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Interspecific Hybridization Among *Vigna* Species to Create New Superior Variety Containing High Protein and Anthocyanin

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Abstract: Genetic variability along with genetic advance of traits is essential for crop improvement. Interspecific hybridization has great potential for the improvement of *Vigna* germplasm, as it creates genetic variation and is a valuable tool for transferring genes controlling traits from one species to another. The main objective of this research was to improve genetic variability and genetic background of *Vigna* germplasm through interspecific hybridization. Four varieties of *Vigna radiata* with different characteristics were crossed with two genotypes of *Vigna umbellata* and four genotypes of *Vigna unguiculata* were crossed with superior variety of *Vigna sesquipedalis*. The hybrids were found to be viable and fertile. Genotypic coefficient of variation and phenotypic coefficient of variation were different among cross combinations and among quantitative traits. There were interspecific hybrids of *V. unguiculata* x *V. sesquipedalis* having good prospect to be developed become hybrid vegetable bean containing high protein and anthocyanin. Through interspecific hybridization and backcrossing of superior hybrids to their parents had improved genetic variability especially number of pods per plant, pod length, plant height, pod texture, and nutrition content. The magnitude of differences between phenotypic coefficient of variation and genotypic coefficient of variation were low for all the observed characters. By crossing among *vigna* species, some superior characters can be obtained.

Key-Words: Anthocyanin, germplasm, hybrids, interspecific hybridization, protein, variability, *vigna*

1 Introduction

Interspecific hybridization is a useful method in plant breeding program to improve genetic variation. The objectives of interspecific hybridization are to transfer one or a few genes from one species to another, to improve genetic variation or genetic background, to achieve new character expression not found in either parent, to produce sterile progeny or new allopolyploid species, and to determine the relationship of one species to another. Hybridization is progressively more recognized as an important process in evolution of plant populations and species [1]. There is a need to look for alien *Vigna* species to diversify and broaden the genetic base of cultivated germplasm of *Vigna* species. Some *Vigna* species can be used as male parents. They are a potential source for resistance to biotic stresses as well as several other yield traits. The percentage of pod setting during crossing among *vigna* species was varying from low to very low [2]. Hybridization is progressively more recognized as an important process in the evolution of plant populations and species.

The *Vigna* genus includes more than 100 wild species, 10 crops have been domesticated from three subgenera, *Vigna*, *Plectrotripsis*, and *eratotropis*. The habitats of wild *Vigna* species could harbor various genes responsible for environmental stress adaptation, which could lead to innovations in agriculture [3]. Some *Vigna* spp are important pulses [4]. The four species of *Vigna*, *Vigna unguiculata* L. Walp., *V. radiata* (L.) Wilczek, *V. sesquipedalis* L. Fruwirth, and *V. umbellata* Thunb. are important food legume crops in parts of Asia [5]. The crops grown in different parts of Indonesia have their extensive cultivation in dry land region. *Vigna unguiculata* (Cowpea) is potential pulses neglected both by farmers and researchers in Indonesia, although this plant is very potential developed through plant breeding. Beside containing high nutrient especially protein content, cowpea especially purple color and redish contained high anthocyanin. To increase variability of protein and anthocyanin sources, it is important to interspecific crossing between cowpea containing high protein and anthocyanin and *Vigna sesquipedalis* (long bean) to obtain protein and

anthocyanin resources through seeds, immature pods, or leaves. By interspecific hybridization both cowpea and long bean improved. The progeny was combining characteristic both parents. Through selection method will be selected progeny with pod harvested immatures vegetable so utilization will be increase, high protein and anthocyanin content, and tolerant to drought stress [6].

Vigna radiata (mungbean) is one of the main crops in Indonesia. It is an excellent source of easily digestible protein which causes low flatulence. This crop is an important short-duration grain legume crop with wide adaptability, requires a low input, and has the ability to improve the soil fertility by fixing Nitrogen. The main problems of mungbean cropping system are low yield and susceptible to many pests. The problems can be overcome through plant breeding program. Genetic variability is essential in plant breeding program especially for characters that will be improved. *Vigna umbellata* (rice bean) has superior characters such as the high pod number per plant and resistance to some pests. Such characters are not found in mungbean. The interspecific hybridization was conducted to combine the superior characters on mungbean and rice bean. Therefore, it is important to study the interspecific hybridization among *Vigna* species to improve genetic variability and genetic background of *Vigna* germplasm [7].

2 Materials and Methods

This research consisted of three activities i.e. (1) interspecific hybridization between *Vigna radiata* (mungbean) and *Vigna umbellata* (ricebean), (2) interspecific hybridization between *Vigna unguicullata* (cowpea) and *Vigna sesquipedalis* (longbean), (3) evaluation among interspecific hybrid populations. This research was conducted in pots set up in a mesh house Agricultural Faculty Mataram University, Mataram for hybridization activities and in irrigated lowland in Narmada, West Lombok, NTB province for field evaluation. The materials used in this research were four superior varieties of mungbean (Kutilang, Vima, Nuri, and Manyar) with different characteristics were crossed with one genotype of ricebean (red seed ricebean) and four genotypes of cowpea (KTUT = Dark orange cowpea, KTUM = orange cowpea, KTCB = big brown cowpea, KTCK = small brown cowpea, KP1 = superior variety of long bean) were crossed with one genotype of long bean (KP1) followed by backcrossing.

Planting for hybridization was conducted periodically in which long days to flowering plants were planted earlier. Method of hybridization was based on IITA Research Guide 42 Hand crossing of cowpeas. Emasculation was conducted in the afternoon for flowers that will be flowering in the next day by removing one third of flowers and removing all pollens. Pollination was conducted in the morning by adhering pollen in the pistil of emasculated flowers. Evaluation of 4 hybrids and 5 parents were set up use a randomized completely block design with ten replications. The observations were recorded on ten percent randomly selected plants from each genotype. The observed traits consisted of plant height, days to maturity, number of pods per plant, number of seeds per pod, pod length, weight of dry seed per plant, pod texture, and nutrition content. The observed data were analyzed using analysis of variance, coefficient of genotypic and phenotypic variability, and analysis of heritability.

3 Results and Discussion

Interspecific hybridization usually increases genetic variability due to the combination of the two different characteristics parents. The genetic variability of a character is a variability caused by genetic factors. The amount of genetic variability is reflected by the coefficient of genetic variability. The coefficient of genetic variability is the square root of the genetic variability divided by the average traits multiplied by 100%. Coefficient of genetic variance in hybrids derived from interspecific hybridization both the crosses between *Vigna radiata* x *Vigna umbellata* (Table 1) and between *Vigna unguicullata* and *Vigna sesquipedalis* (Table 2) was higher than their parents. Quantitative traits observed in interspecific hybridization is only a characteristic that really contrasts between the two parents that were crossed. The characteristics of *V. radiata* (mungbean) are the relatively few number of pods, short flowering date, low seed weight per plant, high protein content, and low carbohydrate content, while *V. umbellata* (ricebean) has the opposite characteristics. The characteristics of *V. unguicullata* (cowpea) was plant height and pod length is relatively short, high protein and anthocyanin content especially the purple pods, otherwise *V. sesquipedalis* has the opposite characteristics (Table 3). The genetic coefficient of hybrids from interspecific hybridization is higher than their parents due to the hybrids occur recombination of traits controlled by different genes

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in the two parents. The magnitude of differences between phenotypic coefficient of variation and genotypic coefficient of variation were low for all the characters.

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Tabel 1. Genotypic and phenotypic variance, coefficient of genotypic variance (CGV), coefficient of phenotypic variance (CPV) and heritability on all observed traits of hybrids (*Vigna radiata* x *Vigna umbellata*)

Quantitative traits	Type of crossing	Genotypic variance	Phenotypic variance	CGV (%)	CPV (%)	Narrow sense heritability	Broad sense heritability
Number of pods	KL x RSR	29.4	72.0	22.2	24.6	34.6	40.8
	V x RSR	26.9	74.3	21.7	24.1	34.2	36.2
	N x RSR	27.3	62.7	23.5	27.3	33.7	43.5
	MR x RSR	21.6	69.5	19.2	21.7	29.9	31.1
	KL	24.9	71.2	21.0	25.8		34.9
	V	29.3	65.1	24.8	27.3		45.0
	N	12.8	34.4	4.8	6.0		37.2
	MR	17.5	52.9	3.5	5.1		33.1
	RSR	9.7	25.8	4.2	5.9		37.6
Days to maturity	KL x RSR	72.3	122.4	21.3	23.9	43.7	59.0
	V x RSR	65.2	112.4	23.6	25.7	32.8	58.9
	N x RSR	68.4	132.1	27.1	29.4	31.8	51.7
	MR x RSR	57.8	131.7	25.8	28.4	34.6	43.8
	KL	83.2	142.6	21.7	23.3		58.3
	V	65.7	146.2	23.6	25.8		45.0
	N	43.1	112.3	9.2	11.1		38.7
	MR	42.6	103.2	15.2	17.4		41.3
	RSR	31.4	115.3	18.3	19.9		27.2
Weight of seeds per plant	KL x RSR	4.42	19.5	19.3	21.9	21.2	22.6
	V x RSR	6.54	19.5	23.2	25.5	31.3	33.5
	N x RSR	8.65	20.4	28.2	29.4	35.8	43.5
	MR x RSR	3.57	19.8	17.0	18.7	10.4	17.8
	KL	4.62	18.8	19.9	22.1		25.0
	V	9.39	21.1	29.5	30.2		44.1
	N	3.16	17.3	23.2	25.1		18.0
	MR	7.94	23.9	24.8	26.4		33.2
	RSR	7.14	23.4	27.3	28.9		30.3
Protein content	KL x RSR	89.3	124.8	37.5	39.6	57.6	71.5
	V x RSR	109.2	159.3	25.1	27.3	67.5	68.5
	N x RSR	80.9	125.2	28.5	29.9	63.2	64.6
	MR x RSR	92.3	136.0	20.9	22.3	65.3	67.9
	KL	87.5	143.6	27.1	28.3		60.9
	V	69.4	104.0	28.7	29.4		66.7
	N	49.3	14.8	20.2	21.3		36.5
	MR	40.9	151.4	18.7	19.8		27.0
	RSR	47.5	141.7	12.1	13.9		33.5
Carbohydrat content	KL x RSR	0.35	0.56	16.0	17.5	27.7	62.6
	V x RSR	0.35	0.63	15.9	17.1	31.8	55.7
	N x RSR	0.31	0.54	15.5	16.8	20.1	57.9
	MR x RSR	0.40	0.51	16.4	17.6	33.5	78.8
	KL	0.28	0.61	15.4	16.9		47.3
	V	0.31	0.59	15.6	17.3		54.0
	N	0.23	0.42	8.6	9.8		54.7
	MR	0.32	0.56	7.5	8.7		57.1
	RSR	0.27	0.41	11.3	12.9		65.8

Note: KL = Kutilang variety, V = Vima variety, N = Nuri variety, MR=Manyar variety, RSR = Red Seed Ricebean

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 Tabel 2. Genotypic and phenotypic variance, coefficient of genotypic variance (cgv), coefficient of phenotypic variance (CPV) and heritability on all observed traits of hybrids (*Vigna unguiculata* x *Vigna sesquipedalis*)

Quantitative traits	Type of crossing	Genotypic variance	Phenotypic variance	CGV (%)	CPV (%)	Narrow sense heritability (%)	Broad sense heritability (%)
Plant height	KTUT x KP1	83.4	156.3	16.5	18.2	41.7	53.4
	KTUM x KP1	102.5	192.9	17.9	19.1	48.4	53.1
	KTCB x KP1	76.5	141.3	13.4	15.2	52.6	54.1
	KTCK x KP1	79.1	147.2	14.8	16.3	49.2	53.8
	KTUT	63.2	132.4	13.4	15.2		47.7
	KTUM	54.9	127.6	6.2	8.3		43.0
	KTCB	55.5	112.6	10.5	11.8		49.3
	KTCK	67.1	134.5	12.1	13.6		49.9
	KP1	62.8	128.7	10.8	11.9		48.8
Pod length	KTUT x KP1	62.1	112.6	25.3	26.4	50.2	55.2
	KTUM x KP1	58.6	115.8	29.3	30.1	41.3	50.6
	KTCB x KP1	65.2	117.9	15.1	16.2	47.9	55.3
	KTCK x KP1	67.9	116.8	16.8	17.9	53.7	58.1
	KTUT	38.7	101.7	17.2	19.1		38.1
	KTUM	52.6	115.8	17.3	19.5		45.4
	KTCB	54.7	107.9	14.9	16.1		50.7
	KTCK	57.1	126.2	15.3	17.8		45.2
	KP1	43.8	98.9	10.9	12.1		44.3
Protein content	KTUT x KP1	7.9	9.4	37.9	39.2	55.6	83.7
	KTUM x KP1	7.1	10.4	39.2	40.5	43.7	68.5
	KTCB x KP1	8.8	11.3	34.8	37.9	55.9	77.5
	KTCK x KP1	8.7	10.5	27.4	28.9	70.7	82.7
	KTUT	8.7	12.7	27.2	29.0		68.5
	KTUM	5.6	15.8	27.3	29.8		35.4
	KTCB	6.7	17.9	24.9	25.7		37.4
	KTCK	6.1	15.5	27.7	29.1		39.4
	KP1	5.8	18.9	20.9	22.3		30.7
Anthocyan in Content	KTUT x KP1	35.8	62.4	26.1	27.7	54.7	57.4
	KTUM x KP1	41.6	69.6	27.8	29.1	46.3	59.8
	KTCB x KP1	48.4	72.2	31.6	33.2	60.5	67.0
	KTCK x KP1	33.6	57.3	27.8	29.1	54.5	58.6
	KTUT	30.8	54.9	14.5	17.3		56.1
	KTUM	28.7	56.4	17.3	19.0		50.9
	KTCB	29.1	51.7	13.2	15.9		56.3
	KTCK	36.7	67.6	15.6	17.8		54.3
	KP1	32.4	55.3	14.1	15.3		58.6

Note: KTUT = Dark orange cowpea, KTUM = orange cowpea, KTCB = big brown cowpea, KTCK = small brown cowpea, KP1 = superior variety of long bean.

Table 3. Protein and anthocyanin content of the observed genotypes

No.	Genotype	Protein content (%)	Anthocyanin content(ppm)
1	KTUT	28.1	12.4
2	KTUM	27.3	54.8
3	KTCB	24.9	7.9
	KTCK	26.1	14.5
4	KP1	17.4	14.1
5	KPU	17.9	51.4
6	GKH-7-13	25.3	34.2
7	GKH-7-16	26.2	42.1
8	GKH-7-39	26.8	32.6
9	GKH-7-45	27.1	29.5
10	GKH-7-58	28.5	36.7
11	GKH-7-62	24.4	26.6
12	GKH-7-79	27.6	56.4
13	GKH-7-85	23.2	23.4
14	GKH-7-117	21.8	28.9

Note: KTH = black cowpea. KTU = purple cowpea. KTC = brown cowpea. KP1 = superior variety of longbean. KPU = purple longbean. GKH = hybrid vegetable bean

The coefficient of genetic variant is highest (39.2%) in the hybrid protein content of purple cowpea from crosses with long beans (KTUT x KP1). Protein content has a high coefficient of variability on all crosses ranged between 20.9% - 39.2% (Table 2). The results of research conducted by [7] showed that the protein content has the effects of heterosis and heterobeltiosis. This means that there is an over dominant event in which the value offspring is better than the average of the two parents or the best parent. The coefficient of genetic variability in parents used is relatively lower than hybrids from interspecific hybridization because the parents used have a narrow genetic background, so that the variability of genotypes among parents used is low

Differences parents used caused different degrees of character combined so that the population characteristics of the offspring will be different. The difference in the characteristics of cross progeny with each other will be easily observed in the F2 population. The amount of genetic variability for diversity characteristic

appearance of a plant can be predicted through analysis of heritability. According to [9]. A gene cannot reveal its nature without a suitable environment. Similarly, the environment cannot produce a trait without the gene that regulates the trait. Therefore, we must be able to recognize whether the variability that seemed mainly due to differences in genes that are owned by different individuals and whether the variability of other properties is mainly due to the environment in which individuals grow. Characters that have a low narrow sense heritability values tend to be less similar to the parental character as much influenced by environmental factors. According to [10]. Climate change or environment of location will cause a change in the nature of the low heritability value. It also means that the properties are not many inherited from the parents to the offspring.

Genotypic coefficient of variation was different among cross combinations and among quantitative traits. Based on the classification by [11], narrow sense heritability values for all genotypes tested were low to slightly high, while the broad sense heritability is low to high. Highest heritability value (70.7%) was achieved by the proteins in a hybrid between a small brown cowpea beans and long bean (KTCK x KP1), while the lowest (10.4%) are the weight of seed per plant on a hybrid of crossing between Manyar variety with red seed ricebean (MR x RSR). Highest broad sense heritability values (83.7%) was also achieved by the protein content in the hybrid from crosses between purple cowpea beans and longbean (KTUT x KP1), while the lowest (18%) was achieved by the weight of seeds per plant on Nuri variety mungbean. Heritability values in hybrids from crosses tend to be higher than the parental variables and on all types of crosses. The existence of high variability for different characters among vigna varieties had been earlier reported by [12], [14]. Crosses between species vigna increase genetic diversity is needed in plant breeding programs

Genetic variability is very important in plant breeding programs because of the absence of genetic variability difficult to get any improvement or progress of selection. Therefore, several attempts have been, are and will continue to be done by plant breeders with a variety of methods, both conventional and unconventional. One way to increase the genetic variability is to make crosses between species that have different characteristics. By interspecific hybridization will be the incorporation of the characters of both parents and

even allow the emergence of a new character [15]. In order to cross between species is useful. breeders should select parents that contain characters that will be improved so that the genetic variability improvement of opportunities available to the character you want to get. The genetic variability in a population of offspring from crosses in the amount reflected by the coefficient of genetic variability varied between the pair cross to another. between one character to another. Differences parents used will produce different characters in the offspring. This is in accordance with the opinion of [13] which states that the level of genetic variability in populations from crosses is determined by genotyping parents used in these crosses. Therefore. the population characteristics of offspring are different from crosses another.

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