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TRIPLE TEST CROSS ANALYSIS OF SOME PHYSIO-MORPHOLOGICAL TRAITS IN BASMATI RICE (*ORYZA SATIVA* L.)

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Abstract

The genetic basis of flag leaf area, days to flowering, seed weight per panicle, biological yield per plant, harvest index and yield per plant were investigated using triple test cross analysis in Basmati rice. Epistasis was detected for all the traits except biological yield per plant. Partition of epistasis into i (additive x additive) and j + l (additive x dominance + dominance x dominance) types showed that epistasis of i and j + l types were involved in the expression of those traits. Expression of epistasis was dependent on particular cultivars. Various lines contributed significant and positive epistatic deviations to the total epistasis. Additive (*D*) and dominance (*H*) genetic components controlled the manifestation of biological yield per plant. However, partial dominance was revealed by degree of dominance $(H/D)^{1/2}$ for this trait. Direction of dominance (*rs.d*) was non-significant for biological yield per plant showing absence of directional dominance. Due to influence of epistatic effects for majority of the traits, recurrent selection may be recommended to develop high yielding Basmati rice varieties.

Introduction

Basmati rice (*Oryza sativa* L. *2n = 2x = 24*) being an exportable commodity, fetches higher prices in the world market. During 2006-07, Pakistan exported 907.9 million tonnes of Basmati rice with foreign exchange return of 422 million US $ (Anon., 2006-07). In spite of its high value and demand around the world, there has not been much progress in the development of high yielding varieties in Pakistan. During the past 10 years, only 2 Basmati rice varieties viz., Basmati 2000 and Shaheen Basmati were released (Ahmad & Akram, 2006) for general cultivation. However, cultivar Super Basmati released in 1996 is predominantly grown over an area of 71% in Basmati growing tract (Anon., 2008-09). One of the main constraints is lack of reliable genetic information about the inheritance of yield and yield components on which breeding methodology is framed out. Different biometrical techniques viz., biparental cross (Comstock & Robinson, 1948, 1952), diallele and partial diallele cross (Hayman, 1954) and line x tester cross (Kempthorne, 1957) have been developed which provide information about additive and dominance genetic variances and fail to produce information about epistasis variance because their procedures are based on certain genetical assumptions including absence of non-alleleic interactions (Mather & Vines, 1952; Ospal, 1956; Sing & Sing, 1976). Some other biometrical tools viz. generation mean analysis (Hayman, 1958; Jinks & Jones 1958), triallele and quadriallele analysis (Rawlings & Cokerham, 1962a & b) and triple test cross (Kerasy & Jinks, 1968) provide information about all three components of genetic variance i.e., additive, dominance and epistatic variances. Triple test cross (TTC) design developed by Kearsey & Jinks (1968) is an extension of North Carolina Design III of Comstock & Robinson (1952) that is applicable to any population irrespective of its mating system and its gene and genotype frequencies (Kearsey & Jinks (1968). In the absence of epistasis TTC also provides

unbiased estimates of additive (*D*) and dominance (*H*) components of genetic variation, degree of dominance [$(H/D)^{1/2}$] as well as the direction of dominance (*rs.d*) with high degree of precision (Kerasy & Jinks, 1968). Ketata *et al*., (1976) suggested a similar model of TTC where testers L_1, L_2 and L_3 (L_3=L_1 x L_2) were crossed to a number of varieties instead of F_2 individuals as proposed by Kerasy & Jinks (1968).

The present study aims to detect epistasis along with estimation of additive and dominance components for different physio-morphological traits; genetically least exploited traits in Basmati rice. The information obtained through present study would help in understanding the genetic basis of the traits studied and making breeding strategy for the development of high yielding cultivar (s) or valuable germplasm in Basmati rice.

Materials and Methods

Triple test cross (TTC) study was conducted from May, 2003 to June, 2008 at nuclear institute for agriculture and Biology (NIAB), Faisalabad, Pakistan. The breeding material comprised of 12 pure Basmati rice genotypes. Among them two genotypes viz, Basmati-385 (parent, P_1) and EL-30-2-1 (parent, P_2) hereafter referred to as tester L_1 and L_2 were crossed to produce F_1 hybrid (Basmati-385 × EL-30-2-1) during 2003. F_1 hybrid was designated as third tester L_3. Testers were used as female parents whereas lines were used as male parents in TTC experiment. Three testers (L_1, L_2 and L_3) and 10 true breeding lines viz., Basmati-370, DM-2, DM-107-4, DM-16-5-1, Kashmir Basmati, Basmati-Pak, Basmati 2000, Super Basmati, Shaheen Basmati and DM-25 were grown in crossing blocks. Depending upon flowering stage, crosses were made according to TTC fashion (Ketata *et al*., 1976). In total, 30 crosses (20 single and 10 three-way) were generated. Seeds were harvested after 25 to 30 days of pollination. Nursery seedlings of 43 genotypes (30 crosses + 13 parents) were raised and then transplanted into the field. The experiment was laid out following randomized complete block design with three replications. Each genotype was planted in two rows of 2 m each. They were surrounded by two rows of non-experimental rice varieties to minimize the boarder effect. Single seedling was planted at a distance of 20 cm each between and within the rows. Fertilizer was applied @ 100 kg ha^{-1} of Nitrogen (N) and 50 kg ha^{-1} of Phosphorus (P). Half of the N was applied at the time of transplanting while the remaining half in two increments: ¼ after 30 days of transplanting and other ¼ after 60 days of transplanting. All P was applied with nursery transplanting. Stable irrigation supply was ensured for raising of good crop. Water level of 2.5 to 4.0 cm at the time of transplanting was gradually increased to 8.0 cm. After interval of 7 to 10 days, irrigation was discontinued for few days to provide proper aeration and then re-continued. After 4 to 5 days of transplanting, Machette 60 EC (Butachlor) at the concentration of 2 l ha^{-1} and Roanstar (Oxadiazon) @ 3.5 l ha^{-1} were applied to eradicate the weeds. Insect leaf roller and stem borer were controlled by using Talstar 10 EC (biphenthrin) and Padan 4G (cartap) at the rate of 500 ml ha^{-1} and 20 kg ha^{-1}, respectively.Ten random plants were selected from each genotype in each replication and data were recorded for flag leaf area, days to flowering, seed weight per panicle (g), biological yield per plant, harvest index (%) and grain yield per plant (g). Triple test cross analysis was done according to modified method (Ketata *et al*., 1976) as illustrated by Singh & Chaudhary (1985) and Khattak *et al*., (2002).

Results and Discussion

Highly significant differences in genotypes, hybrids, parents, lines and testers were noted for all the traits indicating presence of considerable variability among the genotypes

(Table 1). The significant mean squares of P_1 vs. P_2 and $P_1 + P_2$ vs. F_1 for all the characters showed existence of worth of variations between testers (L_1 and L_2). High differences between L_1 and L_2 resulted into expression of high mean performance of their F_1 (L_3) as revealed by significant mean squares due to $P_1 + P_2$ vs. F_1. Since two testers represented highly significant differences for each character, therefore they would provide precise estimates of additive and dominance variance as reported by Kearsey & Jinks (1968). Except harvest index, lines vs. testers were highly significant for all traits. Similarly hybrids vs parents were also highly significant for all characters except harvest index.

Flag leaf area, days to flowering, seed weight per panicle, harvest index and yield per plant showed highly significant total epistasis except biological yield per plant (Table 2). Current results were in conformity to findings of many researchers: In barley, Prakash *et al*., (2004) observed significant epistatic effects for flag leaf area. Epistatic effects were found for days to flowering by Kulshreshtha *et al*., (1993) and Saleem *et al*., (2005b) in rice. Non-allelic interactions were also documented for harvest index (Verma *et al*., 1994) and yield per plant (Saleem *et al*., (2005a) in rice. Although mean squares of biological yield per plant (Table 1) were highly significant yet epistasis (Table 3) could not be detected. The total epistasis calculated as uncorrected genotypes (lines) sum of square $[\sum(L_{1j}+L_{2j}-2L_{3j})2]/n$ (j =genotypes and n = no. of replications) on the total of the replications for 10 degree of freedom was non-significant for this trait. The absence of epistasis for biological yield per plant in present study contradicted with the findings of Pandey & Singh (2003) where they reported presence of non-allelic interaction. Existence of non-allelic interactions found in current study for days to flowering and yield per plant were inconsistent with Ahmed *et al*., (1985) who recorded predominant role of additive gene action from two experiments in rice using TTC analysis. Division of total epistasis into i and j + l types of epistasis indicated the presence of i type and j + l types epistasis for flag leaf area, days to flowering, seed weight per panicle, harvest index and yield per plant (Table 2). The i type epistasis was found to be much larger in magnitude than j + l types of epistasis indicating the predominant role of i type non-allelic interactions in the inheritance of these traits. The results showing i and j + l types epistasis were in complete harmony with Subbaraman & Rangaswamy (1989) for yield per plant. Epistasis of i type for days to flowering was also recorded by Saleem *et al*., (2005b).

The existence of non-allelelic interactions for economic characters might have important inferences in plant breeding. The i type epistasis represents fixable while j + l types show non-fixable portions of genetic variations (Mather, 1949). Ketata *et al*., (1976) proposed that standard hybridization and selection procedures could take benefit of epistasis if it is i type epistasis (additive x additive) whereas j + l types of epistasis (additive x dominance + dominance x dominance) are not fixable by selection under self fertilization, and therefore they would not be favourable for developing pure lines. Ketata *et al*., (1976) and Subbaraman & Rangasamy (1989) reported that j + l types of non-allelic interactions could be useful in the development of hybrids. In the development of pure line cultivars, the masking effect of epistasis is of no importance if selection is postponed until virtually homozygosity is achieved because only additive type of epistasis is present in pure lines (Ketata *et al*., 1976). Because of additive and fixable nature of i type epistasis; it has more importance for the development of pure cultivars than j + l types of epistasis in cereals (Subbaraman & Rangasamy, 1989: Dhiman *et al*., 1999). In Basmati rice and wheat where the production of hybrids on industrial scale is still faraway to be used (Sing *et al*., 2000; Esmail, 2007) the fixable component of i type epistasis could be easily exploited for breeding of high yielding varieties (Subbaraman & Rangasamy, 1989; Esmail, 2007).

The results have indicated presence of i and j + l types of epistasis for six characters including grain yield. In this condition recurrent selection technique is suggested. This technique has also been proposed for non-allelic inherited traits in rice (Subbaraman & Rangaswamy, 1989). However, selection of plants for trait(s) to be improved may be delayed till F_6 or F_8 generations to allow fixation of epistasis effects i.e., fixation of homozygosity for the majority of the loci, as proposed by Subbaraman & Rangaswamy (1989) in rice and Pandey & Singh (2003) in wheat. In case of delayed selection, single seed descent method (SSD) may be the choice of breeders to reduce the space and other inputs. The interaction of total, i type and j + l types of epistasis with replications were non-significant which indicated that these interactions were not sensitive to the environments (replications). These results were in line with those of many researchers (Kulshreshtha *et al*., 1993; Verma *et al*., 1994; Prakash *et al*., 2004; Saleem *et al*., 2005a; Saleem *et al*., 2005b) in barely and rice.

The epistatic deviations of individual lines (Table 3) have showed that lines viz., Super Basmati, Basmati-Pak and DM-107-4 for flag leaf area; Shaheen Basmati and Basmati-Pak for days to flowering; Kashmir Basmati for seed weight per panicle; Super Basmati for harvest index and Basmati 2000 for yield per plant had significant contributions to the total epistasis. All these lines displayed a significant positive role towards the total non-allelic interactions. The remaining lines did not add significantly to the total epistasis. In earlier studies, Ketata *et al*., (1976) and Khattak *et al*., (2001) identified different lines contributing positive and negative roles to the total epistasis. Although epistasis is an integral part of genetic architecture however, it was not detected for biological yield per plant. Ketata *et al*., (1976) stated that epistasis is determined by the nature of genotypes and to some degree by the number of lines used. Pooni *et al*., (1980) highlighted that the best possible experimental size necessary to detect epistasis through TTC depends mostly on gene distribution in the tester parents, therefore several lines and diverse testers (L_1 and L_2) should be employed to detect epistasis.

The estimate of additive and dominance variance, degree of dominance and direction of dominance for the trait showing absence of epistasis has been presented in Table 4. Additive and dominance components were highly significant for biological yield per plant. Degree of dominance [$(H/D)^{1/2}$ = 0.55] showed partial or incomplete dominance for biological yield per plant. Delayed selection of plants having high biological yield per plant would be rewarding. The direction of dominance (rs.d) for biological yield per plant was non-significant which showed that dominant alleles were dispersed between testers; therefore they did not show any proof of directional dominance for this character.

The present study revealed that epistasis is an integral part of genetic system in Basmati rice for flag leaf area, days to flowering, seed weight per panicle, harvest index and grain yield per plant, therefore recurrent selection may be recommended for cultivar development from present TTC population. Additive and dominance components of variation were important for the genetic control of biological yield per plant.

Table 4. Genetic components controlling biological yield per plant in rice genotypes.

Trait	*D*	*H*	$(H/D)^{1/2}$	*rs.d*
Biological yield per plant (g)	1057.41**	321.35**	0.55	-0.46

*, ** = Significant at 0.05 and 0.01 levels of probability, respectively.

References

Ahmad, C.M. and M. Akram. 2006. *Hand Book on rice varieties in Pakistan*. Rice Res. Inst., KSK, Lahore.

Ahmad, L., A.H. Zakri, B.S. Jalani and D. Omar. 1985. Detection of additive and dominance variation in rice. *In*: *Rice Genetics-I. Proc. Int. Rice Genet. Symp*. IRRI, Manila, Philippines. pp.555-564.

Anonymous. 2006-07. *Agricultural Statistics of Pakistan*. Govt. of Pakistan, Ministry of Food and Agriculture and Livestock, Pakistan.

Anonymous. 2008-09. Year-wise area '000' acres & %age of rice in the Punjab for the year 2001-2002 to 2008-2009. Crop Reporting Institute, Lahore.

Comstock, R.E. and H.F. Robinson. 1948. The components of genetic variance in population of biparental progenies and their use in estimating average degree of dominance. *Biometrics*, 4: 254-266.

Comstock, R.E. and H.F. Robinson. 1952. Estimation of average dominance of genes. *In: Heterosis*. Iowa State College Press, Ames: 494-516.

Dhiman, K.C., T. Dawa and T. Dawa. 1999. Genetic architecture of yield traits in bread wheat. *Crop Improv*., 26: 193-197.

Esmail, R.M. 2007. Detection of genetic components through triple test cross and line x tester analysis in bread wheat. *World J. Agric. Sci*., 3(2): 184-190.

Hayman, B.I. 1954. The theory and analysis of diallele crosses. *Genetics*, 39: 789-809.

Hayman, B.I. 1958. The separation of epistatic variation from additive and dominance variation in generation means. *Heredity*, 12: 371-390.

Jinks, J.L. and R.M. Jones. 1958. Estimation of components of heterosis. *Genetics*, 43: 223-234.

Kearsey, M.J. and J.L. Jinks. 1968. A general method of detecting additive, dominance and epistatic variation for biometrical traits. I. Theory. *Heredity*, 23: 403-409.

Kempthorne, O. 1957. *An introduction to genetic statistics*. John Wiley and Sons Inc., New York.

Ketata, H., E.L. Smith, L.K. Edwards and R.W. McNew. 1976. Detection of epistasis, additive and dominance variation in winter wheat. *Crop Sci*., 16: 1-4.

Khattak, G.S.S., M.A. Haq, M. Ashraf and T. McNeilly. 2001. Genetic basis of variation of yield and yield components in mungbean (*Vigna radiata* (L.) Wilczek). *Hereditas*, 134: 211-217.

Khattak, G.S.S., M.A. Haq, M. Ashraf, A.J. Khan and R. Zamir. 2002. Genetic architecture of secondary yield components in mungbean (*Vigna radiata* (L.) Wilczek). *Breeding Sci*., 52: 235-241.

Kulshreshtha, N., S.C. Mani and S. Chandra. 1993. Triple test cross analysis for yield and yield components in rice (*Oryza satva* L.). *Ind. J. Genet*., 53: 243-246.

Mather, K. 1949. *Biometrical Genetics*. Methuen and Co. Ltd., London.

Mather, K. and A. Vines. 1952. The inheritance of height and flowering time in a cross of *Nicotiana rustica*. *Quantitative Inheritance*, pp. 45-80.

Opsal, B. 1956. The discrimination of interactions and linkage in continuous variation. *Biometrics*, 12: 415-432.

Pandey, D. P and M. Singh. 2003. Triple test cross in bred wheat (*Triticum aestivum* L.). *Crop Res. Hisar*, 26: 473-476.

Pooni, H. S., J.L. Jinks and G.S. Pooni. 1980. A general method for the detection and estimation of additive, dominance and epistatic variation for metrical traits. IV. Triple test cross and analysis for normal families and their self. *Heredity*, 44: 177-192.

Prakash, V., D.D. Saini and R.V. Singh. 2004. Estimation of gene effects for grain yield and its components in barley (*Hordeum vulgare* L.). *Ind. J. Genet*., 64(1): 69-70.

Rawlings, J.O. and C.C. Cockerham 1962b. Analysis of double cross hybrid populations. *Biometrics*, 18: 229-244.

Rawlings, J.O. and C.C. Cockerham. 1962a. Triallele analysis. *Crop Sci*., 2: 228-231.

Saleem, M.Y., B.M. Atta, A.A. Cheema and M.A. Haq. 2005a. Genetics of panicle related traits of agronomic importance in rice through triple test cross analysis. *Spain. J. Agric. Res*., 3: 402-409.

Saleem, M.Y., B.M. Atta, A.A. Cheema, Z. Mukhtar and M.A. Haq. 2005b. Detection of epistasis and estimation of additive and dominance components of genetic varation using triple test cross analysis (*Oryza satva* L.). *Cademo de Pesquisa Ser Bio*., Santa Cruz do Sul., 17: 37-50.

Singh, R.J. and G.S. Khush. 2000. *Rice Breeding and Genetics*. Oxford & IBH Publ. Co. New Delhi.

Singh, R.K. and B.D. Chaudhary.1985. *Biometrical Methods in Quantitative Genetics and Analysis*. Kalyani Publ., New Delhi.

Singh, S. and R.B. Singh. 1976. Triple test cross analysis in two wheat crosses. *Heredity*, 37: 173-177.

Subbaraman, N. and S.S.R. Rangaswamy. 1989. Triple test cross analysis in rice. *Euphytica*, 42: 35-40.

Verma, P.K., P.C. Katoch and R.P. Kaushik. 1994. Genetics of harvest index and grain characteristics eliminating and allowing the inadequacy of testers using selfing generation of triple test cross in rice. *Annals Biol*., 10: 216-222.

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