R^2 = 89.9% (p= 0.014) with shoot dry weight at 100 DAS. These could mean that for maize crop, most nutrients were derived from NPK fertilizers while for sorghum, N and P nutrients were derived from the residues of manure contributed from AMF colonization in sorghum roots. Many researchers have also showed the ability of AMF to utilize organic sources to supply N to their host (e.g. Hawkins et al., 2000; Leigh et al., 2009) although it was also found that AMF colonization did not necessarily result in significantly higher N status of the mycorrhizal than non-mycorrhizal hosts (Hawkins et al., 2000).

However, when the correlation analysis was done between averages of colonization levels, biomass and grain yield between maize in the first and sorghum in the second cropping cycle, in general the results show significant and positive coefficients of correlation. For example, correlation of AMF colonization levels between roots of maize and sorghum are highly significant, with an r = + 0.988 (R^2 = 97.6%, p= 0.002). This means that the pattern of differences in AMF colonization levels between roots of maize and sorghum is highly similar. In other words, AMF colonization levels in roots of maize in the first cropping cycle were carried over into the second cropping cycle where sorghum was grown as the rotation crop. This is because both maize and sorghum are hosts of AMF, and both crops have high mycorrhizal dependency (Guo et al., 2013).

In addition, when a t-test of 'Paired Two Sample for Means' was run on the averages of AMF colonization levels and spore number in soil at maturity of the crops between maize and sorghum in Table 2, higher significant values (p<0.01) were seen on sorghum than on maize. This could be due to the buildup of AMF in the soil after maize crop in the first copping cycle was harvested before sorghum was directly seeded without tillage. Even though both crops were grown simultaneously, it was also found that AMF colonization levels were higher on sorghum than on maize (Guo et al., 2013). This means that sorghum can be used to improve mycorrhizal conditions of the soil under a maize-sorghum cropping sequence.

CONCLUSION

Among the mycorrhiza-based fertilization packages tested on the maize-sorghum cropping sequence system at the drylands in North Lombok of Indonesia, the D_2 package, consisting of 60% NPK recommended dose, 12 t/ha cattle manure, and the indigenous AMF inoculation, was found to be the best fertilization package to improve crop yield and soil nutrient availability. This study noted that the AMF development was higher in the sorghum at the second cropping cycle compared to the growth in the maize at the first cropping cycle. This condition led to the higher NP-uptake and soil nutrient availability in subsequent crops, both at the sandy and dry land, with no additional fertilization and mycorrhizal propagules applied.

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24 Apr 2019 15.10

Dear Dr. Tee

Thank you for the update.

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Tee Syin-Ying [Journal Officer (JTAS ScholarOne)] <journal.officer-1@upm.my>
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Dear Assoc. Prof. Dr. Wahyu Astiko,

You are welcome.

Regards,
(Ms. Syin Ying, Tee on behalf of CEE)
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21-May-2019

Dear Assoc. Prof. Astiko,

Manuscript ID JTAS-1651-2018.R2 entitled "Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-uptake and availability on maize-sorghum cropping sequence in Lombok's drylands" which you submitted to the Journal of Tropical Agricultural Science, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.

The reviewer(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript.

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Once again, thank you for submitting your manuscript to the Journal of Tropical Agricultural Science and I look forward to receiving your revision.

Sincerely,
Prof. Dato' Dr. Abu Bakar Salleh
Chief Executive Editor, Journal of Tropical Agricultural Science
executive_editor.pertanika@upm.my, abubakar@upm.edu.my

Reviewer(s)' Comments to Author:

Reviewer: 2

Comments to the Corresponding Author
The manuscript is suitable for publication in the Journal of Tropical Agricultural Science. However, there are still some minor corrections to be made before it can be accepted for publication.

Line 219 - 226 and table (2): The given statements are inaccurate because no statistical comparison has been made to compare the percentage of AMF colonization for 1st crop (Maize) and 2nd crop (sorghum). Comparisons have been made only for different fertilization packages (D0 - D4) for individual crop. It is highly recommended to the authors to perform new statistical analysis to compare % colonization of AMF (60DAS) for maize vs. Sorghum.

Or else, the authors may consider deleting the statements in line 219-226.

wahyu astiko <astiko@unram.ac.id> 23 Mei 2019 14.59
kepada Pertanika, Tee, MOHD, MScHons,, lolitaabas37

Dear Dr. Tee,

We agree to delete the statement in Line 219-226.

Attached the revised manuscript which also has been uploaded at the journal system together with our response to the minor changes.

Thank you so much for your time and kind help.

Best regards,

Assoc. Prof. Dr. Wahyu Astiko
Agroecotechnology
Faculty of Agriculture, University of Mataram

Edited_JA_ Wahyu ...

Running Title:

Mycorrhizal seed-coating on maize-sorghum cropping sequence

Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-uptake and availability on maize-sorghum cropping sequence in Lombok's drylands

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The List of Number of Tables

Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-uptake and availability on maize-sorghum cropping sequence in Lombok's drylands

ABSTRACT

By improving the nutrient uptake and transport, ~~A~~an indigenous arbuscular mycorrhizal fungal (AMF) is expected to improve crops' performance ~~of food crops~~ in sandy ~~and~~ drylands of North Lombok (Indonesia) ~~in~~ during dry seasons. A field experiment was designed with Randomized Complete Block Design and four replications ~~T~~to examine the benefits of mycorrhiza ~~to maize yield~~ at varying doses ~~on~~ of plant nutrition (nitrogen and phosphorus). Total of ~~,~~ 1 kg of the AMF inoculum was applied to 20 kg maize seeds in different fertilization packages of cattle manure (15, 12, 9, and 6 ton/ha) and inorganic fertilizers (80, 60, 40 and 20% NPK recommended dose). A 100% NPK recommended dose was used as the control (200 kg/ha Phonska and 300 kg/ha Urea). After harvest of maize at 100 days after seeding (DAS) and field cleaning from maize debris, sorghum seeds were then planted in cropping cycle 2 with no additional fertilization and inoculum ~~applied~~. Results indicated that the AMF applications to the maize-sorghum cropping sequence increased the AMF colonization rate, soil ~~the~~ N and P status and ~~, N and P~~ uptake, and dry biomass (root, shoot, and grain). The highest correspondence was observed in the crops which utilized a combination of 60% NPK and 12 ton/ha cattle manure, and the performance was higher at ~~day~~ 100 days after seeding. The number of AMF spores increases over the time where colonization rates were found higher in roots of sorghum (60-81%) than maize (55-75%). ~~When grown simultaneously, mycorrhizal colonization rate on sorghum roots (60-81%) was slightly higher than the rate seen on maize root (55-75%).~~ This study suggests that AMF inoculation increases the ~~maize~~ plant yield and improves soil nutrient availability which is very advantageous for the growth of the maize-sorghum subsequent crop in Lombok's drylands.

Keywords: Seed coating, arbuscular mycorrhizal fungi (AMF), maize-sorghum ~~,~~ cropping sequence, cattle manure, plant nutrition.

INTRODUCTION

The northern part of North Lombok regency (Indonesia) is dominated by drylands with sandy soils texture. With a very short and low number of rainy days (December to April, 100-200 mm) per wet month or no rain during the long dry seasons (May to November), no food crops can be cultivated normally especially in the areas without deep wells. Moreover, inadequate phosphorus (P) availability is also one of the factors limiting the productivity of maize and other food crops in the dryland of North Lombok. Maize crop requires very high P; for example, for the production of 10 ton/ha, maize crops need 102 kg/ha of P_2O_5 and 76% of it is transported into the seeds (Calderón-Vázquez et al., 2009). In contrast, plant roots can take up P at only about 8-13% of the amount of P fertilizer applied (Supardi, 1996). Thus, to cope with the unavailability of soil water and P and other essential nutrients in drylands of North Lombok, arbuscular mycorrhizal fungal (AMF) symbiosis has been used as it is expected to improve the performance of food crops especially during the dry seasons. Many have reported that the soil fungi, in symbiosis with their host plants, can help plants to improve water retention and make their host plants more tolerant to drought (Augé, 2004). AMF colonization also increases nutrient uptake from soils and enhances growth and yield of the host plants although it depends on the species of AMF colonizing the host plants (Marulanda et al., 2003).

The most common findings show that the external hyphae of AMF improve P uptake and transport the nutrient to root cortex of their host (Azcón et al., 2003; Feng et al., 2003; George et al., 1995; Koide & Kabir, 2000; Tarafdar & Marschner, 1994; Tawaraya et al., 2006). Although there is no growth improvement of the host plants due to the AMF symbiosis, the amount of P uptake through AMF (mycorrhizal pathway) can be higher than through the host roots or the direct pathway (Smith & Read, 2010). AMF can also help their hosts to increase the uptake of other essential nutrients such as N, K, Ca, Mg, S, Cu, Zn, Mn, Fe, and B when compared with non-mycorrhizal plants (Dhillion & Ampornpan, 1992; Hawkins et al., 2000; Leigh et al., 2009; Menge, 1983). Most of these capabilities of the AM plants are due to the extensive growth of AMF external hyphae beyond the host roots (Drew et al., 2003; Zhu et al., 2001). The length ratio of AMF hyphae to roots in soil can be 100:1 or higher and with the external hyphae, the mycorrhizal roots can explore further (George et al., 1995). By applying the Plenchette's mycorrhizal dependency formula to the shoot dry weight of maize and sorghum (Plenchette et al., 1983), the results showed a high mycorrhizal dependency, both for maize (up to 70.12%) and for sorghum (up to 79.42%) (Guo et al., 2013; Tawaraya, 2003). The findings of the study indicate that the higher the dependent rate is on AMF symbiosis, the more the dry

matter is produced of the crops. With the high porosity and low water retention capacity on dryland and sandy areas (Bruand et al., 2005) in most areas of North Lombok, the levels of crop dependence on symbiosis with AMF could be higher. Moreover, the symbiosis of crops with AMF could significantly reduce nutrient loss from soil through leaching (Cavagnaro et al., 2015).

The establishment of AM symbiosis can be done through inoculation with AMF propagules. Our previous study (Astiko et al., 2013a) showed that the indigenous AMF inoculation in maize plants in sandy soils had positive implications for the improvement of soil properties by increasing the rates of nutrient uptake by maize crop from the soil and improving its grain yield. Our research group has also shown the benefit of this local inoculation in increasing the growth of soybean and its yield by improving P uptake from the soils, compared to those without AMF inoculation (Astiko et al., 2013b). However, as found from other studies, the development of AMF in the soil and its contribution to growth, yield and nutrient uptake of host plants could be influenced by the order of plant species cultivated in sequence in the cropping system (Johnson et al., 1992; Vivekanandan & Fixen, 1991). The studies by Johnson et al. (1992) an Vivekanandan and Fixen (1991) reported that the P uptake and the AMF colonization were higher on maize crops grown following soybean compared to when maize crops were grown following maize or barley.

From the previous study on maize-soybean cropping patterns, it was found that the AMF inoculated in maize crops grown in the first cycle increased root colonization and AMF sporulation which was very advantageous for the growth of the following crop in the cropping sequence (Astiko, 2013). Different AMF populations (colonization and spore counts) were also found between cropping seasons or between crop species in the same cropping season in Central Lombok, Indonesia (Wangiyana et al., 2006). The present study examined the effects of several combinations of fertilizer packages consisting of indigenous AMF bio-fertilizer and varying doses of organic and NPK fertilizers applied to maize crops on N and P status in soil, and uptake by maize and subsequent sorghum crops as well as the growth and yield components of the maize and sorghum crops in a maize-sorghum cropping sequence on drylands in North Lombok, Indonesia.

MATERIALS AND METHODS

Design of the experiment

The field experiment of maize-sorghum cropping sequence in this study was arranged according to the Randomized Complete Block Design (RCBD) with four replications (blocks).

The study was carried out in the Akar-Akar village located in North Lombok regency, Indonesia, from January to August 2016. Treatments involving the use of five fertilizer packages consisting of different combinations of organic, inorganic and indigenous AMF bio-fertilizer were applied only to the maize crop in the first cropping cycle of the maize-sorghum cropping sequence. ~~The details of the treatments or fertilization packages are listed in Table 1.~~ The 100% NPK-only recommended dose (D0) is the farmer's practice of dose for maize by the locals. The NPK's doses were decreased and had been replaced by cattle manure in varying fertilization packages (D1, D2, D3, D4), and added with AMF as listed in Table 1.

Table 1. ***The mycorrhizal-based fertilization packages tested and applied to maize only in the maize-sorghum cropping sequence***

Fertilization packages	Doses of the packages applied to maize plants only (in the cropping cycle 1)	Sorghum (cropping cycle 2)
D_0	NPK only at 100% the recommended doses (RD), i.e. 200 kg/ha Phonska (NPK 15:15:15) and 300 kg/ha Urea	No fertilizer applied
D_1	NPK at 80% RD + 15 ton/ha cattle manure + AMF	No fertilizer applied
D_2	NPK at 60% RD + 12 ton/ha cattle manure + AMF	No fertilizer applied
D_3	NPK at 40% RD + 9 ton/ha cattle manure + AMF	No fertilizer applied
D_4	NPK at 20% RD + 6 ton/ha cattle manure + AMF	No fertilizer applied

A piece of land used in this study was of a sandy soil type (69% sand, 29% silt, and 2% clay, containing an average of 421 AMF spores per 100 g soil), typical of North Lombok area. The land is located at a geographic position of -8.221650, 116.350283, and measured with 13.82 mg/kg of available P, 0.01% Total N, 0.57 cmol/kg of available K, 7.31 cmol/kg of Ca, and 1.21% of C-organic. After being cultivated using minimum tillage and cleared of weeds, the land was split into 20 treatment plots of 7m x 5m based on the RCBD plotting layout. The different fertilization packages consisted of AMF inoculum, organic fertilizer (cattle manure), and inorganic fertilizer (NPK and urea), were applied only to the maize crop in the first cropping cycle.

An indigenous AMF inoculum, ~~i.e.~~ *Glomus mosseae* (the M_{AA01} mycorrhizal isolate including the hyphae and the mycorrhizal spores), which was originally isolated from dryland area (1,500 spores per 20 g of soils) in Akar-Akar village of North Lombok, was applied through seed-coating of maize seeds prior to seeding. Before application, 1 kg of the AMF inoculum was mixed with 300 g of charcoal powder and 90 g of tapioca flour as the carrier materials. Then this inoculum mixture was mixed with 20 kg maize seeds so that the seeds were coated by the inoculum mixture. For cropping cycle 1, the uncoated or AMF coated maize seeds ("Bisma" variety) were planted by dibbling 2 seeds per planting hole at 70 cm x 20 cm plant spacing. The entire dose of cattle manure (organic fertilizer) at different doses depending on the treatments (Table 1) was applied at the time of planting the maize seeds by burying the whole dose in the planting hole in the position below the seeds. The cattle manure variation in the fertilization package is to identify the optimum combinations to benefit the plant growth, increase the nutrient availability at the soils, and support the AMF development. The maximum combination of cattle manure was 15 ton/ha, the limit for transportation. The cattle manure applied in the package was measured with 3.08% Total Nitrogen, pH 6.66, 17.70 mg/kg of available P, 2.31 cmol/kg of available K, 10.45 C/N ration, and 32.2% C-organic. The inorganic (NPK) fertilizers with the recommended doses of 200 kg/ha Phonska (NPK 15:15:15) and 300 kg/ha Urea (46% N) were applied twice, i.e. at 7 DAS (days after seeding) and at 21 DAS, with the amount applied depending on the treatments (Table 1). At 7 DAS, the maize plants were fertilized with a mixture of the entire Phonska and one third of the Urea treatment doses, followed by application of the remaining Urea treatment doses at 21 DAS. The NPK fertilizers were applied in a furrow of 5 cm alongside the maize plant row at 5-7 cm depth before covered with soil.

After harvest of maize at 100 days after seeding (DAS) and field cleaning from maize debris, sorghum seeds were then planted in cropping cycle 2. In cropping cycle 2, the plots were cleared from maize crop debris and weeds before Before the second sequence, the field was left fallow (rest) for 10 days. Seeds of sorghum ("Numbu" variety) were directly seeded by dibbling 2 seeds per planting holes made around the maize stubbles. These sorghum plants were not fertilized nor inoculated with additional AMF inoculum, but only the chopped maize roots containing the AMF mycorrhizal and spread throughout the plot. For both crops, the young maize and sorghum plants were tinned at 7 DAS by leaving one maize or sorghum plant per planting hole. Weeding and soil piling of the maize and sorghum plant base were done at 15 and 30 DAS. Plant protection was done by spraying "OrgaNeem" (an organic pesticide of plant origin containing Azadirachtin extracted from neem leaves) at a concentration of 5 ml OrgaNeem per liter of water. The OrgaNeem solution was applied to both crops (maize or sorghum) from 10 to 60 DAS at a 3-day interval. Harvesting of maize or sorghum crops was done at 100 DAS.

Measurement and data analysis

The variables measured were AMF development, N and P nutrition, and growth and yield of maize and sorghums. The AMF development includes spore counts at 60 and 100 DAS, and root colonization levels at 60 DAS. The N and P nutrition includes concentration of total N and available P in the rhizosphere of maize and sorghum at the time when the vegetative and generative growth found optimum, respectively at 60 and 100 DAS. The crop variables include dry weight (shoots and roots) and yield components (grain).

Laboratory analysis was conducted at Laboratory of Chemistry and Soil Science, Faculty of Agriculture, Universitas Mataram. Soil pH and texture were measured by standard procedures (Imam & Didar, 2005). Determination of total nitrogen in soil was done by destruction with $(NH_4)_2SO_4$ and distillation with NaOH where the $NH_4^+$was determined by indophenol blue colorimetric method and the NH_3 was defined by a titration with 0.05N of H_2SO_4 solution (Page et al., 1982). Total N in plants was measured using spectrophotometric indophenol blue methods with wave length 636 nm after destruction by $(NH_4)_2SO_4$ and distillation with NaOH following the Conway instruction (Lisle et al., 1990). The available Phosphorus in soil and plant was measured using spectrophotometer (λ = 693 nm) after the extraction process using Bray and Kurt I solution (0,025 N HCl + NH4F 0,03 N) (Bray & Kurtz, 1945). Total organic C was measured by oxidation with $K_2Cr_2O_7$ in presence of sulphuric acid (H_2SO_4) following Walkley and Black's method (Horwitz, 2000).

Determination of N was done using the Kjeldhal method and P by using a spectrometer. AMF spore extraction from soil (100 g soil sample) was done using the wet sieving and decanting technique (Brundrett et al., 1996). The spores collected from the 38 µm sieve after the final centrifugation were captured in a filter paper, which were then observed on a Petri dish using a stereo microscope at 40 x magnification, and counted as spore number per 100 g of soil. The perceantage of root colonization was determined using the Gridline Intersect technique (Giovannetti & Mosse, 1980) under a stereo microscope after the root pieces were stained using the clearing and staining method of Brundrett et al. (1996).

Data were analyzed using analysis of two wayvariance (ANOVA) and the Tukey's HSD (Honestly Significant Difference) means tested at 5% level of significance.

RESULTS AND DISCUSSION

AMF development

In terms of root colonization rates by AMF, there were slight differences between maize and sorghum between treatments fertilizer packages. This can be seen from Table 2 which displays the levels of root colonization by the indigenous AMF which are slightly higher on sorghum roots than on maize roots in each treatment, especially in the D3 treatment where it was 77% on sorghum and 65% on maize roots. When grown simultaneously, Carrenho et al. (2007) found higher colonization rates on maize than on sorghum (Carrenho et al., 2007). In

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this study, however, maize and sorghum were grown in sequence, in which sorghum was directly seeded without treatments and without tillage following the harvest of maize plants. All treatments, including AMF inoculation, were applied only to maize plants in the first cropping cycle. Therefore, it means that sorghum in cropping cycle 2 was grown after AMF propagules have built up in the soil during the growth of the maize plants in cropping cycle 1. This is in line with the results reported in the previous study (Arihara & Karasawa, 2000) which revealed that AMF root colonization and shoot dry weight of maize at 58 DAS were affected by the previous crop grown, whether they were host or non-host of AMF. When grown simultaneously, Carrenho et al. (2007) found higher colonization rates on maize than on sorghum (Carrenho et al., 2007). However, the P fertilization did not affect root colonization, especially on maize following AMF host plants.

Table 2. ***Mean spore number (spores per 100 g soil) at 60 and 100 DAS, and colonization rates (%-colonization) on maize and sorghum in a maize-sorghum cropping sequence***

Fertilization packages	AMF on maize (1st crop)			AMF sorghum (2nd crop)		
	Spore per 100 g soil		% colonization	Spore per 100 g soil		% colonization
	60 DAS	100 DAS	60 DAS	60 DAS	100 DAS	60 DAS
D_0	764^{e}	3464^{d}	30^{d}	1231^{e}	3761^{d}	35^{e}
D_1	1059^{d}	3672^{c}	55^{c}	1343^{d}	4942^{b}	60^{d}
D_2	2119^{a}	4327^{a}	75^{a}	2981^{a}	5165^{a}	81^{a}
D_3	1690^{b}	3894^{b}	65^{b}	1881^{b}	4831^{c}	77^{b}
D_4	1294^{c}	3881^{b}	63^{b}	1769^{c}	4819^{c}	68^{c}
HSD 5%	231	13	2.0	109	12	6.5

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for descriptions of the packages.

Specifically, the degree of root colonization by AMF may be higher in the roots of maize fertilized with highly soluble mineral fertilizers (NPK, diammonium phosphate, ammonium sulfate and Urea). On the other hand, the length of extra-radical mycelium and number of AMF spores were significantly higher in maize fertilized with organic fertilizers (Bokashi manure) (Bautista-Cruz et al., 2014). This indicates that organic fertilization is more favorable for spore production compared with conventional fertilization. It can also be seen from Table 2 that AMF spore numbers are higher at harvest of the maize crop (100 DAS) compared with those at 60 DAS, especially on the D_2 treatment. This indicates a high buildup of AMF propagules for the subsequent sorghum crops. This condition seems to favor higher AMF colonization levels in roots of sorghum in the second cropping cycle compared with that of maize in the first cropping cycle. With no tillage treatment, the AMF colonization levels in sorghum may be higher than the levels in maize or bean roots (Alguacil et al., 2008). In addition, AMF colonization rates may be higher in roots of sorghum than in the roots of maize, either inoculated with *Glomus mosseae* or *G. versiforme*, indicating that sorghum is more favorable for AMF development than maize plants (Guo et al., 2013).

There are many factors influencing the degrees of AMF colonization of crop roots as reported by Carrenho et al. (2007). In examining the effects of different phosphorus, liming, organic matter, and soil texture on root colonization of peanuts, sorghum, and maize in a factorial experiment, each factor significantly affected root colonization level, alone or in interaction with other factors (Carrenho et al., 2007). The most surprising results were the significant interaction effects of plant, phosphorous, and organic matter although there were no significant effects of phosphorous (simple superphosphate of 166.6 mg/L (w/v) soil) nor organic matter (22 mg/L (w/v) soil). As for the interaction effect involving maize, there was no significant effect of phosphorous and organic matter on AMF colonization of maize roots. On the other hand, the application of both phosphorous and organic matter significantly reduced AMF colonization on sorghum roots (Carrenho et al., 2007).

In terms of fertilization effects, AMF colonization rate was reported to be higher on crop roots in unfertilized soils, followed by organic and biodynamic, and the least was seen in exclusively mineral fertilized and conventional farming systems. Thus, it was concluded that the amount of soluble P in the soil was the most limiting factor for AMF colonization in roots of those crops (winter wheat, vetch-rye, and grass-clover) (Mäder et al., 2000). Moreover, it was reported that the increasing P level in the growing media from 0.1 to 0.5 mM P significantly reduced AMF colonization in roots of lettuce plants especially with increasing levels of N contents (1, 5, or 9 mM N) (Azcón et al., 2003).

A negative impact on soil condition occur when the accumulation of soil P has increased beyond the requirement of the crops cultivated (Grant et al., 2005). In the study, it seemed that the amount of P input from the NPK fertilizer as applied in the D_2 treatment was most favorable for AMF development in maize crops. In the D_2 treatment, the NPK fertilizer was reduced to 120 kg/ha Phonska (60% of the recommended NPK dose of 200 kg/ha Phonska in the D_0 treatment) combined with an application of 12 ton/ha cattle manure and inoculation with AMF. Among the treatments with AMF in combination with cattle manure, the D_1 treatment had the highest NPK fertilizer dose (Table 1), and this level of NPK fertilization seems to limit the AMF infection and development in maize roots compared with those in the D_2 treatment, resulting in a higher AMF colonization rate on D_2 than on D_1 treatment. Therefore, AMF colonization and spore numbers in maize were highest in the D_2 treatment, especially when compared with the D_1 or D_0 treatment. Using the same indigenous AMF applied to a pot experiment in which the soil for the growing media was taken from the same field in North Lombok, it was found that the highest level of AMF colonization of maize roots was in the treatment with AMF combined with cattle manure compared with AMF combined with NPK fertilizer or phosphate rock (Astiko et al., 2013a). The previous study also reported a significantly higher AMF spore number in plots fertilized with cattle manure or cattle manure combined with mineral fertilizers compared with those fertilized with mineral fertilizers only (Gryndler et al., 2006).

Soil nutrient status and nutrient sorption by maize and sorghum

There were significant effects of the different fertilizer packages on soil nutrient status (N and P contents of the soil) of the rhizosphere of both maize and sorghum crops at 60 and 100 DAS (Table 3). In general, there is a tendency that the highest values of the soil nutrient status, measured either at 60 or 100 DAS, are highest under the D_2 (60% NPK + 12 t/ha manure + AMF) or D_1 (80% NPK + 15 t/ha manure + AMF) treatment. The values of the soil nutrient status in these treatments were higher than those in D_0 (100% NPK only) treatment, although the dose of NPK fertilizers was reduced in the D_1 and D_2 treatments (Table 3).

Table 3. ***Mean concentration of total N and available P of soil in the rhizospheres of maize (1st crop) and sorghum (2nd crop) for each treatment of fertilization packages, measured at 60 and 100 DAS***

Fertilization packages	Total N ($g.kg^{-1}$) at 60 DAS		Available P ($mg.kg^{-1}$) at 60 DAS		Total N ($g.kg^{-1}$) at 100 DAS		Available P ($mg.kg^{-1}$) at 100 DAS	
	maize	sorghum	maize	sorghum	maize	sorghum	maize	sorghum
D_0	1.24^c	1.10^c	14.97^d	11.41^d	1.33^d	1.23^d	17.43^d	10.15^d
D_1	1.45^a	1.29^a	28.44^b	16.25^b	1.69^b	1.38^b	29.51^b	21.58^b
D_2	1.47^a	1.25^{ab}	35.02^a	28.49^a	1.86^a	1.48^a	36.56^a	29.99^a
D_3	1.39^b	1.22^{ab}	17.52^c	14.59^{bc}	1.47^c	1.31^c	19.37^c	18.59^c
D_4	1.33^b	1.20^b	16.29^c	14.25^c	1.42^c	1.31^c	18.53^c	17.32^c
HSD 5%	0.08	0.07	1.31	1.99	0.08	0.06	1.0	2.98

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages.

At the present study, no infiltration data was measured, however it is important to note that ~~S~~since the NPK fertilizers applications~~ed~~, i.e. Phonska and Urea at 7 DAS and Urea at 21 DAS, at sandy lands, are easily dissolved in water, and significant amount of the nutrients could have been loss through infiltration during the rainy season. Previous study shows that the sand content of the cultivated land had a positive correlation with infiltration rate, with an r-value of +0.75 (Patle et al., 2018). Since cattle manure is slow in releasing nutrients, it is possible that at the time of measurement (60 and 100 DAS), the loss of released nutrients from the NPK fertilizers through leaching was much higher in the D_0 than in the other treatments of fertilization packages due to dissolution by rain water during the rainy season. However, there were small increases in nutrient status of the soil from 60 to 100 DAS around the rhizospheres of maize crop, especially in those treated with manure and AMF application. This indicated a slow release of N and P nutrients from manure after application to the maize plants in the first cropping cycle, which could be due to AMF inoculation. Many studies have reported that AMF can mobilize and take N and P from organic matters for their hosts (Feng et al., 2003; Hawkins et al., 2000; Koide & Kabir, 2000; Leigh et al., 2009; Tarafdar & Marschner, 1994), and it can be seen from Table 2 that the highest average of AMF colonization levels in maize roots was in the D_2 treatment. Therefore, N and P uptake in shoots of maize and sorghum was highest in the D_2 treatment (Table 4).

In addition, soil contents of those nutrients corresponded well with the levels of N and P uptake of the crops, i.e. all showing positive correlation coefficients. Although it is not significant for P uptake, the highest values of N and P uptake were also seen in the D_2 treatment for both maize and sorghum (Table 4). The correlation analysis of the mean values obtained at 60 DAS showed a significant correlation between soil N and N sorption in the shoots, with a value of r = + 0.970 (R-square = 94.1%, p = 0.006) for maize plants, and r = + 0.904 (R-square = 81.7%, p = 0.035) for sorghum plants. These indicate those fertilizers in the packages contribute to nutrient contents of the crops, which are significantly different between treatments of fertilization packages (Table 4).

Table 4. ***Mean N and P sorption (mg.g^{-1} plant dry weight) by each crop at 60 DAS for each treatment of fertilization packages***

Fertilization packages	N and P uptake (mg.g^{-1} plant dry weight) by each crop at 60 DAS			
	Maize (1st cropping cycle)		Sorghum (2nd cropping cycle)	
	N	P	N	P
D_0	18.62^{e}	1.71^{d}	12.74^{d}	0.65^{d}
D_1	27.58^{b}	2.60^{b}	18.92^{b}	0.85^{c}
D_2	28.00^{a}	2.72^{a}	20.86^{a}	1.48^{a}
D_3	23.10^{c}	2.41^{c}	17.29^{c}	1.36^{b}
D_4	20.44^{d}	2.41^{c}	17.22^{c}	1.32^{b}
HSD 5%	0.41	0.11	0.07	0.04

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages.

However, P status of the soil did not show a significant correlation with P uptake either by maize or sorghum crop. In spite of that, there is a significant positive correlation between the degree of AMF colonization in the roots and P sorption in the shoot of maize plants, with an r = + 0.909 (R-square = 82.6%, p = 0.032), and that of sorghum plants, with an r = + 0.940 (R-square = 88.4%, p = 0.017). This shows that there were significant contributions of AMF colonization in the roots to the amount of P taken up by the plants, both maize and sorghum. If soil soluble P has not exceeded crop requirements, AMF association will still result in significantly positive effects on P uptake and biomass production because of P requirements of the crops; for optimum growth and yields, crops require P since the early stage of their growth, either from soil, fertilizer application, or from AMF associations (Grant et al., 2005).

Although sorghum crop in the second cropping cycle was not fertilized with manure nor NPK fertilizers, direct seeding of sorghum after harvesting the maize plants without tillage seemed to be a favorable condition in establishing AMF association in the sorghum crop, which resulted in higher AMF colonization rates in sorghum than in maize roots for each fertilizer package (Table 2). The AMF associations seemed to enable the sorghum crops to absorb adequate P and other nutrients from soil and residues of the manure applied in the first cropping cycle even though these sorghum crops were not fertilized. This could occur because of the potential of AMF external hyphae that help the host plants in absorbing and dissolving nutrients from soil and manures and other nutrient pools, as well as other nutrients unavailable for uptake by plant roots (Menge, 1983; Smith & Read, 2010; Tawaraya et al., 2006). Guo et al. (2013) also reported significant effects of AMF inoculation on C, N, P, and K contents (mg/pot) in shoot and roots of maize and sorghum grown in pots filled with mixtures of mine tailing and topsoil, and between the AMF species used, *G. versiforme* showed higher colonization rates which resulted in higher nutrient sorption and biomass of maize and sorghum compared with *G. mosseae* (Guo et al., 2013).

Biomass and yield components of maize and sorghum

In terms of biomass production and yield components of maize and sorghum, there were also significant effects of the fertilization packages in which D_2 treatment presents the highest values of biomass production (Table 5) and yield components (Table 6). This means that the nutrient status of the soils corresponded well with the biomass weight of the crops, indicated by the positive correlation coefficients, although only some of them are significant. The AMF applications to maize crops, observed at 60 DAS, showed a significant correlation to dry shoot weight, N and P status of the soil, and the N and P sorption. In contrast, AMF colonization levels of maize roots only showed significant correlation with root dry weight at 60 DAS, with an r = + 0.912 (R^2 = 83.2%, p= 0.031) and shoot dry weight at maturity (100 DAS), with an r = + 0.892 (R^2 = 79.6%, p= 0.042). This could mean that during the vegetative growth, AMF colonization have focused on improving root growth to increase nutrient sorption during the vegetative growth of maize crops.

Table 5. ***Mean dry biomass weight (g/plant) of maize and sorghum for each treatment of fertilization packages***

Fertilization packages	Dry biomass weights (g/plant) of maize (1st crop) and sorghum (2nd crop)							
	Maize root		Maize shoot		Sorghum root		Sorghum shoot	
	60 DAS	100 DAS	60 DAS	100 DAS	60 DAS	100 DAS	60 DAS	100 DAS
D_0	8.13^d	10.43^e	34.15^d	62.29^e	0.82^d	12.30^d	8.31^d	47.74^e
D_1	15.13^b	22.39^b	61.92^b	109.03^b	2.22^b	22.34^b	16.89^b	95.01^b
D_2	17.12^a	34.56^a	72.86^a	111.25^a	3.37^a	24.44^a	23.53^a	105.14^a
D_3	13.65^c	18.48^c	52.26^c	101.05^c	1.68^c	14.52^c	12.01^c	85.46^c
D_4	13.34^c	15.43^d	51.29^c	95.87^d	1.38^c	14.47^c	11.81^c	66.06^d
HSD 5%	0.31	3.04	0.97	2.21	0.30	0.05	0.20	9.54

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages.

In relation to nutrient uptake, although AMF colonization levels did not show significant correlation with N uptake in shoots of maize or sorghum, they did show a positive significant correlation with P uptake both in shoots of maize and sorghum, with an r = + 0.909 (R^2 = 82.6%, p= 0.032) for maize, and r = + 0.940 (R^2 = 88.4%, p= 0.017) for sorghum. These could be due to the ability of AMF extra-radical hyphae in mobilizing and absorbing P from organic matters for their hosts (Feng et al., 2003; Koide & Kabir, 2000; Tarafdar & Marschner, 1994). Based on the strength of the relationships between AMF colonization levels and P uptake, the value of R^2 is higher in sorghum (R^2 = 88.4%) than in maize (R^2 = 82.6%). This means that the contribution of AMF colonization in roots to P uptake in shoots was higher in sorghum crop at the second cropping cycle than in maize in the first cropping cycle. Since sorghum did not receive any fertilizers, and cattle manure is a slow-release organic fertilizer, it could mean that most P nutrient contained in the shoots, especially in shoots of sorghum in the second cropping cycle, was mostly taken up from the manure under the contribution of AMF colonization in the roots. However, this still needs to be confirmed by further research. This view is supported by the conditions of the study area, which was dominated by sand, and if leaching happened during the rainy season, the loss of dissolved nutrients from the NPK fertilizers applied in the first cropping cycle could be high. Therefore, the main source of nutrients, especially for sorghum crop in the second cropping cycle was the cattle manure applied to the maize crop in the first cropping cycle.

Table 6. ***Mean weights of total dry grains (kg/plot) and 100 dry grains for each crop and each treatment of fertilization packages***

Fertilization packages	Mean dry grain yield (kg/plot) and weight of 100 dry grains (g)			
	Maize in the 1st cropping cycle		Sorghum in the 2nd cropping cycle	
	Grain yield	100 grains	Grain yield	100 grains
D_0	9.60^{e}	22.48^{d}	3.57^{d}	2.73^{d}
D_1	17.40^{b}	26.94^{b}	5.05^{b}	3.01^{b}
D_2	22.80^{a}	28.12^{a}	6.65^{a}	3.61^{a}
D_3	15.60^{c}	25.98^{c}	4.43^{c}	2.90^{c}
D_4	10.20^{d}	24.61^{c}	4.17^{c}	2.81^{cd}
HSD 5%	0.59	1.37	0.26	0.09

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages.

In relation to nitrogen nutrient, there was a very low and non-significant correlation between AMF colonization levels and N uptake in maize shoots with an R^2 of only 41.2%. However, the N uptake of maize at 60 DAS was highly significantly correlated with the N status of the soil with an R^2 = 94.1% (p= 0.006). Moreover, the N soil availability also showed a significant correlation with biomass and grain yield of maize, i.e. with an R^2 = 84.1% (p= 0.029) with grain yield at 60 DAS, R^2 = 92.2% (p= 0.009) with shoot dry weight at 60 DAS, and R^2 = 90.6% (p= 0.012) with shoot dry weight at 100 DAS.

In contrast, for sorghum in the second cropping cycle, the soil N status at 60 DAS showed a significant correlation only with shoot dry weight at 100 DAS. Unlike in maize crop where AMF colonization levels showed a significant and positive correlation with shoot dry weight at maturity (100 DAS), AMF colonization levels in sorghum roots did not show any significant correlation with biomass weight or yield components of sorghum. However, AMF colonization rates in sorghum roots showed a sufficiently high correlation with N uptake in sorghum shoots, with an R^2 = 71.9% (p= 0.069), and N uptake in sorghum shoots showed positive significant correlation with biomass and grain yield of sorghum, i.e. with an R^2 = 80.5% (p= 0.039) with grain yield, R^2 = 83.0% (p= 0.031) with shoot dry weight at 60 DAS, and R^2 = 89.9% (p= 0.014) with shoot dry weight at 100 DAS. No additional fertilizers and inoculums were applied at the cropping cycle 2. These could mean that for maize crop, most nutrients were derived from NPK fertilizers while for sorghum, N and P nutrients were derived from the residues of manure contributed from AMF colonization in sorghum roots. Many researchers have also showed the ability of AMF to utilize organic sources to supply N to their host (e.g. Hawkins et al., 2000; Leigh et al., 2009) although it was also found that AMF colonization did not necessarily result in significantly higher N status of the mycorrhizal than non-mycorrhizal hosts (Hawkins et al., 2000).

However, when the correlation analysis was done between averages of colonization levels, biomass and grain yield between maize in the first and sorghum in the second cropping cycle, in general the results show significant and positive coefficients of correlation. For example, correlation of AMF colonization levels between roots of maize and sorghum are highly significant, with an r = + 0.988 (R^2 = 97.6%, p= 0.002). This means that the pattern of differences in AMF colonization levels between roots of maize and sorghum is highly similar. In other words, AMF colonization levels in roots of maize in the first cropping cycle were carried over into the second cropping cycle where sorghum was grown as the rotation crop. This is because both maize and sorghum are hosts of AMF, and both crops have high mycorrhizal dependency (Guo et al., 2013).

In addition, when a t-test of ‘Paired Two Sample for Means’ was run on the averages of AMF colonization levels and spore number in soil at maturity of the crops between maize and sorghum in Table 2, higher significant values (p<0.01) were seen on sorghum than on maize. This could be due to the buildup of AMF in the soil after maize crop in the first copping cycle was harvested before sorghum was directly seeded without tillage. Even though both crops were grown simultaneously, it was also found that AMF colonization levels were higher on sorghum than on maize (Guo et al., 2013). This means that sorghum can be used to improve mycorrhizal conditions of the soil under a maize-sorghum cropping sequence.

CONCLUSION

Among the mycorrhiza-based fertilization packages tested on the maize-sorghum cropping sequence system at the drylands in North Lombok of Indonesia, the D_2 package, consisting of 60% NPK recommended dose, 12 t/ha cattle manure, and the indigenous AMF inoculation, was found to be the best fertilization package to improve crop yield and soil nutrient availability. This study noted that the AMF development was higher in the sorghum at the second cropping cycle compared to the growth in the maize at the first cropping cycle. This condition led to the higher NP-uptake and soil nutrient availability in subsequent crops, both at the sandy ~~and~~ dry land, with no additional fertilization and mycorrhizal propagules applied.

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Mycorrhizal Seed-coating on Maize-sorghum Cropping Sequence

Indigenous Mycorrhizal Seed-coating Inoculation on Plant Growth and Yield, and NP-uptake and Availability on Maize-sorghum Cropping Sequence in Lombok's Drylands

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The List of Number of Tables

Indigenous Mycorrhizal Seed-coating Inoculation on Plant Growth and Yield, and NP-uptake and Availability on Maize-sorghum Cropping Sequence in Lombok's Drylands

ABSTRACT

By improving the nutrient uptake and transport, an indigenous arbuscular mycorrhizal fungal (AMF) is expected to improve crops' performance in sandy drylands of North Lombok (Indonesia) during dry seasons. A field experiment was designed with Randomized Complete Block Design and four replications to examine the benefits of mycorrhiza at varying doses of plant nutrition (nitrogen and phosphorus). Total of 1 kg of the AMF inoculum was applied to 20 kg maize seeds in different fertilization packages of cattle manure (15, 12, 9 and 6 ton/ha) and inorganic fertilizers (80, 60, 40 and 20% NPK recommended dose). A 100% NPK recommended dose was used as the control (200 kg/ha Phonska and 300 kg/ha Urea). After harvest of maize at 100 days after seeding (DAS) and field cleaning from maize debris, sorghum seeds were then planted in cropping cycle 2 with no additional fertilization and inoculum. Results indicated that the AMF applications to the maize-sorghum cropping sequence increased the AMF colonization rate, soil N and P status and uptake, and dry biomass (root, shoot, and grain). The highest correspondence was observed in the crops which utilized a combination of 60% NPK and 12 ton/ha cattle manure, and the performance was higher at 100 days after seeding. The number of AMF spores increases over the time where colonization rates were found higher in roots of sorghum (60-81%) than maize (55-75%). This study suggests that AMF inoculation increases the plant yield and improves soil nutrient availability which is very advantageous for the growth of the maize-sorghum subsequent crop in Lombok's drylands.

Keywords: Arbuscular mycorrhizal fungi (AMF), cattle manure, maize-sorghum cropping sequence, plant nutrition, seed coating

INTRODUCTION

The northern part of North Lombok regency (Indonesia) is dominated by drylands with sandy soils texture. With a very short and low number of rainy days (December to April, 100-200 mm) per wet month or no rain during the long dry seasons (May to November), no food crops can be cultivated normally especially in the areas without deep wells. Moreover, inadequate phosphorus (P) availability is also one of the factors limiting the productivity of maize and other food crops in the dryland of North Lombok. Maize crop requires very high P; for example, for the production of 10 ton/ha, maize crops need 102 kg/ha of P_2O_5 and 76% of it is transported into the seeds (Calderón-Vázquez et al., 2009). In contrast, plant roots can take up P at only about 8-13% of the amount of P fertilizer applied (Supardi, 1996). Thus, to cope with the unavailability of soil water and P and other essential nutrients in drylands of North Lombok, arbuscular mycorrhizal fungal (AMF) symbiosis has been used as it is expected to improve the performance of food crops especially during the dry seasons. Many have reported that the soil fungi, in symbiosis with their host plants, can help plants to improve water retention and make their host plants more tolerant to drought (Augé, 2004). AMF colonization also increases nutrient uptake from soils and enhances growth and yield of the host plants although it depends on the species of AMF colonizing the host plants (Marulanda et al., 2003).

The most common findings show that the external hyphae of AMF improve P uptake and transport the nutrient to root cortex of their host (Azcón et al., 2003; Feng et al., 2003; George et al., 1995; Koide & Kabir, 2000; Tarafdar & Marschner, 1994; Tawaraya et al., 2006). Although there is no growth improvement of the host plants due to the AMF symbiosis, the amount of P uptake through AMF (mycorrhizal pathway) can be higher than through the host roots or the direct pathway (Smith & Read, 2010). AMF can also help their hosts to increase the uptake of other essential nutrients such as N, K, Ca, Mg, S, Cu, Zn, Mn, Fe and B when compared with non-mycorrhizal plants (Dhillion & Ampornpan, 1992; Hawkins et al., 2000; Leigh et al., 2009; Menge, 1983). Most of these capabilities of the AM plants are due to the extensive growth of AMF external hyphae beyond the host roots (Drew et al., 2003; Zhu et al., 2001). The length ratio of AMF hyphae to roots in soil can be 100:1 or higher and with the external hyphae, the mycorrhizal roots can explore further (George et al., 1995). By applying the Plenchette's mycorrhizal dependency formula to the shoot dry weight of maize and sorghum (Plenchette et al., 1983), the results showed ~~a~~ high mycorrhizal dependency, both for maize (up to 70.12%) and for sorghum (up to 79.42%) (Guo et al., 2013; Tawaraya, 2003). The findings of the study indicate that the higher the dependent rate is on AMF symbiosis, the more the dry

matter is produced of the crops. With the high porosity and low water retention capacity on dryland and sandy areas (Bruand et al., 2005) in most areas of North Lombok, the levels of crop dependence on symbiosis with AMF could be higher. Moreover, the symbiosis of crops with AMF could significantly reduce nutrient loss from soil through leaching (Cavagnaro et al., 2015).

The establishment of AM symbiosis can be done through inoculation with AMF propagules. Our previous study (Astiko et al., 2013a) showed that the indigenous AMF inoculation in maize plants in sandy soils had positive implications for the improvement of soil properties by increasing the rates of nutrient uptake by maize crop from the soil and improving its grain yield. Our research group has also shown the benefit of this local inoculation in increasing the growth of soybean and its yield by improving P uptake from the soils, compared to those without AMF inoculation (Astiko et al., 2013b). However, as found from other studies, the development of AMF in the soil and its contribution to growth, yield and nutrient uptake of host plants could be influenced by the order of plant species cultivated in sequence in the cropping system (Johnson et al., 1992; Vivekanandan & Fixen, 1991). The studies by Johnson et al. (1992) as well as Vivekanandan and Fixen (1991) reported that the P uptake and the AMF colonization were higher on maize crops grown following soybean compared to when maize crops were grown following maize or barley.

From the previous study on maize-soybean cropping patterns, it was found that the AMF inoculated in maize crops grown in the first cycle increased root colonization and AMF sporulation which was very advantageous for the growth of the following crop in the cropping sequence (Astiko, 2013). Different AMF populations (colonization and spore counts) were also found between cropping seasons or between crop species in the same cropping season in Central Lombok, Indonesia (Wangiyana et al., 2006). The present study examined the effects of several combinations of fertilizer packages consisting of indigenous AMF bio-fertilizer and varying doses of organic and NPK fertilizers applied to maize crops on N and P status in soil, and uptake by maize and subsequent sorghum crops as well as the growth and yield components of the maize and sorghum crops in a maize-sorghum cropping sequence on drylands in North Lombok, Indonesia.

MATERIALS AND METHODS

Design of the Experiment

The field experiment of maize-sorghum cropping sequence in this study was arranged according to the Randomized Complete Block Design (RCBD) with four replications (blocks). The study was carried out in the Akar-Akar village located in North Lombok regency, Indonesia, from January to August 2016. Treatments involving the use of five fertilizer packages consisting of different combinations of organic, inorganic and indigenous AMF bio-fertilizer were applied only to the maize crop in the first cropping cycle of the maize-sorghum cropping sequence. The 100% NPK-only recommended dose (D_0) is the farmer's practice of dose for maize by the locals. The NPK's doses were decreased and had been replaced by cattle manure in varying fertilization packages (D_1, D_2, D_3, D_4), and added with AMF as listed in Table 1.

Table 1
The mycorrhizal-based fertilization packages tested and applied to maize only in the maize-sorghum cropping sequence

Fertilization packages	Doses of the packages applied to maize plants only (in the cropping cycle 1)	Sorghum (cropping cycle 2)
D_0	NPK only at 100% the recommended doses (RD), i.e. 200 kg/ha Phonska (NPK 15:15:15) and 300 kg/ha Urea	No fertilizer applied
D_1	NPK at 80% RD + 15 ton/ha cattle manure + AMF	No fertilizer applied
D_2	NPK at 60% RD + 12 ton/ha cattle manure + AMF	No fertilizer applied
D_3	NPK at 40% RD + 9 ton/ha cattle manure + AMF	No fertilizer applied
D_4	NPK at 20% RD + 6 ton/ha cattle manure + AMF	No fertilizer applied

A piece of land used in this study was of a sandy soil type (69% sand, 29% silt and 2% clay, containing an average of 421 AMF spores per 100 g soil), typical of North Lombok area. The land is located at a geographic position of -8.221650, 116.350283, and measured with 13.82 mg/kg of available P, 0.01% Total N, 0.57 cmol/kg of available K, 7.31 cmol/kg of Ca, and 1.21% of C-organic. After being cultivated using minimum tillage and cleared of weeds, the land was split into 20 treatment plots of 7m x 5m based on the RCBD plotting layout. The different fertilization packages consisted of AMF inoculum, organic fertilizer (cattle manure), and inorganic fertilizer (NPK and Urea), were applied only to the maize crop in the first cropping cycle.

An indigenous AMF inoculum, *Glomus mosseae* (the M_{AA01} mycorrhizal isolate including the hyphae and the mycorrhizal spores), which was originally isolated from dryland area (1,500 spores per 20 g of soils) in Akar-Akar village of North Lombok, was applied through seed-coating of maize seeds prior to seeding. Before application, 1 kg of the AMF inoculum was mixed with 300 g of charcoal powder and 90 g of tapioca flour as the carrier

materials. Then this inoculum mixture was mixed with 20 kg maize seeds so that the seeds were coated by the inoculum mixture. For cropping cycle 1, the uncoated or AMF coated maize seeds ("Bisma" variety) were planted by dibbling 2 seeds per planting hole at 70 cm x 20 cm plant spacing. The entire dose of cattle manure (organic fertilizer) at different doses depending on the treatments (Table 1) was applied at the time of planting the maize seeds by burying the whole dose in the planting hole in the position below the seeds. The cattle manure variation in the fertilization package is to identify the optimum combinations to benefit the plant growth, increase the nutrient availability at the soils, and support the AMF development. The maximum combination of cattle manure was 15 ton/ha, the limit for transportation. The cattle manure applied in the package was measured with 3.08% Total Nitrogen, pH 6.66, 17.70 mg/kg of available P, 2.31 cmol/kg of available K, 10.45 C/N ration, and 32.2% C-organic. The inorganic (NPK) fertilizers with the recommended doses of 200 kg/ha Phonska (NPK 15:15:15) and 300 kg/ha Urea (46% N) were applied twice, i.e. at 7 DAS (days after seeding) and at 21 DAS, with the amount applied depending on the treatments (Table 1). At 7 DAS, the maize plants were fertilized with a mixture of the entire Phonska and one third of the Urea treatment doses, followed by application of the remaining Urea treatment doses at 21 DAS. The NPK fertilizers were applied in a furrow of 5 cm alongside the maize plant row at 5-7 cm depth before covered with soil.

After harvest of maize at 100 days after seeding (DAS) and field cleaning from maize debris, sorghum seeds were then planted in cropping cycle 2. Before the second sequence, the field was left fallow (rest) for 10 days. Seeds of sorghum ("Numbu" variety) were directly seeded by dibbling 2 seeds per planting holes made around the maize stubbles. These sorghum plants were not fertilized nor inoculated with additional AMF inoculum, but only the chopped maize roots containing the AMF mycorrhizal and spread throughout the plot. For both crops, the young maize and sorghum plants were tinned at 7 DAS by leaving one maize or sorghum plant per planting hole. Weeding and soil piling of the maize and sorghum plant base were done at 15 and 30 DAS. Plant protection was done by spraying "OrgaNeem" (an organic pesticide of plant origin containing Azadirachtin extracted from neem leaves) at a concentration of 5 ml OrgaNeem per liter of water. The OrgaNeem solution was applied to both crops (maize or sorghum) from 10 to 60 DAS at a 3-day interval. Harvesting of maize or sorghum crops was done at 100 DAS.

Measurement and Data Analysis

The variables measured were AMF development, N and P nutrition, and growth and yield of maize and sorghums. The AMF development includes spore counts at 60 and 100 DAS, and root colonization levels at 60 DAS. The N and P nutrition includes concentration of total N and available P in the rhizosphere of maize and sorghum at the time when the vegetative and generative growth found optimum, respectively at 60 and 100 DAS. The crop variables include dry weight (shoots and roots) and yield components (grain).

Laboratory analysis was conducted at Laboratory of Chemistry and Soil Science, Faculty of Agriculture, University of Mataram. Soil pH and texture were measured by standard procedures (Imam & Didar, 2005). Determination of total nitrogen in soil was done by destruction with $(NH_4)_2SO_4$ and distillation with NaOH where the $NH_4^+$was determined by indophenol blue colorimetric method and the NH_3 was defined by a titration with 0.05N of H_2SO_4 solution (Page et al., 1982). Total N in plants was measured using spectrophotometric indophenol blue methods with wave length 636 nm after destruction by $(NH_4)_2SO_4$ and distillation with NaOH following the Conway instruction (Lisle et al., 1990). The available Phosphorus in soil and plant was measured using spectrophotometer (λ = 693 nm) after the extraction process using Bray and Kurt I solution (0.025 N HCl + NH4F 0.03 N) (Bray & Kurtz, 1945). Total organic C was measured by oxidation with $K_2Cr_2O_7$ in presence of sulphuric acid (H_2SO_4) following Walkley and Black’s method (Horwitz, 2000).

AMF spore extraction from soil (100 g soil sample) was done using the wet sieving and decanting technique (Brundrett et al., 1996). The spores collected from the 38 µm sieve after the final centrifugation were captured in a filter paper, which were then observed on a Petri dish using a stereo microscope at 40 x magnification, and counted as spore number per 100 g of soil. The percentage of root colonization was determined using the Gridline Intersect technique (Giovannetti & Mosse, 1980) under a stereo microscope after the root pieces were stained using the clearing and staining method of Brundrett et al. (1996).

Data were analyzed using analysis of two way ANOVA and the Tukey’s HSD (Honestly Significant Difference) means tested at 5% level of significance.

RESULTS AND DISCUSSION

AMF Development

In this study, however, maize and sorghum were grown in sequence, in which sorghum was directly seeded without treatments and without tillage following the harvest of maize plants. All treatments, including AMF inoculation, were applied only to maize plants in the first cropping cycle. Therefore, it means that sorghum in cropping cycle 2 was grown after AMF propagules have built up in the soil during the growth of the maize plants in cropping cycle 1. This is in line with the results reported in the previous study (Arihara & Karasawa, 2000) which revealed that AMF root colonization and shoot dry weight of maize at 58 DAS were affected by the previous crop grown, whether they were host or non-host of AMF. When grown simultaneously, Carrenho et al. (2007) found higher colonization rates on maize than on sorghum. However, the P fertilization did not affect root colonization, especially on maize following AMF host plants.

Table 2
Mean spore number (spores per 100 g soil) at 60 and 100 DAS, and colonization rates (%-colonization) on maize and sorghum in a maize-sorghum cropping sequence

Fertilization packages	AMF on maize (1st crop)			AMF sorghum (2nd crop)		
	Spore per 100 g soil		% colonization	Spore per 100 g soil		% colonization
	60 DAS	100 DAS	60 DAS	60 DAS	100 DAS	60 DAS
D_0	764^{e}	3464^{d}	30^{d}	1231^{e}	3761^{d}	35^{e}
D_1	1059^{d}	3672^{c}	55^{c}	1343^{d}	4942^{b}	60^{d}
D_2	2119^{a}	4327^{a}	75^{a}	2981^{a}	5165^{a}	81^{a}
D_3	1690^{b}	3894^{b}	65^{b}	1881^{b}	4831^{c}	77^{b}
D_4	1294^{c}	3881^{b}	63^{b}	1769^{c}	4819^{c}	68^{c}
HSD 5%	231	13	2.0	109	12	6.5

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for descriptions of the packages

Specifically, the degree of root colonization by AMF may be higher in the roots of maize fertilized with highly soluble mineral fertilizers (NPK, diammonium phosphate, ammonium sulfate and Urea). On the other hand, the length of extra-radical mycelium and number of AMF spores were significantly higher in maize fertilized with organic fertilizers (Bokashi manure) (Bautista-Cruz et al., 2014). This indicates that organic fertilization is more favorable for spore production compared with conventional fertilization. It can also be seen from Table 2 that AMF spore numbers are higher at harvest of the maize crop (100 DAS) compared with those at 60 DAS, especially on the D_2 treatment. This indicates a high buildup of AMF propagules for the subsequent sorghum crops. This condition seems to favor higher AMF colonization levels in roots of sorghum in the second cropping cycle compared with that of maize in the first cropping

cycle. With no tillage treatment, the AMF colonization levels in sorghum may be higher than the levels in maize or bean roots (Alguacil et al., 2008). In addition, AMF colonization rates may be higher in roots of sorghum than in the roots of maize, either inoculated with *Glomus mosseae* or *Glomus versiforme*, indicating that sorghum is more favorable for AMF development than maize plants (Guo et al., 2013).

There are many factors influencing the degrees of AMF colonization of crop roots as reported by Carrenho et al. (2007). In examining the effects of different phosphorus, liming, organic matter, and soil texture on root colonization of peanuts, sorghum, and maize in a factorial experiment, each factor significantly affected root colonization level, alone or in interaction with other factors (Carrenho et al., 2007). The most surprising results were the significant interaction effects of plant, phosphorous, and organic matter although there were no significant effects of phosphorous (simple superphosphate of 166.6 mg/L (w/v) soil) nor organic matter (22 mg/L (w/v) soil). As for the interaction effect involving maize, there was no significant effect of phosphorous and organic matter on AMF colonization of maize roots. On the other hand, the application of both phosphorous and organic matter significantly reduced AMF colonization on sorghum roots (Carrenho et al., 2007).

In terms of fertilization effects, AMF colonization rate was reported to be higher on crop roots in unfertilized soils, followed by organic and biodynamic, and the least was seen in exclusively mineral fertilized and conventional farming systems. Thus, it was concluded that the amount of soluble P in the soil was the most limiting factor for AMF colonization in roots of those crops (winter wheat, vetch-rye, and grass-clover) (Mäder et al., 2000). Moreover, it was reported that the increasing P level in the growing media from 0.1 to 0.5 mM P significantly reduced AMF colonization in roots of lettuce plants especially with increasing levels of N contents (1, 5, or 9 mM N) (Azcón et al., 2003).

A negative impact on soil condition occur when the accumulation of soil P has increased beyond the requirement of the crops cultivated (Grant et al., 2005). In the study, it seemed that the amount of P input from the NPK fertilizer as applied in the D_2 treatment was most favorable for AMF development in maize crops. In the D_2 treatment, the NPK fertilizer was reduced to 120 kg/ha Phonska (60% of the recommended NPK dose of 200 kg/ha Phonska in the D_0 treatment) combined with an application of 12 ton/ha cattle manure and inoculation with AMF. Among the treatments with AMF in combination with cattle manure, the D_1 treatment had the

highest NPK fertilizer dose (Table 1), and this level of NPK fertilization seems to limit the AMF infection and development in maize roots compared with those in the D_2 treatment, resulting in a higher AMF colonization rate on D_2 than on D_1 treatment. Therefore, AMF colonization and spore numbers in maize were highest in the D_2 treatment, especially when compared with the D_1 or D_0 treatment. Using the same indigenous AMF applied to a pot experiment in which the soil for the growing media was taken from the same field in North Lombok, it was found that the highest level of AMF colonization of maize roots was in the treatment with AMF combined with cattle manure compared with AMF combined with NPK fertilizer or phosphate rock (Astiko et al., 2013a). The previous study also reported a significantly higher AMF spore number in plots fertilized with cattle manure or cattle manure combined with mineral fertilizers compared with those fertilized with mineral fertilizers only (Gryndler et al., 2006).

Soil Nutrient Status and Nutrient Sorption by Maize and Sorghum

There were significant effects of the different fertilizer packages on soil nutrient status (N and P contents of the soil) of the rhizosphere of both maize and sorghum crops at 60 and 100 DAS (Table 3). In general, there is a tendency that the highest values of the soil nutrient status, measured either at 60 or 100 DAS, are highest under the D_2 (60% NPK + 12 t/ha manure + AMF) or D_1 (80% NPK + 15 t/ha manure + AMF) treatment. The values of the soil nutrient status in these treatments were higher than those in D_0 (100% NPK only) treatment, although the dose of NPK fertilizers was reduced in the D_1 and D_2 treatments (Table 3).

Table 3

Mean concentration of total N and available P of soil in the rhizospheres of maize (1st crop) and sorghum (2nd crop) for each treatment of fertilization packages, measured at 60 and 100 DAS

Fertilization packages	Total N ($g.kg^{-1}$) at 60 DAS		Available P ($mg.kg^{-1}$) at 60 DAS		Total N ($g.kg^{-1}$) at 100 DAS		Available P ($mg.kg^{-1}$) at 100 DAS	
	maize	sorghum	maize	sorghum	maize	sorghum	maize	sorghum
D_0	1.24^{c}	1.10^{c}	14.97^{d}	11.41^{d}	1.33^{d}	1.23^{d}	17.43^{d}	10.15^{d}
D_1	1.45^{a}	1.29^{a}	28.44^{b}	16.25^{b}	1.69^{b}	1.38^{b}	29.51^{b}	21.58^{b}
D_2	1.47^{a}	1.25^{ab}	35.02^{a}	28.49^{a}	1.86^{a}	1.48^{a}	36.56^{a}	29.99^{a}
D_3	1.39^{b}	1.22^{ab}	17.52^{c}	14.59^{bc}	1.47^{c}	1.31^{c}	19.37^{c}	18.59^{c}
D_4	1.33^{b}	1.20^{b}	16.29^{c}	14.25^{c}	1.42^{c}	1.31^{c}	18.53^{c}	17.32^{c}
HSD 5%	0.08	0.07	1.31	1.99	0.08	0.06	1.0	2.98

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages

At the present study, no infiltration data was measured, however it is important to note that since the NPK fertilizers applications, i.e. Phonska and Urea at 7 DAS and Urea at 21 DAS, at sandy lands, are easily dissolved in water, and significant amount of the nutrients could have been loss through infiltration during the rainy season. Previous study shows that the sand content of the cultivated land had a positive correlation with infiltration rate, with an r-value of +0.75 (Patle et al., 2018). Since cattle manure is slow in releasing nutrients, it is possible that at the time of measurement (60 and 100 DAS), the loss of released nutrients from the NPK fertilizers through leaching was much higher in the D_0 than in the other treatments of fertilization packages due to dissolution by rain water during the rainy season. However, there were small increases in nutrient status of the soil from 60 to 100 DAS around the rhizospheres of maize crop, especially in those treated with manure and AMF application. This indicated a slow release of N and P nutrients from manure after application to the maize plants in the first cropping cycle, which could be due to AMF inoculation. Many studies have reported that AMF can mobilize and take N and P from organic matters for their hosts (Feng et al., 2003; Hawkins et al., 2000; Koide & Kabir, 2000; Leigh et al., 2009; Tarafdar & Marschner, 1994), and it can be seen from Table 2 that the highest average of AMF colonization levels in maize roots was in the D_2 treatment. Therefore, N and P uptake in shoots of maize and sorghum was highest in the D_2 treatment (Table 4).

In addition, soil contents of those nutrients corresponded well with the levels of N and P uptake of the crops, i.e. all showing positive correlation coefficients. Although it is not significant for P uptake, the highest values of N and P uptake were also seen in the D_2 treatment for both maize and sorghum (Table 4). The correlation analysis of the mean values obtained at 60 DAS showed a significant correlation between soil N and N sorption in the shoots, with a value of r = + 0.970 (R-square = 94.1%, p = 0.006) for maize plants, and r = + 0.904 (R-square = 81.7%, p = 0.035) for sorghum plants. These indicate those fertilizers in the packages contribute to nutrient contents of the crops, which are significantly different between treatments of fertilization packages (Table 4).

Table 4

Mean N and P sorption (mg.g^{-1} plant dry weight) by each crop at 60 DAS for each treatment of fertilization packages

Fertilization packages	N and P uptake (mg.g^{-1} plant dry weight) by each crop at 60 DAS			
	Maize (1st cropping cycle)		Sorghum (2nd cropping cycle)	
	N	P	N	P
D_0	18.62^{e}	1.71^{d}	12.74^{d}	0.65^{d}

D_1	27.58^{b}	2.60^{b}	18.92^{b}	0.85^{c}
D_2	28.00^{a}	2.72^{a}	20.86^{a}	1.48^{a}
D_3	23.10^{c}	2.41^{c}	17.29^{c}	1.36^{b}
D_4	20.44^{d}	2.41^{c}	17.22^{c}	1.32^{b}
HSD 5%	0.41	0.11	0.07	0.04

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages

However, P status of the soil did not show a significant correlation with P uptake either by maize or sorghum crop. In spite of that, there is a significant positive correlation between the degree of AMF colonization in the roots and P sorption in the shoot of maize plants, with an r = + 0.909 (R-square = 82.6%, p = 0.032), and that of sorghum plants, with an r = + 0.940 (R-square = 88.4%, p = 0.017). This shows that there were significant contributions of AMF colonization in the roots to the amount of P taken up by the plants, both maize and sorghum. If soil soluble P has not exceeded crop requirements, AMF association will still result in significantly positive effects on P uptake and biomass production because of P requirements of the crops; for optimum growth and yields, crops require P since the early stage of their growth, either from soil, fertilizer application, or from AMF associations (Grant et al., 2005).

Although sorghum crop in the second cropping cycle was not fertilized with manure nor NPK fertilizers, direct seeding of sorghum after harvesting the maize plants without tillage seemed to be a favorable condition in establishing AMF association in the sorghum crop, which resulted in higher AMF colonization rates in sorghum than in maize roots for each fertilizer package (Table 2). The AMF associations seemed to enable the sorghum crops to absorb adequate P and other nutrients from soil and residues of the manure applied in the first cropping cycle even though these sorghum crops were not fertilized. This could occur because of the potential of AMF external hyphae that help the host plants in absorbing and dissolving nutrients from soil and manures and other nutrient pools, as well as other nutrients unavailable for uptake by plant roots (Menge, 1983; Smith & Read, 2010; Tawaraya et al., 2006). Guo et al. (2013) also reported significant effects of AMF inoculation on C, N, P, and K contents (mg/pot) in shoot and roots of maize and sorghum grown in pots filled with mixtures of mine tailing and topsoil, and between the AMF species used, *G. versiforme* showed higher colonization rates which resulted in higher nutrient sorption and biomass of maize and sorghum compared with *G. mosseae* (Guo et al., 2013).

Biomass and Yield Components of Maize and Sorghum

In terms of biomass production and yield components of maize and sorghum, there were also significant effects of the fertilization packages in which D_2 treatment presents the highest values of biomass production (Table 5) and yield components (Table 6). This means that the nutrient status of the soils corresponded well with the biomass weight of the crops, indicated by the positive correlation coefficients, although only some of them are significant. The AMF applications to maize crops, observed at 60 DAS, showed a significant correlation to dry shoot weight, N and P status of the soil, and the N and P sorption. In contrast, AMF colonization levels of maize roots only showed significant correlation with root dry weight at 60 DAS, with an r = + 0.912 (R^2 = 83.2%, p= 0.031) and shoot dry weight at maturity (100 DAS), with an r = + 0.892 (R^2 = 79.6%, p= 0.042). This could mean that during the vegetative growth, AMF colonization have focused on improving root growth to increase nutrient sorption during the vegetative growth of maize crops.

Table 5
Mean dry biomass weight (g/plant) of maize and sorghum for each treatment of fertilization packages

Fertilization packages	Dry biomass weights (g/plant) of maize (1st crop) and sorghum (2nd crop)							
	Maize root		Maize shoot		Sorghum root		Sorghum shoot	
	60 DAS	100 DAS	60 DAS	100 DAS	60 DAS	100 DAS	60 DAS	100 DAS
D_0	8.13^d	10.43^e	34.15^d	62.29^e	0.82^d	12.30^d	8.31^d	47.74^e
D_1	15.13^b	22.39^b	61.92^b	109.03^b	2.22^b	22.34^b	16.89^b	95.01^b
D_2	17.12^a	34.56^a	72.86^a	111.25^a	3.37^a	24.44^a	23.53^a	105.14^a
D_3	13.65^c	18.48^c	52.26^c	101.05^c	1.68^c	14.52^c	12.01^c	85.46^c
D_4	13.34^c	15.43^d	51.29^c	95.87^d	1.38^c	14.47^c	11.81^c	66.06^d
HSD 5%	0.31	3.04	0.97	2.21	0.30	0.05	0.20	9.54

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages

In relation to nutrient uptake, although AMF colonization levels did not show significant correlation with N uptake in shoots of maize or sorghum, they did show a positive significant correlation with P uptake both in shoots of maize and sorghum, with an r = + 0.909 (R^2 = 82.6%, p = 0.032) for maize, and r = + 0.940 (R^2 = 88.4%, p = 0.017) for sorghum. These could be due to the ability of AMF extra-radical hyphae in mobilizing and absorbing P from organic matters for their hosts (Feng et al., 2003; Koide & Kabir, 2000; Tarafdar & Marschner, 1994). Based on the strength of the relationships between AMF colonization levels and P uptake, the value of R^2 is higher in sorghum (R^2 = 88.4%) than in maize (R^2 = 82.6%). This means that the contribution of AMF colonization in roots to P uptake in shoots was higher in sorghum crop at the second cropping cycle than in maize in the first cropping cycle. Since sorghum did not

receive any fertilizers, and cattle manure is a slow-release organic fertilizer, it could mean that most P nutrient contained in the shoots, especially in shoots of sorghum in the second cropping cycle, was mostly taken up from the manure under the contribution of AMF colonization in the roots. However, this still needs to be confirmed by further research. This view is supported by the conditions of the study area, which was dominated by sand, and if leaching happened during the rainy season, the loss of dissolved nutrients from the NPK fertilizers applied in the first cropping cycle could be high. Therefore, the main source of nutrients, especially for sorghum crop in the second cropping cycle was the cattle manure applied to the maize crop in the first cropping cycle.

Table 6

Mean weights of total dry grains (kg/plot) and 100 dry grains for each crop and each treatment of fertilization packages

Fertilization packages	Mean dry grain yield (kg/plot) and weight of 100 dry grains (g)			
	Maize in the 1st cropping cycle		Sorghum in the 2nd cropping cycle	
	Grain yield	100 grains	Grain yield	100 grains
D_0	9.60^{e}	22.48^{d}	3.57^{d}	2.73^{d}
D_1	17.40^{b}	26.94^{b}	5.05^{b}	3.01^{b}
D_2	22.80^{a}	28.12^{a}	6.65^{a}	3.61^{a}
D_3	15.60^{c}	25.98^{c}	4.43^{c}	2.90^{c}
D_4	10.20^{d}	24.61^{c}	4.17^{c}	2.81^{cd}
HSD 5%	0.59	1.37	0.26	0.09

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages

In relation to nitrogen nutrient, there was a very low and non-significant correlation between AMF colonization levels and N uptake in maize shoots with an R^2 of only 41.2%. However, the N uptake of maize at 60 DAS was highly significantly correlated with the N status of the soil with an R^2 = 94.1% (p = 0.006). Moreover, the N soil availability also showed a significant correlation with biomass and grain yield of maize, i.e. with an R^2 = 84.1% (p = 0.029) with grain yield at 60 DAS, R^2 = 92.2% (p = 0.009) with shoot dry weight at 60 DAS, and R^2 = 90.6% (p = 0.012) with shoot dry weight at 100 DAS.

In contrast, for sorghum in the second cropping cycle, the soil N status at 60 DAS showed a significant correlation only with shoot dry weight at 100 DAS. Unlike in maize crop where AMF colonization levels showed a significant and positive correlation with shoot dry weight at maturity (100 DAS), AMF colonization levels in sorghum roots did not show any

significant correlation with biomass weight or yield components of sorghum. However, AMF colonization rates in sorghum roots showed a sufficiently high correlation with N uptake in sorghum shoots, with an R^2 = 71.9% (p = 0.069), and N uptake in sorghum shoots showed positive significant correlation with biomass and grain yield of sorghum, i.e. with an R^2 = 80.5% (p = 0.039) with grain yield, R^2 = 83.0% (p = 0.031) with shoot dry weight at 60 DAS and R^2 = 89.9% (p = 0.014) with shoot dry weight at 100 DAS. No additional fertilizers and inoculums were applied at the cropping cycle 2. These could mean that for maize crop, most nutrients were derived from NPK fertilizers while for sorghum, N and P nutrients were derived from the residues of manure contributed from AMF colonization in sorghum roots. Many researchers have also showed the ability of AMF to utilize organic sources to supply N to their host (e.g. Hawkins et al., 2000; Leigh et al., 2009) although it was also found that AMF colonization did not necessarily result in significantly higher N status of the mycorrhizal than non-mycorrhizal hosts (Hawkins et al., 2000).

However, when the correlation analysis was done between averages of colonization levels, biomass and grain yield between maize in the first and sorghum in the second cropping cycle, in general the results show significant and positive coefficients of correlation. For example, correlation of AMF colonization levels between roots of maize and sorghum are highly significant, with an r = + 0.988 (R^2 = 97.6%, p = 0.002). This means that the pattern of differences in AMF colonization levels between roots of maize and sorghum is highly similar. In other words, AMF colonization levels in roots of maize in the first cropping cycle were carried over into the second cropping cycle where sorghum was grown as the rotation crop. This is because both maize and sorghum are hosts of AMF, and both crops have high mycorrhizal dependency (Guo et al., 2013).

In addition, when a t-test of 'Paired Two Sample for Means' was run on the averages of AMF colonization levels and spore number in soil at maturity of the crops between maize and sorghum in Table 2, higher significant values (p<0.01) were seen on sorghum than on maize. This could be due to the buildup of AMF in the soil after maize crop in the first copping cycle was harvested before sorghum was directly seeded without tillage. Even though both crops were grown simultaneously, it was also found that AMF colonization levels were higher on sorghum than on maize (Guo et al., 2013). This means that sorghum can be used to improve mycorrhizal conditions of the soil under a maize-sorghum cropping sequence.

CONCLUSION

Among the mycorrhiza-based fertilization packages tested on the maize-sorghum cropping sequence system at the drylands in North Lombok of Indonesia, the D_2 package, consisting of 60% NPK recommended dose, 12 t/ha cattle manure, and the indigenous AMF inoculation, was found to be the best fertilization package to improve crop yield and soil nutrient availability. This study noted that the AMF development was higher in the sorghum at the second cropping cycle compared to the growth in the maize at the first cropping cycle. This condition led to the higher NP-uptake and soil nutrient availability in subsequent crops, both at the sandy dryland, with no additional fertilization and mycorrhizal propagules applied.

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Mycorrhizal Seed-coating on Maize-sorghum Cropping Sequence

Indigenous Mycorrhizal Seed-coating Inoculation on Plant Growth and Yield, and NP-uptake and Availability on Maize-sorghum Cropping Sequence in Lombok's Drylands

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ABSTRACT

By improving the nutrient uptake and transport, an indigenous arbuscular mycorrhizal fungal (AMF) is expected to improve crops' performance in sandy drylands of North Lombok (Indonesia) during dry seasons. A field experiment was designed with Randomized Complete Block Design and four replications to examine the benefits of mycorrhiza at varying doses of plant nutrition (nitrogen and phosphorus). Total of 1 kg of the AMF inoculum was applied to 20 kg maize seeds in different fertilization packages of cattle manure (15, 12, 9 and 6 ton/ha) and inorganic fertilizers (80, 60, 40 and 20% NPK recommended dose). A 100% NPK recommended dose was used as the control (200 kg/ha Phonska and 300 kg/ha Urea). After harvest of maize at 100 days after seeding (DAS) and field cleaning from maize debris, sorghum seeds were then planted in cropping cycle 2 with no additional fertilization and inoculum. Results indicated that the AMF applications to the maize-sorghum cropping sequence increased the AMF colonization rate, soil N and P status and uptake, and dry biomass (root, shoot, and grain). The highest correspondence was observed in the crops which utilized a combination of 60% NPK and 12 ton/ha cattle manure, and the performance was higher at 100 days after seeding. The number of AMF spores increases over the time where colonization rates were found higher in roots of sorghum (60-81%) than maize (55-75%). This study suggests that AMF inoculation increases the plant yield and improves soil nutrient availability which is very advantageous for the growth of the maize-sorghum subsequent crop in Lombok's drylands.

Keywords: Arbuscular mycorrhizal fungi (AMF), cattle manure, maize-sorghum cropping sequence, plant nutrition, seed coating

INTRODUCTION

The northern part of North Lombok regency (Indonesia) is dominated by drylands with sandy soils texture. With a very short and low number of rainy days (December to April, 100-200 mm) per wet month or no rain during the long dry seasons (May to November), no food crops can be cultivated normally especially in the areas without deep wells. Moreover, inadequate phosphorus (P) availability is also one of the factors limiting the productivity of maize and other food crops in the dryland of North Lombok. Maize crop requires very high P; for example, for the production of 10 ton/ha, maize crops need 102 kg/ha of P_2O_5 and 76% of it is transported into the seeds (Calderón-Vázquez et al., 2009). In contrast, plant roots can take up P at only about 8-13% of the amount of P fertilizer applied (Supardi, 1996). Thus, to cope with the unavailability of soil water and P and other essential nutrients in drylands of North Lombok, arbuscular mycorrhizal fungal (AMF) symbiosis has been used as it is expected to improve the performance of food crops especially during the dry seasons. Many have reported that the soil fungi, in symbiosis with their host plants, can help plants to improve water retention and make their host plants more tolerant to drought (Augé, 2004). AMF colonization also increases nutrient uptake from soils and enhances growth and yield of the host plants although it depends on the species of AMF colonizing the host plants (Marulanda et al., 2003).

The most common findings show that the external hyphae of AMF improve P uptake and transport the nutrient to root cortex of their host (Azcón et al., 2003; Feng et al., 2003; George et al., 1995; Koide & Kabir, 2000; Tarafdar & Marschner, 1994; Tawaraya et al., 2006). Although there is no growth improvement of the host plants due to the AMF symbiosis, the amount of P uptake through AMF (mycorrhizal pathway) can be higher than through the host roots or the direct pathway (Smith & Read, 2010). AMF can also help their hosts to increase the uptake of other essential nutrients such as N, K, Ca, Mg, S, Cu, Zn, Mn, Fe and B when compared with non-mycorrhizal plants (Dhillion & Ampornpan, 1992; Hawkins et al., 2000; Leigh et al., 2009; Menge, 1983). Most of these capabilities of the AM plants are due to the extensive growth of AMF external hyphae beyond the host roots (Drew et al., 2003; Zhu et al., 2001). The length ratio of AMF hyphae to roots in soil can be 100:1 or higher and with the external hyphae, the mycorrhizal roots can explore further (George et al., 1995). By applying the Plenchette's mycorrhizal dependency formula to the shoot dry weight of maize and sorghum (Plenchette et al., 1983), the results showed ~~a~~ high mycorrhizal dependency, both for maize (up to 70.12%) and for sorghum (up to 79.42%) (Guo et al., 2013; Tawaraya, 2003). The findings of the study indicate that the higher the dependent rate is on AMF symbiosis, the more the dry

matter is produced of the crops. With the high porosity and low water retention capacity on dryland and sandy areas (Bruand et al., 2005) in most areas of North Lombok, the levels of crop dependence on symbiosis with AMF could be higher. Moreover, the symbiosis of crops with AMF could significantly reduce nutrient loss from soil through leaching (Cavagnaro et al., 2015).

The establishment of AM symbiosis can be done through inoculation with AMF propagules. Our previous study (Astiko et al., 2013a) showed that the indigenous AMF inoculation in maize plants in sandy soils had positive implications for the improvement of soil properties by increasing the rates of nutrient uptake by maize crop from the soil and improving its grain yield. Our research group has also shown the benefit of this local inoculation in increasing the growth of soybean and its yield by improving P uptake from the soils, compared to those without AMF inoculation (Astiko et al., 2013b). However, as found from other studies, the development of AMF in the soil and its contribution to growth, yield and nutrient uptake of host plants could be influenced by the order of plant species cultivated in sequence in the cropping system (Johnson et al., 1992; Vivekanandan & Fixen, 1991). The studies by Johnson et al. (1992) as well as Vivekanandan and Fixen (1991) reported that the P uptake and the AMF colonization were higher on maize crops grown following soybean compared to when maize crops were grown following maize or barley.

From the previous study on maize-soybean cropping patterns, it was found that the AMF inoculated in maize crops grown in the first cycle increased root colonization and AMF sporulation which was very advantageous for the growth of the following crop in the cropping sequence (Astiko, 2013). Different AMF populations (colonization and spore counts) were also found between cropping seasons or between crop species in the same cropping season in Central Lombok, Indonesia (Wangiyana et al., 2006). The present study examined the effects of several combinations of fertilizer packages consisting of indigenous AMF bio-fertilizer and varying doses of organic and NPK fertilizers applied to maize crops on N and P status in soil, and uptake by maize and subsequent sorghum crops as well as the growth and yield components of the maize and sorghum crops in a maize-sorghum cropping sequence on drylands in North Lombok, Indonesia.

MATERIALS AND METHODS

Design of the Experiment

The field experiment of maize-sorghum cropping sequence in this study was arranged according to the Randomized Complete Block Design (RCBD) with four replications (blocks). The study was carried out in the Akar-Akar village located in North Lombok regency, Indonesia, from January to August 2016. Treatments involving the use of five fertilizer packages consisting of different combinations of organic, inorganic and indigenous AMF bio-fertilizer were applied only to the maize crop in the first cropping cycle of the maize-sorghum cropping sequence. The 100% NPK-only recommended dose (D_0) is the farmer's practice of dose for maize by the locals. The NPK's doses were decreased and had been replaced by cattle manure in varying fertilization packages (D_1, D_2, D_3, D_4), and added with AMF as listed in Table 1.

Table 1
The mycorrhizal-based fertilization packages tested and applied to maize only in the maize-sorghum cropping sequence

Fertilization packages	Doses of the packages applied to maize plants only (in the cropping cycle 1)	Sorghum (cropping cycle 2)
D_0	NPK only at 100% the recommended doses (RD), i.e. 200 kg/ha Phonska (NPK 15:15:15) and 300 kg/ha Urea	No fertilizer applied
D_1	NPK at 80% RD + 15 ton/ha cattle manure + AMF	No fertilizer applied
D_2	NPK at 60% RD + 12 ton/ha cattle manure + AMF	No fertilizer applied
D_3	NPK at 40% RD + 9 ton/ha cattle manure + AMF	No fertilizer applied
D_4	NPK at 20% RD + 6 ton/ha cattle manure + AMF	No fertilizer applied

A piece of land used in this study was of a sandy soil type (69% sand, 29% silt and 2% clay, containing an average of 421 AMF spores per 100 g soil), typical of North Lombok area. The land is located at a geographic position of -8.221650, 116.350283, and measured with 13.82 mg/kg of available P, 0.01% Total N, 0.57 cmol/kg of available K, 7.31 cmol/kg of Ca, and 1.21% of C-organic. After being cultivated using minimum tillage and cleared of weeds, the land was split into 20 treatment plots of 7m x 5m based on the RCBD plotting layout. The different fertilization packages consisted of AMF inoculum, organic fertilizer (cattle manure), and inorganic fertilizer (NPK and Urea), were applied only to the maize crop in the first cropping cycle.

An indigenous AMF inoculum, *Glomus mosseae* (the M_{AA01} mycorrhizal isolate including the hyphae and the mycorrhizal spores), which was originally isolated from dryland area (1,500 spores per 20 g of soils) in Akar-Akar village of North Lombok, was applied through seed-coating of maize seeds prior to seeding. Before application, 1 kg of the AMF inoculum was mixed with 300 g of charcoal powder and 90 g of tapioca flour as the carrier

materials. Then this inoculum mixture was mixed with 20 kg maize seeds so that the seeds were coated by the inoculum mixture. For cropping cycle 1, the uncoated or AMF coated maize seeds ("Bisma" variety) were planted by dibbling 2 seeds per planting hole at 70 cm x 20 cm plant spacing. The entire dose of cattle manure (organic fertilizer) at different doses depending on the treatments (Table 1) was applied at the time of planting the maize seeds by burying the whole dose in the planting hole in the position below the seeds. The cattle manure variation in the fertilization package is to identify the optimum combinations to benefit the plant growth, increase the nutrient availability at the soils, and support the AMF development. The maximum combination of cattle manure was 15 ton/ha, the limit for transportation. The cattle manure applied in the package was measured with 3.08% Total Nitrogen, pH 6.66, 17.70 mg/kg of available P, 2.31 cmol/kg of available K, 10.45 C/N ration, and 32.2% C-organic. The inorganic (NPK) fertilizers with the recommended doses of 200 kg/ha Phonska (NPK 15:15:15) and 300 kg/ha Urea (46% N) were applied twice, i.e. at 7 DAS (days after seeding) and at 21 DAS, with the amount applied depending on the treatments (Table 1). At 7 DAS, the maize plants were fertilized with a mixture of the entire Phonska and one third of the Urea treatment doses, followed by application of the remaining Urea treatment doses at 21 DAS. The NPK fertilizers were applied in a furrow of 5 cm alongside the maize plant row at 5-7 cm depth before covered with soil.

After harvest of maize at 100 days after seeding (DAS) and field cleaning from maize debris, sorghum seeds were then planted in cropping cycle 2. Before the second sequence, the field was left fallow (rest) for 10 days. Seeds of sorghum ("Numbu" variety) were directly seeded by dibbling 2 seeds per planting holes made around the maize stubbles. These sorghum plants were not fertilized nor inoculated with additional AMF inoculum, but only the chopped maize roots containing the AMF mycorrhizal and spread throughout the plot. For both crops, the young maize and sorghum plants were tinned at 7 DAS by leaving one maize or sorghum plant per planting hole. Weeding and soil piling of the maize and sorghum plant base were done at 15 and 30 DAS. Plant protection was done by spraying "OrgaNeem" (an organic pesticide of plant origin containing Azadirachtin extracted from neem leaves) at a concentration of 5 ml OrgaNeem per liter of water. The OrgaNeem solution was applied to both crops (maize or sorghum) from 10 to 60 DAS at a 3-day interval. Harvesting of maize or sorghum crops was done at 100 DAS.

Measurement and Data Analysis

The variables measured were AMF development, N and P nutrition, and growth and yield of maize and sorghums. The AMF development includes spore counts at 60 and 100 DAS, and root colonization levels at 60 DAS. The N and P nutrition includes concentration of total N and available P in the rhizosphere of maize and sorghum at the time when the vegetative and generative growth found optimum, respectively at 60 and 100 DAS. The crop variables include dry weight (shoots and roots) and yield components (grain).

Laboratory analysis was conducted at Laboratory of Chemistry and Soil Science, Faculty of Agriculture, University of Mataram. Soil pH and texture were measured by standard procedures (Imam & Didar, 2005). Determination of total nitrogen in soil was done by destruction with $(NH_4)_2SO_4$ and distillation with NaOH where the $NH_4^+$was determined by indophenol blue colorimetric method and the NH_3 was defined by a titration with 0.05N of H_2SO_4 solution (Page et al., 1982). Total N in plants was measured using spectrophotometric indophenol blue methods with wave length 636 nm after destruction by $(NH_4)_2SO_4$ and distillation with NaOH following the Conway instruction (Lisle et al., 1990). The available Phosphorus in soil and plant was measured using spectrophotometer (λ = 693 nm) after the extraction process using Bray and Kurt I solution (0.025 N HCl + NH4F 0.03 N) (Bray & Kurtz, 1945). Total organic C was measured by oxidation with $K_2Cr_2O_7$ in presence of sulphuric acid (H_2SO_4) following Walkley and Black's method (Horwitz, 2000).

AMF spore extraction from soil (100 g soil sample) was done using the wet sieving and decanting technique (Brundrett et al., 1996). The spores collected from the 38 µm sieve after the final centrifugation were captured in a filter paper, which were then observed on a Petri dish using a stereo microscope at 40 x magnification, and counted as spore number per 100 g of soil. The percentage of root colonization was determined using the Gridline Intersect technique (Giovannetti & Mosse, 1980) under a stereo microscope after the root pieces were stained using the clearing and staining method of Brundrett et al. (1996).

Data were analyzed using analysis of two way ANOVA and the Tukey's HSD (Honestly Significant Difference) means tested at 5% level of significance.

RESULTS AND DISCUSSION

AMF Development

In this study, however, maize and sorghum were grown in sequence, in which sorghum was directly seeded without treatments and without tillage following the harvest of maize plants. All treatments, including AMF inoculation, were applied only to maize plants in the first cropping cycle. Therefore, it means that sorghum in cropping cycle 2 was grown after AMF propagules have built up in the soil during the growth of the maize plants in cropping cycle 1. This is in line with the results reported in the previous study (Arihara & Karasawa, 2000) which revealed that AMF root colonization and shoot dry weight of maize at 58 DAS were affected by the previous crop grown, whether they were host or non-host of AMF. When grown simultaneously, Carrenho et al. (2007) found higher colonization rates on maize than on sorghum. However, the P fertilization did not affect root colonization, especially on maize following AMF host plants.

Table 2
Mean spore number (spores per 100 g soil) at 60 and 100 DAS, and colonization rates (%-colonization) on maize and sorghum in a maize-sorghum cropping sequence

Fertilization packages	AMF on maize (1st crop)			AMF sorghum (2nd crop)		
	Spore per 100 g soil		% colonization	Spore per 100 g soil		% colonization
	60 DAS	100 DAS	60 DAS	60 DAS	100 DAS	60 DAS
D_0	764^{e}	3464^{d}	30^{d}	1231^{e}	3761^{d}	35^{e}
D_1	1059^{d}	3672^{c}	55^{c}	1343^{d}	4942^{b}	60^{d}
D_2	2119^{a}	4327^{a}	75^{a}	2981^{a}	5165^{a}	81^{a}
D_3	1690^{b}	3894^{b}	65^{b}	1881^{b}	4831^{c}	77^{b}
D_4	1294^{c}	3881^{b}	63^{b}	1769^{c}	4819^{c}	68^{c}
HSD 5%	231	13	2.0	109	12	6.5

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for descriptions of the packages

Specifically, the degree of root colonization by AMF may be higher in the roots of maize fertilized with highly soluble mineral fertilizers (NPK, diammonium phosphate, ammonium sulfate and Urea). On the other hand, the length of extra-radical mycelium and number of AMF spores were significantly higher in maize fertilized with organic fertilizers (Bokashi manure) (Bautista-Cruz et al., 2014). This indicates that organic fertilization is more favorable for spore production compared with conventional fertilization. It can also be seen from Table 2 that AMF spore numbers are higher at harvest of the maize crop (100 DAS) compared with those at 60 DAS, especially on the D_2 treatment. This indicates a high buildup of AMF propagules for the subsequent sorghum crops. This condition seems to favor higher AMF colonization levels in roots of sorghum in the second cropping cycle compared with that of maize in the first cropping

cycle. With no tillage treatment, the AMF colonization levels in sorghum may be higher than the levels in maize or bean roots (Alguacil et al., 2008). In addition, AMF colonization rates may be higher in roots of sorghum than in the roots of maize, either inoculated with *Glomus mosseae* or *Glomus versiforme*, indicating that sorghum is more favorable for AMF development than maize plants (Guo et al., 2013).

There are many factors influencing the degrees of AMF colonization of crop roots as reported by Carrenho et al. (2007). In examining the effects of different phosphorus, liming, organic matter, and soil texture on root colonization of peanuts, sorghum, and maize in a factorial experiment, each factor significantly affected root colonization level, alone or in interaction with other factors (Carrenho et al., 2007). The most surprising results were the significant interaction effects of plant, phosphorous, and organic matter although there were no significant effects of phosphorous (simple superphosphate of 166.6 mg/L (w/v) soil) nor organic matter (22 mg/L (w/v) soil). As for the interaction effect involving maize, there was no significant effect of phosphorous and organic matter on AMF colonization of maize roots. On the other hand, the application of both phosphorous and organic matter significantly reduced AMF colonization on sorghum roots (Carrenho et al., 2007).

In terms of fertilization effects, AMF colonization rate was reported to be higher on crop roots in unfertilized soils, followed by organic and biodynamic, and the least was seen in exclusively mineral fertilized and conventional farming systems. Thus, it was concluded that the amount of soluble P in the soil was the most limiting factor for AMF colonization in roots of those crops (winter wheat, vetch-rye, and grass-clover) (Mäder et al., 2000). Moreover, it was reported that the increasing P level in the growing media from 0.1 to 0.5 mM P significantly reduced AMF colonization in roots of lettuce plants especially with increasing levels of N contents (1, 5, or 9 mM N) (Azcón et al., 2003).

A negative impact on soil condition occur when the accumulation of soil P has increased beyond the requirement of the crops cultivated (Grant et al., 2005). In the study, it seemed that the amount of P input from the NPK fertilizer as applied in the D_2 treatment was most favorable for AMF development in maize crops. In the D_2 treatment, the NPK fertilizer was reduced to 120 kg/ha Phonska (60% of the recommended NPK dose of 200 kg/ha Phonska in the D_0 treatment) combined with an application of 12 ton/ha cattle manure and inoculation with AMF. Among the treatments with AMF in combination with cattle manure, the D_1 treatment had the

highest NPK fertilizer dose (Table 1), and this level of NPK fertilization seems to limit the AMF infection and development in maize roots compared with those in the D_2 treatment, resulting in a higher AMF colonization rate on D_2 than on D_1 treatment. Therefore, AMF colonization and spore numbers in maize were highest in the D_2 treatment, especially when compared with the D_1 or D_0 treatment. Using the same indigenous AMF applied to a pot experiment in which the soil for the growing media was taken from the same field in North Lombok, it was found that the highest level of AMF colonization of maize roots was in the treatment with AMF combined with cattle manure compared with AMF combined with NPK fertilizer or phosphate rock (Astiko et al., 2013a). The previous study also reported a significantly higher AMF spore number in plots fertilized with cattle manure or cattle manure combined with mineral fertilizers compared with those fertilized with mineral fertilizers only (Gryndler et al., 2006).

Soil Nutrient Status and Nutrient Sorption by Maize and Sorghum

There were significant effects of the different fertilizer packages on soil nutrient status (N and P contents of the soil) of the rhizosphere of both maize and sorghum crops at 60 and 100 DAS (Table 3). In general, there is a tendency that the highest values of the soil nutrient status, measured either at 60 or 100 DAS, are highest under the D_2 (60% NPK + 12 t/ha manure + AMF) or D_1 (80% NPK + 15 t/ha manure + AMF) treatment. The values of the soil nutrient status in these treatments were higher than those in D_0 (100% NPK only) treatment, although the dose of NPK fertilizers was reduced in the D_1 and D_2 treatments (Table 3).

Table 3
Mean concentration of total N and available P of soil in the rhizospheres of maize (1st crop) and sorghum (2nd crop) for each treatment of fertilization packages, measured at 60 and 100 DAS

Fertilization packages	Total N (g.kg^{-1}) at 60 DAS		Available P (mg.kg^{-1}) at 60 DAS		Total N (g.kg^{-1}) at 100 DAS		Available P (mg.kg^{-1}) at 100 DAS	
	maize	sorghum	maize	sorghum	maize	sorghum	maize	sorghum
D_0	1.24^{c}	1.10^{c}	14.97^{d}	11.41^{d}	1.33^{d}	1.23^{d}	17.43^{d}	10.15^{d}
D_1	1.45^{a}	1.29^{a}	28.44^{b}	16.25^{b}	1.69^{b}	1.38^{b}	29.51^{b}	21.58^{b}
D_2	1.47^{a}	1.25^{ab}	35.02^{a}	28.49^{a}	1.86^{a}	1.48^{a}	36.56^{a}	29.99^{a}
D_3	1.39^{b}	1.22^{ab}	17.52^{c}	14.59^{bc}	1.47^{c}	1.31^{c}	19.37^{c}	18.59^{c}
D_4	1.33^{b}	1.20^{b}	16.29^{c}	14.25^{c}	1.42^{c}	1.31^{c}	18.53^{c}	17.32^{c}
HSD 5%	0.08	0.07	1.31	1.99	0.08	0.06	1.0	2.98

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages

At the present study, no infiltration data was measured, however it is important to note that since the NPK fertilizers applications, i.e. Phonska and Urea at 7 DAS and Urea at 21 DAS, at sandy lands, are easily dissolved in water, and significant amount of the nutrients could have been loss through infiltration during the rainy season. Previous study shows that the sand content of the cultivated land had a positive correlation with infiltration rate, with an r-value of +0.75 (Patle et al., 2018). Since cattle manure is slow in releasing nutrients, it is possible that at the time of measurement (60 and 100 DAS), the loss of released nutrients from the NPK fertilizers through leaching was much higher in the D_0 than in the other treatments of fertilization packages due to dissolution by rain water during the rainy season. However, there were small increases in nutrient status of the soil from 60 to 100 DAS around the rhizospheres of maize crop, especially in those treated with manure and AMF application. This indicated a slow release of N and P nutrients from manure after application to the maize plants in the first cropping cycle, which could be due to AMF inoculation. Many studies have reported that AMF can mobilize and take N and P from organic matters for their hosts (Feng et al., 2003; Hawkins et al., 2000; Koide & Kabir, 2000; Leigh et al., 2009; Tarafdar & Marschner, 1994), and it can be seen from Table 2 that the highest average of AMF colonization levels in maize roots was in the D_2 treatment. Therefore, N and P uptake in shoots of maize and sorghum was highest in the D_2 treatment (Table 4).

In addition, soil contents of those nutrients corresponded well with the levels of N and P uptake of the crops, i.e. all showing positive correlation coefficients. Although it is not significant for P uptake, the highest values of N and P uptake were also seen in the D_2 treatment for both maize and sorghum (Table 4). The correlation analysis of the mean values obtained at 60 DAS showed a significant correlation between soil N and N sorption in the shoots, with a value of r = + 0.970 (R-square = 94.1%, p = 0.006) for maize plants, and r = + 0.904 (R-square = 81.7%, p = 0.035) for sorghum plants. These indicate those fertilizers in the packages contribute to nutrient contents of the crops, which are significantly different between treatments of fertilization packages (Table 4).

Table 4
Mean N and P sorption (mg.g^{-1} plant dry weight) by each crop at 60 DAS for each treatment of fertilization packages

Fertilization packages	N and P uptake (mg.g^{-1} plant dry weight) by each crop at 60 DAS			
	Maize (1st cropping cycle)		Sorghum (2nd cropping cycle)	
	N	P	N	P
D_0	18.62^{e}	1.71^{d}	12.74^{d}	0.65^{d}

D_1	27.58[b]	2.60[b]	18.92[b]	0.85[c]
D_2	28.00[a]	2.72[a]	20.86[a]	1.48[a]
D_3	23.10[c]	2.41[c]	17.29[c]	1.36[b]
D_4	20.44[d]	2.41[c]	17.22[c]	1.32[b]
HSD 5%	0.41	0.11	0.07	0.04

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages

However, P status of the soil did not show a significant correlation with P uptake either by maize or sorghum crop. In spite of that, there is a significant positive correlation between the degree of AMF colonization in the roots and P sorption in the shoot of maize plants, with an r = + 0.909 (R-square = 82.6%, p = 0.032), and that of sorghum plants, with an r = + 0.940 (R-square = 88.4%, p = 0.017). This shows that there were significant contributions of AMF colonization in the roots to the amount of P taken up by the plants, both maize and sorghum. If soil soluble P has not exceeded crop requirements, AMF association will still result in significantly positive effects on P uptake and biomass production because of P requirements of the crops; for optimum growth and yields, crops require P since the early stage of their growth, either from soil, fertilizer application, or from AMF associations (Grant et al., 2005).

Although sorghum crop in the second cropping cycle was not fertilized with manure nor NPK fertilizers, direct seeding of sorghum after harvesting the maize plants without tillage seemed to be a favorable condition in establishing AMF association in the sorghum crop, which resulted in higher AMF colonization rates in sorghum than in maize roots for each fertilizer package (Table 2). The AMF associations seemed to enable the sorghum crops to absorb adequate P and other nutrients from soil and residues of the manure applied in the first cropping cycle even though these sorghum crops were not fertilized. This could occur because of the potential of AMF external hyphae that help the host plants in absorbing and dissolving nutrients from soil and manures and other nutrient pools, as well as other nutrients unavailable for uptake by plant roots (Menge, 1983; Smith & Read, 2010; Tawaraya et al., 2006). Guo et al. (2013) also reported significant effects of AMF inoculation on C, N, P, and K contents (mg/pot) in shoot and roots of maize and sorghum grown in pots filled with mixtures of mine tailing and topsoil, and between the AMF species used, *G. versiforme* showed higher colonization rates which resulted in higher nutrient sorption and biomass of maize and sorghum compared with *G. mosseae* (Guo et al., 2013).

Biomass and Yield Components of Maize and Sorghum

In terms of biomass production and yield components of maize and sorghum, there were also significant effects of the fertilization packages in which D_2 treatment presents the highest values of biomass production (Table 5) and yield components (Table 6). This means that the nutrient status of the soils corresponded well with the biomass weight of the crops, indicated by the positive correlation coefficients, although only some of them are significant. The AMF applications to maize crops, observed at 60 DAS, showed a significant correlation to dry shoot weight, N and P status of the soil, and the N and P sorption. In contrast, AMF colonization levels of maize roots only showed significant correlation with root dry weight at 60 DAS, with an r = + 0.912 (R^2 = 83.2%, p= 0.031) and shoot dry weight at maturity (100 DAS), with an r = + 0.892 (R^2 = 79.6%, p= 0.042). This could mean that during the vegetative growth, AMF colonization have focused on improving root growth to increase nutrient sorption during the vegetative growth of maize crops.

Table 5
Mean dry biomass weight (g/plant) of maize and sorghum for each treatment of fertilization packages

Fertilization packages	Dry biomass weights (g/plant) of maize (1st crop) and sorghum (2nd crop)							
	Maize root		Maize shoot		Sorghum root		Sorghum shoot	
	60 DAS	100 DAS	60 DAS	100 DAS	60 DAS	100 DAS	60 DAS	100 DAS
D_0	8.13^{d}	10.43^{e}	34.15^{d}	62.29^{e}	0.82^{d}	12.30^{d}	8.31^{d}	47.74^{e}
D_1	15.13^{b}	22.39^{b}	61.92^{b}	109.03^{b}	2.22^{b}	22.34^{b}	16.89^{b}	95.01^{b}
D_2	17.12^{a}	34.56^{a}	72.86^{a}	111.25^{a}	3.37^{a}	24.44^{a}	23.53^{a}	105.14^{a}
D_3	13.65^{c}	18.48^{c}	52.26^{c}	101.05^{c}	1.68^{c}	14.52^{c}	12.01^{c}	85.46^{c}
D_4	13.34^{c}	15.43^{d}	51.29^{c}	95.87^{d}	1.38^{c}	14.47^{c}	11.81^{c}	66.06^{d}
HSD 5%	0.31	3.04	0.97	2.21	0.30	0.05	0.20	9.54

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages

In relation to nutrient uptake, although AMF colonization levels did not show significant correlation with N uptake in shoots of maize or sorghum, they did show a positive significant correlation with P uptake both in shoots of maize and sorghum, with an r = + 0.909 (R^2 = 82.6%, p = 0.032) for maize, and r = + 0.940 (R^2 = 88.4%, p = 0.017) for sorghum. These could be due to the ability of AMF extra-radical hyphae in mobilizing and absorbing P from organic matters for their hosts (Feng et al., 2003; Koide & Kabir, 2000; Tarafdar & Marschner, 1994). Based on the strength of the relationships between AMF colonization levels and P uptake, the value of R^2 is higher in sorghum (R^2 = 88.4%) than in maize (R^2 = 82.6%). This means that the contribution of AMF colonization in roots to P uptake in shoots was higher in sorghum crop at the second cropping cycle than in maize in the first cropping cycle. Since sorghum did not

receive any fertilizers, and cattle manure is a slow-release organic fertilizer, it could mean that most P nutrient contained in the shoots, especially in shoots of sorghum in the second cropping cycle, was mostly taken up from the manure under the contribution of AMF colonization in the roots. However, this still needs to be confirmed by further research. This view is supported by the conditions of the study area, which was dominated by sand, and if leaching happened during the rainy season, the loss of dissolved nutrients from the NPK fertilizers applied in the first cropping cycle could be high. Therefore, the main source of nutrients, especially for sorghum crop in the second cropping cycle was the cattle manure applied to the maize crop in the first cropping cycle.

Table 6

Mean weights of total dry grains (kg/plot) and 100 dry grains for each crop and each treatment of fertilization packages

Fertilization packages	Mean dry grain yield (kg/plot) and weight of 100 dry grains (g)			
	Maize in the 1st cropping cycle		Sorghum in the 2nd cropping cycle	
	Grain yield	100 grains	Grain yield	100 grains
D_0	9.60^{e}	22.48^{d}	3.57^{d}	2.73^{d}
D_1	17.40^{b}	26.94^{b}	5.05^{b}	3.01^{b}
D_2	22.80^{a}	28.12^{a}	6.65^{a}	3.61^{a}
D_3	15.60^{c}	25.98^{c}	4.43^{c}	2.90^{c}
D_4	10.20^{d}	24.61^{c}	4.17^{c}	2.81^{cd}
HSD 5%	0.59	1.37	0.26	0.09

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages

In relation to nitrogen nutrient, there was a very low and non-significant correlation between AMF colonization levels and N uptake in maize shoots with an R^2 of only 41.2%. However, the N uptake of maize at 60 DAS was highly significantly correlated with the N status of the soil with an R^2 = 94.1% (p = 0.006). Moreover, the N soil availability also showed a significant correlation with biomass and grain yield of maize, i.e. with an R^2 = 84.1% (p = 0.029) with grain yield at 60 DAS, R^2 = 92.2% (p = 0.009) with shoot dry weight at 60 DAS, and R^2 = 90.6% (p = 0.012) with shoot dry weight at 100 DAS.

In contrast, for sorghum in the second cropping cycle, the soil N status at 60 DAS showed a significant correlation only with shoot dry weight at 100 DAS. Unlike in maize crop where AMF colonization levels showed a significant and positive correlation with shoot dry weight at maturity (100 DAS), AMF colonization levels in sorghum roots did not show any

significant correlation with biomass weight or yield components of sorghum. However, AMF colonization rates in sorghum roots showed a sufficiently high correlation with N uptake in sorghum shoots, with an R^2 = 71.9% (p = 0.069), and N uptake in sorghum shoots showed positive significant correlation with biomass and grain yield of sorghum, i.e. with an R^2 = 80.5% (p = 0.039) with grain yield, R^2 = 83.0% (p = 0.031) with shoot dry weight at 60 DAS and R^2 = 89.9% (p = 0.014) with shoot dry weight at 100 DAS. No additional fertilizers and inoculums were applied at the cropping cycle 2. These could mean that for maize crop, most nutrients were derived from NPK fertilizers while for sorghum, N and P nutrients were derived from the residues of manure contributed from AMF colonization in sorghum roots. Many researchers have also showed the ability of AMF to utilize organic sources to supply N to their host (e.g. Hawkins et al., 2000; Leigh et al., 2009) although it was also found that AMF colonization did not necessarily result in significantly higher N status of the mycorrhizal than non-mycorrhizal hosts (Hawkins et al., 2000).

However, when the correlation analysis was done between averages of colonization levels, biomass and grain yield between maize in the first and sorghum in the second cropping cycle, in general the results show significant and positive coefficients of correlation. For example, correlation of AMF colonization levels between roots of maize and sorghum are highly significant, with an r = + 0.988 (R^2 = 97.6%, p = 0.002). This means that the pattern of differences in AMF colonization levels between roots of maize and sorghum is highly similar. In other words, AMF colonization levels in roots of maize in the first cropping cycle were carried over into the second cropping cycle where sorghum was grown as the rotation crop. This is because both maize and sorghum are hosts of AMF, and both crops have high mycorrhizal dependency (Guo et al., 2013).

In addition, when a t-test of ‘Paired Two Sample for Means’ was run on the averages of AMF colonization levels and spore number in soil at maturity of the crops between maize and sorghum in Table 2, higher significant values (p<0.01) were seen on sorghum than on maize. This could be due to the buildup of AMF in the soil after maize crop in the first copping cycle was harvested before sorghum was directly seeded without tillage. Even though both crops were grown simultaneously, it was also found that AMF colonization levels were higher on sorghum than on maize (Guo et al., 2013). This means that sorghum can be used to improve mycorrhizal conditions of the soil under a maize-sorghum cropping sequence.

CONCLUSION

Among the mycorrhiza-based fertilization packages tested on the maize-sorghum cropping sequence system at the drylands in North Lombok of Indonesia, the D_2 package, consisting of 60% NPK recommended dose, 12 t/ha cattle manure, and the indigenous AMF inoculation, was found to be the best fertilization package to improve crop yield and soil nutrient availability. This study noted that the AMF development was higher in the sorghum at the second cropping cycle compared to the growth in the maize at the first cropping cycle. This condition led to the higher NP-uptake and soil nutrient availability in subsequent crops, both at the sandy dryland, with no additional fertilization and mycorrhizal propagules applied.

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Mycorrhizal Seed-coating on Maize-sorghum Cropping Sequence

Indigenous Mycorrhizal Seed-coating Inoculation on Plant Growth and Yield, and NP-uptake and Availability on Maize-sorghum Cropping Sequence in Lombok's Drylands

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ABSTRACT

By improving the nutrient uptake and transport, an indigenous arbuscular mycorrhizal fungal (AMF) is expected to improve crops' performance in sandy drylands of North Lombok (Indonesia) during dry seasons. A field experiment was designed with Randomized Complete Block Design and four replications to examine the benefits of mycorrhiza at varying doses of plant nutrition (nitrogen and phosphorus). Total of 1 kg of the AMF inoculum was applied to 20 kg maize seeds in different fertilization packages of cattle manure (15, 12, 9 and 6 ton/ha) and inorganic fertilizers (80, 60, 40 and 20% NPK recommended dose). A 100% NPK recommended dose was used as the control (200 kg/ha Phonska and 300 kg/ha Urea). After harvest of maize at 100 days after seeding (DAS) and field cleaning from maize debris, sorghum seeds were then planted in cropping cycle 2 with no additional fertilization and inoculum. Results indicated that the AMF applications to the maize-sorghum cropping sequence increased the AMF colonization rate, soil N and P status and uptake, and dry biomass (root, shoot, and grain). The highest correspondence was observed in the crops which utilized a combination of 60% NPK and 12 ton/ha cattle manure, and the performance was higher at 100 days after seeding. The number of AMF spores increases over the time where colonization rates were found higher in roots of sorghum (60-81%) than maize (55-75%). This study suggests that AMF inoculation increases the plant yield and improves soil nutrient availability which is very advantageous for the growth of the maize-sorghum subsequent crop in Lombok's drylands.

Keywords: Arbuscular mycorrhizal fungi (AMF), cattle manure, maize-sorghum cropping sequence, plant nutrition, seed coating

INTRODUCTION

The northern part of North Lombok regency (Indonesia) is dominated by drylands with sandy soils texture. With a very short and low number of rainy days (December to April, 100-200 mm) per wet month or no rain during the long dry seasons (May to November), no food crops can be cultivated normally especially in the areas without deep wells. Moreover, inadequate phosphorus (P) availability is also one of the factors limiting the productivity of maize and other food crops in the dryland of North Lombok. Maize crop requires very high P; for example, for the production of 10 ton/ha, maize crops need 102 kg/ha of P_2O_5 and 76% of it is transported into the seeds (Calderón-Vázquez et al., 2009). In contrast, plant roots can take up P at only about 8-13% of the amount of P fertilizer applied (Supardi, 1996). Thus, to cope with the unavailability of soil water and P and other essential nutrients in drylands of North Lombok, arbuscular mycorrhizal fungal (AMF) symbiosis has been used as it is expected to improve the performance of food crops especially during the dry seasons. Many have reported that the soil fungi, in symbiosis with their host plants, can help plants to improve water retention and make their host plants more tolerant to drought (Augé, 2004). AMF colonization also increases nutrient uptake from soils and enhances growth and yield of the host plants although it depends on the species of AMF colonizing the host plants (Marulanda et al., 2003).

The most common findings show that the external hyphae of AMF improve P uptake and transport the nutrient to root cortex of their host (Azcón et al., 2003; Feng et al., 2003; George et al., 1995; Koide & Kabir, 2000; Tarafdar & Marschner, 1994; Tawaraya et al., 2006). Although there is no growth improvement of the host plants due to the AMF symbiosis, the amount of P uptake through AMF (mycorrhizal pathway) can be higher than through the host roots or the direct pathway (Smith & Read, 2010). AMF can also help their hosts to increase the uptake of other essential nutrients such as N, K, Ca, Mg, S, Cu, Zn, Mn, Fe and B when compared with non-mycorrhizal plants (Dhillion & Ampornpan, 1992; Hawkins et al., 2000; Leigh et al., 2009; Menge, 1983). Most of these capabilities of the AM plants are due to the extensive growth of AMF external hyphae beyond the host roots (Drew et al., 2003; Zhu et al., 2001). The length ratio of AMF hyphae to roots in soil can be 100:1 or higher and with the external hyphae, the mycorrhizal roots can explore further (George et al., 1995). By applying the Plenchette's mycorrhizal dependency formula to the shoot dry weight of maize and sorghum (Plenchette et al., 1983), the results showed ~~a~~ high mycorrhizal dependency, both for maize (up to 70.12%) and for sorghum (up to 79.42%) (Guo et al., 2013; Tawaraya, 2003). The findings of the study indicate that the higher the dependent rate is on AMF symbiosis, the more the dry

matter is produced of the crops. With the high porosity and low water retention capacity on dryland and sandy areas (Bruand et al., 2005) in most areas of North Lombok, the levels of crop dependence on symbiosis with AMF could be higher. Moreover, the symbiosis of crops with AMF could significantly reduce nutrient loss from soil through leaching (Cavagnaro et al., 2015).

The establishment of AM symbiosis can be done through inoculation with AMF propagules. Our previous study (Astiko et al., 2013a) showed that the indigenous AMF inoculation in maize plants in sandy soils had positive implications for the improvement of soil properties by increasing the rates of nutrient uptake by maize crop from the soil and improving its grain yield. Our research group has also shown the benefit of this local inoculation in increasing the growth of soybean and its yield by improving P uptake from the soils, compared to those without AMF inoculation (Astiko et al., 2013b). However, as found from other studies, the development of AMF in the soil and its contribution to growth, yield and nutrient uptake of host plants could be influenced by the order of plant species cultivated in sequence in the cropping system (Johnson et al., 1992; Vivekanandan & Fixen, 1991). The studies by Johnson et al. (1992) as well as Vivekanandan and Fixen (1991) reported that the P uptake and the AMF colonization were higher on maize crops grown following soybean compared to when maize crops were grown following maize or barley.

From the previous study on maize-soybean cropping patterns, it was found that the AMF inoculated in maize crops grown in the first cycle increased root colonization and AMF sporulation which was very advantageous for the growth of the following crop in the cropping sequence (Astiko, 2013). Different AMF populations (colonization and spore counts) were also found between cropping seasons or between crop species in the same cropping season in Central Lombok, Indonesia (Wangiyana et al., 2006). The present study examined the effects of several combinations of fertilizer packages consisting of indigenous AMF bio-fertilizer and varying doses of organic and NPK fertilizers applied to maize crops on N and P status in soil, and uptake by maize and subsequent sorghum crops as well as the growth and yield components of the maize and sorghum crops in a maize-sorghum cropping sequence on drylands in North Lombok, Indonesia.

MATERIALS AND METHODS

Design of the Experiment

The field experiment of maize-sorghum cropping sequence in this study was arranged according to the Randomized Complete Block Design (RCBD) with four replications (blocks). The study was carried out in the Akar-Akar village located in North Lombok regency, Indonesia, from January to August 2016. Treatments involving the use of five fertilizer packages consisting of different combinations of organic, inorganic and indigenous AMF bio-fertilizer were applied only to the maize crop in the first cropping cycle of the maize-sorghum cropping sequence. The 100% NPK-only recommended dose (D_0) is the farmer's practice of dose for maize by the locals. The NPK's doses were decreased and had been replaced by cattle manure in varying fertilization packages (D_1, D_2, D_3, D_4), and added with AMF as listed in Table 1.

Table 1
The mycorrhizal-based fertilization packages tested and applied to maize only in the maize-sorghum cropping sequence

Fertilization packages	Doses of the packages applied to maize plants only (in the cropping cycle 1)	Sorghum (cropping cycle 2)
D_0	NPK only at 100% the recommended doses (RD), i.e. 200 kg/ha Phonska (NPK 15:15:15) and 300 kg/ha Urea	No fertilizer applied
D_1	NPK at 80% RD + 15 ton/ha cattle manure + AMF	No fertilizer applied
D_2	NPK at 60% RD + 12 ton/ha cattle manure + AMF	No fertilizer applied
D_3	NPK at 40% RD + 9 ton/ha cattle manure + AMF	No fertilizer applied
D_4	NPK at 20% RD + 6 ton/ha cattle manure + AMF	No fertilizer applied

A piece of land used in this study was of a sandy soil type (69% sand, 29% silt and 2% clay, containing an average of 421 AMF spores per 100 g soil), typical of North Lombok area. The land is located at a geographic position of -8.221650, 116.350283, and measured with 13.82 mg/kg of available P, 0.01% Total N, 0.57 cmol/kg of available K, 7.31 cmol/kg of Ca, and 1.21% of C-organic. After being cultivated using minimum tillage and cleared of weeds, the land was split into 20 treatment plots of 7m x 5m based on the RCBD plotting layout. The different fertilization packages consisted of AMF inoculum, organic fertilizer (cattle manure), and inorganic fertilizer (NPK and Urea), were applied only to the maize crop in the first cropping cycle.

An indigenous AMF inoculum, *Glomus mosseae* (the M_{AA01} mycorrhizal isolate including the hyphae and the mycorrhizal spores), which was originally isolated from dryland area (1,500 spores per 20 g of soils) in Akar-Akar village of North Lombok, was applied through seed-coating of maize seeds prior to seeding. Before application, 1 kg of the AMF inoculum was mixed with 300 g of charcoal powder and 90 g of tapioca flour as the carrier

materials. Then this inoculum mixture was mixed with 20 kg maize seeds so that the seeds were coated by the inoculum mixture. For cropping cycle 1, the uncoated or AMF coated maize seeds ("Bisma" variety) were planted by dibbling 2 seeds per planting hole at 70 cm x 20 cm plant spacing. The entire dose of cattle manure (organic fertilizer) at different doses depending on the treatments (Table 1) was applied at the time of planting the maize seeds by burying the whole dose in the planting hole in the position below the seeds. The cattle manure variation in the fertilization package is to identify the optimum combinations to benefit the plant growth, increase the nutrient availability at the soils, and support the AMF development. The maximum combination of cattle manure was 15 ton/ha, the limit for transportation. The cattle manure applied in the package was measured with 3.08% Total Nitrogen, pH 6.66, 17.70 mg/kg of available P, 2.31 cmol/kg of available K, 10.45 C/N ration, and 32.2% C-organic. The inorganic (NPK) fertilizers with the recommended doses of 200 kg/ha Phonska (NPK 15:15:15) and 300 kg/ha Urea (46% N) were applied twice, i.e. at 7 DAS (days after seeding) and at 21 DAS, with the amount applied depending on the treatments (Table 1). At 7 DAS, the maize plants were fertilized with a mixture of the entire Phonska and one third of the Urea treatment doses, followed by application of the remaining Urea treatment doses at 21 DAS. The NPK fertilizers were applied in a furrow of 5 cm alongside the maize plant row at 5-7 cm depth before covered with soil.

After harvest of maize at 100 days after seeding (DAS) and field cleaning from maize debris, sorghum seeds were then planted in cropping cycle 2. Before the second sequence, the field was left fallow (rest) for 10 days. Seeds of sorghum ("Numbu" variety) were directly seeded by dibbling 2 seeds per planting holes made around the maize stubbles. These sorghum plants were not fertilized nor inoculated with additional AMF inoculum, but only the chopped maize roots containing the AMF mycorrhizal and spread throughout the plot. For both crops, the young maize and sorghum plants were tinned at 7 DAS by leaving one maize or sorghum plant per planting hole. Weeding and soil piling of the maize and sorghum plant base were done at 15 and 30 DAS. Plant protection was done by spraying "OrgaNeem" (an organic pesticide of plant origin containing Azadirachtin extracted from neem leaves) at a concentration of 5 ml OrgaNeem per liter of water. The OrgaNeem solution was applied to both crops (maize or sorghum) from 10 to 60 DAS at a 3-day interval. Harvesting of maize or sorghum crops was done at 100 DAS.

Measurement and Data Analysis

The variables measured were AMF development, N and P nutrition, and growth and yield of maize and sorghums. The AMF development includes spore counts at 60 and 100 DAS, and root colonization levels at 60 DAS. The N and P nutrition includes concentration of total N and available P in the rhizosphere of maize and sorghum at the time when the vegetative and generative growth found optimum, respectively at 60 and 100 DAS. The crop variables include dry weight (shoots and roots) and yield components (grain).

Laboratory analysis was conducted at Laboratory of Chemistry and Soil Science, Faculty of Agriculture, University of Mataram. Soil pH and texture were measured by standard procedures (Imam & Didar, 2005). Determination of total nitrogen in soil was done by destruction with $(NH_4)_2SO_4$ and distillation with NaOH where the $NH_4^+$was determined by indophenol blue colorimetric method and the NH_3 was defined by a titration with 0.05N of H_2SO_4 solution (Page et al., 1982). Total N in plants was measured using spectrophotometric indophenol blue methods with wave length 636 nm after destruction by $(NH_4)_2SO_4$ and distillation with NaOH following the Conway instruction (Lisle et al., 1990). The available Phosphorus in soil and plant was measured using spectrophotometer (λ = 693 nm) after the extraction process using Bray and Kurt I solution (0.025 N HCl + NH4F 0.03 N) (Bray & Kurtz, 1945). Total organic C was measured by oxidation with $K_2Cr_2O_7$ in presence of sulphuric acid (H_2SO_4) following Walkley and Black's method (Horwitz, 2000).

AMF spore extraction from soil (100 g soil sample) was done using the wet sieving and decanting technique (Brundrett et al., 1996). The spores collected from the 38 µm sieve after the final centrifugation were captured in a filter paper, which were then observed on a Petri dish using a stereo microscope at 40 x magnification, and counted as spore number per 100 g of soil. The percentage of root colonization was determined using the Gridline Intersect technique (Giovannetti & Mosse, 1980) under a stereo microscope after the root pieces were stained using the clearing and staining method of Brundrett et al. (1996).

Data were analyzed using analysis of two way ANOVA and the Tukey's HSD (Honestly Significant Difference) means tested at 5% level of significance.

RESULTS AND DISCUSSION

AMF Development

In this study, however, maize and sorghum were grown in sequence, in which sorghum was directly seeded without treatments and without tillage following the harvest of maize plants. All treatments, including AMF inoculation, were applied only to maize plants in the first cropping cycle. Therefore, it means that sorghum in cropping cycle 2 was grown after AMF propagules have built up in the soil during the growth of the maize plants in cropping cycle 1. This is in line with the results reported in the previous study (Arihara & Karasawa, 2000) which revealed that AMF root colonization and shoot dry weight of maize at 58 DAS were affected by the previous crop grown, whether they were host or non-host of AMF. When grown simultaneously, Carrenho et al. (2007) found higher colonization rates on maize than on sorghum. However, the P fertilization did not affect root colonization, especially on maize following AMF host plants.

Table 2

Mean spore number (spores per 100 g soil) at 60 and 100 DAS, and colonization rates (%-colonization) on maize and sorghum in a maize-sorghum cropping sequence

Fertilization packages	AMF on maize (1st crop)			AMF sorghum (2nd crop)		
	Spore per 100 g soil		% colonization	Spore per 100 g soil		% colonization
	60 DAS	100 DAS	60 DAS	60 DAS	100 DAS	60 DAS
D_0	764^{e}	3464^{d}	30^{d}	1231^{e}	3761^{d}	35^{e}
D_1	1059^{d}	3672^{c}	55^{c}	1343^{d}	4942^{b}	60^{d}
D_2	2119^{a}	4327^{a}	75^{a}	2981^{a}	5165^{a}	81^{a}
D_3	1690^{b}	3894^{b}	65^{b}	1881^{b}	4831^{c}	77^{b}
D_4	1294^{c}	3881^{b}	63^{b}	1769^{c}	4819^{c}	68^{c}
HSD 5%	231	13	2.0	109	12	6.5

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for descriptions of the packages

Specifically, the degree of root colonization by AMF may be higher in the roots of maize fertilized with highly soluble mineral fertilizers (NPK, diammonium phosphate, ammonium sulfate and Urea). On the other hand, the length of extra-radical mycelium and number of AMF spores were significantly higher in maize fertilized with organic fertilizers (Bokashi manure) (Bautista-Cruz et al., 2014). This indicates that organic fertilization is more favorable for spore production compared with conventional fertilization. It can also be seen from Table 2 that AMF spore numbers are higher at harvest of the maize crop (100 DAS) compared with those at 60 DAS, especially on the D_2 treatment. This indicates a high buildup of AMF propagules for the subsequent sorghum crops. This condition seems to favor higher AMF colonization levels in roots of sorghum in the second cropping cycle compared with that of maize in the first cropping

cycle. With no tillage treatment, the AMF colonization levels in sorghum may be higher than the levels in maize or bean roots (Alguacil et al., 2008). In addition, AMF colonization rates may be higher in roots of sorghum than in the roots of maize, either inoculated with *Glomus mosseae* or *Glomus versiforme*, indicating that sorghum is more favorable for AMF development than maize plants (Guo et al., 2013).

There are many factors influencing the degrees of AMF colonization of crop roots as reported by Carrenho et al. (2007). In examining the effects of different phosphorus, liming, organic matter, and soil texture on root colonization of peanuts, sorghum, and maize in a factorial experiment, each factor significantly affected root colonization level, alone or in interaction with other factors (Carrenho et al., 2007). The most surprising results were the significant interaction effects of plant, phosphorous, and organic matter although there were no significant effects of phosphorous (simple superphosphate of 166.6 mg/L (w/v) soil) nor organic matter (22 mg/L (w/v) soil). As for the interaction effect involving maize, there was no significant effect of phosphorous and organic matter on AMF colonization of maize roots. On the other hand, the application of both phosphorous and organic matter significantly reduced AMF colonization on sorghum roots (Carrenho et al., 2007).

In terms of fertilization effects, AMF colonization rate was reported to be higher on crop roots in unfertilized soils, followed by organic and biodynamic, and the least was seen in exclusively mineral fertilized and conventional farming systems. Thus, it was concluded that the amount of soluble P in the soil was the most limiting factor for AMF colonization in roots of those crops (winter wheat, vetch-rye, and grass-clover) (Mäder et al., 2000). Moreover, it was reported that the increasing P level in the growing media from 0.1 to 0.5 mM P significantly reduced AMF colonization in roots of lettuce plants especially with increasing levels of N contents (1, 5, or 9 mM N) (Azcón et al., 2003).

A negative impact on soil condition occur when the accumulation of soil P has increased beyond the requirement of the crops cultivated (Grant et al., 2005). In the study, it seemed that the amount of P input from the NPK fertilizer as applied in the D_2 treatment was most favorable for AMF development in maize crops. In the D_2 treatment, the NPK fertilizer was reduced to 120 kg/ha Phonska (60% of the recommended NPK dose of 200 kg/ha Phonska in the D_0 treatment) combined with an application of 12 ton/ha cattle manure and inoculation with AMF. Among the treatments with AMF in combination with cattle manure, the D_1 treatment had the

highest NPK fertilizer dose (Table 1), and this level of NPK fertilization seems to limit the AMF infection and development in maize roots compared with those in the D_2 treatment, resulting in a higher AMF colonization rate on D_2 than on D_1 treatment. Therefore, AMF colonization and spore numbers in maize were highest in the D_2 treatment, especially when compared with the D_1 or D_0 treatment. Using the same indigenous AMF applied to a pot experiment in which the soil for the growing media was taken from the same field in North Lombok, it was found that the highest level of AMF colonization of maize roots was in the treatment with AMF combined with cattle manure compared with AMF combined with NPK fertilizer or phosphate rock (Astiko et al., 2013a). The previous study also reported a significantly higher AMF spore number in plots fertilized with cattle manure or cattle manure combined with mineral fertilizers compared with those fertilized with mineral fertilizers only (Gryndler et al., 2006).

Soil Nutrient Status and Nutrient Sorption by Maize and Sorghum

There were significant effects of the different fertilizer packages on soil nutrient status (N and P contents of the soil) of the rhizosphere of both maize and sorghum crops at 60 and 100 DAS (Table 3). In general, there is a tendency that the highest values of the soil nutrient status, measured either at 60 or 100 DAS, are highest under the D_2 (60% NPK + 12 t/ha manure + AMF) or D_1 (80% NPK + 15 t/ha manure + AMF) treatment. The values of the soil nutrient status in these treatments were higher than those in D_0 (100% NPK only) treatment, although the dose of NPK fertilizers was reduced in the D_1 and D_2 treatments (Table 3).

Table 3

Mean concentration of total N and available P of soil in the rhizospheres of maize (1st crop) and sorghum (2nd crop) for each treatment of fertilization packages, measured at 60 and 100 DAS

Fertilization packages	Total N ($g.kg^{-1}$) at 60 DAS		Available P ($mg.kg^{-1}$) at 60 DAS		Total N ($g.kg^{-1}$) at 100 DAS		Available P ($mg.kg^{-1}$) at 100 DAS	
	maize	sorghum	maize	sorghum	maize	sorghum	maize	sorghum
D_0	1.24^{c}	1.10^{c}	14.97^{d}	11.41^{d}	1.33^{d}	1.23^{d}	17.43^{d}	10.15^{d}
D_1	1.45^{a}	1.29^{a}	28.44^{b}	16.25^{b}	1.69^{b}	1.38^{b}	29.51^{b}	21.58^{b}
D_2	1.47^{a}	1.25^{ab}	35.02^{a}	28.49^{a}	1.86^{a}	1.48^{a}	36.56^{a}	29.99^{a}
D_3	1.39^{b}	1.22^{ab}	17.52^{c}	14.59^{bc}	1.47^{c}	1.31^{c}	19.37^{c}	18.59^{c}
D_4	1.33^{b}	1.20^{b}	16.29^{c}	14.25^{c}	1.42^{c}	1.31^{c}	18.53^{c}	17.32^{c}
HSD 5%	0.08	0.07	1.31	1.99	0.08	0.06	1.0	2.98

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages

At the present study, no infiltration data was measured, however it is important to note that since the NPK fertilizers applications, i.e. Phonska and Urea at 7 DAS and Urea at 21 DAS, at sandy lands, are easily dissolved in water, and significant amount of the nutrients could have been loss through infiltration during the rainy season. Previous study shows that the sand content of the cultivated land had a positive correlation with infiltration rate, with an r-value of +0.75 (Patle et al., 2018). Since cattle manure is slow in releasing nutrients, it is possible that at the time of measurement (60 and 100 DAS), the loss of released nutrients from the NPK fertilizers through leaching was much higher in the D_0 than in the other treatments of fertilization packages due to dissolution by rain water during the rainy season. However, there were small increases in nutrient status of the soil from 60 to 100 DAS around the rhizospheres of maize crop, especially in those treated with manure and AMF application. This indicated a slow release of N and P nutrients from manure after application to the maize plants in the first cropping cycle, which could be due to AMF inoculation. Many studies have reported that AMF can mobilize and take N and P from organic matters for their hosts (Feng et al., 2003; Hawkins et al., 2000; Koide & Kabir, 2000; Leigh et al., 2009; Tarafdar & Marschner, 1994), and it can be seen from Table 2 that the highest average of AMF colonization levels in maize roots was in the D_2 treatment. Therefore, N and P uptake in shoots of maize and sorghum was highest in the D_2 treatment (Table 4).

In addition, soil contents of those nutrients corresponded well with the levels of N and P uptake of the crops, i.e. all showing positive correlation coefficients. Although it is not significant for P uptake, the highest values of N and P uptake were also seen in the D_2 treatment for both maize and sorghum (Table 4). The correlation analysis of the mean values obtained at 60 DAS showed a significant correlation between soil N and N sorption in the shoots, with a value of r = + 0.970 (R-square = 94.1%, p = 0.006) for maize plants, and r = + 0.904 (R-square = 81.7%, p = 0.035) for sorghum plants. These indicate those fertilizers in the packages contribute to nutrient contents of the crops, which are significantly different between treatments of fertilization packages (Table 4).

Table 4
Mean N and P sorption (mg.g^{-1} plant dry weight) by each crop at 60 DAS for each treatment of fertilization packages

Fertilization packages	N and P uptake (mg.g^{-1} plant dry weight) by each crop at 60 DAS			
	Maize (1st cropping cycle)		Sorghum (2nd cropping cycle)	
	N	P	N	P
D_0	18.62^{e}	1.71^{d}	12.74^{d}	0.65^{d}

D_1	27.58^{b}	2.60^{b}	18.92^{b}	0.85^{c}
D_2	28.00^{a}	2.72^{a}	20.86^{a}	1.48^{a}
D_3	23.10^{c}	2.41^{c}	17.29^{c}	1.36^{b}
D_4	20.44^{d}	2.41^{c}	17.22^{c}	1.32^{b}
HSD 5%	0.41	0.11	0.07	0.04

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages

However, P status of the soil did not show a significant correlation with P uptake either by maize or sorghum crop. In spite of that, there is a significant positive correlation between the degree of AMF colonization in the roots and P sorption in the shoot of maize plants, with an r = + 0.909 (R-square = 82.6%, p = 0.032), and that of sorghum plants, with an r = + 0.940 (R-square = 88.4%, p = 0.017). This shows that there were significant contributions of AMF colonization in the roots to the amount of P taken up by the plants, both maize and sorghum. If soil soluble P has not exceeded crop requirements, AMF association will still result in significantly positive effects on P uptake and biomass production because of P requirements of the crops; for optimum growth and yields, crops require P since the early stage of their growth, either from soil, fertilizer application, or from AMF associations (Grant et al., 2005).

Although sorghum crop in the second cropping cycle was not fertilized with manure nor NPK fertilizers, direct seeding of sorghum after harvesting the maize plants without tillage seemed to be a favorable condition in establishing AMF association in the sorghum crop, which resulted in higher AMF colonization rates in sorghum than in maize roots for each fertilizer package (Table 2). The AMF associations seemed to enable the sorghum crops to absorb adequate P and other nutrients from soil and residues of the manure applied in the first cropping cycle even though these sorghum crops were not fertilized. This could occur because of the potential of AMF external hyphae that help the host plants in absorbing and dissolving nutrients from soil and manures and other nutrient pools, as well as other nutrients unavailable for uptake by plant roots (Menge, 1983; Smith & Read, 2010; Tawaraya et al., 2006). Guo et al. (2013) also reported significant effects of AMF inoculation on C, N, P, and K contents (mg/pot) in shoot and roots of maize and sorghum grown in pots filled with mixtures of mine tailing and topsoil, and between the AMF species used, *G. versiforme* showed higher colonization rates which resulted in higher nutrient sorption and biomass of maize and sorghum compared with *G. mosseae* (Guo et al., 2013).

Biomass and Yield Components of Maize and Sorghum

In terms of biomass production and yield components of maize and sorghum, there were also significant effects of the fertilization packages in which D_2 treatment presents the highest values of biomass production (Table 5) and yield components (Table 6). This means that the nutrient status of the soils corresponded well with the biomass weight of the crops, indicated by the positive correlation coefficients, although only some of them are significant. The AMF applications to maize crops, observed at 60 DAS, showed a significant correlation to dry shoot weight, N and P status of the soil, and the N and P sorption. In contrast, AMF colonization levels of maize roots only showed significant correlation with root dry weight at 60 DAS, with an r = + 0.912 (R^2 = 83.2%, p= 0.031) and shoot dry weight at maturity (100 DAS), with an r = + 0.892 (R^2 = 79.6%, p= 0.042). This could mean that during the vegetative growth, AMF colonization have focused on improving root growth to increase nutrient sorption during the vegetative growth of maize crops.

Table 5
Mean dry biomass weight (g/plant) of maize and sorghum for each treatment of fertilization packages

Fertilization packages	Dry biomass weights (g/plant) of maize (1st crop) and sorghum (2nd crop)							
	Maize root		Maize shoot		Sorghum root		Sorghum shoot	
	60 DAS	100 DAS	60 DAS	100 DAS	60 DAS	100 DAS	60 DAS	100 DAS
D_0	8.13^{d}	10.43^{e}	34.15^{d}	62.29^{e}	0.82^{d}	12.30^{d}	8.31^{d}	47.74^{e}
D_1	15.13^{b}	22.39^{b}	61.92^{b}	109.03^{b}	2.22^{b}	22.34^{b}	16.89^{b}	95.01^{b}
D_2	17.12^{a}	34.56^{a}	72.86^{a}	111.25^{a}	3.37^{a}	24.44^{a}	23.53^{a}	105.14^{a}
D_3	13.65^{c}	18.48^{c}	52.26^{c}	101.05^{c}	1.68^{c}	14.52^{c}	12.01^{c}	85.46^{c}
D_4	13.34^{c}	15.43^{d}	51.29^{c}	95.87^{d}	1.38^{c}	14.47^{c}	11.81^{c}	66.06^{d}
HSD 5%	0.31	3.04	0.97	2.21	0.30	0.05	0.20	9.54

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages

In relation to nutrient uptake, although AMF colonization levels did not show significant correlation with N uptake in shoots of maize or sorghum, they did show a positive significant correlation with P uptake both in shoots of maize and sorghum, with an r = + 0.909 (R^2 = 82.6%, p = 0.032) for maize, and r = + 0.940 (R^2 = 88.4%, p = 0.017) for sorghum. These could be due to the ability of AMF extra-radical hyphae in mobilizing and absorbing P from organic matters for their hosts (Feng et al., 2003; Koide & Kabir, 2000; Tarafdar & Marschner, 1994). Based on the strength of the relationships between AMF colonization levels and P uptake, the value of R^2 is higher in sorghum (R^2 = 88.4%) than in maize (R^2 = 82.6%). This means that the contribution of AMF colonization in roots to P uptake in shoots was higher in sorghum crop at the second cropping cycle than in maize in the first cropping cycle. Since sorghum did not

receive any fertilizers, and cattle manure is a slow-release organic fertilizer, it could mean that most P nutrient contained in the shoots, especially in shoots of sorghum in the second cropping cycle, was mostly taken up from the manure under the contribution of AMF colonization in the roots. However, this still needs to be confirmed by further research. This view is supported by the conditions of the study area, which was dominated by sand, and if leaching happened during the rainy season, the loss of dissolved nutrients from the NPK fertilizers applied in the first cropping cycle could be high. Therefore, the main source of nutrients, especially for sorghum crop in the second cropping cycle was the cattle manure applied to the maize crop in the first cropping cycle.

Table 6

Mean weights of total dry grains (kg/plot) and 100 dry grains for each crop and each treatment of fertilization packages

Fertilization packages	Mean dry grain yield (kg/plot) and weight of 100 dry grains (g)			
	Maize in the 1st cropping cycle		Sorghum in the 2nd cropping cycle	
	Grain yield	100 grains	Grain yield	100 grains
D_0	9.60^{e}	22.48^{d}	3.57^{d}	2.73^{d}
D_1	17.40^{b}	26.94^{b}	5.05^{b}	3.01^{b}
D_2	22.80^{a}	28.12^{a}	6.65^{a}	3.61^{a}
D_3	15.60^{c}	25.98^{c}	4.43^{c}	2.90^{c}
D_4	10.20^{d}	24.61^{c}	4.17^{c}	2.81^{cd}
HSD 5%	0.59	1.37	0.26	0.09

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages

In relation to nitrogen nutrient, there was a very low and non-significant correlation between AMF colonization levels and N uptake in maize shoots with an R^2 of only 41.2%. However, the N uptake of maize at 60 DAS was highly significantly correlated with the N status of the soil with an R^2 = 94.1% (p = 0.006). Moreover, the N soil availability also showed a significant correlation with biomass and grain yield of maize, i.e. with an R^2 = 84.1% (p = 0.029) with grain yield at 60 DAS, R^2 = 92.2% (p = 0.009) with shoot dry weight at 60 DAS, and R^2 = 90.6% (p = 0.012) with shoot dry weight at 100 DAS.

In contrast, for sorghum in the second cropping cycle, the soil N status at 60 DAS showed a significant correlation only with shoot dry weight at 100 DAS. Unlike in maize crop where AMF colonization levels showed a significant and positive correlation with shoot dry weight at maturity (100 DAS), AMF colonization levels in sorghum roots did not show any

significant correlation with biomass weight or yield components of sorghum. However, AMF colonization rates in sorghum roots showed a sufficiently high correlation with N uptake in sorghum shoots, with an R^2 = 71.9% (p = 0.069), and N uptake in sorghum shoots showed positive significant correlation with biomass and grain yield of sorghum, i.e. with an R^2 = 80.5% (p = 0.039) with grain yield, R^2 = 83.0% (p = 0.031) with shoot dry weight at 60 DAS and R^2 = 89.9% (p = 0.014) with shoot dry weight at 100 DAS. No additional fertilizers and inoculums were applied at the cropping cycle 2. These could mean that for maize crop, most nutrients were derived from NPK fertilizers while for sorghum, N and P nutrients were derived from the residues of manure contributed from AMF colonization in sorghum roots. Many researchers have also showed the ability of AMF to utilize organic sources to supply N to their host (e.g. Hawkins et al., 2000; Leigh et al., 2009) although it was also found that AMF colonization did not necessarily result in significantly higher N status of the mycorrhizal than non-mycorrhizal hosts (Hawkins et al., 2000).

However, when the correlation analysis was done between averages of colonization levels, biomass and grain yield between maize in the first and sorghum in the second cropping cycle, in general the results show significant and positive coefficients of correlation. For example, correlation of AMF colonization levels between roots of maize and sorghum are highly significant, with an r = + 0.988 (R^2 = 97.6%, p = 0.002). This means that the pattern of differences in AMF colonization levels between roots of maize and sorghum is highly similar. In other words, AMF colonization levels in roots of maize in the first cropping cycle were carried over into the second cropping cycle where sorghum was grown as the rotation crop. This is because both maize and sorghum are hosts of AMF, and both crops have high mycorrhizal dependency (Guo et al., 2013).

In addition, when a t-test of ‘Paired Two Sample for Means’ was run on the averages of AMF colonization levels and spore number in soil at maturity of the crops between maize and sorghum in Table 2, higher significant values (p<0.01) were seen on sorghum than on maize. This could be due to the buildup of AMF in the soil after maize crop in the first copping cycle was harvested before sorghum was directly seeded without tillage. Even though both crops were grown simultaneously, it was also found that AMF colonization levels were higher on sorghum than on maize (Guo et al., 2013). This means that sorghum can be used to improve mycorrhizal conditions of the soil under a maize-sorghum cropping sequence.

CONCLUSION

Among the mycorrhiza-based fertilization packages tested on the maize-sorghum cropping sequence system at the drylands in North Lombok of Indonesia, the D_2 package, consisting of 60% NPK recommended dose, 12 t/ha cattle manure, and the indigenous AMF inoculation, was found to be the best fertilization package to improve crop yield and soil nutrient availability. This study noted that the AMF development was higher in the sorghum at the second cropping cycle compared to the growth in the maize at the first cropping cycle. This condition led to the higher NP-uptake and soil nutrient availability in subsequent crops, both at the sandy dryland, with no additional fertilization and mycorrhizal propagules applied.

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Indigenous Mycorrhizal Seed-coating Inoculation on Plant Growth and Yield, and NP-uptake and Availability on Maize-sorghum Cropping Sequence in Lombok's Drylands

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ABSTRACT

By improving the nutrient uptake and transport, an indigenous arbuscular mycorrhizal fungal (AMF) is expected to improve crops' performance in sandy drylands of North Lombok (Indonesia) during dry seasons. A field experiment was designed with Randomized Complete Block Design and four replications to examine the benefits of mycorrhiza at varying doses of plant nutrition (nitrogen and phosphorus). Total of 1 kg of the AMF inoculum was applied to 20 kg maize seeds in different fertilization packages of cattle manure (15, 12, 9 and 6 ton/ha) and inorganic fertilizers (80, 60, 40 and 20% NPK recommended dose). A 100% NPK recommended dose was used as the control (200 kg/ha Phonska and 300 kg/ha Urea). After harvest of maize at 100 days after seeding (DAS) and field cleaning from maize debris, sorghum seeds were then planted in cropping cycle 2 with no additional fertilization and inoculum. Results indicated that the AMF applications to the maize-sorghum cropping sequence increased the AMF colonization rate, soil N and P status and uptake, and dry biomass (root, shoot, and grain). The highest correspondence was observed in the crops which utilized a combination of 60% NPK and 12 ton/ha cattle manure, and the performance was higher at 100 days after seeding. The number of AMF spores increased over the time where colonization rates were found higher in roots of sorghum (60-81%) than maize (55-75%). This study suggests that AMF inoculation increases the plant yield and improves soil nutrient availability

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which is very advantageous for the growth of the maize-sorghum subsequent crop in Lombok's drylands.

Keywords: Arbuscular mycorrhizal fungi (AMF), cattle manure, maize-sorghum cropping sequence, plant nutrition, seed coating

INTRODUCTION

The northern part of North Lombok regency (Indonesia) is dominated by drylands with sandy soils texture. With a very short and low number of rainy days (December to April, 100-200 mm) per wet month or no rain during the long dry seasons (May to November), no food crops can be cultivated normally especially in the areas without deep wells. Moreover, inadequate phosphorus (P) availability is also one of the factors limiting the productivity of maize and other food crops in the dryland of North Lombok. Maize crop requires very high P; for example, for the production of 10 ton/ha, maize crops need 102 kg/ha of P_2O_5 and 76% of it is transported into the seeds (Calderón-Vázquez et al., 2009). In contrast, plant roots can take up P at only about 8-13% of the amount of P fertilizer applied (Supardi, 1996). Thus, to cope with the unavailability of soil water and P and other essential nutrients in drylands of North Lombok, arbuscular mycorrhizal fungal (AMF) symbiosis has been used as it is expected to improve the performance of food crops especially during the dry seasons. Many have reported that the soil fungi, in symbiosis with their host plants, can help plants to improve water retention and make their host plants more tolerant to drought (Augé, 2004). AMF colonization also increases nutrient uptake from soils and enhances growth and yield of the host plants although it depends on the species of AMF colonizing the host plants (Marulanda et al., 2003).

The most common findings show that the external hyphae of AMF improve P uptake and transport the nutrient to root cortex of their host (Azcón et al., 2003; Feng et al., 2003; George et al., 1995; Koide & Kabir, 2000; Tarafdar & Marschner, 1994; Tawaraya et al., 2006). Although there is no growth improvement of the host plants due to the AMF symbiosis, the amount of P uptake through AMF (mycorrhizal pathway) can be higher than through the host roots or the direct pathway (Smith & Read, 2010). AMF can also help their hosts to increase the uptake of other essential nutrients such as N, K, Ca, Mg, S, Cu, Zn, Mn, Fe and B when compared with non-mycorrhizal plants (Dhillion & Ampornpan, 1992; Hawkins et al., 2000; Leigh et al., 2009; Menge, 1983). Most of these capabilities of the AM plants are due to the extensive growth of AMF external hyphae beyond the host roots (Drew et al., 2003; Zhu et al., 2001). The length ratio of AMF hyphae to roots in soil can be 100:1 or higher and with the external hyphae, the mycorrhizal roots can explore further (George et al., 1995). By applying the Plenchette's mycorrhizal dependency formula to the shoot dry weight of maize and sorghum (Plenchette et al., 1983), the results showed ~~a~~ high mycorrhizal dependency, both for maize (up to 70.12%) and for sorghum (up to 79.42%) (Guo et al., 2013;

Tawaraya, 2003). The findings of the study indicate that the higher the dependent rate is on AMF symbiosis, the more the dry matter is produced of the crops. With the high porosity and low water retention capacity on dryland and sandy areas (Bruand et al., 2005) in most areas of North Lombok, the levels of crop dependence on symbiosis with AMF could be higher. Moreover, the symbiosis of crops with AMF could significantly reduce nutrient loss from soil through leaching (Cavagnaro et al., 2015).

The establishment of AM symbiosis can be done through inoculation with AMF propagules. Our previous study (Astiko et al., 2013a) showed that the indigenous AMF inoculation in maize plants in sandy soils had positive implications for the improvement of soil properties by increasing the rates of nutrient uptake by maize crop from the soil and improving its grain yield. Our research group has also shown the benefit of this local inoculation in increasing the growth of soybean and its yield by improving P uptake from the soils, compared to those without AMF inoculation (Astiko et al., 2013b). However, as founded from other studies, the development of AMF in the soil and its contribution to growth, yield and nutrient uptake of host plants could be influenced by the order of plant species cultivated in sequence in the cropping system (Johnson et al., 1992; Vivekanandan & Fixen, 1991). The studies by Johnson et al. (1992) as well as Vivekanandan and Fixen (1991) reported that the P uptake and the AMF colonization were higher on maize crops grown following soybean compared to when maize crops were grown following maize or barley.

From the previous study on maize-soybean cropping patterns, it was found that the AMF inoculated in maize crops grown in the first cycle increased root colonization and AMF sporulation which was very advantageous for the growth of the following crop in the cropping sequence (Astiko, 2013). Different AMF populations (colonization and spore counts) were also found between cropping seasons or between crop species in the same cropping season in Central Lombok, Indonesia (Wangiyana et al., 2006). The present study examined the effects of several combinations of fertilizer packages consisting of indigenous AMF bio-fertilizer and varying doses of organic and NPK fertilizers applied to maize crops on N and P status in soil, and uptake by maize and subsequent sorghum crops as well as the growth and yield components of the maize and sorghum crops in a maize-sorghum cropping sequence on drylands in North Lombok, Indonesia.

MATERIALS AND METHODS

Design of the Experiment

The field experiment of maize-sorghum cropping sequence in this study was arranged according to the Randomized Complete Block Design (RCBD) with four replications (blocks). The study was carried out in the Akar-Akar village located in North Lombok regency, Indonesia, from January to August 2016. Treatments involving the use of five fertilizer packages consisting of different combinations of organic, inorganic and indigenous AMF bio-fertilizer were applied only to the maize crop in the first cropping cycle of the maize-sorghum cropping sequence. The 100% NPK-only recommended dose (D_0) is the farmer's practice of dose for maize by the locals. The NPK's doses were decreased and had been replaced by cattle manure in varying fertilization packages (D_1, D_2, D_3, D_4), and added with AMF as listed in Table 1.

Table 1
The mycorrhizal-based fertilization packages tested and applied to maize only in the maize-sorghum cropping sequence

Fertilization packages	Doses of the packages applied to maize plants only (in the cropping cycle 1)	Sorghum (cropping cycle 2)
D_0	NPK only at 100% the recommended doses (RD), i.e. 200 kg/ha Phonska (NPK 15:15:15) and 300 kg/ha Urea	No fertilizer applied
D_1	NPK at 80% RD + 15 ton/ha cattle manure + AMF	No fertilizer applied
D_2	NPK at 60% RD + 12 ton/ha cattle manure + AMF	No fertilizer applied
D_3	NPK at 40% RD + 9 ton/ha cattle manure + AMF	No fertilizer applied
D_4	NPK at 20% RD + 6 ton/ha cattle manure + AMF	No fertilizer applied

A piece of land used in this study was of a sandy soil type (69% sand, 29% silt and 2% clay, containing an average of 421 AMF spores per 100 g soil), typical of North Lombok area. The land is located at a geographic position of -8.221650, 116.350283, and measured with 13.82 mg/kg of available P, 0.01% Total N, 0.57 cmol/kg of available K, 7.31 cmol/kg of Ca, and 1.21% of C-organic. After being cultivated using minimum tillage and cleared of weeds, the land was splitted into 20 treatment plots of 7m x 5m based on the RCBD plotting layout. The different fertilization packages consisted of AMF inoculum, organic fertilizer (cattle manure), and inorganic fertilizer (NPK and Urea), were applied only to the maize crop in the first cropping cycle.

An indigenous AMF inoculum, *Glomus mosseae* (the M_{AA01} mycorrhizal isolate including the hyphae and the mycorrhizal spores), which was originally isolated from dryland area (1,500 spores per 20 g of soils) in Akar-Akar village of North Lombok, was applied through seed-coating of maize seeds prior to seeding. Before application, 1 kg of the AMF inoculum was mixed with 300 g of charcoal powder and 90 g of tapioca flour as the carrier materials. Then this inoculum mixture was mixed with 20 kg maize seeds so that the seeds were coated by the inoculum mixture. For cropping cycle 1, the uncoated or AMF coated maize seeds ("Bisma" variety) were planted by dibbling 2 seeds per planting hole at 70 cm x 20 cm plant spacing. The entire dose of cattle manure (organic fertilizer) at different doses depending on the treatments (Table 1) was applied at the time of planting the maize seeds by burying the whole dose in the planting hole in the position below the seeds. The cattle manure variation in the fertilization package is to identify the optimum combinations to benefit the plant growth, increase the nutrient availability at the soils, and support the AMF development. The maximum combination of cattle manure was 15 ton/ha, the limit for transportation. The cattle manure applied in the package was measured with 3.08% Total Nitrogen, pH 6.66, 17.70 mg/kg of available P, 2.31 cmol/kg of available K, 10.45 C/N ration, and 32.2% C-organic. The inorganic (NPK) fertilizers with the recommended doses of 200 kg/ha Phonska (NPK 15:15:15) and 300 kg/ha Urea (46% N) were applied twice, i.e. at 7 DAS (days after seeding) and at 21 DAS, with the amount applied depending on the treatments (Table 1). At 7 DAS, the maize plants were fertilized with a mixture of the entire Phonska and one third of the Urea treatment doses, followed by application of the remaining Urea treatment doses at 21 DAS. The NPK fertilizers were applied in a furrow of 5 cm alongside the maize plant row at 5-7 cm depth before covered with soil.

After harvest of maize at 100 days after seeding (DAS) and field cleaning from maize debris, sorghum seeds were then planted in cropping cycle 2. Before the second sequence, the field was left fallow (rest) for 10 days. Seeds of sorghum ("Numbu" variety) were directly seeded by dibbling 2 seeds per planting holes made around the maize stubbles. These sorghum plants were not fertilized nor inoculated with additional AMF inoculum, but only the chopped maize roots containing the AMF mycorrhizal and spread throughout the plot. For both crops, the young maize and sorghum plants were tinned at 7 DAS by leaving one maize or sorghum plant per planting hole. Weeding and soil piling of the maize and sorghum plant base were done at 15 and 30 DAS. Plant protection was done by spraying "OrgaNeem" (an organic pesticide of plant origin containing Azadirachtin extracted from neem leaves) at a concentration of 5 ml OrgaNeem per liter of water. The OrgaNeem solution was applied to both crops (maize or sorghum) from 10 to 60 DAS at a 3-day interval. Harvesting of maize or sorghum crops was done at 100 DAS.

Measurement and Data Analysis

The variables measured were AMF development, N and P nutrition, and growth and yield of maize and sorghums. The AMF development includes spore counts at 60 and 100 DAS, and root colonization levels at 60 DAS. The N and P nutrition includes concentration of total N and available P in the rhizosphere of maize and sorghum at the time when the vegetative and generative growth found optimum, respectively at 60 and 100 DAS. The crop variables include dry weight (shoots and roots) and yield components (grain).

Laboratory analysis was conducted at Laboratory of Chemistry and Soil Science, Faculty of Agriculture, University of Mataram. Soil pH and texture were measured by standard procedures (Imam & Didar, 2005). Determination of total nitrogen in soil was done by destruction with $(NH_4)_2SO_4$ and distillation with NaOH where the $NH_4^+$was determined by indophenol blue colorimetric method and the NH_3 was defined by a titration with 0.05N of H_2SO_4 solution (Page et al., 1982). Total N in plants was measured using spectrophotometric indophenol blue methods with wave length 636 nm after destruction by $(NH_4)_2SO_4$ and distillation with NaOH following the Conway instruction (Lisle et al., 1990). The available Phosphorus in soil and plant was measured using spectrophotometer (λ = 693 nm) after the extraction process using Bray and Kurt I solution (0.025 N HCl + NH4F 0.03 N) (Bray & Kurtz, 1945). Total organic C was measured by oxidation with $K_2Cr_2O_7$ in presence of sulphuric acid (H_2SO_4) following Walkley and Black's method (Horwitz, 2000).

AMF spore extraction from soil (100 g soil sample) was done using the wet sieving and decanting technique (Brundrett et al., 1996). The spores collected from the 38 µm sieve after the final centrifugation were captured in a filter paper, which were then observed on a Petri dish using a stereo microscope at 40 x magnification, and counted as spore number per 100 g of soil. The percentage of root colonization was determined using the Gridline Intersect technique (Giovannetti & Mosse, 1980) under a stereo microscope after the root pieces were stained using the clearing and staining method of Brundrett et al. (1996).

Data were analyzed using analysis of two way ANOVA and the Tukey's HSD (Honestly Significant Difference) means tested at 5% level of significance.

RESULTS AND DISCUSSION

AMF Development

In this study, however, maize and sorghum were grown in sequence, in which sorghum was directly seeded without treatments and without tillage following the harvest of maize plants. All treatments, including AMF inoculation, were applied only to maize plants in the first cropping cycle. Therefore, it means that sorghum in cropping cycle 2 was grown after AMF propagules have built up in the soil during the growth of the maize plants in cropping cycle 1. This is in line with the results reported in the previous study (Arihara & Karasawa, 2000) which revealed that AMF root colonization and shoot dry weight of maize at 58 DAS were affected by the previous crop grown, whether they were host or non-host of AMF. When grown simultaneously, Carrenho et al. (2007) found higher colonization rates on maize than on sorghum. However, the P fertilization did not affect root colonization, especially on maize following AMF host plants.

Specifically, the degree of root colonization by AMF may be higher in the roots of maize fertilized with highly soluble mineral fertilizers (NPK, diammonium phosphate, ammonium sulfate and Urea). On the other hand, the length of extra-radical mycelium and number of AMF spores were significantly higher in maize fertilized with organic fertilizers (Bokashi manure) (Bautista-Cruz et al., 2014). This indicates that organic fertilization is more favorable for spore production compared with conventional fertilization. It can also be seen from Table 2 that AMF spore numbers are higher at harvest of the maize crop (100 DAS) compared with those at 60 DAS, especially on the D_2 treatment. This indicates a high buildup of AMF propagules for the

Table 2
Mean spore number (spores per 100 g soil) at 60 and 100 DAS, and colonization rates (%-colonization) on maize and sorghum in a maize-sorghum cropping sequence

Fertilization packages	AMF on maize (1st crop)			AMF sorghum (2nd crop)		
	Spore per 100 g soil		% colonization 60 DAS	Spore per 100 g soil		% colonization 60 DAS
	60 DAS	100 DAS		60 DAS	100 DAS	
D_0	764[e]	3464[d]	30[d]	1231[e]	3761[d]	35[e]
D_1	1059[d]	3672[c]	55[c]	1343[d]	4942[b]	60[d]
D_2	2119[a]	4327[a]	75[a]	2981[a]	5165[a]	81[a]
D_3	1690[b]	3894[b]	65[b]	1881[b]	4831[c]	77[b]
D_4	1294[c]	3881[b]	63[b]	1769[c]	4819[c]	68[c]
HSD 5%	231	13	2.0	109	12	6.5

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for descriptions of the packages

subsequent sorghum crops. This condition seems to favor higher AMF colonization levels in roots of sorghum in the second cropping cycle compared with that of maize in the first cropping cycle. With no tillage treatment, the AMF colonization levels in sorghum may be higher than the levels in maize or bean roots (Alguacil et al., 2008). In addition, AMF colonization rates may be higher in roots of sorghum than in the roots of maize, either inoculated with *Glomus mosseae* or *Glomus versiforme*, indicating that sorghum is more favorable for AMF development than maize plants (Guo et al., 2013).

There are many factors influencing the degrees of AMF colonization of crop roots as reported by Carrenho et al. (2007). In examining the effects of different phosphorus, liming, organic matter, and soil texture on root colonization of peanuts, sorghum, and maize in a factorial experiment, each factor significantly affected root colonization level, alone or in interaction with other factors (Carrenho et al., 2007). The most surprising results were the significant interaction effects of plant, phosphorous, and organic matter although there were no significant effects of phosphorous (simple superphosphate of 166.6 mg/L (w/v) soil) nor organic matter (22 mg/L (w/v) soil). As for the interaction effect involving maize, there was no significant effect of phosphorous and organic matter on AMF colonization of maize roots. On the other hand, the application of both phosphorous and organic matter significantly reduced AMF colonization on sorghum roots (Carrenho et al., 2007).

In terms of fertilization effects, AMF colonization rate was reported to be higher on crop roots in unfertilized soils, followed by organic and biodynamic, and the least was seen in exclusively mineral fertilized and conventional farming systems. Thus, it was concluded that the amount of soluble P in the soil was the most limiting factor for AMF colonization in roots of those crops (winter wheat, vetch-rye, and grass-clover) (Mäder et al., 2000). Moreover, it was reported that the increasing P level in the growing media from 0.1 to 0.5 mM P significantly reduced AMF colonization in roots of lettuce plants especially with increasing levels of N contents (1, 5, or 9 mM N) (Azcón et al., 2003).

A negative impact on soil condition occur when the accumulation of soil P has increased beyond the requirement of the crops cultivated (Grant et al., 2005). In the study, it seemed that the amount of P input from the NPK fertilizer as applied in the D_2 treatment was most favorable for AMF development in maize crops. In the D_2 treatment, the NPK fertilizer was reduced to 120 kg/ha Phonska (60% of the recommended NPK dose of 200 kg/ha Phonska in the D_0 treatment) combined with an application of 12 ton/ha cattle manure and inoculation with AMF. Among the treatments with AMF in combination with cattle manure, the D_1 treatment had the highest NPK fertilizer dose (Table 1), and this level of NPK fertilization seems to limit the AMF infection and development in maize roots compared with those in the D_2 treatment, resulting in a higher AMF colonization rate on D_2 than on D_1 treatment. Therefore, AMF colonization and spore numbers in maize were highest in the D_2 treatment, especially when compared with the D_1 or D_0 treatment. Using the same indigenous AMF applied to a pot experiment in which the soil for the growing media was taken from the same field in North Lombok, it was found that the highest level of AMF colonization of maize roots was in the treatment with AMF combined with cattle manure compared with AMF combined with NPK fertilizer or phosphate rock (Astiko et al., 2013a). The previous study also reported a significantly higher AMF spore number in plots fertilized with cattle manure or cattle manure combined with mineral fertilizers compared with those fertilized with mineral fertilizers only (Gryndler et al., 2006).

Soil Nutrient Status and Nutrient Sorption by Maize and Sorghum

There were significant effects of the different fertilizer packages on soil nutrient status (N and P contents of the soil) of the rhizosphere of both maize and sorghum crops at 60 and 100 DAS (Table 3). In general, there is a tendency that the highest values of the soil nutrient status, measured either at 60 or 100 DAS, are highest under the D_2 (60% NPK + 12 t/ha manure + AMF) or D_1 (80% NPK + 15 t/ha manure + AMF) treatment. The values of the soil nutrient status in these treatments were higher than those in D_0 (100% NPK only) treatment, although the dose of NPK fertilizers was reduced in the D_1 and D_2 treatments (Table 3).

At the present study, no infiltration data was measured, however it is important to note that since the NPK fertilizers applications, i.e. Phonska and Urea at 7 DAS and Urea at 21 DAS, at sandy lands, are easily dissolved in water, and significant amount of the nutrients could have been loss through infiltration during the rainy season. Previous study shows that the sand content of the cultivated land had a positive correlation with infiltration rate, with an

Table 3
Mean concentration of total N and available P of soil in the rhizospheres of maize (1st crop) and sorghum (2nd crop) for each treatment of fertilization packages, measured at 60 and 100 DAS

Fertilization packages	Total N ($g.kg^{-1}$) at 60 DAS		Available P ($mg.kg^{-1}$) at 60 DAS		Total N ($g.kg^{-1}$) at 100 DAS		Available P ($mg.kg^{-1}$) at 100 DAS	
	maize	sorghum	maize	sorghum	maize	sorghum	maize	sorghum
D_0	1.24^c	1.10^c	14.97^d	11.41^d	1.33^d	1.23^d	17.43^d	10.15^d
D_1	1.45^a	1.29^a	28.44^b	16.25^b	1.69^b	1.38^b	29.51^b	21.58^b
D_2	1.47^a	1.25^{ab}	35.02^a	28.49^a	1.86^a	1.48^a	36.56^a	29.99^a
D_3	1.39^b	1.22^{ab}	17.52^c	14.59^{bc}	1.47^c	1.31^c	19.37^c	18.59^c
D_4	1.33^b	1.20^b	16.29^c	14.25^c	1.42^c	1.31^c	18.53^c	17.32^c
HSD 5%	0.08	0.07	1.31	1.99	0.08	0.06	1.0	2.98

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages

r-value of +0.75 (Patle et al., 2018). Since cattle manure is slow in releasing nutrients, it is possible that at the time of measurement (60 and 100 DAS), the loss of released nutrients from the NPK fertilizers through leaching was much higher in the D_0 than in the other treatments of fertilization packages due to dissolution by rain water during the rainy season. However, there were small increases in nutrient status of the soil from 60 to 100 DAS around the rhizospheres of maize crop, especially in those treated with manure and AMF application. This indicated a slow release of N and P nutrients from manure after application to the maize plants in the first cropping cycle, which could be due to AMF inoculation. Many studies have reported that AMF can mobilize and take N and P from organic matters for their hosts (Feng et al., 2003; Hawkins et al., 2000; Koide & Kabir, 2000; Leigh et al., 2009; Tarafdar & Marschner, 1994), and it can be seen from Table 2 that the highest average of AMF colonization levels in maize roots was in the D_2 treatment. Therefore, N and P uptake in shoots of maize and sorghum was highest in the D_2 treatment (Table 4).

In addition, soil contents of those nutrients corresponded well with the levels of N and P uptake of the crops, i.e. all showing positive correlation coefficients. Although it is not significant for P uptake, the highest values of N and P uptake were also seen in the D_2 treatment for both maize and sorghum (Table 4). The correlation analysis of the mean values obtained at 60 DAS showed a significant correlation between soil N and N sorption in the shoots, with a value of r = + 0.970 (R-square = 94.1%, p = 0.006) for maize plants, and r = + 0.904 (R-square = 81.7%, p = 0.035) for sorghum plants. These indicate those fertilizers in the packages contribute to nutrient contents of the crops, which are significantly different between treatments of fertilization packages (Table 4).

However, P status of the soil did not show a significant correlation with P uptake either by maize or sorghum crop. In spite of that, there is a significant positive correlation

Table 4
Mean N and P sorption (mg.g^{-1} plant dry weight) by each crop at 60 DAS for each treatment of fertilization packages

Fertilization packages	N and P uptake (mg.g^{-1} plant dry weight) by each crop at 60 DAS			
	Maize (1st cropping cycle)		Sorghum (2nd cropping cycle)	
	N	P	N	P
D_0	18.62^e	1.71^d	12.74^d	0.65^d
D_1	27.58^b	2.60^b	18.92^b	0.85^c
D_2	28.00^a	2.72^a	20.86^a	1.48^a
D_3	23.10^c	2.41^c	17.29^c	1.36^b
D_4	20.44^d	2.41^c	17.22^c	1.32^b
HSD 5%	0.41	0.11	0.07	0.04

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages

between the degree of AMF colonization in the roots and P sorption in the shoot of maize plants, with an r = + 0.909 (R-square = 82.6%, p = 0.032), and that of sorghum plants, with an r = + 0.940 (R-square = 88.4%, p = 0.017). This shows that there were significant contributions of AMF colonization in the roots to the amount of P taken up by the plants, both maize and sorghum. If soil soluble P has not exceeded crop requirements, AMF association will still result in significantly positive effects on P uptake and biomass production because of P requirements of the crops; for optimum growth and yields, crops require P since the early stage of their growth, either from soil, fertilizer application, or from AMF associations (Grant et al., 2005).

Although sorghum crop in the second cropping cycle was not fertilized with manure nor NPK fertilizers, direct seeding of sorghum after harvesting the maize plants without tillage seemed to be a favorable condition in establishing AMF association in the sorghum crop, which resulted in higher AMF colonization rates in sorghum than in maize roots for each fertilizer package (Table 2). The AMF associations seemed to enable the sorghum crops to absorb adequate P and other nutrients from soil and residues of the manure applied in the first cropping cycle even though these sorghum crops were not fertilized. This could occur because of the potential of AMF external hyphae that help the host plants in absorbing and dissolving nutrients from soil and manures and other nutrient pools, as well as other nutrients unavailable for uptake by plant roots (Menge, 1983; Smith & Read, 2010; Tawaraya et al., 2006). Guo et al. (2013) also reported significant effects of AMF inoculation on C, N, P, and K contents (mg/pot) in shoot and roots of maize and sorghum grown in pots filled with mixtures of mine tailing and topsoil, and between the AMF species used, *G. versiforme* showed higher colonization rates which resulted in higher nutrient sorption and biomass of maize and sorghum compared with *G. mosseae* (Guo et al., 2013).

Biomass and Yield Components of Maize and Sorghum

In terms of biomass production and yield components of maize and sorghum, there were also significant effects of the fertilization packages in which D_2 treatment presents the highest values of biomass production (Table 5) and yield components (Table 6). This means that the nutrient status of the soils corresponded well with the biomass weight of the crops, indicated by the positive correlation coefficients, although only some of them were significant. The AMF applications to maize crops, observed at 60 DAS, showed a significant correlation to dry shoot weight, N and P status of the soil, and the N and P sorption. In contrast, AMF colonization levels of maize roots only showed significant correlation with root dry weight at 60 DAS, with an r = + 0.912 (R^2 = 83.2%, p= 0.031) and shoot dry weight at maturity (100 DAS), with an r = + 0.892 (R^2 = 79.6%, p= 0.042). This could mean that during the vegetative growth, AMF colonization have focused on improving root growth to increase nutrient sorption during the vegetative growth of maize crops.

In relation to nutrient uptake, although AMF colonization levels did not show significant correlation with N uptake in shoots of maize or sorghum, they did show a positive significant correlation with P uptake both in shoots of maize and sorghum, with an r = + 0.909 (R^2 = 82.6%, p = 0.032) for maize, and r = + 0.940 (R^2 = 88.4%, p = 0.017) for sorghum. These could be due to the ability of AMF extra-radical hyphae in mobilizing and absorbing P from organic matters for their hosts (Feng et al., 2003; Koide & Kabir, 2000; Tarafdar & Marschner, 1994). Based on the strength of the relationships between AMF colonization levels and P uptake, the value of R^2 is higher in sorghum (R^2 = 88.4%) than in maize (R^2 = 82.6%). This means that the contribution of AMF colonization in roots to P uptake in shoots was higher in sorghum crop at the second cropping cycle than in maize in the first cropping cycle. Since sorghum did not receive any fertilizers, and cattle manure is a

Table 5
Mean dry biomass weight (g/plant) of maize and sorghum for each treatment of fertilization packages

Fertilization packages	Dry biomass weights (g/plant) of maize (1st crop) and sorghum (2nd crop)							
	Maize root		Maize shoot		Sorghum root		Sorghum shoot	
	60 DAS	100 DAS	60 DAS	100 DAS	60 DAS	100 DAS	60 DAS	100 DAS
D_0	8.13^d	10.43^e	34.15^d	62.29^e	0.82^d	12.30^d	8.31^d	47.74^e
D_1	15.13^b	22.39^b	61.92^b	109.03^b	2.22^b	22.34^b	16.89^b	95.01^b
D_2	17.12^a	34.56^a	72.86^a	111.25^a	3.37^a	24.44^a	23.53^a	105.14^a
D_3	13.65^c	18.48^c	52.26^c	101.05^c	1.68^c	14.52^c	12.01^c	85.46^c
D_4	13.34^c	15.43^d	51.29^c	95.87^d	1.38^c	14.47^c	11.81^c	66.06^d
HSD 5%	0.31	3.04	0.97	2.21	0.30	0.05	0.20	9.54

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages

slow-release organic fertilizer, it could mean that most P nutrient contained in the shoots, especially in shoots of sorghum in the second cropping cycle, was mostly taken up from the manure under the contribution of AMF colonization in the roots. However, this still needs to be confirmed by further research. This view is supported by the conditions of the study area, which was dominated by sand, and if leaching happened during the rainy season, the loss of dissolved nutrients from the NPK fertilizers applied in the first cropping cycle could be high. Therefore, the main source of nutrients, especially for sorghum crop in the second cropping cycle was the cattle manure applied to the maize crop in the first cropping cycle.

In relation to nitrogen nutrient, there was a very low and non-significant correlation between AMF colonization levels and N uptake in maize shoots with an R^2 of only 41.2%. However, the N uptake of maize at 60 DAS was highly significantly correlated with the N status of the soil with an R^2 = 94.1% (p = 0.006). Moreover, the N soil availability also showed a significant correlation with biomass and grain yield of maize, i.e. with an R^2 = 84.1% (p = 0.029) with grain yield at 60 DAS, R^2 = 92.2% (p = 0.009) with shoot dry weight at 60 DAS, and R^2 = 90.6% (p = 0.012) with shoot dry weight at 100 DAS.

In contrast, for sorghum in the second cropping cycle, the soil N status at 60 DAS showed a significant correlation only with shoot dry weight at 100 DAS. Unlike in maize crop where AMF colonization levels showed a significant and positive correlation with shoot dry weight at maturity (100 DAS), AMF colonization levels in sorghum roots did not show any significant correlation with biomass weight or yield components of sorghum. However, AMF colonization rates in sorghum roots showed a sufficiently high correlation with N uptake in sorghum shoots, with an R^2 = 71.9% (p = 0.069), and N uptake in sorghum shoots showed positive significant correlation with biomass and grain yield of sorghum, i.e. with an R^2 = 80.5% (p = 0.039) with grain

Table 6
Mean weights of total dry grains (kg/plot) and 100 dry grains for each crop and each treatment of fertilization packages

Fertilization packages	Mean dry grain yield (kg/plot) and weight of 100 dry grains (g)			
	Maize in the 1st cropping cycle		Sorghum in the 2nd cropping cycle	
	Grain yield	100 grains	Grain yield	100 grains
D_0	9.60^e	22.48^d	3.57^d	2.73^d
D_1	17.40^b	26.94^b	5.05^b	3.01^b
D_2	22.80^a	28.12^a	6.65^a	3.61^a
D_3	15.60^c	25.98^c	4.43^c	2.90^c
D_4	10.20^d	24.61^c	4.17^c	2.81^{cd}
HSD 5%	0.59	1.37	0.26	0.09

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages

yield, R^2 = 83.0% (p = 0.031) with shoot dry weight at 60 DAS and R^2 = 89.9% (p = 0.014) with shoot dry weight at 100 DAS. No additional fertilizers and inoculums were applied at the cropping cycle 2. These could mean that for maize crop, most nutrients were derived from NPK fertilizers while for sorghum, N and P nutrients were derived from the residues of manure contributed from AMF colonization in sorghum roots. Many researchers have also shown the ability of AMF to utilize organic sources to supply N to their host (e.g. Hawkins et al., 2000; Leigh et al., 2009) although it was also found that AMF colonization did not necessarily result in significantly higher N status of the mycorrhizal than non-mycorrhizal hosts (Hawkins et al., 2000).

However, when the correlation analysis was done between averages of colonization levels, biomass and grain yield between maize in the first and sorghum in the second cropping cycle, in general the results show significant and positive coefficients of correlation. For example, correlation of AMF colonization levels between roots of maize and sorghum are highly significant, with an r = + 0.988 (R^2 = 97.6%, p = 0.002). This means that the pattern of differences in AMF colonization levels between roots of maize and sorghum is highly similar. In other words, AMF colonization levels in roots of maize in the first cropping cycle were carried over into the second cropping cycle where sorghum was grown as the rotation crop. This is because both maize and sorghum are hosts of AMF, and both crops have high mycorrhizal dependency (Guo et al., 2013).

In addition, when a t-test of ‘Paired Two Sample for Means’ was run on the averages of AMF colonization levels and spore number in soil at maturity of the crops between maize and sorghum in Table 2, higher significant values (p<0.01) were seen on sorghum than on maize. This could be due to the buildup of AMF in the soil after maize crop in the first copping cycle was harvested before sorghum was directly seeded without tillage. Even though both crops were grown simultaneously, it was also found that AMF colonization levels were higher on sorghum than on maize (Guo et al., 2013). This means that sorghum can be used to improve mycorrhizal conditions of the soil under a maize-sorghum cropping sequence.

CONCLUSION

Among the mycorrhiza-based fertilization packages tested on the maize-sorghum cropping sequence system at the drylands in North Lombok of Indonesia, the D_2 package, consisting of 60% NPK recommended dose, 12 t/ha cattle manure, and the indigenous AMF inoculation, was found to be the best fertilization package to improve crop yield and soil nutrient availability. This study noted that the AMF development was higher in the sorghum at the second cropping cycle compared to the growth in the maize at the first cropping cycle. This condition led to the higher NP-uptake and soil nutrient availability in subsequent crops, both at the sandy dryland, with no additional fertilization and mycorrhizal propagules applied.

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