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# PROCEEDINGS

*The 2<sup>nd</sup> Society for Indonesian Biodiversity*  
**INTERNATIONAL CONFERENCE**  
**VOLUME 2, JULY 2013**  
**ISSN 2252-617X**

**SIGNIFICANCE OF CLIMATE CHANGE ON BIODIVERSITY  
IN SUSTAINING THE GLOBE**

**Lombok, West Nusa Tenggara, Indonesia, 6 - 8 November 2012**

**Editors :**

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**Prof. I Komang Damar Jaya, Ph.D (University of Mataram)**  
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## **PREFACE**

Continuing The 1<sup>st</sup> International Conference on Biodiversity in 2011 that was held in Solo, The Society For Indonesian Biodiversity proudly held The 2<sup>nd</sup> International Conference on Biodiversity which was focus on issues related to "Significance of Climate Change on Biodiversity in Sustaining The Globe. This conference has attracted significant numbers of participants from scientist, government agencies, NGO, and other experts from 5 different countries (Australia, New Zealand, United States of America, Malaysia, and Indonesia). This event was expected to promote innovations in the real research on biodiversity to tackle biodiversity loss that rapidly occurred in our life.

The proceeding is the continuation of the 1<sup>st</sup> proceeding issued by the Society for Indonesian Biodiversity. The proceeding contain all oral and poster presented on the 2<sup>nd</sup> ICB 2012 in Lombok, Indonesia. Papers presented in this proceeding comprised wide ranges of issues regarding climate change impacts of agricultural and forestry biodiversity, fresh water, coastal and marine diversity, as well as economic and community biodiversity.

On behalf of The Society for Indonesian Biodiversity, we would like to thank to all authors, paper reviewers, editorial team, organizing committee, local government, and sponsors for their contribution and involvement in this conference.

Mataram, July 2013

Sutamo  
Chairman of the Society for Indonesian Biodiversity

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## GENETIC IMPROVEMENT OF VIGNA GERMPLASM THROUGH INTERSPECIFIC HYBRIDIZATION

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### ABSTRACT

The purpose of this study was to improve the genetic quality of several types of legumes of the genus *Vigna* through inter-species hybridization. The ultimate goal of this activity is to obtain a new superior variety of hybrid vegetable pea which contains high protein and anthocyanin, is tolerant to drought and has high production. This research was conducted for two years. The activities of the first year are: 1). Back crosses between superior hybridized species and their two parents, 2). Evaluation of all existing genotypes and selection of superior individuals in the F<sub>2</sub> population. Data were analyzed by analysis of diversity, LSD, estimation of narrow and broad meaning heritability, correlation analysis of both genotypic and phenotypic, and determining the success rate of crosses. The results of this study are: 1). Several hybrids resulting from crosses between cowpea and long bean species have the potential to be developed into superior varieties containing high protein and anthocyanins, 2). By backcrossing superior hybrids with their two parents and continuing the selection, there is an improvement in genetic quality. 3). All observed variables had narrow and broad categories of heritability, except flowering age which had high heritability and number of seeds per plant had low heritability. 4). The number of pods per plant has a significant positive correlation with yield so that it can be used as a selection criterion for yield improvement.

Keywords: protein, anthocyanins, hybridization between species

### INTRODUCTION

West Nusa Tenggara Province has a lot of plant genetic resources, especially legumes. Therefore, this legume plant has the potential to be developed into a new superior variety through a plant breeding program. Hybrid vegetable peas can be obtained through hybridization between cowpea species (*Vigna unguiculata* L. Walp.) and long beans (*Vigna sesquipedalis* L. Fruwirth). Cowpea is a potential legume that has not received much attention from both farmers and researchers in Indonesia, even though its potential is enormous. In addition to its high nutritional content, especially as a source of protein, cowpea, especially those that are purple and reddish in color, contain high anthocyanins, and are able to grow both on dry land and other marginal lands. Dry land or marginal land in Indonesia is very wide, so that cowpea, especially local varieties that are adaptive, have the potential to be developed.

Local cowpea in Indonesia has high genetic diversity both qualitatively and quantitatively, so it has the potential to be developed into new superior varieties by improving its genetic quality through breeding programs (Anyia and Herzog, 2004; Karsono, 1998; Ujianto, et al. 2003). Despite its advantages, cowpea has a weakness, namely its hard pods so that the young pods cannot be used as a vegetable. The genetic quality of cowpea, especially the characteristics of its pods, can be done through hybridization with other species, namely long beans. Hybridization between cowpea and long bean will produce hybrid vegetable beans which contain high protein and anthocyanins. Sources of protein and anthocyanins can be found in seeds, young pods and leaves. If the cowpea pods can be used as vegetables, it means they can be harvested young, besides adding alternatives for farmers, it can also provide other benefits. By harvesting earlier, it is usually still fresh green so that it can be used as animal feed which has a high nutritional content or can also be used as green manure. Besides that, the intensity of planting in one year can be higher because the harvesting period is shorter, so that in the same unit of time more sources of protein can be obtained (Bressani, 1985; Singh, et al., 2003).

A cheap and easy-to-obtain source of protein is everyone's dream, especially the poor. Sources of protein can come from animals (animal protein) or plants (vegetable protein). At this time, animal protein sources are very expensive, difficult to reach by the less fortunate. On the other hand, vegetable protein sources, especially those from legumes, are relatively cheap and affordable for the less fortunate. Besides their high protein content, legumes, especially those that are purplish and reddish in color, contain anthocyanins which are very beneficial for the health and defense of the human body. Local varieties of cowpea have a variety of protein content in the range of 22-31% and purplish or reddish cowpea has a high anthocyanin content. Other advantages of local cowpea are tolerance to drought, variety in quantitative characteristics, especially yield and harvest age so that it has the potential to be developed through plant breeding programs. Despite its advantages, cowpea has a weakness, namely the young pods are stiff so they are not suitable for vegetables. Long beans have the characteristics of pods that are soft, crunchy, pods and the distance between the seeds is long so it is ideal for vegetables, but the stems are very tall which are vines and twists so that they require a ramp in their cultivation. These properties are not possessed by cowpea. To combine the superior characteristics of each parent, cross-species hybridization can be carried out, namely between cowpea (*Vigna unguiculata* L. Walp.) and (*Vigna sesquipedalis* L. Fruwirth). (Aremu et al., 2007; Suryadi, et al., 2003 ; Umaharan, et al., 1997; Gomathinayagam, et al., 1998). Combining the characteristics of these two different species will produce a hybrid vegetable bean that has the combined characteristics of both its parents, both desirable and undesirable. With the right selection methods and criteria, breeders can choose hybrids that have the desired traits.

The development of hybrid vegetable peas containing high protein and anthocyanin through an effective and efficient breeding program requires genetic information, especially on the traits to be improved. This is important because character improvement through breeding programs takes a long time, effort and costs a lot.

## MATERIAL AND METHOD

This research is a continuation of previous research. Assembling legume varieties with high protein and anthocyanin content suitable for dry land and high production, cowpea with a soft pod texture, and long beans with a strong tree texture without stems will be carried out for 2 years consisting of 5 stages of activity. . For the first year the activity consists of the first (crossback) and second (genetic analysis) stages

In the first stage of the experiment, a backcross was carried out between F1 selected as the female parent and crossed with the female parent (P1) as the male parent to obtain BC1.1. and with the male parent (P2) as the male to get BC1.2. At this stage F1 populations were also planted to obtain F2 population seeds to be used for testing.

The second stage of the experiment was genetic analysis by evaluating all existing populations (P1, P2, F1, F2, BC1.1. and BC1.2). The experimental method used in the fourth stage of the activity was a complete randomized block design with 3 repetitions. Observations were more focused on protein and anthocyanin content, pod characteristics, namely pod softness, pod length and circumference, number of seeds per pod, seed and pod color. In addition, the number of pods per plant, seed weight per plant and plant height were also observed. At this stage, selection was also carried out to select superior individuals in the F2 population to be used as planting material for the next generation.

Percobaan tahap kedua yaitu analisis genetik dengan mengevaluasi semua populasi yang ada (P1, P2, F1, F2, BC1.1. dan BC1.2). Metode percobaan yang digunakan pada kegiatan tahap keempat yaitu rancangan acak kelompok lengkap dengan ulangan 3 kali. Pengamatan lebih difokuskan pada kandungan protein dan anthosianin, karakteristik polong yaitu kelunakan polong, panjang dan lingkaran polong, jumlah biji per polong, warna biji dan polong. Disamping itu juga diamati jumlah polong per tanaman, berat biji per tanaman dan tinggi tanaman. Pada tahap ini juga dilakukan seleksi untuk memilih individu-individu yang unggul pada populasi F2 untuk dijadikan bahan pertanaman pada generasi berikutnya.

## RESULTS AND DISCUSSION

The hybrid individuals selected as a result of the evaluation of crossbreeds between 4 cowpea local varieties of NTB and 4 long beans were backcrossed with their parents, both female and male parents. The results of backcrossing are characterized and evaluated by involving parents and their hybrids to determine the characteristics of the backcrossed population. This evaluation and characterization activity was carried out in pots with relatively homogeneous environmental conditions so that the genetic superiority of the individuals being evaluated could be identified. In general, by backcrossing between selected hybrids and their parents, genetic quality improves. This is indicated by the improvement in the value of the quantitative properties observed under relatively uniform environmental conditions.

Evaluation of all existing populations, namely the population of female parents (P1), male parents (P2), hybridized first offspring (F1), backcrossed with female parents (BC1.1.), backcrossed to male parents (BC1.2). and the second generation population (F2) is carried out in the field, namely on farmer's land in order to obtain real data according to natural conditions. This evaluation is intended to obtain a comprehensive picture of the genetic results of crossing between these species. In this evaluation, the number of F2 populations is far greater than the other populations because segregation occurs in the F2 population. Evaluation of segregated populations requires a large population so that all of them are represented and there is no drift of genetic diversity due to the small number of populations used which is commonly called genetic drift.

Table 1. Backcross Success Rate between First Offspring (F1) and The two parents are Cowpeas (KT) and Long Beans (KP)

No.	Genotype	Number of Flowers Crossed	Number of Pods	Success Percentage (%)
1	(KTU x KPH) x KTU	20	9	45,0
2	(KTU x KPH) x KPH	18	11	61,1
3	(KTU x KPJ) x KTU	18	10	55,6
4	(KTU x KPJ) x KPJ	19	11	57,9
5	(KTC x KPH) x KTC	17	8	47,1
6	(KTC x KPH) x KPH	20	7	35,0
7	(KTC x KPJ) x KTC	20	9	45,0
8	(KTC x KPJ) x KPJ	20	7	35,0
9	(KTP x KPH) x KTP	18	8	44,4
10	(KTP x KPH) x KPH	20	8	40,0
11	(KTP x KPJ) x KTP	17	8	47,1
12	(KTP x KPJ) x KPJ	20	9	45,0
13	(KTB x KPH) x KTB	19	7	36,8
14	(KTB x KPH) x KPH	20	9	45,0
15	(KTB x KPJ) x KTB	17	7	41,2
16	(KTB x KPJ) x KPJ	20	9	45,0

For the purposes of genetic analysis and also to recover the traits, the two parents were backcrossed. The success rate of the cross is not much different from the cross between cowpea and long bean. The success rate of crosses is slightly better because the offspring are already heterozygous so the chances of joining are greater. This is in accordance with the results of crosses conducted by Fatokun and Singh (2007) which showed that the



success rate of crossing heterozygous populations obtained from intraspecies crosses was higher than that of homozygous population crosses. Fewer backcrosses were made because they were needed in small amounts compared to the F2 population. Back-cross with cowpeas to produce a population of BC.1.1. and backcrossed with broad beans to produce a population of BC.1.2. to use genetic analysis for the traits of interest.

The advantage of this hybrid vegetable bean is its high protein and antho-cyanin content. This hybrid vegetable bean is the result of a cross between the local variety of West Nusa Tenggara cowpea species and long beans. Combining the superior traits of the two parents produces a hybrid that is better than the two parents. Cowpeas, especially those with purple pods and seeds, have a high protein and anthocyanin content, especially compared to long beans. The weakness of cowpea is the texture of the pods which are stiff and short so that the young pods cannot be harvested for vegetables. Long beans, although the protein and anthocyanin content is rather low, the texture of the pods is soft and crunchy and long. By crossing these two different species, a hybrid vegetable bean was produced which had a high protein and anthocyanin content with a soft texture, the pod length was shorter than the long bean but much longer than the cowpea.

The hybrid vegetable bean is the result of a cross between a cowpea, which has light purple pods and seeds, and a long bean, which has black seeds and dark green pods. The highest protein content was found in cowpea KTC, with brown seeds of 30.5 followed by KTB and KTU of 28.9 and 27.2, while the highest anthocyanin content was achieved in KTU, namely cowpea with purple seeds of 71.4 ppm. followed by KTC with brown seeds of 58.7 ppm. This is in accordance with the results of research by Lazcano et al. (2001) who showed that purple carrots contained higher levels of anthocyanins than the others. Crosses between cowpea which has a high protein content and long beans produce offspring whose protein content ranges between the two parents. This is possibly caused by the action of genes that control protein levels that are not fully dominant or intermediate and there is more than one controlling gene. This is in accordance with the results of genetic analysis conducted by Shi, et al. (1999) which showed that protein levels in rice were controlled by several genes and the gene action was not completely dominant.

The results of the evaluation showed that the protein and anthocyanin contents varied widely. Parents who have high protein or anthocyanin content tend to produce offspring with higher protein and anthocyanin content. The cross between KTC and KPH, although the protein content was lower than the other two, in the F2 population the anthocyanin content was higher, namely 89.7 ppm, exceeding the two parents so that the results of this cross have good prospects to be developed into legume varieties that have both protein and anthocyanin. tall. The protein content varies from one type of bean to another, especially the Vigna group. According to Turkova and Klozova (1985) this difference is mainly due to differences in lysine content. Likewise, the anthocyanin content of nuts is also different. This is mainly because the color of both the seeds and pods is also different so that the anthocyanin content also varies. The crosses of KTU with KPJ and KPH and KTC with KPH and KPJ have good prospects to be developed into legumes with high anthocyanin content.

Table 2. Protein and Anthocyanin Content, , and Average Length Parental Pods and Crosses between Cowpea (KT) and Peanut

No.	Genotype	Protein Content (%)	Anthocyanin Content (ppm)	Pod Texture	Average Pod Length (cm)
1	KTU	27,2	71,4	Stiff	15,46

2	KTC	30,5	58,7	Rigid	14,66
3	KTP	24,6	12,6	Rigid	16,17
4	KTB	28,9	21,4	Rigid	16,48
5	KPH	18,5	13,8	Soft	52,53
6	KPJ	17,6	21,6	Soft	56,87
7	KPP	20,4	9,6	Soft	59,23
8	KPC	19,4	10,4	Soft	53,22
9	KTU x KPH	24,5	45,5	Soft	36,50
10	KTU x KPJ	23,9	56,4	Soft	37,50
11	KTU x KPP	24,9	21,6	Soft	38,25
12	KTU x KPC	24,1	37,8	Soft	30,25
13	KTC x KPH	27,6	41,1	Stiff	38,00
14	KTC x KPJ	26,8	38,9	Stiff	38,00
15	KTC x KPP	28,7	11,2	Stiff	37,50
16	KTC x KPC	28,2	22,6	Stiff	28,50
17	KTP x KPH	21,6	8,8	Stiff	37,75
18	KTP x KPJ	21,2	6,7	Stiff	36,00
19	KTP x KPP	22,1	3,4	Stiff	31,50
20	KTP x KPC	21,8	5,6	Stiff	40,75
21	KTB x KPH	23,4	9,8	Stiff	36,25
22	KTB x KPJ	22,6	10,3	Stiff	28,25
23	KTB x KPP	23,5	6,2	Stiff	37,00
24	KTB x KPC	23,9	4,4	Stiff	30,50

### CONCLUSION

From the results of the first year's research, the following conclusions can be drawn:

1. Several hybrids resulting from crosses between cowpea and long bean species have the potential to be developed into superior varieties containing high protein and anthocyanins,
2. By back-crossing superior hybrids with the two parents and continuing the selection, genetic quality is improved by obtaining superior lines containing high protein and anthocyanin and improving the texture of the pods. The hybrid lines resulting from crosses of cowpea and black long bean (F2.1.2) have good prospects for development into new superior varieties containing high protein and anthocyanin and soft pod texture.
3. All the observed variables had moderate heritability in the narrow sense and broad categories except for flowering age which had a high heritability and the number of seeds per plant had a low heritability value.
4. The number of pods per plant has a significant positive correlation with yields, both genotypic and phenotypic, so that it can be used as a selection criterion for yield improvement.

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