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GENE ACTION STUDIES FOR PROTEIN QUALITY TRAITS IN *ZEA MAYS* L. UNDER NORMAL AND DROUGHT CONDITIONS

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Abstract

A complete 8 × 8 diallel set (parents and F_1 hybrids) involving eight maize inbred lines was planted in replicated trials. Genetic components D and H (H_1, H_2) showed that inheritance of quality traits i.e. protein, tryptophan and lysine percentage was under the control of additive effects with partial-dominance under normal as well as drought stress conditions. Inbred line $NCMLD_4$ contained maximum number of dominant genes for protein and tryptophan percentage across water regimes. Additive gene action together with high narrow sense heritability suggested that improvement in maize for these traits through early generation selection can prove fruitful.

Key words: Maize, gene action, quality protein maize, additive-dominance model, heritability

Introduction

Maize is widely used as food for human beings, feed for livestock and provides raw materials for food industry world over. It is a potential source of protein for human and animals as it contains 7-13 percent protein (Moro *et al.*, 1996). Maize, being a staple food in many African countries, provides people with 21-58 percent and 17-60 percent of their daily requirement of calories and protein respectively (Krivanek *et al.*, 2007). However, protein in maize endosperm, like most cereal proteins, are mostly deficient in essential amino acids, particularly lysine, tryptophan and methionine (Segal *et al.*, 2003). Lysine and tryptophan ranges between 1.6-2.6 percent and 0.2-0.5 percent respectively of the total grain protein (Krivanek *et al.*, 2007). This nutritional deficiency is a global concern, particularly for the countries where maize is a staple food and used as major source of protein (Zaidi *et al*., 2008). Numerous strategies have been embarked to improve amino acid balance in total grain protein in maize. However, a major advancement was the identification of *opaque-2* mutation (*o2*). The *o2* mutants showed enhanced vital amino acid contents with undesirable pleotropic effects on agronomic traits (Mertz *et al.*, 1964). Lower yields, vulnerability to diseases and mechanical damage due to soft endosperm discouraged the use of *o2* mutants in maize breeding. Discovery and development of hard endosperm *o2* grains by CIMMYT's scientists was another milestone which boosted breeding for quality protein maize (QPM). Quality protein maize can help alleviate human undernourishment as it contains the *o2* mutation which imparts increased lysine and tryptophan (60-100 percent) and higher biological value (about 80 percent) than ordinary maize (Pixely & Bjarnason, 2002; Zaidi *et al.*, 2008).

After remediation of the pleotropic effects, quite a few challenges threaten QPM research and its dissemination to the farmers despite of the potential promises of its benefits. Apart from other factors, drought stress presents a major productivity barrier for maize. Knowledge of genetic makeup of QPM populations as well as genetic mechanisms controlling various traits of interest becomes important in breeding maize for quality protein under variable environmental stresses like drought. The Hayman's diallel analysis approach can serve valuable in selection and breeding for quality protein maize, superior hybrids and synthetics under drought conditions (Hussain *et al.*, 2009). The diallel analysis provides information regarding additive and dominance variances, expected environmental component of variation, distribution of genes among the parents, proportion of positive and negative genes, maternal and reciprocal effects along various genetic ratios such as average degree of dominance, ratio of dominant and recessive genes in the parents. This information is certainly valuable to develop selection criterion and strategy to improve particular crop trait(s).

Materials and Methods

Eight maize inbred lines $NCMLQ_1$, $NCMLQ_2$, $NCMLQ_3$, $NCMLQ_4$, $NCMLD_1$, $NCMLD_2$, $NCMLD_3$, and $NCMLD_4$ were planted during kharif season and crosses were made in all possible combinations. These parental lines constitute a diverse group of genotypes as these lines were derived from QPM (OPV) trials introduced from CIMMYT Africa and were screened for drought response at flowering. These parental lines along with their 56 F_1 hybrid combinations were sown during next year's spring season in two separate trials for normal and restricted irrigations following randomized complete block design in a plot size of 6 m^2 having two rows of four meter length, in three replications. To induce drought stress to our trial, irrigation was withdrawn following the flowering stage, whereas normal irrigations were applied to the stress free portion of our experiment. Upon maturity, guarded plants were selected for data recording of following quality parameters:

Protein percentage: After removing embryos, 25-30 kernels were milled using an Udy Cyclone Mill to prepare the flour. Later, crude protein percentages were determined using Kjeldhal's method of nitrogen estimation as described by Pearson (1976).

Tryptophan percentage. Tryptophan percentages were determined by HPLC procedure as described in "Quality Protein Maize Breeding Manual" by Vivik *et al.* (2008). Photo-spectrometer readings for tryptophan were made on 560 nm and following formula was used to calculate percentages:

$$\text{Quality index (QI)} = \frac{\text{Photo – Spectrometer reading at 560 nm}}{\text{Protein percentage of specific genotype}} \times 100$$

Lysine percentage: Lysine percentages were determined according to HPLC procedure explained in "Quality Protein Breeding Manual" by Vivik *et al.* (2008).

After recording the data, analysis of variance (Steel & Torrie, 1980) was carried out to determine variance among the genotypes for traits under study, followed by two scaling test i.e., regression analysis and analysis of Wr + Vr, Wr – Vr to establish the adequacy of the data sets for additive-dominance model. Afterwards, Hayman's genetic analysis (Hayman, 1954a, 1954b) for determination of first and second degree components of variation.

Results and Discussion

Analysis of variance (Table 1) established significant differences among genotypes for each trait under both irrigation regimes. Two scaling tests for validity of additive-dominance model fitted the data set partially adequate for further computations of components of variation regarding protein, tryptophan and lysine percentage (Table 2). Depictions of Hayman's analysis of variance are shown in Table 3. Significant values of 'a' and 'b' made prediction for the involvement of additive and non-additive effects in inheritance of protein, tryptophan and lysine in maize kernel endosperm under normal as well as water curtailed trials. Maternal effects were interacting in the heredity of protein as exposed by significant values of 'c' under both irrigation regimes in contrast to tryptophan and lysine which showed non-significant 'c' values in both experimental trials. Reciprocal effects 'd' were non-significant for all traits in both trials.

Interplay of dominance gene action H (H_1, H_2) was imparted under normal conditions for protein percentage unlike both additive D and dominance H (H_1, H_2) gene actions under stressed conditions (Table 4). The greater values of H than D indicated that dominance gene action was overriding additive type of inheritance. However, negative values of F showed that positive alleles were less frequent. The ratio of uv ($H_2/4H_1$) was less than 0.25 which suggested that there was equal distribution of genes among parents for protein percentage. Visual analysis revealed by the Wr-Vr graph (Fig 1a, 1b) helped in determining that this trait was under the control of partial dominance as positive intercept of the regression line was observed. The genetic component $(H_1/D)^{1/2}$ i.e., average degree of dominance affirmed the role of partial dominance as its values were less than unity for protein percentage. Configuration of array points along regression plot assigned maximum dominant genes to the line $NCMLD_4$ affixing itself near the origin while $NCMLQ_3$ and $NCMLQ_4$ were the parents with maximum number of recessive genes at both irrigation regimes. Present findings agreed to the results of Kumar *et al.* (2002) who also reported same type of gene action for protein content in maize kernels unlike to those inferred by Joshi *et al.* (1998) and Irshad-ul-Haq (2010) who were in favour of over dominance for the same character.

Table 1. Mean squares of protein quality traits in maize in 8 × 8 diallel cross under normal and drought stress conditions.

	df	Protein %	Tryptophan %	Lysine %
Source (under normal conditions)				
Replications	2	15.24**	0.001n.s	0.005n.s
Genotypes	63	0.40**	0.009**	0.058**
Error	126	0.02	0.001	0.004
Source (under water stress conditions)				
Replications	2	0.13**	0.002**	0.012n.s
Genotypes	63	0.63**	0.004**	0.065**
Error	126	0.006	0.0002	0.005

Table 2. Two scaling test for validity of hypothesis for adequacy of data set on protein quality traits to simple additive-dominance model in maize 8 × 8 diallel cross.

Plant Characters	Trials	t^2Values	*b*-values		Remarks
			b=0	*b*=1	
Protein percentage	Normal	4.90n.s	9.730**	2.758*	Partial adequate model
	Drought	4.76n.s	6.197**	2.997*	Partial adequate model
Tryptophan percentage	Normal	0.05 ns	3.875**	0.482 ns	Adequate model
	Drought	2.21 ns	5.534**	2.184*	Partial adequate model
Lysine percentage	Normal	8.72 ns	7.020**	3.933**	Partial adequate model
	Drought	1.75 ns	3.701**	2.253*	Partial adequate model

Table 3. Hayman's ANOVA for protein quality traits in maize 8 × 8 diallel cross under normal and drought stress conditions.

Mean square values				
S.O.V (under normal conditions)	**df**	**Protein %**	**Tryptophan %**	**Lysine %**
Replications	2	16.24**	0.0011*	0.005ns
a	7	2.99**	0.052**	0.365**
b	28	0.19**	0.008**	0.040**
b_1	1	0.49**	0.016**	0.329**
b_2	7	0.11**	0.009**	0.035**
b_3	20	0.21**	0.008**	0.027**
c	7	0.12**	0.00094ns	0.001ns
d	21	0.002ns	0.00059ns	0.001ns
Error	126	0.007	0.0010	0.0041
Total	191			
S.O.V (under drought conditions)				
Replications	2	0.13**	0.002**	0.012ns
a	7	4.64**	0.024**	0.371**
b	28	0.21**	0.0036**	0.050**
b_1	1	0.71**	0.0491**	0.449**
b_2	7	0.15**	0.0038**	0.020**
b_3	20	0.21**	0.0012**	0.041**
c	7	0.14**	0.0003ns	0.002ns
d	21	0.003ns	0.0001ns	0.003ns
Error	126	0.006	0.0002	0.005
Total	191			

Table 4. First and second degree statistics of genetic variation for various morphological traits in 8x8 diallel cross in maize.

	Protein percentage				**Tryptophan percentage**				**Lysine percentage**			
	Normal		**Drought**		**Normal**		**Drought**		**Normal**		**Drought**	
D	0.17	±0.14	0.27	±0.02	0.007	±0.001	0.003	±0.0003	0.034	±0.002	0.034	±0.003
H_1	0.147	±0.03	0.175	±0.04	0.005	±0.0012	0.002	±0.0006	0.032	±0.005	0.033	±0.006
H_2	0.12	±0.02	0.138	±0.04	0.004	±0.001	0.002	±0.0005	0.024	±0.005	0.028	±0.005
F	-0.048	±0.03	-0.080	±0.05	0.005	±0.001	0.002	±0.0006	0.012	±0.005	0.008	±0.006
h^2	0.072	±0.02	0.103	±0.03	0.002	±0.001	0.007	±0.0004	0.049	±0.003	0.063	±0.004
E	0.0023	±0.005	0.0020	±0.006	0.0001	±0.0002	0.00007	±0.0001	0.0009	±0.001	0.0009	±0.001
$(H_1/D)^{1/2}$	0.93		0.81		0.94		0.95		0.96		0.98	
KD/KR	0.43		0.41		0.69		0.68		0.60		0.56	
h^2/H_2	0.66		0.85		0.47		0.64		2.35		2.55	
H	-0.27		0.32		-0.04		-0.08		-0.22		-0.25	
uv $(H_2/4H_1)$	0.21		0.20		0.17		0.18		0.19		0.22	
D/(D+E)	0.99		0.99		0.98		0.98		0.97		0.97	
h^2b	0.99		0.99		0.96		0.96		0.96		0.96	
h^2n	0.79		0.84		0.62		0.61		0.68		0.65	

D: Additive Variance, H_1: Dominance Variance 1, H_2: Dominance Variance 2, $h^{2:}$ Dominance Effects (as the algebraic sum over all loci in heterozygous phase), E: Environmental component of variation, F: Product of additive by dominance effects, $(H_1/D)^{1/2}$: Average degree of dominance, uv $(H_2/4H_1)$: Balance of positive and negative genes, KD/KR: Proportion of dominant genes, D/(D+E): True sense heritability, h^2/H_2: Number of effective factors, h: Average direction of dominance, h^2b: Broad sense heritability, h^2n: Narrow sense heritability

It was evident that additive and non-additive genes were effective jointly for tryptophan percentage as inferred by significance of D and H (H_1, H_2) variances (Table 4). Yet, greater values of additive variance D than for the dominance variances H (H_1, H_2) put more emphasis on the additive gene effects. Symmetric distribution of genes among parents was specified by the ratio values of $H_2/4H_1$ which were less than 0.25 for tryptophan percentage under both irrigation regimes. Genetic component $(H_1/D_1)^{1/2}$ accounted for numeric values of 0.94 and 0.95 abounded dominance to the level of mere partial-dominance which was clearly directed by the positive intercept of the Wr-axis by the regression line (Fig. 2a, 2b). The graphs showed that $NCMLD_4$ and $NCMLD_3$ shared the maximum dominant alleles in both set of experiments, whereas, $NCMLQ_1$ and$NCMLQ_3$ carried maximum recessive alleles in normal and drought trials respectively, as they were located far from the origin. Results reported by Ngaboyisonga *et al.* (2008) are in agreement with our results for tryptophan percentage. The estimates of narrow sense heritability h^2n were 62% under normal and 61% under water stressed conditions.

Significance of D and H (H_1, H_2) variances (Table 4) revealed that both additive and dominance type of gene actions were involved in determining inheritance of lysine content in maize kernels, still, additive gene effects were more pronounced as greater values of D titled the balance towards vital role of additive gene effects than dominance. This role of additive under drought conditions was further advocated by non-significance of F value. Distribution of genes among parents remained symmetrical as $H_2/4H_1$ ratios remained less than 0.25 under both trials. Average degree of dominance $(H_1/D)^{1/2}$ values being less than unity affixed partial dominance to lysine percentage. Partial dominance for this trait has also been reported by Irshad-ul-Haq (2010). The same was observed in the Wr-Vr graphs (Fig. 3a, 3b) as positive intersect of regression line declared partial dominance in normal and drought conditions. Scenario of the array point distribution along regression line revealed that inbred lines $NCMLQ_4$ and $NCMLD_3$ were the parents which carried maximum of the dominant alleles under normal and drought conditions respectively, whereas $NCMLQ_1$ was the line with maximum recessive alleles in these trials.

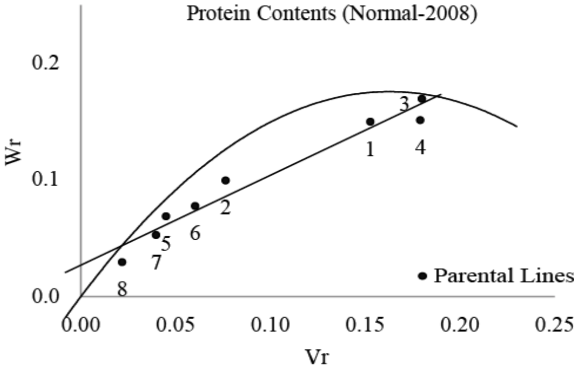

Fig. 1a. Wr-Vr graph for protein percentage under normal conditions.

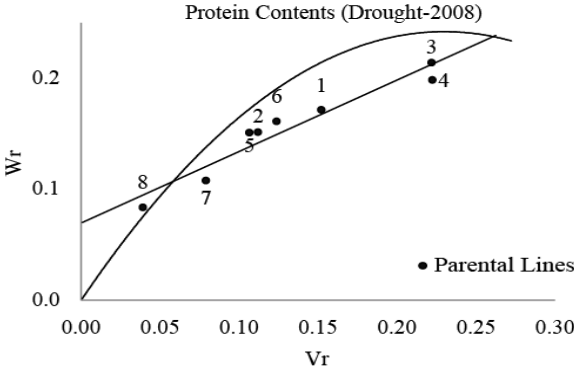

Fig. 1b. Wr-Vr graph for protein percentage under drought conditions.

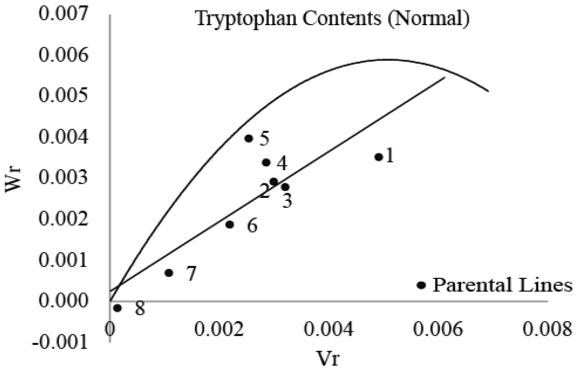

Fig. 2a. Wr-Vr graph for tryptophan percentage under normal conditions.

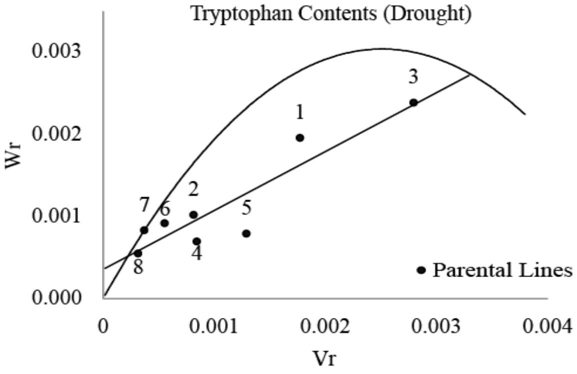

Fig. 2b. Wr-Vr graph for tryptophan percentage under drought conditions.

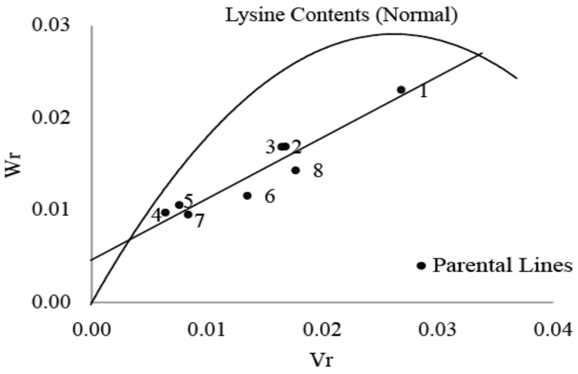

Fig. 3a. Wr-Vr graph for lysine percentage under normal conditions.

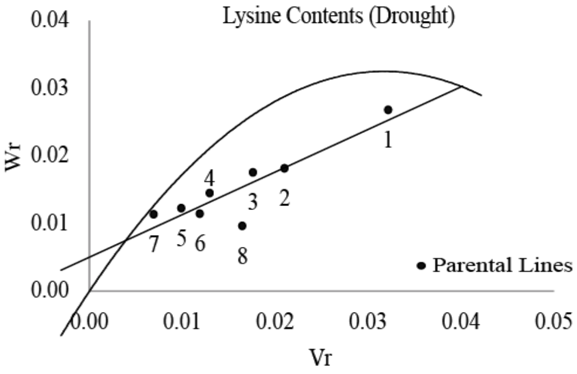

Fig. 3b. Wr-Vr graph for lysine percentage under drought conditions.

Conclusion

Additive gene action with partial dominance was the mode of inheritance for protein, lysine and tryptophan percentage in maize under drought stress conditions and for lysine and tryptophan under normal irrigation supply. This inheritance pattern together with high narrow sense heritability for all traits suggests that improvement for these traits through selections in early generation could prove feasible.

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References

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

Hayman, B.I. 1954b. The theory and analysis of variance of diallel tables. *Biometrics.* 10: 235

Hussain, I., M. Ahsan, M. Saleem and A. Ahmad. 2009. Gene action studies for agronomic traits in maize under normal and water stress conditions. *Pak. J. Sci.,* 46(2): 107-112.

Irshad-ul-Haq, M. *Genetic regulation for yield and quality attributes in maize.* 2010. PhD research thesis. PMAS Arid Agric. Univ., Rawalpindi, Pakistan.

Joshi, V.N., N.K. Pandiya and R.B. Dubey. 1998. Heterosis and combining ability for quality and yield in early maturing single cross of maize (*Zea mays* L.). *Ind. J. Genet. Plant Breed.,* 58(4): 519-524.

Krivanek, A.F., H. de Groote, N.S. Gunaratna, A.O. Diallo and D. Friesen. 2007. Breeding and disseminating quality protein maize (QPM) for Africa. *Afri. J. Biotechnol.,* 6(4): 312-324.

Kumar, R.S., E. Satyanarayana and P. Shanthi. 2002. Evaluation of high oil maize (*Zea mays* L.) hybrids for agronomic, yield and quality parameters. Proceeding of the 8th Asian Regional Maize Workshop, Bangkok, Thailand: August 5-8.

Mertz, E.T., L.S. Bates and O.E. Nelson. 1964. Mutant gene that changes protein composition and increases lysine content of maize endosperm. *Science*, 145: 279-280.

Moro, G. L., J. E. Habben, B.R. Hamaker and B.A. Larkins. 1996. Characterization of the variability in lysine content for normal and opaque-2 maize endosperm. *Crop Sci.,* 36: 1651-1659.

Ngaboyisonga, C., K. Njoroge, D. Kirubi and S.M. Githiri. 2008. Effects of field conditions, low nitrogen and drought on genetic parameters of protein and tryptophan concentrations in grain of quality protein maize. *Int. J. Pl. Prod.,* 2(2): 137-152.

Pearson, D. 1976. *Chemical Analysis of Foods*. (7th Ed) Livingstone, London, Churchill.

Pixley K.V. and M.S. Bjarnason. 2002 Stability of grain yield, endosperm modification, and protein quality of hybrid and open-pollinated quality protein maize (QPM) cultivars. *Crop Sci*., 42: 1882-1890.

Segal, G., R.T. Song and J. Messing. 2003. A new opaque variant of maize by a single dominant RNA-interference-inducing transgene. *Genetics,* 165: 387-397.

Steel, R.G.D and J.H. Torrie. 1980.*Principles and Procedures of Statistics. A biometrical approach*.2nd Ed., McGraw Hill Inter. Book Co. Tokyo, Japan.

Vivek, B.S., A.F. Krivanek, N. Palacios-Rojas, S. Twumasi-Afriyie and A.O. Diallo. 2008. *Breeding Quality Protein Maize (QPM): Protocols for Developing QPM Cultivars*. Mexico, D.F.: CIMMYT.

Zaidi, P.H., Mehrajuddin, M.L. Jat, K. Pixley, R.P. Singh and Sain Dass. 2008. Resilient maize for improved and stable productivity of rain-fed environment of South and South-East Asia. Paper presented in 10th Asian Regional Maize Workshop, 20-23 October, 2008, Makassar, Indonesia.

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