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Evaluation of Genetic Variability and Genetic Advance to Qualitative and Quantitative For Selection of Expected Lines of Purple Common Bean (Phaseolus Vulgaris L)

Very Andriani[1], Andy Soegianto[2], Kuswanto[3]
[1,] Center for Plant Variety Protection and Agricultural Permits, Secretariat General, Ministry of Agriculture Jl. Harsono RM No. 3 Bulding B of 5th Floor, Ragunan, South Jakarta
[2, 3] Faculty of Agriculture, University of Brawijaya Jl. Veteran Malang 65145

***Abstract**: In order to obtain superior varieties of bean that contain high antioxidant content and high yield, a crossbred between local parent varieties (Mantili, Gilik Ijo and Gogo Kuning) by the introduction varieties (Purple Queen and Cherooke Sun). Evaluation and selection are the main activities that must be done after obtaining high variability initial population. Objectives of the research were to evaluate genetic variability and genetic advance, as well as to select the expected lines of purple pod common beans and high yield in F3 and F4 populations. From the previous research, 42 expected lines have been selected that based on pedigree selection method, and result of the evaluation showed that 42 lines, which were tested in F3 population, have high genetic variability on qualitative characters (growth type, pod color, pod shape, and the pod texture), therefore, further selection is required to obtain the homogenous lines qualitatively. Results of the selection on F3 population showed 13 expected lines and after being tested, they showed variability of growth type, pod color, pod shape, and texture of the pod, despite uniform lines have been found qualitatively. On qualitatively uniform lines, a selection may be done in order to obtain high yield lines. On characters of numbers of pod and weight of pod per plant, they had moderate variability, high expected genetic advance and heritability, so that selection on those characters can still be done. Results of the selection on F4 population showed some qualitatively homogenous expected lines and they can be used as further breeding materials, such as PQGK1.1 and PQGI 169.1.*

***Keywords:** common bean, phaseolus vulgaris, genetic variability, genetic advance*

I. Introduction

Common beans (Phaseolus vulgaris L.) are vegetables, which are mostly consumed by society because they contain high nutrients and cheap. It is admitted that common beans contain high protein, carbohydrate, fibers, and isoflavonoid (Anderson, et al., 1999), besides that, common beans contain iron, phosphor, magnesium, mangaan, zinc, and calcium (Broughton, *et al*., 2003). The demand of common beans for consumption in Indonesia has increased along with the rapid growth of population. The growth of common beans production had increased in 2007 – 2010, for about 0.12%, but the consumption growth was about 0.26%, the growth of common beans availability per capita - 0.34 %, and average of the imported growth rate for common beans was 6.59% (Center of Data and Information System of Agriculture, 2012).

Today, the commercial beans at the market, which is mostly consumed by the public, is the green pod beans, whereas in fact the beans have wider ranges of variability and each of them has specific and superior properties. Beans breeding that were intended to increase the yield has not enough; therefore an idea emerges to combine the superior properties of local beans and introduction through a set of crops breeding to increase yield and nutritive contents (high anthosianin).

This research is intermediate of the previous research, in which a crossbred has been done between local beans derived from Surakarta that have high yield and green pod (Mantili, Gogo Kuning and Gilik Ijo) and the introduction beans that contain anthosianin and have purple pod (Purple Queen), as well as high beta-carotene and have yellow pod (Cherokee Sun) and result of the evaluation showed that the descendant (progeny) as a result of crossbred between local beans and Cherokee Sun have maternal effect, but not with the Purple Queen, in which the resulted descendants (progeny) have purple pods (Oktarisna, *et al*,. 2013).

It is presumed that the purple pod beans contain high anthosianin. Anthosianin plays as antioxidant because it could avoid any free radicals; prevent any cell damages that could initiate the emergence of various diseases, particularly coronary attack and stroke, with high antioxidant content. According to Kuswanto (2012), anthosianin could protect the body cells from internal attack, such as instable emotion and stress, so that it could inhibit cell aging and makes the skin smoother. Anthosianin plays important role for the crops itself, synthesis in petal as trap for pollinator insects, synthesis in fruits and seeds as seed dispersion (Holton and Cornish, 1995). In legumes with purple pods are avoided by aphids due to most parts of the stems, leaves, and pods have trichome, which could reduce the decreasing production due to some disease infection (Hardinaningsih, 2012).

Study on genetic variability is important to be known in genetic improvement program because it would give information about the effect of heterocyst and separate them in recombinant, increase possibility in gaining superior genotype in progeny (Silva *et al*., 2008 in Cabral *et al* ., 2011). Result of the evaluation on F2 population showed variability, heritability, and high genetic advance on characters of pod color, numbers of pod per plant, and weight of pods per plant (Handayani, 2014), so that those characters should be selected to choose the potential lines for further breeding materials. High heritability and genetic variability will determine success of the selection. The higher genetic variability, the greater opportunity is to obtain lines or varieties that conform to the expected criteria. Heritability estimation is useful to find out the relative value of the selection based on phenotypic expression of the different characters (Safavi *et al*., 2011). Heritability has also shown whether a character is being controlled by genetic factor or environmental factor, therefore it can be found out to the extent of the character could be passed on to the next descendant. The estimation value of the selection advance to estimate the extent of increasing that will be reached from the selected characters. So that, the selection will run more effectively on characters that have high estimation values for heritability and genetic advance because of little environmental effect.

II. Material and Method

The research had been conducted for two planting seasons, 1) September – December 2013 (planting F3), 2) January - April 2014 (planting F4) in Batu Malang, East Java, which comprised of several stages: 1) individual selection on F2 population as planting material for F3, 2) evaluation of variability and selection on F3 population as planting material for F4, 3) evaluation of variability and selection of F4 population to obtain potential lines as breeding materials for the next generation.

Planting materials, which were used in planting season 1, were resulted from selection of pedigree on F2 and in planting season 2 used planting materials as a result of selection on pedigree of F3 as presented in Table 1. Selection on F2 population was 10% out of total individuals that have purple pods and high yields, while selection on F3 was 30% out of population that have purple pods and high yields. The selected lines were planted as single plant without replication along with 5 parental varieties and 1 comparative variety on the same field.

Table 1. The result of pedigree selection lines on F2 and F3

42 lines as a result of pedigree selection on F2 include :
PQM.2, PQM.11, PQM.19, MPQ.27, MPQ.36, MPQ.39
GKPQ.1, GKPQ.7, GKPQ.12, GKPQ.19, GKPQ.31, GKPQ.36, GKPQ.44, GKPQ.50
PQGK.1, MCS.1, 9, 10, 13
GIPQ.1, GIPQ.4, GIPQ.12, GIPQ.19, GIPQ.21, GIPQ.23, GIPQ.25, GIPQ.35, GIPQ.39, GIPQ.41, PQGI.46, PQGI.169
GKCS.6, GKCS.11, GKCS.49, GKCS.53, GKCS.54, GKCS.56, GKCS.57, GKCS.83, GKCS.91, GKCS.97, GKCS.108
13 lines as a result of pedigree selection on F3 include :
GKPQ.12.4, GKPQ.19.4, PQGK.1.12
GIPQ.12.2, GIPQ.19.10, GIPQ.23.10, GIPQ.35.11, PQGI.169.1
GKCS.6.6, GKCS.54.11, GKCS.97.2, GKCS.108.1, MCS.13.3

Notes: PQM is the crossbred line between Purple Queen x Mantili, MPQ is the crossbred line between Mantili x Purple Queen, GKPQ is the crossbred line between Gogo Kuning x Purple Queen, PQGK is the crossbred line between Purple Queen x Gogo Kuning, GIPQ is the crossbred line between Gilik Ijo x Purple Queen, PQGI is the crossbred line between Purple Queen x Gilik Ijo, GKCS is the crossbred line between Gogo Kuning x Cherokee Sun, MCS is the crossbred line between Mantili x Cherokee Sun. Numbers following the name of lines show the individual number of the selected plant based on the pedigree method.

Plant breeding has been done such as in common beans of creeping type, in general, and observation has been done on qualitative characters (growing type, stem color, leaf color, whether anthosianin coloration in leaf exists or not, standard color of the flower, pod color, pod shape, and texture of the pod), and quantitative characters (days of flowering, days of harvest time, numbers of pod per plant, pod's length, diameter of the pod, weight per pod, and weight of pods per plant).

The qualitative data was analyzed descriptively by calculating percentage of morphological character variability on each population and cluster analysis based on the method of UPGMA (Unweighted Pair-Cluster Method Arithmetic) through the program of MVSP (Multi Variate Statistical Package) version 3.22 along with simple matching coefficient. Objective of such cluster analysis was to find out the extent of relationship and closeness of the genetic distance between individuals in population. Different genetic distance between individuals in population described the genetic variability. Such grouping of individuals that have similar characters will facilitate the selection process.

The quantitative data was analyzed by calculating the mean values, range, standard deviation and variability. Estimation of the variability components comprised of phenotypic variability, genotypic variability, and environmental variability. The phenotypic variability is variability of each population of generation; it is presumed that the environmental variability is derived from mean values of phenotypic variability of both parents and genetic variability is the differences between phenotypic variability and environmental variability. Measurement of variability coefficient is counted in accordance with equation : KKG = $\frac{\sqrt{\sigma^2 g}}{X}$ x 100%, criteria of assessment for genetic variability according to Knight (1979) Narrow : 0-10%, Medium:10-20%, and Wide:>20%. The estimation value of heritability is counted in accordance with equation (Singh and Chaudary 1979), $h^2 = \frac{\sigma^2 g}{\sigma^2 F}$, criteria of assessment for heritability according to Stanfield (1983), high: $x > 0,50$, medium: $0,2 \leq x \leq 0,5$ and low: $0 \leq x \leq 0,2$. Analysis of the genetic advance is counted in accordance with equation, $R = i. h^2.\sigma p$. R = genetic advance, i = intensity of the selection, h^2 = heritability and σp = standard deviation of phenotypic variability. Percentage of the expected genetic advance is counted using the equation: KGH= $\frac{R}{x}$ x 100%, in which KGH = expected genetic advance, R = selection advance and x = mean. According to Hadiati and Rostini (2003), criteria of the expected genetic advance included high: > 14.1%, medium: 7,1 – 14%, and low: 0 - 7%. In fact, genetic advance is the difference between the genetic advance of population after selection and the expected genetic advance before selection.

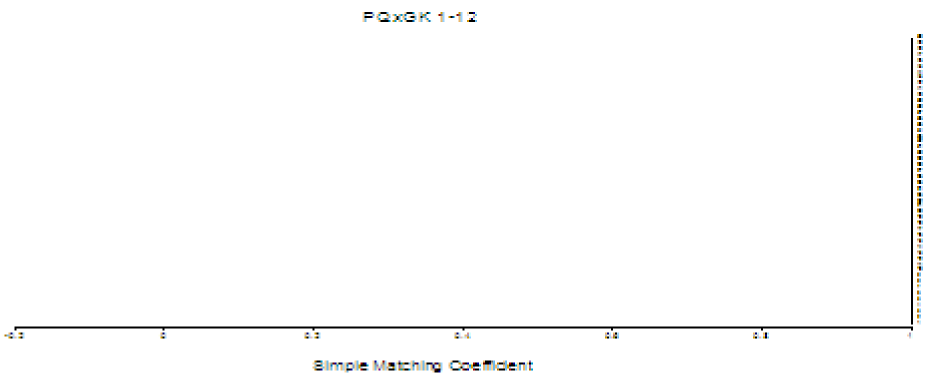
III. Result And Discussion

Qualitative Character Variability

Results of the research showed high variability on characters of growth type, pod color, pod shape, and pod texture, particularly in the descendant population as a result of crossbred between local parents and the Cherokee Sun. These were due to both parents having extremely different and allele segregation has still occurred in the heterozygote lines. The previous research stated that crossbred between local parents and Cherokee Sun showed “maternal effect“, but the crossbred between local parents and Purple Queen did not show “maternal effect” on pod color (Oktarisna, 2013), on descendant (progeny) of Mantili x Cherokee Sun was presumed to have epistasis that control the purple color even though both parents have green and yellow pods (Handayani, 2014).

Dendogram as a result of cluster analysis on F3 population showed qualitative character variability, both intra-lines and inter-lines. The individual plant in each line has still joined in different groups that indicated that the population has still varied, so that further selection is required to obtain homogenous lines qualitatively. Pod color determines the success of beans breeding that contains high anthosianin. Paramitha (2014) states that legumes with Dark Grayish Purple pods contain the highest anthosianin in comparison with other pods. Diverse colors of pod in F3 are divided into 7 groups, such as deep purple (Greyed Purple Group N 186 A-B), light purple (Greyed Purple Group N186 C-D), greenish purple (Greyed Purple Group N 187 A-B), purplish green (Greyed Green Group 189 A-B), reddish green, greenish red (Greyed Red Group 181 A-D), green (Green Group 141).

Results of the individual selection, based on color character of purple pod and high yield, have brought about 13 lines as planting material of F4, and after being evaluated, the results showed character variability in growth type, pod shape, and the pod texture. All populations have purple pods (Greyed Purple Group N 186 A and Greyed Purple Group N 186 C). Dendogram showed that PQGK.1.12 and PQGI.169.1 lines have uniformity on 8 qualitative characters, which have been observed (Figure 1).



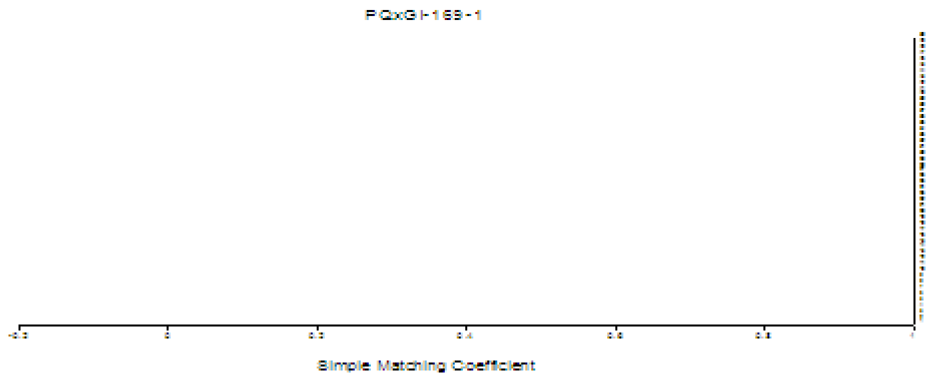


Figure 1. Dendogram of cluster analysis result on PQGI.169.1 and PQGK.1.12 population towards 8 qualitative characters that being observed.

The pod shape has changed as shown on F3, which was previously dominant round and fine texture of the surface pod becoming medium, but on F4 tended to be varied from fine, medium, and coarse (Figure 2). It was presumed as a result of selection. Selection on F3 was only based on individual that having the character of purple pod and high yield without considering shape and texture of the pod. It is presumed that selection on pod color could affect shape and texture of the pod, moreover if the character was controlled by the same genes or the connected genes. Syukur et al. (2009) stated that, in general, selection on one character is easier to be done but it can affect other characters. It may occur if those characters are controlled by the same genes or the connected genes. Besides that, numbers of individual, which were planted in each F3 population, are limited so that a character could not well-expressed. In order to make the selection process of F5 to be more effective, the homogenous seeds could be mixed (bulk) so that variability of the pod shape and pod texture would be high. High variability in shapes and textures of the pod on F5 population has provided great opportunity to choose superior genotypes as expected. Allard (1960) stated that modification of pedigree selection can be done by pure pedigree selection method, so that F4 would be followed by bulk of the selected plants. On F5, emphasizing on selection has changed totally from observable properties to subjective properties, which are more difficult to be determined. Such properties sometimes are easily observed if per plant was treated in many rows than just a single row. According to Kuswanto (2006), in order to increase variability between lines in F5 population of legumes as a result of crossbred, the seeds were mixed from 1-2 pods of one plant to another, and followed by genetic variability test between the available bulk populations. The change in shape and texture of the pods could be caused by the change of environmental factors, even though the qualitative characters are only controlled by the simple gene and not easily affected by the environment. In the same phenomena, the environmental condition for growing (length of days and temperature) during the emergence has affected on the emergence of multi-foliolate expression on the growth of soybean (Orf et al. (2006)) in Musalamah and Suyatmo (2006).

Figure 2. Variability of pod shape 1) round, 2) slightly flattened, 3) flat

The pod shape and pod texture of beans is one of the determinant factors of the consumer's desire. Permadi and Dini (2000) stated that consumers prefer the shape of round pods to the flat pods, relative even surface, massive with the pod length of 15-22 cm, so that those characters are important in breeding purple common beans.

Quantitative Character Variability

Tables 2 and 3 show the tested lines on F3 and F4 populations that have high genetic variability on numbers of pod and weight of pods per plant. A research by Nechifor et al. (2011) also showed high genetic variability on numbers of pod per plant and weight of pods per plant.

Yield quality of the plants is affected by numbers of component, such as pod length, pod diameter, pod weight, and numbers of pod. For the characters of pod length and pod diameter, they showed low variability along with lower value of the genetic variance than the environmental variance, which meant that both characters were dominantly affected by the environmental factors. Selection on both characters could be done tightly, but the results would not be effective. Pod length, pod diameter, and weight per pod are the quantitative characters, which are controlled by polygenic and easily affected by environment. For characters of early flowering and early harvesting, they showed low genetic variability, so that the selection would be ineffective to be done. Sofie et al. (2011) stated that 42 germplasm of "exotic" beans, which were grown at the valley of Kashmir in order to obtain superior genotype of beans "under temperate condition" have genetic variability for days of flowering (13,304) and days of harvesting (13,430) or they are categorized as low – medium.

Mean for numbers of pod, pod length, pod weight, and weight of pod per plant on F4 population are lower than on F3 population (Table 2 and 3). This is due to the environmental factor where the plants grown, F4, is less supporting the optimal growth of the beans. The climatic condition (rainfall, humidity, high wind velocity), impact of eruption from Mount Kelud and high infection of disease have inhibited the beans to grow optimal and could not produce maximum pods. As comparison, 5 parental varieties and 1 national superior variety of beans, Lebat, were planted and the yield reduced for almost 30-40% numbers of pod per plant and weight of pod per plant from traits that they possessed.

Besides that, the seed as planting material on F4 population derived from 1 individual plant. If the plant does not have any resistant to the environmental stress, it would have difficulty to adapt, so that it produced less pods and it would affect on weight of pods per plant. According to Kuswanto (2006), natural selection will occur in each population due to inability of the plant to overcome the environmental stress. The plants that grow and produce pods are plants, which could overcome the environmental stress naturally. The increasing mean values of pod weight per plant will be followed by numbers of pod per plant. However, the increasing length and diameter of the pods could not increase weight of pods per plant. Many factors have affected them, and one of them is the existence of connected genes. The connection between characters or traits/properties from one to another could simultaneously affect other characters that have close relationship.

Heritability and Genetic Advance

Effectiveness of the selection is not only determined by greater genetic variability, but also affected by high heritability value and the genetic advance. Heritability is a genetic effect of a phenotypic appearance that can be bequeathed from the parents to the descendants (progeny). High heritability shows great genetic variations and less environmental variation. The expected genetic advance is a shift of population's median value from condition of the population to condition after selection, under assumption of differential quantity (Aryana, 2010). In the wide sense, heritability is important to find out the effect of additive genes can be bequeathed to their descendant (progeny) (Bello, 2012).

Result of the research showed that high heritability and genetic advance were seen on characters for numbers of pod per plant and weight of pods per plant. High heritability values, followed by high selection response, are a result of additive genes. On the contrary, trait or property that has high heritability and low selection response, was affected by non additive genes (dominant, epistasis). An analogous research on beans was reported by Nechifor et al. (2011) that numbers of pod per plant and numbers of seed per pod have medium heritability values and high genetic advance. The genetic (additive gene) factors are more dominant than the environmental factors.

Breeding to improve the characters can be done through selection process on characters that have high heritability values and genetic advance (Mohamed et al., 2012, Selvaraj et a.l, 2011). Selection on F3 population has increased the genetic advance on days of flowering, as proved that almost all lines have early flowering than before. If the flowering time comes earlier, the harvest time will be sped up. The increasing genetic advance on a character, as a result of selection, is not always followed by the increasing genetic advance of other characters. However, it is affected by some other factors, such as: selection or choosing the individual, which is going to be planted, extreme environmental factors, and genes connection (interrelated genes).

Table 2. Mean, variance, heritability, and the expected genetic advance of F3 population

Value	Character						
	Early flowering (dap)	Early harvest time (dap)	Σ pod/plant	Pod length (cm)	Pod diameter (cm)	Weight per pod (gram)	Weight pods per plant (gram)
Mean	33,06	45,03	53,87	13,94	0,66	7,10	368,15
Phenotypic variance (%)	2,18	2,96	66,68	2,59	0,00	0,73	3.065,82
Environment variance (%)	1,23	1,52	19,72	1,54	0,00	0,32	1.021,25
Genetic variance (%)	0,95	1,44	46,96	1,05	0,00	0,41	2.044,57
Coefficient of Genetic Variability (%)	2,96	2,66	12,72	7,36	4,11	9,00	12,28
Heritability	0,44	0,49	0,70	0,41	0,20	0,56	0,67
Genetic Advance	3,44	3,27	18,79	8,26	3,20	11,86	17,65

Note : CGV based on Knight (1979) 0-10% = low, 10-20%=medium, >20% =high, heritability based on Stanfield (1983) 0≤x≤0.2 = low 0,2≤x≤0.5 = medium, x>0.50 = high, GA based on Hadiati et al. (2003), 0-7% = low, 7-14% = medium and >14,1 = high

Table 3. Mean, variance, heritability, and the expected genetic advance of F4 population

Value	Character						
	Early flowering (dap)	Early harvest time (dap)	Σ pod/plant	Pod length (cm)	Pod diameter (cm)	Weight per pod (gram)	Weight pods per plant (gram)
Mean	31,84	44,84	49,92	12,83	0,66	6,09	306,80
Phenotypic variance	1,92	0,54	71,65	1,85	0,01	0,56	2.579,84
Environmental variance	0,90	0,35	27,33	0,90	0,01	0,44	1.021,70
Genetic variance	1,02	0,19	44,32	0,95	0,00	0,12	1.558,14
Coefficient of Genetic Variability	3,18	1,37	13,34	7,59	4,51	5,75	12,87
Heritability	0,53	0,35	0,62	0,51	0,08	0,22	0,60
Genetic Advance	4,08	0,97	18,46	9,57	2,27	4,73	17,60

Note : CGV based on Knight (1979) 0-10% = low, 10-20%=medium, >20% =high, heritability based on Stanfield (1983) 0≤x≤0.2 = low 0,2≤x≤0.5 = medium, x>0.50 = high, GA based on Hadiati et al. (2003), 0-7% = low, 7-14% = medium and >14,1 = high

Table 4. Mean for numbers of pod and weight of pod per plant on F4

Lines	Mean	σ^2p	σ^2e	σ^2g	CPV (%)	CGV (%)	h²	GA (%)
(Lines)	(Mean)	(Phenotip variance)	(Environment variance)	(Genetic variance)	(Coefficient of Phenotip variance)	(Coefficient of Genetic variance)	(Heritability)	(Genetic Advance)
Numbers of pod per plant								
GKCS.6.6	61,57	107,36	43,38	63,97	174,36	103,90	0,60	17,65
GKCS.54.11	52,50	291,53	43.38	248,1	555,29	472,66	0,85	48,72
GKCS.97.2	45,63	89,55	43.38	46,2	196,27	101,19	0,52	18,82
GKCS.108.1	52,94	164,76	43.38	121,9	311,20	229,26	0,74	31,43
GIPQ.12.2	58,13	311,27	72.72	238,6	535,44	410,35	0,77	40,94
GIPQ.23.10	48,39	81,43	72.72	8,71	168,28	18,00	0,11	3,51
GIPQ.35.11	57,75	174,72	72.72	102,0	302,55	176,63	0,58	23,52
GIPQ.19.10	31,25	75,33	72.72	2,61	238,67	8,27	0,03	1,68
PQGI.169.1	45,50	85,63	72.72	12,91	188,20	28,38	0,15	5,40
GKPQ.12.4	50,85	218,56	76.97	141,6	429,80	278,43	0,65	33,15
GKPQ.19.4	49,16	136,14	76.97	59,2	276,95	120,36	0,43	18,16
PQGK.1.12	36,44	101,73	76.97	24,8	279,19	67,94	0,24	11,86
MCS.13.13	58,82	386,77	55.21	331,6	669,41	573,86	0,86	51,36

Weight of pod per plant (gram)

GKCS.6.6	337,36	7.064,18	2.606,75	4.457,43	2.093,98	1.321,28	0,63	27,67
GKCS.54.11	325,04	11.442,81	2.606	8.836,06	3.520,40	2.718,43	0,77	44,73
GKCS.97.2	271,75	4.309,71	2.606	1.702,96	1.585,91	626,67	0,40	16,80
GKCS.108.1	305,03	8.651,10	2.606	6.044,35	2.836,16	1.981,57	0,70	37,50
GIPQ.12.2	419,33	23.617,07	4.208	19.408,48	5.632,09	4.628,44	0,82	53,01
GIPQ.23.10	311,27	5.052,04	4.208	843,45	1.623,04	270,97	0,17	6,71
GIPQ.35.11	372,75	26.549,74	4.208	22.341,15	7.122,75	5.993,67	0,84	64,74
GIPQ.19.10	227,32	5.657,84	4.208	1.449,25	2.469,82	632,64	0,26	14,80

PQGI.169.1	318,63	5.450,56	4.208,59	1.241,96	1.679,66	382,73	0,23	9,12
GKPQ.12.4	263,34	6.698,80	3.692	3.006,68	2.543,82	1.141,76	0,45	24,55
GKPQ.19.4	231,98	3.810,57	3.692	118,45	1.616,35	50,24	0,03	1,43
PQGK.1.12	305,70	9.740,87	3.692	6.048,75	3.186,45	1.978,68	0,62	35,28
MCS.13.13	298,91	19.019,76	3.254,04	15.765,72	6.279,17	5.204,89	0,83	66,42

Note : CGV based on Knight (1979) 0-10% = low, 10-20%=medium, >20% =high, heritability based on Stanfield (1983) $0\leq x\leq 0.2$ = low $0,2\leq x\leq 0.5$ = medium, x>0.50 = high, GA based on Hadiati et al. (2003), 0-7% = low, 7-14% = medium and >14,1 = high

IV. Conclusion and Suggestion

1- High variability has been shown on qualitative character, such as pod color, pod shape, and texture of the pod, so that a selection on those characters is required in order to obtain homogenous lines. On quantitative characters, medium variability can be shown in numbers of pod per plant and weight per pod per plant, so that further selection is required in order to obtain lines that having high yield.

2- In homogenous lines, based on qualitative characters such as PQGK.1, PQGI.169, may follow consumer's preferential test and anthosianin test, by choosing individual that has high yield character.

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