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Effects of field conditions, low nitrogen and drought on genetic parameters of protein and tryptophan concentrations in grain of quality protein maize

Claver Ngaboyisonga*, Kiarie Njoroge, Duncan Kirubi, Sam. M. Githiri

University of Nairobi, Faculty of Agriculture, Department of Plant Science and Crop Protection, Nairobi, Kenya
*Corresponding authors. E-mail: cngaboyisonga@hotmail.com

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Abstract

Quality Protein Maize (QPM) has about twice the amount of lysine and tryptophan of normal maize and hence represents an important tool of correcting its deficiency in protein quality. However, the effects of low nitrogen and drought on genetic parameters such as gene action and combining abilities of protein quantity and quality of QPM are not known. To study how these genetic parameters are affected by field conditions, low nitrogen and drought, eight inbred lines were acquired from Centro Internacional Del Mejoramiento de Maiz y Trigo (CIMMYT) and used to generate single cross hybrids with North Carolina Design II procedures. The single crosses were evaluated at Kiboko in Kenya in 2006 under optimum, low nitrogen and drought environments. Observations were performed on protein and tryptophan concentrations in grain. Results showed that, the gene action of protein concentration was predominantly of additive and maternal natures whereas that of tryptophan concentration was predominantly of non-additive nature. Field conditions, low nitrogen, and drought changed the proportions of genetic effects. Field conditions suppressed maternal effects for protein concentration, but induced non-additive effects for both traits. Low nitrogen reduced additive and maternal effects on protein concentration while it reduced non-additive effects on tryptophan concentration. Drought reduced non-additive effects on both protein and tryptophan concentrations in grain. By changing the proportion of genetic effects, environments changed magnitudes and directions of general (GCAs) and specific (SCAs) combining abilities.

Keywords: Drought; Low nitrogen; Optimum environment; Protein and tryptophan concentrations in grain; QPM.

Introduction

Maize is an important source of protein in human lives (Anonymous, 1988). Nutritionally, maize is deficient in two essential amino acids: lysine and tryptophan (Bhatia and Rabson, 1987). However, QPM contains in general more than 55% of tryptophan and

lysine compared to normal maize (Prasanna et al., 2001) and hence represents a valuable option to correct maize deficiency in protein quality. QPM was developed by combining the genetic systems of the gene mutant *opaque-2* (o_2) (Mertz et al., 1964) and genetic endosperm modifiers (Prasanna et al., 2001; Vasal, 2001; Vasal, 2000).

The genetic system of the o_2 gene is qualitative. However, because it is recessive, its effects are expressed in the endosperm when three alleles, two from female parent and one from male parent are present. It increases lysine and tryptophan in endosperm by acting on the four types of storage proteins in maize endosperm: albumins, globulins, zeins, and glutelins. Zeins contain low lysine with 0.1g/100g while glutelins are considerably rich in lysine with 2g/100 g or more (Lin et al., 1997; Misra et al., 1975). The o_2 mutant increases the level of lysine and tryptophan by suppressing or reducing the synthesis of zeins and increasing that of glutelins (Damerval and de Vienne, 1993; Habben et al., 1993).

The o_2 gene adversely affects several important agronomic traits including kernel characteristics. It adversely affects the accumulation of dry matter resulting in lower yields due to increased endosperm size. The kernel phenotype is changed in a soft, chalk, and dull appearance. Kernels dry slowly following physiological maturity of the grain and have a higher incidence of ear rots. Other changes include larger germ size and low kernel density (Lin et al., 1997; Moro et al., 1995).

Genetic modifiers are genes capable of altering the expression of other genes at different loci in the genome (Thain and Hickman, 2003). The o_2-endosperm genetic modifiers alter the undesirable correlated effects of o_2 gene. The parties of the endosperm modified are vitreous and hard instead of being opaque and soft (Villegas et al., 1992; Bjarnason et al., 1988). The o_2-modified endosperm varieties have agronomic characteristics comparable with those of normal maize. However, endosperm modification of QPM is accompanied by slight increase in total proteins and slight decrease in lysine and tryptophan (Vasal, 2000; Bjarnason and Vasal, 1992).

Current effort on QPM is to increase its cultivation in the regions, especially in Sub-Saharan Africa, experiencing problems of malnutrition and where maize is the staple crop. In these regions, however, maize is frequently produced under environmental stresses, among which, low soil nitrogen and drought are the most important. Impacts of low nitrogen and drought on grain yield of normal maize have been extensively studied (Edmeades et al., 2006). However, those impacts on protein quality and quantity of QPM have not yet been studied at any extent. The objective of this study was assess and to estimate impacts of field conditions, low nitrogen and drought on genetic effects of gene action and on combining abilities of protein and tryptophan concentrations in grain.

Materials and methods

Eight lines were received from CIMMYT-Kenya and were grouped into female and male parents by taking care to include in each group of parents two lines with high protein quality and two other with low quality (Table 1). They were used to produce 16 single cross hybrids with North Carolina Design II (NCDII) during the October 2004-February 2005 cropping season. The 16 single crosses were evaluated at KARI-Kiboko station (2°25 S, 37°75 E, 975 meters above sea level) in Kenya in October 2005- February cropping season, under optimum, low nitrogen and drought environments.

Table 1. Pedigrees, protein and tryptophan concentrations (%) in grain of lines involved in crossing.

Name	Pedigree	Tryptophan concentration in grain (%)	Protein quality
FEP2	[CML202/CML144]F2-23-3-1-B*4	0.095	High
FEP4	[CML202/CML144]F2-66-2-3-B*4	0.074	Low
FEP5	[CML205/CML182]-B-47-1-B*3	0.068	Low
FEP6	[CML389/CML176]B-11-1-B*3	0.109	High
MAP1	[CML205/CML176]-B-2-1-B*3	0.091	High
MAP3	[CML389/CML176]B-29-2-B*3^{+}	0.098	High
MAP4	[CML389/GQL5]B-22-1-B*3	0.060	Low
MAP6	[CML159/[MSR/POOL9]C1F2-205-1(OSU23i)-5-3-X-X-1-B-B]-B-10-1-B*3	0.067	Low

The optimum environment received irrigation throughout the season and fertilizers were applied by supplying 64 kg N/ha and 46 P/ha at planting, 46 kg N/ha four weeks after planting and 46 kg/ha seven weeks after planting. The low nitrogen environment was achieved by not top-dressing nitrogen fertilizers during the season. However, a starter nitrogen of 18 kg/ha was applied at planting to allow uniform germination, emergence and early seedling growth. Phosphorus was applied at 46 kg/ha at planting while irrigation was provided during the cropping season. Field was thoroughly cleaned during plowing and all plant residues removed. Drought environments were obtained by stopping irrigation one week before flowering. The field received 64 kg N/ha and 46 P/ha at planting, 46 kg N/ha four weeks after planting and 46 kg/ha seven weeks after planting like optimum environment.

The experimental design was a Randomized Complete Blocks Design (RCBD) with three replications. The plot was made of two rows of 5-m length with the distance between rows and hills of 0.75 m and 0.25 m respectively. Planting was performed by two seeds per hill and a thinning three weeks after planting reduced the stand at one plant per hill. Thus, a planting density of 53000 plants/ha was achieved.

Before planting, Furadan®5G (composition: 5% w/w carbonfuran, 10% inert) was applied in rows and covered with little soil to control soil, germination and seedling pests. Additionally, an insecticide called "Buldock" was applied two times: three weeks after planting and six weeks after planting to control stem borers that are the major biological constraint to maize at Kiboko. Weeding was performed, as it was required.

The ears harvested in each plot were dried at constant weight and five best ears were chosen. Approximately six kernels from each selected ear, having regular sizes were taken from the middle of the cob and formed a bulk of 30 kernels for each plot. The 30 kernels for each plot were sent to CIMMYT-Cereal Quality Laboratory in Mexico for quality protein analysis. Moreover, F_1 kernels from all crosses obtained after crossing nursery were also sent for protein quality analysis.

The determination of protein content and quality followed the procedures described by Villegas (1975) and Villegas et al. (1984). The grain samples were finely grounded, the resulting flour was defatted, and concentration of nitrogen (%) and tryptophan (%) in grain were calorimetrically determined. The protein concentration in grain (%) was obtained by multiplying nitrogen concentration with a factor of 6. 35.

The analysis of variance for genetic analysis and the linear regression were performed using GenStat computer package program (VSN Int., 2007). The genetic analysis was performed in each environment, including F_1 kernels, and across all four environments following the model of a cross-classification design where the sources of variation of crosses were subdivided into those of female parents (female additive effects), male parents (male additive effects), and females×males (non-additive). Linear regression of parents onto crosses was used to estimate the relationship between parents and their crosses.

Results

Genetic variation of protein and tryptophan concentrations in grain in F_1 kernels he analysis of variance in F_1 kernels revealed highly significant ($P<0.001$) differences between crosses for both protein and tryptophan concentration in grain, indicating that genetic variation was highly significant. Furthermore, it showed highly significant differences between female and male parents ($P<0.001$) for protein concentration and simply significant differences ($P<0.05$) for tryptophan concentration. Thus, female and male additive effects were significant for both protein and tryptophan content in grain. The interaction "females×males" was highly significant ($P<0.001$) and therefore non-additive effects were highly significant for the two traits (Tables 2 and 3).

The $F=MS_F/MS_M$ was not significant ($P>0.05$) indicating that maternal effects were not significant. The proportion of female additive effects (SS_{FEP}/SS_{CRO}) was 70.8% for protein concentration and 13.4% for tryptophan content while the percentage of male additive effects (SS_{MAP}/SS_{CRO}) was 13.8% and 21% of genetic effects respectively. Hence, total additive effects [$(SS_{FEP}+SS_{MAP})/SS_{CRO}$] accounted for 84.6% and 34.4% while non-additive effects ($SS_{F\times M}/SS_{CRO}$) formed 15.4% and 65.6% of genetic effects respectively. The proportion of maternal effects [$(SS_{FEP}-SS_{MAP})/SS_{CRO}$] estimated as the difference between female and male additive effects was 56.9% for protein concentration. They represented high proportion of additive effects although they were not significant. The total additive effects comprised thus, 56.9% of maternal effects and 27.7% of real additive effects ($2SS_{MAP}/SS_{CRO}$). The proportion of maternal effects was negative for tryptophan indicating that they were completely absent. Genetic effects underlying protein concentration in grain included 27.7% of real additive effects, 56.9% maternal effects, and 15.4% non-additive effects whereas that controlling tryptophan concentration comprised 35% of additive effects and 65% of non-additive effects. Therefore, the protein concentration was essentially governed by maternal and additive effects although maternal effects were not significant whereas tryptophan concentration was essentially controlled by non-additive effects.

The female parent 4 (FEP4) had positive and highest GCA of 1.37% for protein content and the male 1 (MAP1) had a GCA of 0.51% among the male parents. Specifically, FEP4 crossed with MAP1 gave the best SCA of 0.73%. Other best combinations for protein concentration were S13, S24, and S34 because they had a SCA superior to 0.40% (Table 5).

Table 2. Analysis of variance for combining abilities in F1 kernels, under optimum, low nitrogen and drought environments for protein concentration in grain (%).

Sources	DF	F1 kernels		Optimum		Low nitrogen		Drought	
		MS	F	MS	F	MS	F	MS	F
REP	1	3.8×10^{-4}	0.31^{NS}	1.2×10^{-4}	0.19^{NS}	0.03	1.34^{NS}	0.03	1.02^{NS}
CRO	15	2.69	2215^{***}	1.20	1873^{***}	1.27	53.95^{***}	0.66	24.44^{***}
FEP (F)	3	9.55	7837^{***}	0.52	810^{***}	1.09	46.49^{***}	0.33	12.14^{***}
MAP (M)	3	1.87	1536^{***}	2.51	3910^{***}	0.95	40.5^{***}	2.08	77.18^{***}
F/M	3/3	-	5.11^{NS}	-	0.21^{NS}	-	1.15^{NS}	-	0.16^{NS}
F×M	9	0.69	568^{***}	0.99	1548^{***}	1.43	60.91^{***}	0.30	10.96^{***}
Error	15	1.2×10^{-3}	-	6.410^{-4}	-	0.02	-	0.03	-

***: Significance at P<0.001, **: Significance at P<0.01, *: Significance at P<0.05
^{NS}: No Significance (P>0.05), REP: Replications, CRO: Crosses
FEP: Female parents, MAP: Male parents, SS=MS×DF

Table 3. Analysis of variance for combining abilities in F1 kernels, under optimum, low nitrogen and drought environments for tryptophan concentration in grain (%).

Sources	DF	F1 kernels		Optimum		Low nitrogen		Drought	
		MS	F	MS	F	MS	F	MS	F
REP	1	1.7×10^{-5}	0.42^{NS}	5.3×10^{-6}	0.13^{NS}	4.3×10^{-5}	3.91^{NS}	6.6×10^{-5}	1.65^{NS}
CRO	15	2.0×10^{-4}	5.21^{***}	2.5×10^{-4}	6.28^{***}	1.7×10^{-4}	15.45^{***}	2.7×10^{-4}	6.75^{***}
FEP (F)	3	1.4×10^{-4}	3.48^{*}	1.6×10^{-4}	4.03^{*}	3.9×10^{-4}	35.92^{***}	6.8×10^{-4}	16.92^{***}
MAP (M)	3	2.1×10^{-4}	5.46^{*}	7.8×10^{-5}	1.96^{NS}	1.7×10^{-4}	15.79^{***}	2.5×10^{-4}	6.22^{**}
F/M	3/3	-	0.64^{NS}	-	2.05^{NS}	-	2.29^{NS}	-	2.72^{NS}
F×M	9	2.2×10^{-4}	5.70^{**}	3.4×10^{-4}	8.47^{***}	9.7×10^{-5}	8.51^{***}	1.4×10^{-4}	3.53^{*}
Error	15	3.9×10^{-5}	-	4.0×10^{-5}	-	1.1×10^{-5}	-	4.0×10^{-5}	-

***: Significance at P<0.001, **: Significance at P<0.01*, Significance at P<0.05
^{NS}: No Significance (P>0.05), REP: Replications, CRO: Crosses
FEP: Female parents, MAP: Male parents, SS=MS×DF

The female parent 2 (FEP2) and the male parent 2 (MAP2) had the highest GCAs of 0.00615% and 0.0047% for tryptophan concentration in grain. Among female parents, only line 2 (FEP2) had positive GCA for tryptophan concentration in grain; other three lines had negative and non-significant GCAs. The male parents 3 (MAP3) and 4 (MAP4) had the lowest GCAs of -0.0047% and -0.0043%. Hewer, best SCAs were obtained by crossing FEP1 and MAP3 at one hand and FEP3 and MAP4 at the other (Table 6). The linear regression slope (b=0.82±0.25) of female parents onto crosses for protein concentration in grain was highly significant (P<0.001), demonstrating a high and positive relationship between female parents and crosses. Female parents with high protein content produced crosses with high protein concentration and those with low protein content gave crosses with low protein content (Table 6 and Figure 1).

Table 4. Combined analysis of variance for combining abilities for protein and tryptophan concentration (%) in grain

Sources	DF	Protein concentration in grain			Tryptophan concentration in grain		
		SS	MS	F	SS	MS	F
ENV (E)	3	197.21	65.74	4433^{***}	0.0117	0.004	120.86^{***}
E/REP	4	0.06	0.01	1.13^{NS}	3.4×10^{-3}	8.5×10^{-4}	26.20^{***}
CRO (C)	15	31.67	2.11	161.6^{***}	4.2×10^{-3}	2.8×10^{-4}	8.55^{***}
FEP (F)	3	13.29	4.43	339.1^{***}	1.5×10^{-3}	5.1×10^{-4}	15.83^{***}
MAP (M)	3	14.70	4.90	374.8^{***}	1.4×10^{-3}	4.8×10^{-4}	14.92^{***}
F/M	3/3	-	-	0.90^{NS}	-	-	1.06^{NS}
F×M	9	3.68	0.41	31.30^{***}	1.2×10^{-3}	1.3×10^{-4}	4.00^{***}
E×C	45	55.67	1.24	94.66^{***}	9.3×10^{-3}	2.1×10^{-4}	6.37^{***}
E×F	9	21.15	2.35	179.86^{***}	2.6×10^{-3}	2.9×10^{-4}	8.84^{***}
E×M	9	7.52	0.84	63.96^{***}	6.8×10^{-4}	7.6×10^{-5}	2.34^{*}
E×F×M	27	26.99	1.00	76.49^{***}	6.0×10^{-3}	2.2×10^{-4}	6.88^{***}
Error	60	0.78	0.01	-	1.9×10^{-3}	3.2×10^{-5}	-

***: significance at P<0.001, **: Significance at P<0.01, *: significance at P<0.05
NS: Not significant (P>0.05), ENV: Environments, REP: Replications
CRO: Crosses, FEP: Female parents, MAP: Male parents

The linear regression slope (b=0.47±0.22) of male parents on crosses for protein concentration was significant (P<0.05), denoting a positive relation between male parents and crosses, although at a lower extent than female parents. The slope (b=1.06±0.27) of linear regression of mid-parents (mean of female and male parents for each cross) on crosses was highly significant (P<0.01), implying a very high and positive relationship between mid-parents and crosses; high mid-parents gave crosses with very high protein content while low mid-parents produced crosses with less protein content (Table 7 and Figure 1). The regression slopes of female, male and mid-parents were not significantly different (Table 7).

Linear regression slopes of female, male and mid-parents onto crosses for tryptophan concentration were very small and not significant (P>0.05), implying absence of any relationship between parents and crosses confirming absence of maternal effects and predominance of non-additive effects in controlling tryptophan concentration in grain. The high amount of tryptophan content in crosses resulted in a combination of specific parents regardless their tryptophan concentration in grain (Table 7 and Figure 2).

Table 5. Estimates of GCAs and SCAs for protein concentration (%) in grain.

Items	F1 kernels	Optimum	Low nitrogen	Drought	All combined
FEP1	-1.170***	0.291***	-0.133***	-0.178**	-0.297***
FEP2	0.293***	0.057***	0.461***	-0.053NS	0.189***
FEP3	-0.495***	-0.326***	-0.414***	-0.06NS	-0.324***
FEP4	1.372***	-0.022**	0.086NS	0.291***	0.432***
MAP1	0.512***	0.260***	0.242***	0.541***	0.389***
MAP2	0.208***	0.432***	0.180***	-0.084NS	0.184***
MAP3	-0.622***	0.127***	0.086NS	0.205***	-0.051*
MAP4	-0.097***	-0.818***	-0.508***	-0.662***	-0.522***
SCA11	-0.014NS	0.678***	-0.532***	0.334**	0.116***
SCA12	-0.270***	0.881***	-0.062***	-0.728***	-0.045 NS
SCA13	0.440***	-0.627***	-0.218***	0.045NS	-0.09***
SCA14	-0.155***	-0.931***	0.812***	0.350***	0.018 NS
SCA21	-0.657***	-0.275***	1.531***	0.147NS	0.187***
SCA22	-0.043*	-0.260***	-0.625***	0.147NS	-0.195***
SCA23	0.157***	0.482***	-0.312***	0.045NS	0.093***
SCA24	0.542***	0.053**	-0.594***	-0.338**	-0.084***
SCA31	-0.059**	0.108***	-1.093***	-0.221*	-0.316***
SCA32	0.245***	-0.314***	0.219***	0.091NS	0.06 NS
SCA33	-0.675***	-0.479***	0.781***	0.021NS	-0.088***
SCA34	0.490***	0.686***	0.094NS	0.108NS	0.344***
SCA41	0.730***	-0.510***	0.094NS	-0.260**	0.013 NS
SCA42	0.068**	-0.307***	0.469***	0.490***	0.180***
SCA43	0.078**	0.623***	-0.25***	-0.111NS	0.085***
SCA44	-0.877***	0.193***	-0.312***	-0.119NS	-0.279***

***: significance at P<0.001, **: Significance at P<0.01, *: significance at P<0.05
NS: Not significant (P>0.05)

Effects of field conditions, low nitrogen, and drought on genetic variation of protein and tryptophan concentrations in grain

The analysis of variance performed in each environment showed highly significant differences (P<0.001) between crosses for protein concentration, indicating highly genetic variation. Female and male parents were significantly different, implying that both female and male additive effects were highly significant (P<0.001). The $F=MS_F/MS_M$ was not significant (P>0.05), revealing that maternal effects were not significant. The interaction "Females×Males" was highly significant (P<0.001) denoting that non-additive effects were

highly significant making protein concentration in grain trait to be controlled by additive and non-additive effects (Table 2).

Table 6. Estimates of GCAs and SCAs for tryptophan concentration (%) in grain.

Items	F1 kernels	Optimum	Low nitrogen	Drought	All combined
FEP1	-0.0013^{NS}	0.0042*	0.0087***	0.0126***	0.0060*
FEP2	0.0061**	-0.0062**	0.0009^{NS}	-0.0088***	-0.0019^{NS}
FEP3	-0.0018^{NS}	-0.0003^{NS}	-0.0083***	0.0006^{NS}	-0.0024^{NS}
FEP4	-0.0031^{NS}	0.0025^{NS}	-0.0013^{NS}	-0.0043*	-0.0015^{NS}
MAP1	0.0042*	0.0000^{NS}	0.0031**	0.0083***	0.0039**
MAP2	0.0047*	0.0038^{NS}	0.0044***	-0.0026^{NS}	0.0026^{NS}
MAP3	-0.0047*	0.0000^{NS}	-0.0018^{NS}	-0.0021^{NS}	-0.0021^{NS}
MAP4	-0.0043*	-0.0038^{NS}	-0.0057***	-0.0037^{NS}	-0.0043**
SCA11	0.0039^{NS}	0.0020^{NS}	0.0054***	-0.0082*	0.0006^{NS}
SCA12	-0.0041^{NS}	0.0170***	-0.0059***	0.0027^{NS}	0.0023^{NS}
SCA13	0.0138**	-0.0250***	-0.0062***	0.0027^{NS}	-0.0038^{NS}
SCA14	-0.0136**	0.0060^{NS}	0.0067***	0.0028^{NS}	0.0008^{NS}
SCA21	-0.007^{NS}	0.0020^{NS}	0.0027***	0.0037^{NS}	0.0008^{NS}
SCA22	-0.008*	-0.0090**	0.0073***	0.0041^{NS}	-0.0012^{NS}
SCA23	-0.0086*	0.0090**	-0.0039***	-0.0084*	-0.0027^{NS}
SCA24	0.0235***	-0.0020^{NS}	-0.0061***	0.0070*	0.0030^{NS}
SCA31	0.0024^{NS}	0.0010^{NS}	-0.0091***	0.0103**	0.0012^{NS}
SCA32	0.0059^{NS}	0.0020^{NS}	0.0001^{NS}	0.0012^{NS}	0.0022^{NS}
SCA33	-0.0067^{NS}	0.0000^{NS}	0.0063***	-0.0073*	-0.0021^{NS}
SCA34	-0.0016^{NS}	-0.0040^{NS}	0.0027^{NS}	-0.0042^{NS}	-0.0014^{NS}
SCA41	0.0007^{NS}	-0.0060^{NS}	0.0009^{NS}	-0.0058^{NS}	-0.0026^{NS}
SCA42	0.0062^{NS}	-0.0100**	-0.0014^{NS}	-0.0079*	-0.0033^{NS}
SCA43	0.0015^{NS}	0.0160***	0.0038***	0.0131**	0.0084*
SCA44	-0.0083*	0.0000^{NS}	-0.0033^{NS}	0.0007^{NS}	-0.0025

***: significance at P<0.001, **: Significance at P<0.01, *: significance at P<0.05
^{NS}: Not significant (P>0.05)

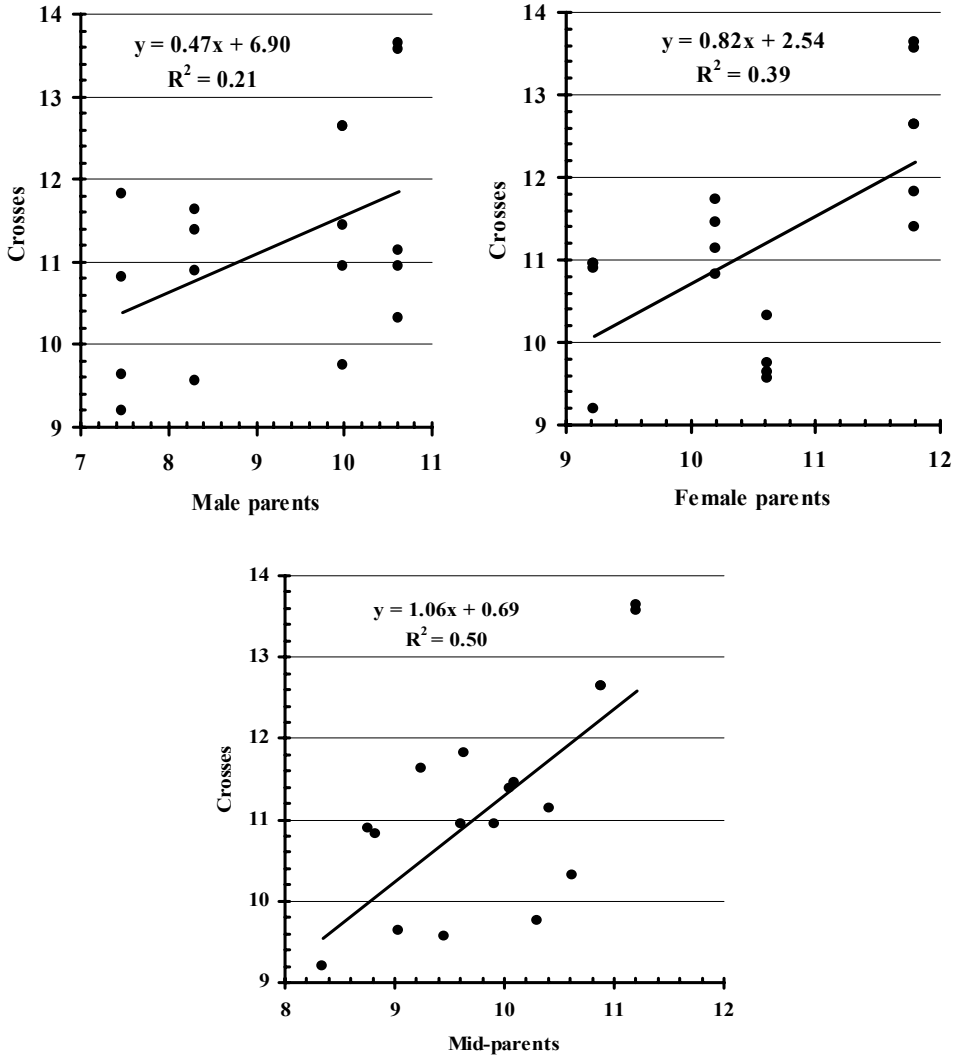

Figure 1. Regression of parents onto crosses for protein concentration (%) in grain.

Female additive effects constituted 8.7% under optimum condition, 17.2% under low nitrogen deficiency, and 10% under drought. Male additive effects fractions were 41.8% under optimum environment, 15% under low nitrogen, and 62.8% under drought. Hence, total additive effects formed 50.5% under optimum environment, 32.3% under low nitrogen, and 72.8% under drought. Non-additive effects accounted for 49.5%, 67.7%, and 27.2% of genetic effects respectively. Therefore, total additive effects were reduced by 34.2% under optimum condition, by 52.4% under low nitrogen and by 11.9% under

drought in comparison with F1 kernels. At the same time non-additive effects increased the by same amount. Maternal effects were completely absent (negative proportions) under optimum and drought conditions and accounted for as little as 2.2% under low nitrogen.

The analysis of variance performed in each environment indicated highly significant differences (P<0.001) between crosses for tryptophan concentration, implying highly genetic variation. Female additive effects were significant (P<0.05) under optimum environment, and highly significant (P<0.001) under low nitrogen and drought. Male additive effects were highly significant (P<0.001) under low nitrogen and drought, but not significant (P>0.05) under optimum condition. Hence, under optimum condition, additive female effects were essentially maternal effects because male additive effects were not significant Additionally, $F=MS_F/MS_M$ was not significant (P>0.05), implying non-significance of maternal effects for tryptophan concentration. Non-additive effects ("Females×Males") were highly significant (P<0.05) in all environments (Table 3).

Female additive effects formed 12.7% of genetic effects under optimum condition, 45.8% under low nitrogen, and 50.4% under drought. Male additive effects constituted for 6.2% (negligible) under optimum condition, 20% under low nitrogen, and 18.5% under drought. Total additive effects represented 18.9% under optimum condition, 65.9% under low nitrogen, and 68.9% under drought whereas non-additive effects formed 81.1% of genetic variation under optimum environment, 34.1% under low nitrogen, and 31.1% under drought. Thus, non-additive effects increased under optimum compared to F_1 kernels by 15.8%, but under low nitrogen, they decreased by 31.2% and under drought by 34.2%. At the same time, additive effects decreased or increased the same amount.

Combined analysis of variance across all environments showed highly significant differences (P<0.001) between crosses for both protein and tryptophan concentrations in grain, hence genetic variation was highly significant. Furthermore, the interaction "Environments×Crosses" was highly significant (P<0.001) indicating that the genetic variation changed with environments (Table 4).

Table 7. Regression slopes by regressing female, male, and mid-parents onto crosses and for protein and tryptophan concentrations (%) in grain and comparison of those slopes.

Items	Protein content in grain			Tryptophan content in grain		
	b	S.E.	T	b	S.E.	T
Female parents	0.82	0.25	3.28^{**}	0.00	0.12	0.04^{NS}
Male parents	0.47	0.22	2.14^{*}	0.11	0.11	0.98^{NS}
Mid-parents	1.06	0.27	4.00^{***}	0.18	0.16	1.16^{NS}
Female vs. male parents	0.35	0.33	1.05^{NS}	-0.11	0.16	-0.68^{NS}
Female vs. mid-parents	0.24	0.37	0.65^{NS}	0.18	0.20	0.90^{NS}
Male vs. mid-parents	0.59	0.35	1.69^{NS}	0.07	0.19	0.36^{NS}

***: significance at P<0.001, **: Significance at P<0.01, *: significance at P<0.05
^{NS}: Not significant (P>0.05), S.E. Standard error, T: t test

Across environments for protein and tryptophan concentrations, female additive (female parents) and male additive (male parents) effects were highly significant (P<0.001). $F=MS_F/MS_M$ was not significant for the two traits and hence maternal effects were not significant. Moreover, non-additive ("Females×Males") effects were highly significant (P<0.001). Female additive effects formed 42% of genetic effects for protein concentration and 36.6% for tryptophan concentration while male additive effects formed 46.4% protein concentration and 34.1% for tryptophan concentration. Additive effects represented 88.4% of genetic effects for protein concentration and 70.7% for tryptophan concentration whereas non-additive effects constituted 11.6% and 29.3%.

Interaction "Environment × Crosses" and its components, "Environments × Females", Environments × Males", and "Environments × Females ×Males", were significant (P<0.05) for the two traits indicating that genetic effects and their components (female and male additive and non-n additive effects) interacted significantly with environments (Table 4).

The GCAs of several lines were changed in directions and magnitudes when evaluated under various environments for both protein and tryptophan concentrations in grain. Despite exceptions, the GCAs gradually reduced in absolute values when environments changed from F_1 kernels to optimum, low nitrogen and drought (Tables 5 and 6).

Across environments and for protein concentration, FEP2, FEP4, MAP1 and MAP2 had positive and highly significant GCAs whereas FEP1, FEP3, MAP3 and MAP4 had negative and significant GCAs (Table 5). For tryptophan concentration, FEP1 had positive and significant GCAs whereas other female parents had negative and non-significant GCAs. MAP2 had positive and significant GCA whereas MAP4 had negative and significant GCA. The other two male parents had non-significant GCAs (Table 6). The signs of GCAs for tryptophan content as well as for protein concentration changed from one environment to another (Tables 5 and 6).

SCAs of several crosses changed in directions and magnitude from one environment to another. Changes were more apparent under optimum condition than under low nitrogen and drought. Across environments, the cross S34 had the highest SCA of 0.344% protein concentration. Its parents (FEP3 and MAP4) had across environments highly significant and negative GCAs. Across environments, the cross S43 had the highest, positive and significant SCA for tryptophan concentration in grain. Furthermore, all crosses had non-significant SCAs across environments for tryptophan concentration, except the cross S43 (Table 6).

Discussion

The results showed that protein concentration in grain in QPM, like many other traits in normal maize (Lee et al., 2005; Menkir and Ayodele, 2005; Hallauer and Miranda, 1998) was controlled by additive and non-additive effects of gene action, but additive effects were predominant over non-additive. Furthermore, because maternal effects formed high proportions of genetic effects, protein concentration was almost exclusively under control of additive and maternal effects. The results also demonstrated that tryptophan concentration in grain was controlled by both additive and non-additive effects but unlike protein concentration and many other traits in normal maize, non-additive effects were predominant over additive whereas maternal effects were completely absent.

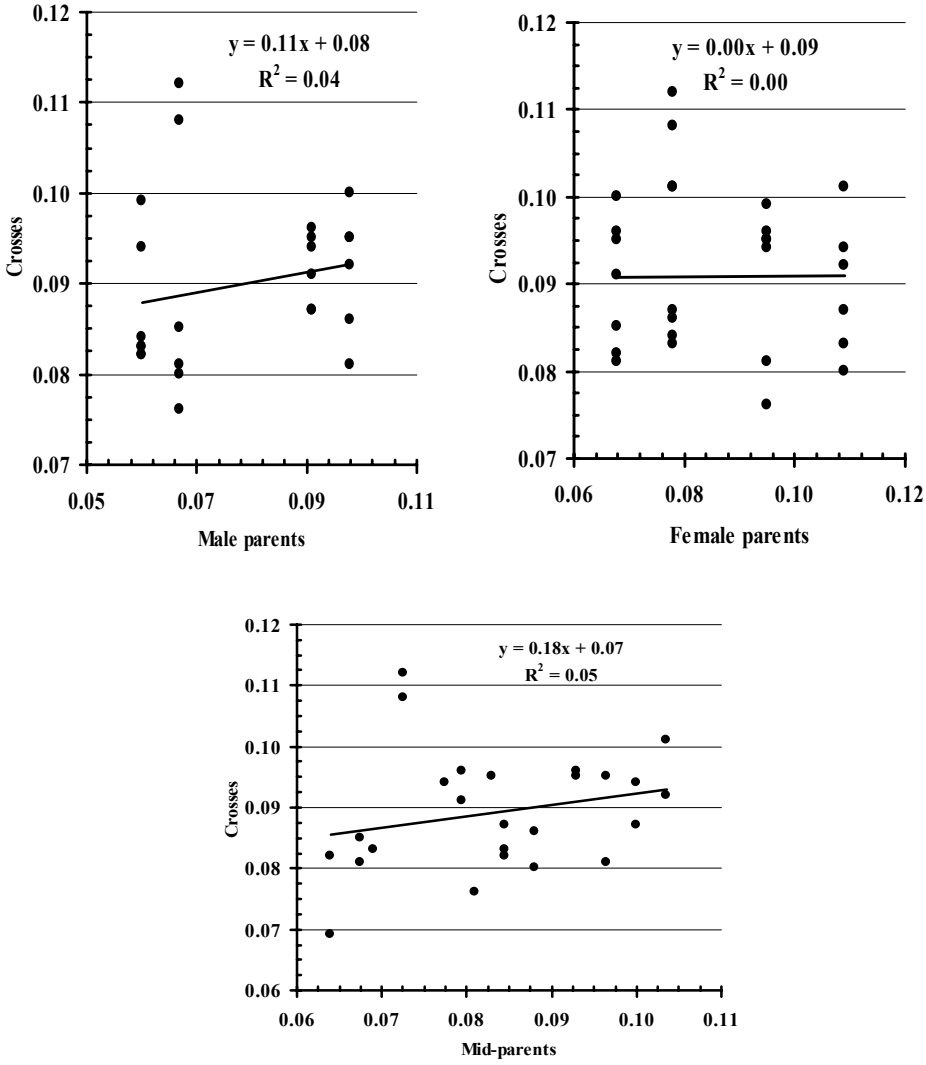

Figure 2. Regression of parents onto crosses for tryptophan concentration (%) in grain.

Maternal effects result from the influence of specific maternal genotype such as extra-nuclear genes and tissues like endosperm of female parent to its offspring. It is known, that the female parent provides two alleles and the male parent one allele of a locus for the synthesis of endosperm (Poehlman and Sleper, 1995), and thus, the female parent may influence more importantly the synthesis of endosperm in its offspring than male parent. Maternal effects appear to be importantly involved in control of protein concentration in grain of QPM like some traits in normal maize especially those linked to tolerance and

resistance to insects (Dhiwayo et al., 2005; Derera et al., 2001), but they are completely absent in control of tryptophan concentration.

The linear regression of female, male and mid-parents on crosses showed that parents with high levels of protein content in grain transmitted this character to their offspring. This was even important when mid-parent was high. However, the linear regression of parents onto crosses for tryptophan concentration strongly demonstrated the absence of relationship between levels of tryptophan in parent and tryptophan concentration in their offspring. This confirmed the non-additive nature of tryptophan concentration because the levels in the offspring resulted from interactions between increasing alleles of same loci in one parent and decreasing alleles in the other (dominance) or interaction between alleles of different loci (epistasis). Therefore, dominance and epistatic effects played an important role in the expression of tryptophan in crosses.

The selection for protein and tryptophan concentrations in QPM is done simultaneously. However, in QPM, the primarily interest is protein quality, i.e. levels of tryptophan; therefore selection of parents should be based on their performance in hybrid combination for tryptophan content, but not on the levels of tryptophan in the parental lines or on the basis of protein concentration. Due to non-additive nature of tryptophan content, demonstrated in this study, reciprocal recurrent methods may be the most appropriate methods of selection and improvement for tryptophan concentration as they increase the SCA of the parents and select offspring based on their performance in crosses (Hallauer and Miranda, 1988).

Field conditions (optimum condition) reduced additive effects, especially female additive effects and hence maternal effects and elevated at the same time non-additive effects. Moreover, low nitrogen further reduced additive effects of protein concentration in comparison with F1 kernels and even in comparison of optimum environment. Drought, unlike optimum and low nitrogen conditions slightly reduced additive effects for protein concentration in grain in comparison with F_1 kernels.

The reduction of additive effects for protein concentration with concomitant rise of non-additive effects when F_1 kernels were evaluated in field conditions seemed to work in two ways. The fist appeared to have involved the reduction of maternal effects or the activation of some effects common to female and male parents, but expressed in F_1 kernels for female parents and in field condition for male parents. The second way, which seemed to be more plausible, considered reduction of additive effects and rise of non-additive effects as result of activation enhancement and induction of inter-allelic interactions (non-additive effects) which were not possible under non-field conditions. Intuitively, field conditions acted probably more importantly or exclusively on epistasis (interactions between alleles from different loci) than on dominance (interactions between increasing and decreasing alleles of the same locus). Globally, additive effect proportion decreased while non-additive effect proportion increased.

Low nitrogen in the fields for protein concentration, reduced and/or inactivated additive as well as non-additive effects, but acted more importantly on additive effects, especially by suppressing maternal effects present in F_1 kernels and of non-additive effects generated by field conditions. Additive and non-additive effects decreased, but additive effects decreased more importantly so that non-additive looked as if they were elevated. Drought suppressed and/or inactivated completely several inter-allelic interactions and hence reduced non-

additive effects for protein concentration. Further, it suppressed completely maternal effects and hence reduced additive effects as well.

Field conditions with appropriate levels of nutrients and water further increased non-additive effects on tryptophan concentration by activating, enhancing, inducing, or by providing necessary conditions to the expression of inter-allelic interactions (dominance and epistasis) which could not be expressed in non-field conditions. Intuitively for tryptophan concentration, it is more likely that field conditions activated more on epistasis (interactions between alleles from different loci) than on dominance (interactions between decreasing and increasing alleles of the same locus). Low nitrogen and drought suppressed the non-additive effects with the global result of increasing the percentage of additive effects for tryptophan concentration. They suppressed or indistinctively reduced inter-allelic interactions.

The effects of field conditions, low nitrogen, and drought on proportions of various genetic effects have therefore consequences on GCAs of parental lines and SCAs of crosses. By suppressing or increasing no-additive effects in crosses, environments increase, decrease, and even change signs of GCAs and SCAs.

Conclusion

Genetic variation of protein concentration is controlled by additive and maternal effects while that tryptophan is almost exclusively under control of non-additive effects. Due to its non-additive nature, selection of parents should be based on their performance in hybrid combination for tryptophan content, but not on the levels of tryptophan in the parental lines or based on protein concentration. Field conditions induce the expression of inter-allelic interactions and hence they increase the proportion in genetic variation for both traits, but they suppress some of maternal effects generated by extra-nuclear inheritance and endosperm tissue on protein concentration. Low nitrogen affects additive and maternal effects for protein concentration while it affects non-additive effects for tryptophan concentration. Drought affects non-additive effects for both protein and tryptophan concentrations in grain but affects also maternal effects for protein concentration. By changing the proportion of genetic effects, environments changed magnitudes and directions of of GCAs and SCAs.

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