

IMPROVING STORAGE ROOT PROTEIN CONTENT IN SWEET POTATO THROUGH OPEN-MATING POLLINATION

Sri Umi Lestari^{*)}, Ricky Indri Hapsari and Sutoyo

Faculty of Agriculture, Tribhuwana Tungadewi University
Jl. Telaga Warna Blok C Tlogomas Malang 65144 East Java Indonesia
^{*)} Corresponding author Phone: +62-341-565500 E-mail: sriumi.lestari@yahoo.com

Received: November 2, 2011/ Accepted: October 9, 2012

ABSTRACT

A field experiment was conducted at the Research Station of Brawijaya University from April to August 2009. An augmented randomized complete block design with seven blocks was utilized, seven parental clones as standard treatments and seven half-sib families as augmented treatments. Each block consisted of seven parental clones plus one randomly assigned family of half-sibs. In addition, the first block contained 29 accessions, the 2nd block had 120, the 3rd block had 194, the 4th block had 45, the 5th block had 28, the 6th block had 186, and the 7th block had 108. Ten cuttings of each parental was planted in each block, along with a single plant of each accession. There were five improved clones identified with high protein content and high yield, ranging from 6.00-8.21% dwb and 1.95 to 2.73 kg/plant, respectively. Broad-sense heritability and genetic advance of storage root weight, storage root number, percentage dry matter and protein content were high, whereas vegetative biomass and harvest index were low. The results showed that storage root weight, storage root number, percentage dry matter and protein content could be used as the basis for a crop improvement program in sweet potato.

Keywords: broad-sense heritability, expected genetic advance, improved clones, high yield, protein content, sweet potato

INTRODUCTION

Sweet potatoes (*Ipomoea batatas*) are usually known as a highly efficient crop producing carbohydrates (Kozai *et al.*, 1999), but generally have very low protein content (Bradbury, 1989),

on average less than 2.5% on a dry weight basis (dwb) for the storage root (Basuki *et al.*, 2002). Improvement of the quality traits, such as protein content, can be done if the traits showed variability (Peterson *et al.*, 1998). Lestari and Basuki (2007) stated that a wide range of protein content in a sweet potato population could be obtained by means of biparental crosses between five high yielding clones (D67, 73-6/2, Boko, JP-23, and JP-33) and two introductory clones (Beniazuma and BIS-214). This population had a protein content from 0 – 15.42% dwb.

Open-mating pollination of seven clones (D67, 73-6/2, Boko, JP-23, JP-33, Beniazuma and BIS-214) generated 1870 seeds, producing a number of accessions for a base population of sweet potato (Lestari *et al.*, 2009). However, this population has not been evaluated for its agronomic and genetic performance. New accessions with high yield (≥ 0.75 kg/plant) and high protein content ($\geq 7\%$ dwb) will be selected from this population. Food materials having 10-15% protein energy (PE) ratio can be used as sources of protein (Bradbury, 1989). To reach a PE ratio value of 10%, a sweet potato clone has to contain at least 3% protein. However, AVRDC recommended improving the protein content to greater than 7% (AVRDC, 1982). For genetic performance, the genetic variability, coefficient of genetic variation, heritability and genetic advance of the population should be evaluated. According to Fisher *et al.* (1989), the selection response of the traits depends on genetic variability and heritability. Likewise, a coefficient of genetic variation and genetic advance are also needed by breeders for selection of new genotypes and crop improvement programs (Murthy *et al.*, 2010).

In earlier studies, Johnson *et al.* (1955) proved that the estimate of heritability together with the genetic advance was more beneficial

Accredited SK No.: 81/DIKTI/Kep/2011

<http://dx.doi.org/10.17503/Agrivita-2012-34-3-p225-232>

than just using heritability value in estimating the effects of a selection. High genetic advance coupled with high heritability estimates offers the most suitable conditions for selection (Larik *et al.*, 1989). Heritability and genetic advance on yield potential and quality are prerequisites for effective crop improvement (Waqar-UI-Haq *et al.*, 2008). This study reports the performance of improved clones and the genetic variation, heritability, and genetic advance in sweet potato populations resulting from an open-mating pollination.

MATERIALS AND METHODS

Testing Materials

Seven hundred and ten sweet potato accessions from an open-mating pollination of seven parental clones were evaluated. This accessions were classified in seven half-sib families according to their female parent: D67-OP (29 accessions), 73-6/2-OP (120 accessions), Boko-OP (194 accessions), JP-23-OP (45 accessions), JP-33-OP (28 accessions), Beniazuma-OP (186 accessions), and BIS-214-OP (108 accessions). Five female parent clones (D67, 73-6/2, Boko, JP-23, and JP-33) among the seven parental clones had a high yield (0.70-1.14 kg/plant) and two other clones (Beniazuma and BIS-214) had a protein content ranging between 4.90 - 6.00% dwb.

Experimental Design

This experiment was conducted at the Research Station of Brawijaya University, Malang, Indonesia, from April to August (in the dry season) in 2009. A field experiment arranged in an Augmented Randomized Complete Block Design with seven blocks. All accessions (710 genotypes), comprising seven half-sib populations, were used as test cultivars with the seven female parents as standard cultivars. The test cultivars were planted as single plants distributed to each of seven blocks at random according to their half-sib family. Therefore, each block was comprised of all parental clones and a half-sib family. Ten cuttings of each parental clone were planted in each block, along

with a single plant of each accession at a spacing of 75 cm in the row and 100 cm between rows. The number of rows in each block was not same, but was dependent on the number of accessions in each half-sib population. The first block from D67-OP contained 29 accessions, the 2nd block from 73-6/2-OP had 120, the 3rd block from Boko-OP had 194, the 4th block from JP-23-OP had 45, the 5th block from JP-33-OP had 28, the 6th block from Beniazuma-OP had 186, and the 7th block from BIS-214-OP had 108. Several traits such as fresh weight of storage roots, storage root number, % dry matter, protein content, biomass fresh weight and harvest index were measured from all parental clones and a single plant of each accession. Harvest index was measured as the ratio between storage root dry weight and total fresh weight, reported as the percent dry weight.

Data Analysis

The analysis of standard cultivars carried out to estimate the influence of environmental factors (σ^2_E), whereas the test cultivars used to estimate the phenotypic variance (σ^2_P). The analysis of variance based on a technique proposed by Sharma (2006) (Table 1). If there was a significant difference among the tested cultivars, selected genotypes (\bar{Y}_v) were compared against the average of the standard cultivars (\bar{Y}_c) by the value of Least Significant Increase (LSI). If $\bar{Y}_v > (\bar{Y}_c + LSI)$, a significance at level $\alpha = 0.05$ or at $\alpha = 0.01$ was evident (Petersen, 1994), and the selected genotypes were the improved clones.

$$LSI = t_{\alpha} \sqrt{\frac{2 \times M_6}{n}}$$

where: t_{α} = t value from Student's t Distribution.

According to Ram *et al.* (1997), environmental variance (σ^2_E) obtained from a mean square of error of the experiments with standard cultivars and is equal to M_6 (Table 1).

Table 1. Analysis of variance based on the augmented randomized complete block design (Sharma, 2006)

Source of variation	df	Mean Square	Computed F
Block	(b - 1)	M ₁	M ₁ /M ₆
Entries	(c + v - 1)	M ₂	M ₂ /M ₆
C (standard cultivar)	(c - 1)	M ₃	M ₃ /M ₆
V (tested cultivar)	(v - 1)	M ₄	M ₄ /M ₆
C x V	1	M ₅	M ₅ /M ₆
Error	(b - 1)(c - 1)	M ₆	
Total	N - 1		

$$CV = \frac{\sqrt{M_6}}{\bar{Y}} \times 100; \quad CV \sigma_g^2 = \frac{\sqrt{\sigma_g^2}}{\bar{Y}} \times 100; \quad \bar{Y} = \text{mean of a trait measurement}$$

The following steps, the phenotype variance (σ_P^2) calculated using the procedure of Ram *et al.* (1997) :

Calculating the effect of the j^{th} block = (b_j),

$$(b_j) = \frac{1}{C} (Y_{.j} - \sum \bar{Y}_{ij} - \sum \chi_{ij})$$

where:

C = No. of standard cultivars (= 7)

$Y_{.j}$ = Block total over standard cultivars (C) and tested cultivars (V)

\bar{Y}_{ij} = Means of standard cultivars

χ_{ij} = Total of values of tested cultivars in j^{th} block

Adjusted value for a tested cultivar = Phenotypic value (x_i)

$$x_i = x_i - b_j$$

where:

x_i = Observed value (unadjusted)

b_j = Block effect

Phenotypic variance (σ_P^2),

$$\sigma_P^2 = \frac{1}{v-1} \left\{ \sum x_i^2 - (\sum x_i)^2 / v \right\}$$

$$\text{Genotypic variance } (\sigma_g^2) = \sigma_P^2 - MS_E = \sigma_P^2 - M_6$$

$$\text{Broad-sense heritability } (H^2) = \sigma_g^2 / \sigma_P^2;$$

$$\text{Expected Genetic Advance } (\Delta G) = i \sigma_P H^2$$

Where $i = 1.40$ for a 20% selection differential.

The values of H^2 and ΔG were jointly used to determine the observed traits can be used as the basis for selection in the sweet potato breeding program.

RESULTS AND DISCUSSION

Half-sib Population Distribution

The distribution within seven half-sib families of sweet potato showed a wide diversity of storage root protein content and root yield (Figure 1). The wide diversity of the traits was also indicated by the coefficients of genetic variation of 27.51% and 58.40% (Table 2) and genetic variance of 0.37 ± 0.21 and 0.19 ± 0.12 (Table 4), respectively.

From the distribution within half-sib populations (Figure 1), five clones found having combined storage root protein content ($\geq 6\%$ dwb) and high yield potential (± 1.50 kg/plant). Both of traits were phenotypic value of protein content and storage root yield.

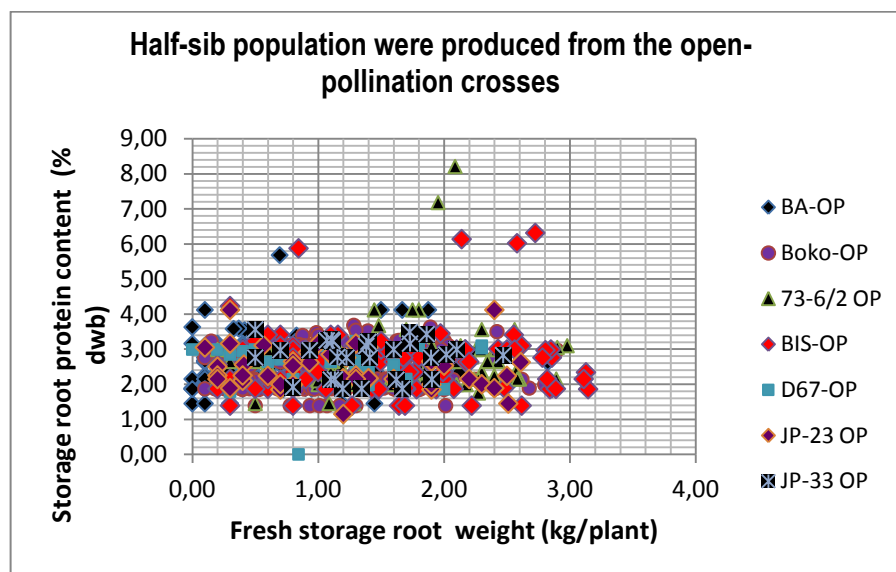


Figure 1. Half-sib population produced through open-mating pollination among seven clones

Table 2. ANOVA of the protein content and fresh storage root weight/plant in half-sib populations

Source of variation	df	Mean Square of	
		Storage root protein content (% dwb)	Fresh storage root weight (kg/plant)
Block	6	10.12**	8.93**
Entries	716	1.71**	0.57**
C (standard cultivars)	6	19.55**	0.34**
V (tested cultivars)	709	1.21**	0.57**
C x V	1	248.64**	0.45**
Error	36	0.04	0.01
Total			
		CV = 20.03	CV = 11.47
		CV σ^2_g = 27.51	CV σ^2_g = 58.40
		\bar{Y} = 2.05	\bar{Y} = 0.89

Remarks: **) significant differences at $\alpha = 1\%$ level

The Augmented Randomized Complete Block Design used in a trial provided a high accuracy as indicated by relatively low coefficients of experimental variation (CV) for both traits, ranging from 11% to 20% (Table 2). Thus, the use of the Augmented Randomized Complete Block Design considered sufficient for an experiment as a preliminary evaluation, with a number of seeds that are usually not sufficient for an experimental design with replication. Federer and Agavarao (Petersen, 1994) further

described that the Augmented Randomized Complete Block Design was developed by Federer (1961). Using sugarcane, Federer (1961) proposed the application of the Augmented Design as a solution to address the need to limit the number of genotypes used in testing in a field. The Augmented Design is the expansion of the use of the Randomized Block Design using standard cultivars grown in all the blocks. New cultivars planted in each block with no replication. Statistical analysis of the standard cultivars then

used to correct the performance of new cultivars for a valid comparison.

Performance of Improved Clones

The protein content and fresh storage root yield of sweet potato showed highly significant difference among the tested cultivars (Table 2). Performance of some accessions was very encouraging because they showed high protein content and high-yield. Yield of the accessions varied from 0 to 3.15 kg/plant and protein content varied from 0 to 8.21% dwb (Fig.1). Some of the accessions (D67 and 73-6/2) were compared reasonably well with the high yield check cultivars, which averaged 0.85 and 1.14 kg storage root yield/plant, respectively, whereas check cultivars with high protein content, Beniazuma and BIS-214, ranging from 4.86 to 6.00% dwb. Of the entries, 44.37 % yielded higher than 1.1 kg/plant but only 0.70 % had a protein content higher than 6% dwb. There were only five clones with both high protein content and high yield. These clones were BIS OP-4, BIS OP-45, BIS OP-61, 73-6/2 OP-5 and 73-6/2 OP-8, and the all generated from female BIS-214 and 73-6/2 plants.

When the female parent clones carrying the protein content traits (Beniazuma and BIS-214) and having high yield potential (D67 and

73-6/2) compared with the improved clones, the five selected clones showed highly significant difference from their female parent (Table 3). This was demonstrated by the mean of the traits that were larger than the means of the standard cultivars ($\bar{Y}_V > \bar{Y}_C + LSI$). As these values derived from the data of this single plant experiment, further testing with greater replication trials are still required.

The storage root protein content of the improved clones ranged from 6% to 8% dwb (Table 3), encouraging for efforts to improve sweet potato varieties having high protein content for Indonesia. Currently, the average protein content of sweet potato varieties in Indonesia is less than 2.5% dwb (Basuki *et al.*, 2002), while the improved clones had a 3-fold greater protein content. Further research needed to evaluate the stability of the protein content of the improved clones when planted in several locations and seasons.

Furthermore, phenotypic values can be verified by how much genetic variance existed for the traits concerned. The genetic analysis of the observed traits are presented in Table 4. There was wide genetic diversity for all traits except biomass fresh weight and harvest index.

Table 3. Protein content and fresh storage root weight of five improved clones

Clone	Protein Content (% dwb)	Fresh Storage Root Weight (kg/plant)
BIS OP-4	6.32**	2.73**
BIS OP-45	6.02 *	2.58**
BIS OP-61	6.14**	2.14**
73 OP-5	8.21**	2.09**
73 OP-8	7.18**	1.95**
$\bar{Y}_C + LSI_{0,05}$		
$\bar{Y}_C + LSI_{0,01}$ (Beniazuma)	4.93	
	4.95	
$\bar{Y}_C + LSI_{0,05}$		
$\bar{Y}_C + LSI_{0,01}$ (BIS-214)	6.07	
	6.09	
$\bar{Y}_C + LSI_{0,05}$		
$\bar{Y}_C + LSI_{0,01}$ (D67)		0.89
		0.90
$\bar{Y}_C + LSI_{0,05}$		
$\bar{Y}_C + LSI_{0,01}$ (73-6/2)		1.18
		1.19

Remarks: *) significant differences at 5% level; **) significant differences at $\alpha = 1\%$ level

Estimation of Genetic Variance Components

There is a need to know whether genetic variations of a trait significantly different and its variation were wide or narrow. There was a large genetic variability for all traits except biomass fresh weight and harvest index, with greater than one or two standard errors ($\sigma_{\sigma^2_g}$) (Table 4). Pinaria *et al.* (1995) stated that if $\sigma_{\sigma^2_g}^2 > \sigma_{\sigma^2_g}$ or $\sigma_{\sigma^2_g}^2 > 2 \sigma_{\sigma^2_g}$, genetic variability is large, but if $\sigma_{\sigma^2_g}^2 \leq \sigma_{\sigma^2_g}$, there is narrow genetic diversity.

From open-mating pollination with high yielding parent clones and high protein clones generated a wide genetic variation of both traits. Thus, to create extensive genetic variation in protein content and high yield through open-mating pollination can involve parental clones Beniazuma and BIS-214 because they are introductory clones. As indicated by Li (1982),

crossing with introductory clones provided extensive genetic variation for generating new clones.

Estimates of Heritability and Genetic Advance

Estimation of genetic parameters for key traits is necessary in order to improve the traits, included heritability and expected genetic advance. Estimates of heritability provide information about how much of a character transmitted to subsequent generations. Information on heritability and genetic advance can give guidelines for breeders in predicting the behavior of key characters in offspring and estimate how much they respond to selection. The availability of the estimated value of heritability, together with the genetic advance for each trait, is a prerequisite for effective crop improvement (Waqar-UI-Haq *et al.*, 2008).

Table 4. Genetic variance (\pm standard errors) of fresh storage root weight, storage root number, % dry weight, protein content, biomass fresh weight, and harvest index

Observed trait	$\sigma_{\sigma^2_g}^2 \pm \sigma_{\sigma^2_g}$	$\sigma_{\sigma^2_g}^2 \pm 2 \sigma_{\sigma^2_g}$	Criteria
Fresh storage root weight (kg/plant)	0.19 ± 0.12	0.19 ± 0.24	Wide (*)
Storage root number (root number/plant)	0.93 ± 0.39	0.93 ± 0.78	Wide (**)
% Dry weight	23.32 ± 2.34	23.32 ± 4.68	Wide (**)
Protein content (% dwb)	0.37 ± 0.21	0.37 ± 0.42	Wide (*)
Biomass fresh weight (kg/plant)	0.00 ± 0.16	0.00 ± 0.32	Narrow (Ns)
Harvest index	0.00 ± 0.13	0.00 ± 0.26	Narrow (Ns)

Remarks: ($\sigma_{\sigma^2_g}^2$) = genotypic variance ($=\sigma_P^2 - M_6$); $\sigma_{\sigma^2_g}$ = standar errors of genotypic variance, ns = not significant; * = genetic variance significant at 5% level >1 standard error; ** = genetic variance significant at 1% level > 2 standard error

Table 5. Estimated broad-sense heritability (H^2), expected genetic advance (ΔG) and mean of traits measurement in sweet potato

Traits	H^2	Criteria	CV $\sigma_{\sigma^2_g}^2$ (%)	ΔG^*	\bar{Y}	Criteria
Fresh storage root weight/plant	0.93	high	58.40	0.59 (106.57%)	0.55	high
Number of storage roots/plant	0.87	high	59.21	1.26 (47.55%)	2.64	high
% Dry matter of storage roots	0.58	high	88.56	5.16 (17.35%)	29.73	high
Storage root protein content (% dwb)	0.90	high	27.51	0.81 (16.59%)	4.86	high
Biomass fresh weight/plant	0.00	low	0.00	0.00 (0.00%)	1.04	low
Harvest index	0.00	low	0.00	0.00 (0.00%)	44.40	low

Remarks: H^2 = Broad-sense heritability; CV $\sigma_{\sigma^2_g}^2$ = Coefficient of genetic variation; ΔG = expected genetic advance
* ΔG in unit parameter of each observation and the percent stated in (...); \bar{Y} = mean of a trait measurement

Broad-sense heritability and expected genetic advance of each trait presented in Table 5. Heritability values describe the genetic variance proportion of the phenotype variance of each trait. Heritability values for the fresh storage root weight, storage root number, percentage dry matters, and protein content were high, greater than 50%. According to McWhirter (1979), the value of heritability can be classified into high heritability ($H^2 > 50\%$), medium heritability ($20\% < H^2 < 50\%$), and low heritability ($H^2 < 20\%$). Estimates of heritability are used as a tool to describe the reliability of a phenotype, and could help plant breeders make selections of a particular trait when that trait has a large heritability (Unche *et al.*, 2008).

The genetic advance of the traits was high except for biomass per plant and harvest index. Based upon the opinions of Fehr (1987) stating that if the value of ΔG is 0 - 3.30%, genetic advance is low, if it is 3.31 - 6.60 %, genetic advance is slightly lower, if it is 6.61 - 10 %, genetic advance is rather high, and if it is $> 10\%$, genetic advance is higher. Thus, based on heritability and ΔG , genetic advance should be considered high for storage root fresh weight, storage root number, percentage dry matter and protein content. High genetic advance coupled with high heritability estimates offer the most suitable conditions for selection (Larik *et al.*, 1989). Therefore, the availability of good information on heritability and genetic advance in observed traits is a prerequisite for effective crop improvement (Waqar-UI-Haq *et al.*, 2008).

CONCLUSIONS

Five improved clones (BIS OP-4, BIS OP-45, BIS OP-61, 73-6/2 OP-5 and 73-6/2 OP-8) selected from half-sib populations which were generated through open-mating pollination in preliminary trials, had high protein content (6.00-8.21% dwb) and high yield (1.95-2.73 kg/plant). The fresh storage root weight, storage root number, percentage dry matter, and protein content could used as the basis for a crop improvement program in sweet potato.

ACKNOWLEDGMENTS

We thank to the Directorate General of Higher Education, the Ministry of National Education of The Republic Indonesia for financial support through the competitive research grants in 2009 and 2010.

REFERENCES

- AVRDC.1982. Progress report for 1981. Shan-hua, Tainan, Taiwan, Republic of China. pp. 84
- Basuki, N., Harijono, Damanhuri, S.S. Antarlina, and Y.Widodo. 2002. Nutrient identification in sweet potato germplasm to support agro-industry (*In Indonesian*). Jurnal Ilmu-ilmu Hayati (Life Sciences) 14 (1): 94-105.
- Bradbury, J.H. 1989. Chemical composition of cooked and uncooked sweet potato and its significance for human nutrition. *In* Sweet potato research and development for small farmers. Ed. by K.T. Mackay, M.K. Palomar, and R.T. Sanico. SEAMEO - SEARCA, Laguna, The Philippines. p.183-195.
- Federer, W.T.1961. Augmented designs with one-way elimination of heterogeneity. *Biometrics* 17: 447-473.
- Fehr, W.R. 1987. Principles of cultivar development, theory and technique. Macmillan Publishing Co. New York.
- Fisher, R.A., L. O'brien and K.J. Quail. 1989. Early generation selection in Wheat II Grain quality. *Aust.J.Agric.Res.* 40(6): 1133-1142.
- Johnson, H.W., H.F. Robinson and R.E. Comstock. 1955. Genotypic and phenotypic correlation in soybean and their implications in selection. *Agron.J.* 47: 477-483.
- Kozai, T., C.Chun, K.Ohyama, T.Hoshi, F. Afreen, S.Zobayed, and C.Kubota. 1999. Transplant production in closed systems with artificial lighting for solving global issues on Environment Conservation, Food, Resource and Energy. p. 31-45. *In* Proc. ACESYS 111 Conference, Rutgers University, Piscataway, NJ.

- Larik, A.S., H.M.I. Hafiz and A.M. Khushk. 1989. Estimation of genetic parameters in wheat populations derived from inter-cultivar hybridization. *Pakphyton*.1: 51-56.
- Lestari, S.U., R.I. Hapsari, and Sutoyo. 2009. Evaluation and selection of open-mating pollination in protein rich of sweetpotato (*In Indonesian*). Research Report on Competitive Grant for First Year. University Tribhuwana Tungadewi. Malang.
- Lestari, S.U. dan N. Basuki. 2007. Breeding of sweet potato clones for high protein yield (*In Indonesian*). *Buana Sains*. 7(1): 61-70.
- Li, I. 1982. Breeding for increased protein content in sweetpotatoes. Ed. by R.L. Villareal and T.D. Griggs. Proceeding of the first international symposium. AVRDC.
- McWhirter, K.S. 1979. Breeding of cross-pollinated crops. *In*: G.M. Halloran, R. Knight, K.S. McWhirter, D.H.B. Sparrow, (Eds.). *Plant Breeding*. Australian Vice-Chancellors Committee Brisbane. pp. 255
- Murthy, B.C.K., H.P. Puttaraju and S. Hittalmani. 2010. Genetic variability and correlation studies in selected mulberry (*Morus* spp.) germplasm accessions. *Electronic Journal of Plant Breeding*, 1(3): 351-355.
- Peterson, C.J., R.A. Graybosch, D.R. Shelton and P.S. Banziger. 1998. Baking quality of hard winter wheat: response of cultivars to environment in the great plains. *Euphytica*. 100: 157-162.
- Pinaria, S., A. Baihaki, R. Setiamihardja and A.A. Daradjad. 1995. Genetic variability for biomass characters in 53 soybean genotypes (*In Indonesian*). *Zuriat* 6(2): 88-92.
- Ram, B., B.S. Chaudary, and D.K. Yadav. 1997. General and specific selection indices for single stool stages of selection in sugarcane. *Euphytica* 95: 39-44.
- Sharma, J.R. 2006. Statistical and biometrical techniques in plant breeding. Reprint. New Age International Ltd. Publishers, New Delhi.
- Unche P.B, M.B., Misal, S.B. Borgaonkar, G.V. Godhawale, B.D. Chavan, and D.R. Sawant. 2008. Genetic variability studies in sweet sorghum (*Sorghum bicolor* L. Moench). *Int. J. Plant Sci.* 3(1): 16-18.
- Waqar-Ul-Haq, M.F. Malik, M. Rashid, M. Munir and Z. Akram. 2008. Evaluation and estimation of heritability and genetic advancement for yield related attributes in wheat lines. *Pak.J.Bot.* 40(4): 1699-1702.