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MORPHOGENETIC VARIATION OF SHALLOT (A*llium cepa* L. Aggregatum Group)

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ABSTRACT

There are many shallot cultivars cultivated in Java with varying greatly morphological traits and yield. Morphological and yield variation indicate that there are genetic variation and varying in resistance to pest and disease. One of major disease that cause yield losses of shallot is Fusarium Basal Rot (FBR) caused by *Fusarium oxysporum* f. sp. c*epae* (Foc). The pathogen could cause yield losses of shallot in field up to 90%.

The number of sixteen shallot cultivars were collected and studied for determining polymorphisms of nuclear based on Random Amplified Polymorphism DNA (RAPD) and the morphological traits. Potted research was conducted at greenhouse from December to February 2012, in Department of Agriculture, Universitas Gadjah Mada (UGM), Yogyakarta. Four shallot cultivars were selected for study the response to Foc under biofertilizer application. They were Kuning, Trisula, Tiron, and Crok cultivars. The field experiment was carried out from June to August 2012 at the Agricultural Training, Research and Development Station (ATRD/KP4) in Kalitirto, Sleman, Yogyakarta. The research design was split plot 4 x 4, with three replications. The plots consisted of shallot which cultivated in Foc inoculation, biofertilizer application, combination of Foc inoculation and biofertilizer application, and without any treatments. The subplot consisted of shallot cultivars. All data were statistically analyzed using the variance analysis. Standard error was tested to determine the significant differences among treatment means.

Similarity coefficient among shallot cultivars as revealed by UPGMA cluster analysis of RAPD markers generated to molecular dendogram. The similarity of genetic dendogram ranged from 0.85 to 0.66 and separated of cultivars into two groups. Based on morphological analysis, there were variations of all variable that tested. Mophological dendogram made possible to identify four group.

Fusarium Basal Rot (FBR) incidence caused by seedborne was 6.94%. Biofertilizer application could not decrease significantly FBR incidence but it could increase number of bulb per plant in Crok and Kuning cultivars. FBR incidence with 43.75% caused by Foc inoculation was significantly decreasing plant height, number of bulbs, diameter of bulbs, and length of bulbs. However, yield of shallot decreased significantly from 1.05

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kg/m^2 to 0.63 kg/m^2 when the shallot plantation was inoculated by Foc. The shallot plantation was inoculated by Foc under biofertilizer application did not show significantly decreasing FBR incidence and increasing the yield. FBR incidence and yield of Trisula, Crok and Tiron cultivars did not show difference significantly from Kuning cultivar as susceptible to Foc.

Key words: *cultivar, shallot, RAPD, Fusarium oxysporum f. sp. Cepae (Foc), biofertilizer*

INTRODUCTION

Shallot (*Allium cepa* L. Aggregatum Group) is a botanical variety which produces aggregation of bulbs (Jones and Mann, 1963). In Indonesia, consumers prefer shallot as bulb for the main seasoning ingredients in food or culinary qualities. There are many shallot cultivars are cultivated in Java with varying greatly morphological traits and yield (Anonymous, 2011). Morphological and yield variation indicate that there are genetic variation and varying in resistance to pest and disease.

Study of genetic resources based on morphological trait is essential in plant breeding as it reveals important trait to plant breeders. However, morphological trait does not always reflect real genetic trait because of genetic and environment interaction. It possibly influences plant growth and genes expression (Hartl *et al.*, 1988).

Currently, molecular markers are considered the best tools in genetic studies due to the possibility to differentiate at the DNA level. Random amplified polymorphic DNA (RAPD) technique has been commonly used because its simplicity, easy, low cost, requirement for only a small quantity of DNA and the ability in generate numerous polymorphisms (Bardakci, 2001).

In this case, study of morphological and genetic variation is important for conserving, evaluating, and utilizing genetic resources; and for studying the diversity of pre-breeding and breeding germplasm. Study of genetic variation is necessary for identifying diverse parental combination with high genetic variation for selection.

MATERIAL AND METHODS

Fresh bulbs of sixteen different cultivars were collected. All cultivars of shallot were cultivated in the greenhouse, Faculty of Agriculture, Gadjah Mada University, Yogyakarta from December 2011 to February 2012. Two plants per cultivar were collected were used for genetic analysis. Twenty plants per cultivar were collected for morphological data.

0.2 g of young leaf materials were ground in 2% CTAB in a mortar, and 1.5 ml 2% CTAB was added to each of the samples. The extracts of samples were incubated at 65°C water bath for 30 minutes by shaking gently every ten minutes. The samples were centrifuged for 5 min at 5,000 rpm in a microcentrifuge. After the supernatant was collected, then it was added 300 µl CIAA (chloroform/ isoamyl alchohol (24:1, v/v)), and centrifuged for 10 min at 10,000 rpm. It was reprecipitated with cold 96% ethanol overnight. The sample was centrifuged at 12,000 rpm for 10 minutes. The pellet was rinsed with cold 70% ethanol and was centrifuged for 10 minutes at 12,000 rpm. After the supernatant was removeed, DNA pellet allowed to air dry until only slightly moist for an hour. It was added with aqua bides ±20-30µl. Finally, it was store at 20°C.

Amplification was carried out using five primers. The primers detail is shown in Table 1.

Table 1. Primers used for RAPD analysis

CMN-A45	5'-TGGCCTCTTGGA-3'	Arifin *et al.,* 2000
CMN-A53	5'-GACGCCCATTAT-3'	
OPA-1	5'-CAGGCCCTTC-3'	Phuong *et al*., 2006
OPA-11	5'-CAATCGCCGT-3'	Peredes *et al*., 2008
UBC-391	5'-GCGAACCTCG-3'	Asili, 2010

The reaction mixture (25µl) for PCR was composed with 12 µl PCR kit, 0.5 primer DNA, 1 µl 20 ng template DNA, and 11 µl dW. The amplification was carried out in a Program Temp Control System (for

preheating 4 minutes 95°C; forty two cycles denaturation 1 minutes 94°C, annealing 1 minutes 37°C, extension 1.5 minutes 72°C, and final extension 7 minutes 72°C).

Eight µl of the reaction mixture was separated by electrophoresis on 1.2% agarose gel in Tris Base buffer (1x) at 80 volt for 60 minutes. The gel electrophoresis was placed in ethidium bromide for 15 minutes and then the gel was photographed above UV light. The electrophoresis gel was carried out at least twice to obtain reproducible bands.

Polymorphic DNA fragments were scored as either present (1) or absent (0). Only distinct and reproducible fragments were scored. Binary matrix was used to estimate the genetic similarities between pairs, by similarity matching coefficient. These similarity coefficients were used to construct dendrogram using the unweighted pair group method with arithmetic averages (UPGMA), through the NTSYS Version 2.1 (Microsoft windows based Freeware for population Genetic Analysis) program.

The experiment was completely randomized design. The experiment was involved one factor which was cultivar. The details of data recorded consist of plant height, number of leaf per plant, leaf greenness, number of tillages per plant, number of bulbs per plant, weight of bulb, diameter of bulb, length of bulb, color scale, total soluble solids (TSS) content of bulb and disease incidence.

Morphological clustering was used to construct morphological dendogram using SAS 9.1 program. Analyses of variance (ANOVA) were carried out for all variables to check the differences in their mean values among the groups obtained from the clustering stage and complemented with a Duncan's Multiple Range Test would be tested to determine the significant differences among their means (Mallor *et al.*, 2011).

RESULT AND DISCUSSION

A total of 68 fragments were generated using five primers. The number of polymorphic fragments varied from 9 to 18 in the size ranging from 150 to 1700 bases pairs (bps). The five primers assessed were polymorphic (Table 2).

Table 2. Details of RAPD analysis of 16 different cultivars of shallot

Primer	Total number of fragments	Number of polymorphic fragments	Percentage of polymorphism	Molecular weight range (bps)
CMN-A59	18	18	100	150 - 1400
UBC-391	18	18	100	150 – 1700
OPA-11	9	9	100	125 – 800
CMN-A45	9	9	100	200 – 1000
OPA-1	15	14	93.75	200 - 1100

Figure 2.3 presented the molecular dendogram based on similarity coefficient among shallot cultivars as revealed by UPGMA cluster analysis of RAPD markers. The dendogram showed a clear separation into two groups. The first group included a total of 5 cultivars. The second group clustered 11 cultivars.

Cluster 1 consisted of Bima, Crok, Tiron, Biru and Pilip cultivars. The percentage of the similiarity ranged between 0.72 to 0.86. Bima cultivar is local cultivar from Central Java whereas Crok, Tiron and Biru cultivars are local from Yogyakarta. Pilip cultivar is introduction cultivar from Philipine which is cultivated in Yogyakarta. Cluster 1 was clearly separated into two sub group. Sub group 1 consist of Sumenep cultivar and the others cultivars generated into sub group 2. In this case, Sumenep cultivar belongs to *Allium × wakegi* Araki was clearly separated from group one into sub group one.

Cluster 2 was formed by Bauji, Trisula, Katumi, Kuning, Mentes,

Pikatan, Sanraen, Sembrani and Sumenep cultivars. Bauji and Manjung are local cultivar from East Java whereas Bima and Kuning cultivars are local cultivar from Central Java. Katumi and Sembrani cultivasr are hybrid cultivar. Sanraen, Mentes, Pancasona, Pikatan and Trisula cultivars will be released by Balitsa. The percentage of the similiarity ranged between 0.715 and 0.87.

RAPD analysis were conducted to establish the phylogenetic relationship among collected accession in Indonesia by Arifin *et al.* (2000). The research resulted that the grouping of shallot cultvars separated into two main group.

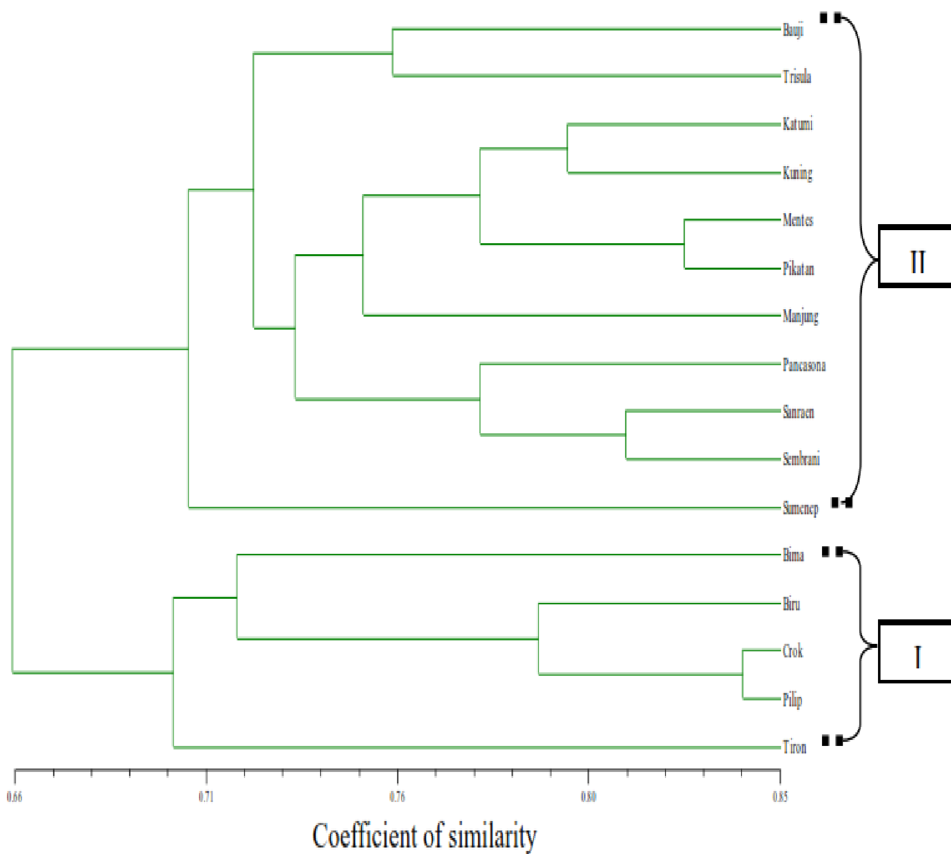

Figure 1. Molecular dendogram constructed from the results of similarity matching based on RAPD analysis.

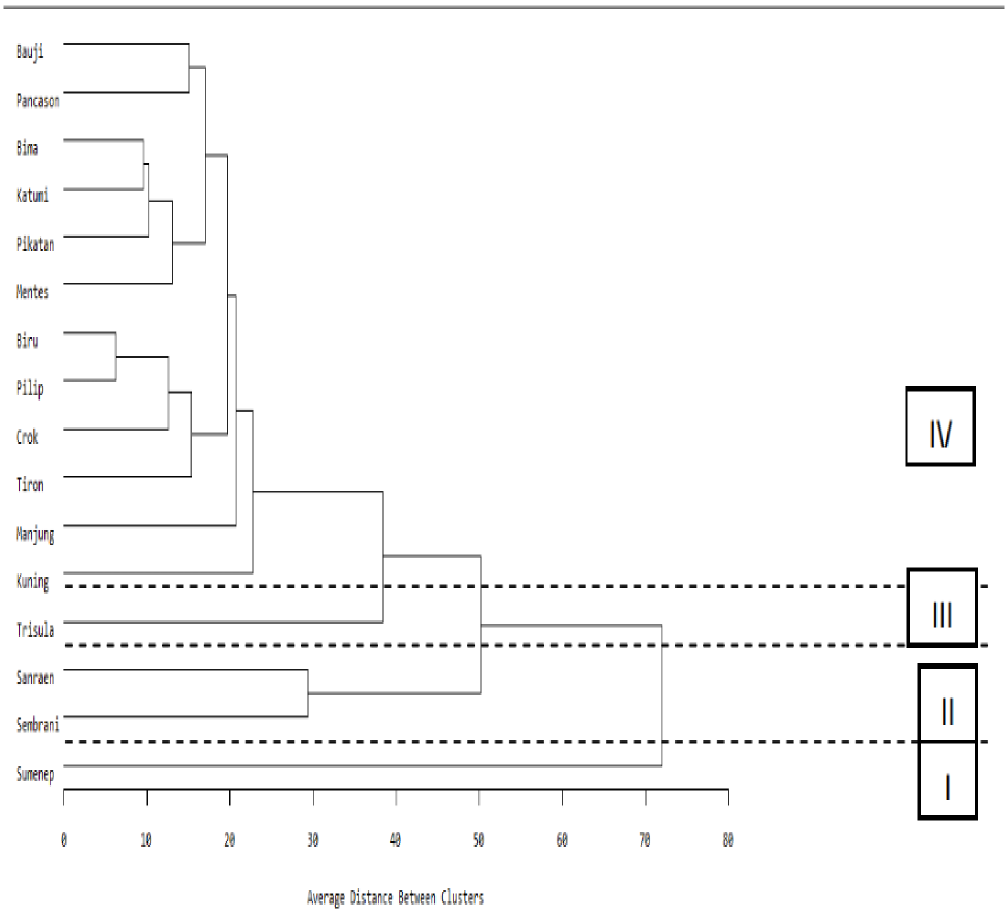

Figure 2. Morphological dendogram based on quantitative traits of sixteen cultivars of shallot.

Analysis of variance for plant height, number of leaf, leaf grenness, number of tillage, number of bulbs per plant, weight of bulbs per plant, diameter of bulb, length of bulbs, total soluble solid content, skin and fleshy color scale revealed significant difference among sixteen different shallot cultivars (Table 2).

Figure 2 showed the morphological dendogram based on average distance among cultivars. The dendogram made possible to identify four groups. First group consisted of Sumenep cultivar. Second group was made

up Sembrani and Sanraen cultivar. Third group was most characterized of Trisula cultivar. The others cultivars were made up of fourth group.

Table 2. Morphological traits of sixteen shallot cultivars

Cultivar	Height (cm)	No. of Leaves plant^{-1}	SPAD	No. of bulbs plant^{-1}	Weight of bulbs plant^{-1} (gram)	Diameter of bulb (mm)	Length of bulb (mm)	Shape index	Total soluble solids content (^{0}Brix)
Bauji	34.6 bcde	15.7 ef	40.3 ef	9.0 cd	13.07 b	12.30 c	21.71 de	0.57 bcde	14.7 cd
Bima	32.9 ef	17.4 de	41.0 de	8.9 cd	10.02 d	10.68 ef	20.93 de	0.52 efg	17.8 a
Biru	36.6 b	23.2 bc	42.2 cd	9.9 abc	10.47 d	10.97 de	20.75 de	0.53 cdef	17.0 ab
Crok	34.4 cde	26.1 a	40.1 ef	10.7 a	11.82 bcd	11.53 cde	19.48 f	0.60 b	15.1 bcd
Katumi	32.1 f	16.1 ef	39.1 fgh	9.9 abc	11.07 cd	10.74 ef	20.32 def	0.53 cdef	13.3 e
Kuning	33.5 def	16.9 de	40.6 ef	9.2 bc	12.57 bc	11.76 cde	21.97 de	0.54 cdef	16.6 abc
Manjung	36.2 bc	14.8 f	39.3 fgh	7.2 e	11.59 bcd	12.14 cd	26.04 b	0.47 g	14.7 cd
Mentes	32.7 ef	17.2 de	38.6 f	9.3 bc	11.09 cd	11.39 cde	21.04 de	0.54 cde	15.7 bcd
Pancasona	33.7 def	17.1 de	40.1 efg	7.8 de	11.70 bcd	11.20 cde	20.23 ef	0.56 bcde	14.3 d
Pikatan	32.9 ef	14.3 f	41.1 de	5.2 f	10.02 d	11.13 cde	21.14 de	0.52 defg	14.5 cd
Pilip	39.2 a	21.8 c	38.6 ghi	10.3 ab	10.62 cd	11.46 cde	22.39 d	0.51 efg	15.0 bcd
Sanraen	36.5 bc	18.3 d	42.8 c	5.9 f	18.58 a	21.22 a	30.13 a	0.71 a	15.0 bcd
Sembrani	36,3 bc	17.4 de	46.1 a	7.3 e	19.18 a	18.33 b	31.30 a	0.59 bc	14.8 cd
Sumenep	25.4 g	24.3 ab	44.1 b	5.9 f	7.10 e	9.71 f	18.29 g	0.35 h	18.1 a
Tiron	32.6 ef	23.4 bc	37.6 i	10.4 ab	10.11 d	10.61 ef	19.32 f	0.58 bcd	14.8 cd
Trisula	35.2 bcd	18.1 d	38.2 hi	7.3 e	11.67 bcd	12.07 cd	24.76 c	0.49 fg	15.0 bcd
CV (%)	5.82	10.15	3.50	14.9	15.32	9.61	9.91	9.59	10.13

Note: Mean separation within columns by Duncan's multiple range test. Values followed by the same letter within columns did not show significant differences at 0.05 probability level

Table 3 was carried out for all variables to check the differences in their mean values among the groups obtained from the clustering stage. Group 1 was mostly characterized by the shortest plant height, the lightest of bulb diameter, the lowest bulb shape index, the lightest weight of bulb, the lowest both of redness and yellowness of fleshy color scale, and the highest leaf white spot incidence. Group 2 showed the highest of bulb diameter, bulb length, shape index and weight of bulb. Group 3 was formed by medium bulb diameter, the highest of red direction skin color scale of bulb and fleshy color scale and the lowest yellow direction of fleshy color scale of bulb. It means Trisula cultivar is the most redness than the others. Group 4 mainly included by medium size of bulb.

Tabel 3. Morphological values for each cluster

	Cluster			
	I	II	III	IV
N[1)]	1	2	1	12
Height plant (cm)	**25.4 b**	36.4 a	35.2 a	34.3 a
Number of leaves plant^{-1}	24.3 a	17.9 a	18.1 a	18.6 a
Leaf greenness	44.12 a	44.45 a	38.24 b	39.89 b
Sum of tillage plant^{-1}	**7.4 a**	4.6 b	4.1 b	5.9 ab
Number of bulbs plant^{-1}	5.89 a	6.61 a	7.33 a	8.99 a
Diameter of bulb (mm)	**9.71 c**	**19.77 a**	**12.07 b**	11.33 bc
Length of bulb (mm)	27.76 ab	**30.71 a**	24.76 bc	**21.19 c**
Shape index of bulb	**0.35 c**	**0.65 a**	0.53 b	0.49 b
Weight of bulbs (gram)	**7.10 c**	**18.88 a**	11.67 b	11.18 b
TSS content (^{0}Brix)	17.30 a	15.69 a	14.89 a	15.65 a
Skin scale color				
a* (redness)	14.68 b	20.12 ab	**26.24 a**	22.27 ab
b* (yellowness)	9.92 a	3.4 a	0.83 a	7.64 a
Fleshy scale color				
a* (redness)	**10.59 b**	16.34 ab	**24.45 a**	14.42 ab
b* (yellowness)	**-0.49 a**	-4.40 ab	**-7.98 b**	-3.98 ab
Soft rot incidence percentage (%)	0 b	25 a	40 a	7 b
Leaf yellow strip, low sum of tiller, irregular leaf growth incidence percentage (%)	0 a	15 a	0 a	3.75 a
Fusarium incidence percentage (%)	0 a	0 a	0 a	0.83 a
Leaf white spotted incidence percentage (%)	**60 a**	0 b	0 b	0 b

Notes: Mean separation within rows by Duncan's multiple range test. Values followed by the same letter within columns did not show significant differences at 0.05 probability level.[1)] Number of accessions per cluster

In this study, RAPD technique was succesfully utilized to determine the genetic relationship among sixteen different cultivars. This research tested five primers resulted in satisfactory amplification fragments with a polymorphism rate of 93.75% - 100%. The variations in fragments pattern as well as the total number of fragments amplified in different cultivars from 9 to 18 fragments. The size of fragments ranged from 150 to 1700 bases pairs (bps). Similarity coefficient among shallot cultivars as revealed by UPGMA cluster analysis of RAPD markers generated to molecular dendogram. The similarity of genetic dendogram ranged from 0.85 to 0.66 and separated of cultivars into two groups. First group consisted of Bima, Crok, Tiron, Biru and Pilip cultivars. Second group was formed by Bauji, Trisula, Katumi, Kuning, Mentes, Pikatan, Sanraen, Sembrani and Sumenep cultivars. RAPD analysis were conducted to establish the phylogenetic relationship among collected

accession in Indonesia by Arifin *et al.* (2000). The research resulted that the grouping of shallot cultivars grown in Java separated into two main group.

Analysis of variance for plant height, number of leaves, leaf grenness, number of tillage, number of bulbs per plant, weight of bulbs per plant, diameter of bulb, length of bulbs, total soluble solid content, skin and fleshy color scale revealed significant difference among sixteen different shallot cultivars. Mophological dendogram made possible to identify four group.

Some of cultivars remained the same group in both genetic and morphological dendogram. Biru, Crok, Pilip and Tiron cultivars were grouped in first cluster of genetic dendogram which were reflected medium size bulb in fourth cluster of morphological dendogram. Sanraen and Sembrani cultivars were close similarity genetic which showed big size grouped in the morphological dendogram. The grouping of shallot by RAPD analysis may thus reflect part of the morphological traits of shallot. The other case, some of cultivars remained the different between genetic and morphological dendogram. There is possible reason. According to Hart *et al.* (1988), morphological traits are influenced by environmental condition so it showed considerable variation, due to unknown mechanism of genetic control and great environmental effects in the process of trait expression. The result reflected that morphological traits were not always reliable in estimating genetic relationship among shallot cultivars. It should be use mainly of genetic variation for discrimination.

Plant breeders often select hybridization targets with large differences in morphological traits, rarely considering their genetic background. Therefore, breeders often have to trying to get ideal traits due to some hybridization combination distinct morphological differences and possible share high genetic similarity in their genome. It is necessary for plant breeders to explore plant genetic diversities at the genome level.

CONCLUSION

1. There were genetic and morphological variation among sixteen different shallot cultivar. Similarity of genetic dendogram among sixteen different cultivars showed high variation ranging between 0.85 to 0.66 and separated into two groups whereas mophological dendogram made possible to identify four group.
2. Morphological traits were not always reliable in estimating genetic relationship among sixteen different shallot cultivars. It should be use mainly of genetic variation for discrimination.

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