GENETIC ANALYSIS OF WATER-LOGGING TOLERANCE IN TROPICAL MAIZE (*ZEA MAYS* L.)

P.H. Zaidi[1,*,•], P. Maniselvan[1], A. Srivastava[2,#], Poonam Yadav[1], R.P. Singh[1]

[1] *Directorate of Maize Research, Pusa Campus, New Delhi-110012, India*
[2] *CIMMYT- ARMP, C/o ICRISAT, Patancheru 502 324, India*

ABSTRACT - Approximately 80% of maize (*Zea mays* L.) in South and Southeast Asia is grown as a rain-fed crop, where temporary excessive soil moisture or water-logging during the summer-rainy season is one of the major production constraints in large areas of this region. The present genetic study analyzed the tolerance of tropical maize to water-logging stress. Elite maize inbred lines with known stable performance in terms of improved grain yield under water-logging stress were crossed using half-diallel (7 x 7) and line × tester (8 × 3) mating designs, in which four lines were common in both the mating designs. F1 progenies (excluding reciprocals) and their parents were evaluated under managed water-logging stress at knee high stage (V7-8 growth stage) at the maize research farm, Indian Agricultural Research Institute, New Delhi, India (28.4°N, 77.1°E, 228.1 masl). In addition, the same set of entries was simultaneously evaluated under a normal moisture regime. Analysis showed that both general combining ability (GCA) and specific combining ability (SCA) effects were statistically significant. However, the GCA effect was comparatively higher (P<0.01) than the SCA effect (P<0.05). This result suggests that both additive and non-additive factors affect the expression of tolerance to water-logging stress in tropical maize. Analysis of the diallel and L × T dataset showed that water-logging tolerance in maize followed an additive-dominance genetic model, with additive gene effects dominating. Our findings suggest that reciprocal recurrent selection would be an effective approach for improving water-logging tolerance in tropical maize. Evaluating S1 progeny *per se* and their test-crosses under managed water-logging stress, discarding susceptible fraction and combining the selected best lines in terms of *per se* and test-cross performance could result in improved water-logging tolerant population. The new lines derived from the improved population could be used in developing water-logging tolerant synthetic varieties to exploit the additive gene effects and hybrids to exploit the non-additive gene action.

KEY WORDS: Maize; Water-logging; Excess soil moisture; Genetics; Inheritance.

INTRODUCTION

Inability of non-wetland crop species, including maize, to withstand excessive soil moisture conditions in the rhizosphere, caused by water-logging or any other factor, results in substantial yield losses. Maize crops grown during the summer-rainy season in the tropics occasionally face extreme climatic conditions and a variety of biotic and abiotic pressures that limit yield potential. Among abiotic stresses, water-logging, caused by contingent flooding, continuous rainfall coupled with inadequate drainage or a high water table, is one of the most important constraints for maize production in Asia and many other parts of the world. In South and Southeast Asia alone, over 18% of the total maize growing areas are frequently affected by floods and water-logging problems (ZAIDI *et al.*, 2009).

Excessive moisture or submergence leads to reduced gas exchange between root tissues and the atmosphere because the diffusion rate of gases in flooded soil is approximately 100 times lowers than in air (KENNEDY *et al.,* 1992). Respiration by plant roots, soil micro-flora and fauna leads to a rapid exhaustion of soil oxygen, resulting in hypoxia followed by anoxia. Unlike rice plants, maize plants have no naturally occurring air spaces in their roots. Therefore, as a result of the gradual decline in oxygen, plant roots suffer hypoxia (low oxygen) followed by anoxia (no oxygen) when faced with prolonged (>3 days) excess soil moisture (DENNIS *et al.*, 2000; ZAIDI and SINGH, 2002). However, the extent

• Present address: CIMMYT-ARMP, C/o ICRISAT, Patancheru 502 324, India.

Present address: Monsanto India Ltd., Gaziabad, India.

* For correspondence (phzaidi@cgiar.org).

of damage due to water-logging stress varies significantly with the developmental stage of the crop. Previous studies have shown that maize is comparatively more susceptible to water-logging from the early seedling stage to the tasseling stage (MUKHTAR et al., 1990; ZAIDI et al., 2004). However, significant genetic variability has been observed in the tolerance of maize to water-logging stress (TORBERT et al., 1993; RATHORE et al., 1996, 1997; ZAIDI et al., 2002, 2003, 2007a). This variability could be exploited to develop maize varieties tolerant to contingent/intermittent water-logging stress during the summer-rainy season in the tropics.

An effective breeding strategy for developing water-logging tolerant cultivars primarily depends on a sound knowledge and understanding of the inheritance mechanism of the stress tolerance in tropical maize. Studies on the combining ability of water-logging stress tolerance in Indian maize have been attempted (KHERA et al., 1990; HOSSAIN, 2001), however, only limited information on some location-specific germplasm is available. We selected maize inbred lines from wide genetic background, including Indian maize program and CIMMYT lines from diverse sources. Objective of the present study was to determine the genetics of inheritance of water-logging tolerance in tropical maize, which might be applicable on wide genetic background.

MATERIALS AND METHODS

Germplasm

Maize inbred lines used to generate two sets of experimental crosses (diallel and line × tester) were selected from tropical/subtropical line (S_5-S_n) evaluation trials (Table 1). The trials that identified tolerant sources of germplasm for water-logging stress were conducted during 1998-2003 at maize research farm, Indian Agricultural Research Institute, New Delhi, India (28.4°N, 77.1°E, 228.1 masl). Germplasm screened in the line evaluation trials included CM-lines from the All India Coordinated Maize Improvement Program (AICMIP), advanced generation elite lines from the Regional Research Station, Haryana Agriculture University, India, and CML-lines from the International Maize and Wheat Improvement Center (CIMMYT) Tropical and Subtropical Maize Program. Beginning from *Kharif* 1998, an average of 100 lines were screened under managed water-logging stress every year, and the top-ranking 10-15% entries were selected on the basis of their superior performance under water-logging stress. Since the lines had never been exposed to water-logging on this scale before, none of the lines showed good tolerance to the stress when screening began. However, significant genotypic variability was found for water-logging tolerance. Individual tolerant plants from superior entries were tagged, and the line was maintained through intra-entry sib-mating, and planted ear-to-row in the next

season. This procedure was followed for 4-6 seasons, with susceptible fractions being systematically discarded. The procedure was continued until the line became consistent in its performance under water-logging stress. From the 11 lines and 3 testers used in the present study, 5 lines were from CIMMYT, while 6 lines and all 3 testers were from the Indian Maize Program. CML-311 was derived from CIMMYT's sub-tropical maize population 500 and CML-327 and CML-425 from tropical population 45 and pool 31, respectively. CML-429 and CA00106 were derived from an early maturity downy mildew resistant (EYDMR) population from CIMMYT's Asian Regional Maize Program (CIMMYT, 1998). The Indian maize line WL8-B-B-B-1 was derived from CM-119, while WL18-B-B-B-4, WL18-B-B-B-6, WL28-B-B-B-2 and CM-118 were derived from a yellow flint population PTR (Puerto). WL36-B-B-B-4 was derived from an open pollinated variety CM-501, which originated from Suwan-1c7 population. The tester line CM-123 was derived from an Indian yellow flint population H3191-3, HKI-323 were derived from CIMMYT's tropical maize pool 28 and HKI-1105 from the hybrid Cargil 633 (KAUL et al., 2009).

Two sets of experimental crosses (diallel and line × tester) were generated during the winter cycle of 2003-04 using inbred lines and testers with established reaction to water-logging stress (Table 1). In the half-diallel set, seven inbred lines were crossed using all possible combinations (excluding reciprocals) and a total of 21 hybrid combinations were harvested with a sufficient amount of seed. For the line × tester (L × T) set, eight lines were crossed with three testers from opposite heterotic groups (KEMPTHORNE, 1957) and 24 hybrid combinations were then harvested for further study.

TABLE1 - *Reaction of the parental lines used in the study on genetics of water-logging stress tolerance in maize.*

Inbred lines	Standard pedigree	Reactions
Lines in diallel		
WL8-B-B-B-1	CM-119	Tolerant
WL18-B-B-B-6	CM-118-2	Highly tolerant
WL28-B-B-B-2	CM-118-3	Tolerant
WL36-B-B-B-4	CM-501	Susceptible
CML-425	P31C4S5B-6-##-BBBB	Tolerant
CML-429	EYDMRGC5-S2-BB-3-1-B*4	Highly tolerant
CML-311	S89500F2-2-2-2-B*5	Tolerant
Lines in L x T		
CML327	P45C6F83-3-1-B*5	Tolerant
WL8-B-B-B-1	CM-119	Tolerant
WL18-B-B-B-6	CM-118-2	Highly tolerant
WL28-B-B-B-2	CM-118-3	Tolerant
CA00106	EY-DMRGC5-S2-B-B-2-1-B*5	Tolerant
CM118	CM-118-1	Susceptible
CM311	S89500F2-2-2-2-B*5	Tolerant
WL18-B-B-B-4	CM-118-4	Tolerant
Testers		
HKI-1105	Cargil 633-⊗-⊗-⊗-⊗-⊗-⊗-B	Tolerant
HKI-323	(P28/TSR)-33-2-7-1-2-B-F-⊗-⊗-B	Susceptible
CM123	H3191-3-⊗-⊗-⊗-⊗-⊗-B*5	Susceptible

Experimental details and stress treatment

Experiments were conducted during the *Kharif* (summer-rainy season) of 2004 and 2005 at the maize research farm, Indian Agricultural Research Institute, New Delhi, India (28.4°N, 77.1°E, 228.1 masl). The experimental station soil is characterized as sandy loam with a pH of 7.8. All entries were planted in the field using an Alpha (0, 1) lattice design (PATTERSON and WILLIAMS, 1976) with two replications. Experimental crosses and parental lines were evaluated in the same trials under managed water-logging conditions, keeping one inbred border row on both sides of the parental line plots. Same set was also planted under normal moisture regimes. Planting was done during the last week of June and all entries were over sown and thinned to one plant per hill at the V_2 growth stage to give a population density of 66, 600 plants ha^{-1}. Each entry was planted in a one row plot; 3.0 m long, with 20 cm spacing within and 75 cm between rows. Before planting, 60 kg nitrogen (N) ha^{-1} in the form of urea, 60 kg phosphorous ha^{-1}, as single super phosphate, 40 kg potassium ha^{-1}, as muriate of potash, and 10 kg zinc, as zinc sulfate, were applied as a basal dose. Second and third doses of N (each 30 kg N ha^{-1}) were side-dressed at the knee-high and tasseling stages. Pre-emergence application of pendimethalin and atrazine (both at 0.75 kg ha^{-1} a.i., tank mixed) were used for weed management in the experimental plots. Experiments were kept free from insects, weeds and diseases using recommended post-emergence chemical management and optimal agronomic practices.

The water-logging stress treatment was applied at the knee-high stage (V_{7-8} growth stage) continuously for 7 days. Water-logging experiments were conducted in a field specifically designed and leveled for this purpose. In each bed, two drainage pipes were fixed at 10.5 cm height above the ground. Initially the treatment plots were completely filled with water, and maintained as such for seven days at a depth of 10±0.5cm by supplying water at a rate that exceeded infiltration and evaporation. After completion of the stress treatment the field was completely drained.

Observations

In vivo chlorophyll concentration of the uppermost fully expanded leaves was determined for 10 plants per plot at one week after release of the stress, using a Minolta SPAD-502 chlorophyll meter. Results were averaged to give a single plot value. Brace root node was recorded on 10 plants, at 50% male flowering, by counting the number of aboveground nodes bearing brace roots. Days from planting to anthesis and silking, indicated by 50% of plants having one extruded anther or producing one visible silk, were recorded daily by visual observations during the flowering period. Anthesis-silking interval (ASI) was calculated as the difference between the number of days to 50% silking and 50% anthesis. Under water-logging stress, few highly susceptible lines failed to reach 50% silking, resulting in barren plants. In such cases maximum days to 50% silking in the trial was considered as days to 50% silking of those genotypes, and the same was used for calculation of ASI. At maturity, ears were harvested, excluding two plants closest to the alley at both ends of the row. Ears were dried to a constant moisture level and grain yield was recorded on a shelled grain basis and adjusted to 15% grain moisture.

Statistical analysis

Analysis of variance (ANOVA) was calculated using ALPHA-program (CIMMYT, 1999). Lattice-adjusted means were computed for each trial. The combined analysis was conducted using Gen-Stat v.8, which indicated that year and year × genotype effects were non-significant. Data for both years were pooled after further testing the homogeneity of error variance using Hartley's F_{max} test (OTT, 1988). Half-diallel analysis was conducted using GRIFFING's (1956) Method 2 (Model II). Both the half-diallel and the L × T analysis were performed using SPAR1 statistical software.

RESULTS

Under water-logging stress the inbred lines selected for the present study had highly significant genotypic variability for all traits, including ASI (P<0.01), chlorophyll content (P<0.01), stress-induced brace roots (P<0.01) and grain yield (P<0.001) (Table 2). However, the genotypic variation was not significant under normal moisture conditions, except in the case of grain yield (P<0.05). The tolerant lines showed a many fold increase in brace root development under water-logging, while the increase was nominal for the susceptible lines. Genotypic variability among the lines was highly significant (P<0.001) for stress-induced brace development; however, the genotype × environment interaction effects were statistically non-significant. Leaf chlorophyll content varied significantly among the lines even under normal moisture. However, variation increased under water-logged conditions. Susceptible lines showed more than 50% loss in chlorophyll content under water-logging, while most of the tolerant lines had relatively less chlorophyll loss (Table 2), and faster recovery after the stress was removed (data not shown). Water-logging significantly affected female flowering (data not shown), which resulted in an average increase in the ASI by 3.6 days. This ranged from 1.6 days in WL28-B-B-B-2 to 7.4 days in CM-118. The tolerant lines were, however, able to maintain ASI close to or less than 5 days under water-logging stress. The overall effect of water-logging stress was eventually expressed in terms of a high yield penalty, which ranged from 29.8% (WL18-B-B-B-6) to 94.2% (CM-118). Mean grain yield of the lines under water-logging stress ranged from 0.17 (CM-118) to 2.11 t ha^{-1} (CML-429) and mean grain yield of testers used in the L × T analysis ranged from 0.22 (HKI-323) to 1.42 (HKI-1105). Genotypic variability for grain yield was also significant under normal moisture (P<0.05); however, under water-logging stress it increased many fold and the variation was very significant (P<0.001). Grouping of inbred lines in terms of their reactions under water-logging stress (highly

TABLE 2 - *Mean of grain yield and other traits of inbred lines under water-logging and normal moisture.*

Pedigree	Anthesis-silking interval (days)		Nodes with brace roots		Chlorophyll (SPAD unit)		Grain yield (t/ha)	
Lines	WL	NM	WL	NM	WL	NM	WL	NM
CML 327	4.9	1.2	2.2	1.0	33.9	51.2	1.60	2.74
WL8-B-B-B-1	5.3	2.1	2.2	0.0	34.6	50.0	1.09	3.01
WL18-B-B-B-6	3.0	1.2	3.6	1.0	39.4	40.1	2.03	2.89
WL28-B-B-B-2	3.6	2.0	2.6	0.7	33.2	45.6	1.44	2.56
WL36-B-B-B-4	6.9	0.8	0.8	0.0	23.4	48.6	0.19	2.97
CML-425	3.0	0.6	2.4	1.0	40.2	49.5	1.78	3.01
CML-429	3.7	1.7	3.2	0.7	36.7	44.0	2.11	3.13
CA 00106	6.0	0.4	3.1	0.3	37.5	44.3	1.28	2.59
CM118	8.3	0.9	1.2	0.3	33.5	45.2	0.17	2.91
CML-311	4.8	1.4	3.0	0.7	36.2	41.2	1.63	2.86
WL18-B-B-B-4	4.0	0.3	2.7	1.0	45.2	55.2	1.12	2.95
Testers								
HKI1105	5.3	0.9	2.1	0.7	31.2	49.5	1.42	3.16
HKI 323	11.4	0.9	1.2	0.3	22.9	46.3	0.22	3.01
CM123	10.3	0.3	0.4	0.0	29.7	40.1	0.24	2.93
Mean	4.7	1.1	2.2	0.6	34.1	46.5	1.20	2.91
L.S.D.	1.03	ns	1.08	ns	11.03	7.92	0.97	1.17
F-significance for line	**	–	**	–	**	–	***	*
F-significance for line x environment	*	–	—	–	*	–	*	*

*, ** Significant difference at P <0.05 and 0.01, respectively, and ns indicates non-significant difference.
WL = Water-logging; NM = Normal moisture.

tolerant, tolerant or susceptible) was most apparent for the effect on grain yield, and to a significant extent a similar trend was also observed for other agronomic traits.

Lines used in diallel analysis showed highly significant (SE=0.25**, significant at P<0.01) genotypic variability in terms of grain yield under water-logging, and ranged from 0.19 (WL36-B-B-B-4) to 2.11 t ha^{-1} (CML-429) (Table 2). ANOVA of the diallel dataset with respect to grain yield under water-logging revealed significant general combining ability (GCA) and specific combining ability (SCA) effects (Table 3). However, the effect of GCA was comparatively higher (P<0.01) than SCA (P<0.05). GCA estimate of individual lines for grain yield under water-logging stress indicated that out of seven lines, the effects were significant in the cases of WL18-B-B-B-6 and WL28-B-B-B-2 at P<0.05 and in the cases of WL36-B-B-B-4 and CML-429 at P<0.01 (Table 4). However, the GCA effects were negative in the cases of WL28-B-B-B-2 and WL36-B-B-B-4, which indicates their contribution towards susceptibility. Data

TABLE 3 - *ANOVA of diallel set of data for combining ability for water-logging stress.*

Sources	d. f.	MSS
GCA	6	44.46**
SCA	21	10.92*
Error	27	1.26

* and ** indicate statistical significance at P<0.05 and P<0.01, respectively.

analysis for SCA estimates showed that most of the crosses in diallel exhibited significant effects (Table 4). All crosses with CML-429 showed positive and highly significant SCA effects (P<0.01), including the cross with the susceptible line WL36-B-B-B-4, which also had a positive and significant SCA effect at P<0.05. However, the SCA effect on the crosses which had another highly tolerant line (WL18-B-B-B-6) was comparatively smaller than when CML-429 was one of the parents in the cross. All crosses

TABLE 4 - *Estimates of general combining ability (GCA), specific combining ability (SCA) effects, mean yields and estimated components of variance involved in diallel analysis for water-logging stress.*

Parents	WL8-B-B-B-1	WL18-B-B-B-6	WL28-B-B-B-2	WL36-B-B-B-4	CML425	CML429	CML311
WL8-B-B-B-1	-0.11	0.42*	0.11	-2.01**	0.35*	0.82**	0.22
WL18-B-B-B-6	4.56 (30.1)	0.36*	-0.06	0.48*	0.45*	1.28**	0.55*
WL28-B-B-B-2	4.24 (35.6)	4.08 (41.2)	-0.42*	-1.98**	-0.08	0.65**	-1.02**
WL36-B-B-B-4	2.13 (69.7)	3.24 (55.7)	1.98 (72.8)	-0.81**	0.57*	0.59*	-1.09**
CML-425	4.49 (35.7)	4.59 (42.8)	4.06 (43.3)	3.05 (56.4)	0.22	0.93**	0.84**
CML-429	4.96 (34.8)	5.12 (29.8)	4.79 (31.4)	3.56 (50.8)	4.97 (38.9)	0.67**	0.79**
CML-311	4.36 (45.6)	4.06 (49.1)	3.14 (53.3)	2.98 (59.3)	4.98 (34.4)	4.12 (41.3)	0.09

Diagonal represents GCA effects; figure above diagonal represents SCA effects, figure below diagonal represents mean yield under water-logging stress. Figures in parenthesis are yield losses (%) under water-logging stress.
* and ** indicate statistical significance at P<0.05 and P<0.01, respectively.
Standard errors: S.E. (gi) = 0.15; S.E. (gi-gj) = 0.24; S.E. (Sij) = 0.38; S.E. (Sij-Sik) = 0.67; S.E. (SijSki) = 0.40.

showed positive and significant SCA effects at P<0.05, except for the WL18-B-B-B-6 × CML-429 (P<0.01) cross and the cross between WL18-B-B-B-6 × WL28-B-B-B-2 which had a negative and not significant SCA effect. In the case of the susceptible line (WL36-B-B-B-4), out of a total of six crosses, three showed strong negative SCA effects (P<0.01). However, the other three crosses of WL36-B-B-B-4 with CML-429, CML-425 and WL18-B-B-B-6 had positive and significant (P<0.05) SCA effects. Among others, the crosses which had CML-425 (tolerant) as one of the parents showed positive and significant SCA effects, except CML-425 × WL28-B-B-B-2 which had a negative SCA which was not significant.

Comparison of the performance of hybrids with their parents under water-logging stress indicated that, in general, parental lines showed relatively more susceptibility to the stress than their hybrid progenies (Table 2 and Table 4). Mean grain yield of F1s under water-logging ranged from 1.98 (WL36-B-B-B-4 × WL28-B-B-B-2) to 5.12 t ha^{-1} (CML-429 × WL18-B-B-B-6). The crosses involving highly tolerant lines (WL18-B-B-B-6 and CML-429) were relatively less susceptible to the water-logging stress. That is, mean yield loss was at a minimum (37.8%) when CML-429 was one of the parents, followed by WL18-B-B-B-6 (41.4%). On the other hand, the F1s with WL-36-B-B-B-4 (a susceptible line) as one of the parents had a maximum mean

TABLE 5 - *ANOVA of combining ability for parents and hybrid involved in line x tester analysis and estimated components of variance.*

Source	d.f.	MSS
Replication	1	6.502
Parents (P)	10	6.492*
Lines	7	8.285**
Testers	2	1.451*
Lines vs. Testers	1	4.021**
Crosses (C)	23	1.231**
Lines x Testers	14	3.823*
P vs. C	1	21.532**
Error	34	0.274

σ^2GCA	0.304
σ^2SCA	0.115
σ^2A (F=1)	3.228
σ^2D (F=1)	1.275

Contribution of lines = 35.99%*
Contribution of testers = 9.22%
Contribution of line x tester = 54.78%**
* and ** indicate statistical significance at P<0.05 and P<0.01, respectively.

yield loss (60.8%) under water-logged conditions. Among other lines, CML-425 also showed a promising contribution as a parent, with 41.9% mean yield loss in F1s under water-logging.

ANOVA for L × T analysis, with respect to grain yield under water-logging (Table 5) revealed significant variation among parents (P<0.05). The variation among lines was strong (P<0.01) compared to that among testers (P<0.05). The lines versus testers variance was highly significant (P<0.01), indicating that testers were highly divergent from lines, which satisfies the choice of testers. The variance for parents versus hybrids was also highly significant (P<0.01). This indicates significant average heterosis, i.e. difference between mean yield of parents (1.08 t ha^{-1}) and crosses (3.13 t ha^{-1}). Variation among the F1 progenies was highly significant (P<0.01), in which lines contributed 35.99% (P<0.05) and the lines × tester interaction component was significantly apportioned 54.78% (P<0.01). However, the contribution of testers to variation among F1 progenies (9.23%) was not statistically significant. The additive variance (σ^2A) was greater than non-additive (σ^2D), indicating that reciprocal recurrent selection aimed at accumulating favorable alleles in heterotic populations might be a better choice for improving water-logging tolerance in maize.

GCA effects of lines ranged from -2.03 (CM118) to 1.23 (WL18-B-B-B-6) and testers from -0.49 (CM123) to 0.82 (HKI1105) (Table 6). The effects were statistically significant in the case of all the lines, except WL18-B-B-B-4 and CML-311. However, among the three testers only HKI-1105 showed significant positive GCA effects. Among the six lines with significant GCA effects, two (CM-118 and WL8-B-B-B-1) showed negative GCA effects. Significant positive GCA effects indicated their ability to confer water-logging tolerance to their progeny. In SCA analysis most of the crosses did not have significant effects (Table 7). The L × T variance was highly significant (Table 5), which resulted in specific cross combinations distinctly superior over others. Out of a total of 24 crosses seven showed significant positive SCA effects, while another four showed significant negative SCA effects. Among the crosses with significant positive SCA effects, six crosses with the tolerant tester (HKI-1105) showed highly significant (P<0.01) SCA effects, while one was significant at P<0.05.

In the L × T set, mean grain yield of F1 progenies under water-logging stress (Table 7) ranged from 1.13 (CM-118 × CM-123) to 4.62 t ha^{-1} (CML-327 × HKI-1105). In general, all crosses, except WL18-B-B-B-6 × HKI323 and CM118 × HKI1105, with tolerant testers performed relatively well under water-logging stress, as compared to crosses with

TABLE 6 - *General combining ability (GCA) effects and means yield under water-logging stress in the L x T analysis.*

Parents	GCA effects	Mean
Lines		
CML327	0.93**	1.60
WL8-B-B-B-1	-0.85*	1.09
WL18-B-B-B-6	1.23**	2.03
WL28-B-B-B-2	0.76*	1.44
CA00106	0.89*	1.28
CM118	-2.03**	0.17
CM311	-0.62	1.27
WL18-B-B-B-4	-0.25	1.12
Testers		
HKI1105	0.82*	1.42
HKI323	-0.33	0.22
CM123	-0.49	0.24
LSD at	P = 0.05	P = 0.01
GCA (lines)	0.41	0.54
GCA (testers)	0.23	0.30

* and ** indicate statistical significance at P<0.05 and P<0.01, respectively.

susceptible testers. The crosses between tolerant lines × tolerant testers had a relatively good level of water-logging tolerance. All top-ranking crosses, with yields ranging from 3.79 (WL18-B-B-B-4 × HKI1105) to 4.62 t ha^{-1} (CML-327 × HKI-1105), were crosses between highly tolerant/tolerant lines and tolerant testers (HKI-1105). The two worst performing crosses under water-logging, were crosses between susceptible lines (CM-118) and susceptible testers (CM-123 and HKI-323). Yield losses under water-logging stress ranged from 38.6% (CML-327 × HKI-1105) to 88.2% (CM-118 × CM-123). Mean yield reduction in F1 progenies in water-logged conditions between tolerant × tolerant crosses was 39.9%, whereas in the crosses between tolerant × susceptible, susceptible × tolerant and susceptible × susceptible crosses this reduction was 58.2%, 85.8% and 88.9%, respectively.

Mean grain yield of hybrids, generated from crosses of inbred lines with different doses of tolerance to water-logging, is presented in Table 8. Mean yield was highest in F1 progenies of highly tolerant (HT) × HT lines, while it was lowest in susceptible (S) × S crosses. Mean yield of the crosses between HT × tolerant (T) and T × T was almost equal, and both were significantly higher than the
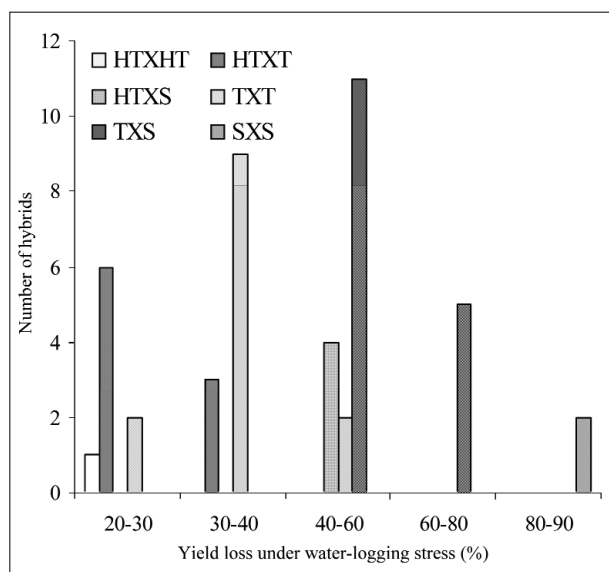
FIGURE 1 - Frequency distribution of yield losses (%) under water-logging in hybrids involving lines with different levels of tolerance to water-logging stress (HT = highly tolerant, T = tolerant and S = susceptible).

crosses generated between T × S and S × S lines. Means for secondary traits, including ASI, nodes with brace roots and chlorophyll contents under water-logging also followed a similar trend. Out of a total of eight hybrids with yield losses less than 30%, four were crosses between the HT × T, one between HT × HT and the other two were T × T (Fig. 1). However, maximum number of hybrids (17) grouped in the range of 40-60% yield losses under water-logging, which included two hybrids of T × T, four of HT × S and 11 hybrids of T × S crosses. The two crosses with yield losses >80% under water-logging were cross between S × S and other five crosses with 60-80% yield loss under stress were cross between T × S lines.

DISCUSSION

Genetics of water-logging stress tolerance has been studied for various non-wetland crop species (Cao et al., 1992; Boru et al., 2001; Collaku and Harrison, 2005). In the case of tropical maize, some progress has been made in identifying the QTL associated with secondary traits in maize under water-logging (Fazhan et al., 2007; Mano et al., 2005, 2009), however little is known about the heritability of tolerance to water-logging stress in tropical

maize. Understanding the genetic inheritance of water-logging tolerance is pre-requisite for developing tolerant varieties suitable for growing in water-logging-prone environments in the tropics.

Inbred lines selected for the diallel and L × T studies showed highly significant genotypic variability under water-logging stress for all traits, including grain yield (Table 2). Genotypic variability with respect to water-logging tolerance in maize has been reported in previous studies (Torbert et al., 1993; Rathore et al., 1998; Zaidi et al., 2004, 2007b). A water-logging-induced increase in brace root development in tolerant genotypes was identified as one of the stress-adaptive traits in maize under water-logging stress (Subbaiah and Sach, 2003; Mano et al., 2005; Zaidi et al., 2007b,c). The significant decrease in leaf chlorophyll contents under water-logging stress (Table 2) is identified as one of the first stress symptoms (Zaidi and Singh, 2001; Zhou et al., 2004), which may be related to nitrogen deficiency caused by leaching and denitrification of the soil nitrogen (Rathore et al., 1996). This may be due to a super-oxide radical mediated increase in chlorophyll breakdown (Yan et al., 1995). Water-logging stress resulted in a significant increase in the number of days ASI in all genotypes (Table 2). However, this increase was comparatively larger in susceptible lines. In general, effects of various abiotic stresses, including water-logging (Zaidi et al., 2004, 2007b), drought (Bolaños and Edmeades, 1996), and nitrogen deficiency (Banziger and Laffitte, 1997), on ASI were found to be largely based on delayed silking, not on anthesis. Reduced current photosynthesis in susceptible genotypes under excessive moisture stress (Huang et al., 1994) apparently reduces assimilates for silk growth and cob development. In general, inbred lines (Table 2) showed relatively more susceptibility to water-logging stress than their hybrid progenies (Table 4 and 7). This highlights the contribution of heterosis in stress tolerance (Dass et al., 1997). In our previous study (Zaidi et al., 2007a) we found that the contribution of heterosis, both mid- and best-parent, increased under water-logging for most traits, including grain yield.

Diallel and L × T analyses with respect to grain yield under water-logging stress indicated a lack of complete dominance in the F1 progenies, as varying degrees of reactions were observed in HT × S and T × S crosses (Table 4 and 7). These observations strongly suggest the presence of a polygenic system controlling water-logging tolerance, with partial

TABLE 7 - *Specific combining ability (SCA) effects and mean yield under water-logging stress in the L x T analysis.*

Lines/Testers	HKI-1105		HKI-323		CM-123	
	SCA effects	Grain yield (t ha^{-1})	SCA effects	Grain yield (t ha^{-1})	SCA effects	Grain yield (t ha^{-1})
CML327	1.35**	4.62	-0.51	3.08	-0.84*	2.76
WL8-B-B-B-1	1.12**	3.53	-0.85*	2.95	-0.27	2.53
WL18-B-B-B-6	1.31**	4.58	-0.26	3.46	-1.05**	3.15
WL28-B-B-B-2	1.01**	4.21	-0.4	3.06	-0.61	2.99
CA00106	1.30**	4.50	-0.74	3.47	-0.56	3.14
CM118	0.52	1.92	-0.28	1.09	-0.24	1.13
CM311	1.04**	4.24	-0.46	2.89	-0.58	3.02
WL18-B-B-B-4	0.97*	3.97	-0.17	3.24	-0.80*	2.40
LSD at	P=0.05	P= 0.01				
Sij	0.382	0.483				

* and ** indicate statistical significance at P<0.05 and P<0.01, respectively.

TABLE 8 - *Mean of grain yield (t ha^{-1}) and secondary traits of hybrids under water-logging stress for different combinations of inbred lines with different levels of tolerance to water-logging stress.*

Traits	Cross	HTxHT	HTxT	HTxS	TxT	TxS	SxS
Grain yield (t/ha)	Diallel	5.12	4.51	3.40	4.21	2.97	-
	LxT	-	4.20	2.50	3.60	2.17	0.98
Anthesis silking interval (d)	Diallel	3.20	2.96	4.52	3.57	6.12	-
	LxT	-	1.59	4.02	2.55	5.46	5.19
Nodes with brace roots	Diallel	3.11	3.24	2.19	2.58	2.37	-
	LxT	-	3.27	1.99	2.16	2.01	1.16
Chlorophyll (SPAD units)	Diallel	39.60	42.30	33.20	41.60	31.48	-
	LxT	-	38.46	36.20	36.21	37.38	24.37

HT = highly tolerant; T = tolerant; S = susceptible.

dominance of tolerance over susceptibility. Previous studies on other crops also suggested that water-logging appears to be quantitatively inherited (BORU *et al.*, 2001). Also in the case of maize, water-logging at the seedling stage showed continuous variation in an F2 population and the trait is considered to be quantitative in nature (MANO *et al.*, 2005). Diallel analysis showed that for grain yield under water-logging stress both GCA and SCA effects were significant, but the GCA effect was comparatively stronger (P<0.01) than SCA (P<0.05). This suggests that both additive and non-additive factors govern the expression of tolerance to water-logging stress in tropical maize. Although, the additive component

was pre-dominant in the expression of grain yield under stress, there also appears to be a significant contribution of non-additive genetic effects. Results of both the diallel and L × T analysis clearly suggest that water-logging tolerance in maize is governed by an additive-dominance genetic model, with additive gene effects dominating. ZHOU *et al.* (2004) also reported that the inheritance of water-logging tolerance traits in barley followed an additive-dominant genetic model. In a study on tropical maize KHERA *et al.* (1990) also suggested that tolerance to excess soil moisture is genetically controlled and both additive and non-additive gene actions are important. Similar observations were also made by HOSSAIN

(2001), where it was reported that under water-logged conditions variances due to GCA were higher than SCA. CAO *et al.* (1994) reported that water-logging tolerance in wheat genotypes was mainly governed by additive factors and it was also affected by non-additive ones. These findings suggest that recurrent selection may also be an effective approach to increase the tolerance level of tropical maize to water-logging.

Among parental lines used in the study, few lines showed highly significant positive GCA effects (Table 2 and 4) and the crosses involving those lines had superior performance under stress (Table 2 and 7). However, the contribution of tolerant lines to stress tolerance was reduced when combined with susceptible lines. All hybrids with a good level of water-logging tolerance were crosses between HT × HT, HT × T or T × T lines, whereas all susceptible hybrids were crosses between S × S or T × S (Fig. 1). In our previous study on the relationship between line and cross performance under water-logging stress we found a strong and significant relationship between grain yield of best-parent and F1 progenies (ZAIDI *et al.*, 2007a). Similar results were reported from studies on European corn (PRESTERL *et al.*, 2002) and tropical maize (SHORT and EDMEADES, 1991; ZAIDI *et al.*, 2003) under low nitrogen stress, and under high population density (EL-LAKANY and RUSSELL, 1971). Means of secondary traits, including ASI, nodes with brace roots and chlorophyll contents under water-logging also followed a similar trend, indicating a strong relationship between grain yield and these secondary traits under water-logging. In our previous studies we also found a significant correlation of yield with some stress-adaptive secondary traits, including ASI, brace root and chlorophyll contents (ZAIDI *et al*., 2004; ZAIDI *et al.*, 2007c). Similar observations were reported by other researchers for maize under water-logging (RATHORE *et al*., 1998; KHERA *et al.*, 1990) and other abiotic stresses (BOLAÑOS and EDMEADES, 1996; BANZIGER and LAFITTE, 1997).

Our findings suggest that both additive and non-additive gene actions are important for water-logging tolerance in tropical maize. However, additive gene action is comparatively more important, which along with heterosis significantly contributed to water-logging tolerance. Therefore, reciprocal recurrent selection under managed water-logging stress conditions may be an effective approach to increase the level of tolerance to water-logging in tropical maize. Selection in early generation for *per se* and test-cross performance under managed water-logging stress could be very efficient in discarding the susceptible progenies. Water-logging tolerant lines derived from improved populations through reciprocal recurrent selection could be used in developing water-logging tolerant synthetic varieties and hybrids.

REFERENCES

BANZIGER M., H.R. LAFITTE, 1997 Efficiency of secondary traits for improving maize for low-nitrogen target environments. Crop Sci. **37**: 482-485.

BOLAÑOS J., G.O. EDMEADES, 1996 The importance of anthesis-silking interval in breeding for drought tolerance in tropical maize. Field Crop Res. **48**: 65-80.

BORU G., M.V. GINKEL, W.E. KRONSTAD, L. BOERSMA, 2001 Expression and inheritance of tolerance to water-logging stress in wheat. Euphytica **117**: 91-98.

CAO Y., S.B. CAI, W. ZHU, E. XIONG, X.U. FANG, 1994 Combining ability analysis of water-logging tolerance and main agronomic traits in wheat. Jiangsu J. Agric. Sci. **11**: 11-15.

CAO Y., S.B. CAI, W. ZHU, X.U. FANG, 1992 Genetic evaluation of Y, traits in wheat. Sci. Agric. Sinica **27**: 40-55.

CIMMYT, 1998 A complete listing of improved maize germplasm from CIMMYT. Maize Program special report. CIMMYT, Mexico, D.F.

CIMMYT MAIZE PROGRAM, 1999 A user's manual for field book 5.1/7.1 and ALPHA. Mexico, D.F., CIMMYT.

COLLAKU A., S.A. HARRISON, 2005 Heritability of water-logging tolerance in wheat. Crop Sci. **45**: 722-727.

DASS S., N.N. SINGH, Y.P. DANG, A.K. DHAWAN, 1997 Morpho-physiological basis of breeding drought and low-N tolerant maize genotypes in India. pp. 107-111. *In:* G.O. Edmeades, M. Bänziger, H.R. Mickelson, C.B. Pena-Valdivia (Eds.), Developing drought and low-N tolerant maize. Proc. Symposium, Match 25-29, 1996, CIMMYT, El-Batan, Mexico, D.F.,

DENNIS E.S., R. DOLFERUS, M. ELLIS, M. RAHMAN, Y. YU, F.U. HOEREN, A. GROVER, K.P. ISMOND, A.G. GOOD, W.J. PEACOCK, 2000 Molecular strategies for improving water-logging tolerance in plants. J. Exp. Bot. **51**: 89-97.

EL-ELAKANY M.A., W.A. RUSSELL, 1971 Effectiveness of selection in successive generations of maize inbred progenies for improvement of hybrid yield. Crop Sci. **11**: 703-706.

FAZHAN Q., Z. YONGLIAN, A. ZILI, X. SHANGZHONG, 2007 Mapping of QTL Associated with Water-logging Tolerance during the Seedling Stage in Maize. Ann. Botany **99:** 1067-1081.

GRIFFING B., 1956 Concept of general and specific combining ability in relation to diallel crossing system. Aust. J. Biol. Sci. **9:** 463-493.

HOSSAIN F., 2001 Studies on combining ability for water-logging tolerance in Maize (*Zea mays* L.) Thesis, M.Sc.(Ag.), GBPU-AT, Pantnagar, Distt. U.S. Nagar, India.

HUANG B.R., J.W. JOHNSON, D.C. BRIDGES, 1994 Growth, physiological and anatomical response of two wheat genotypes to water-logging and nutrient supply. J. Exp. Bot. **45:** 193-202.

KAUL J., S. DASS, J.C. SHEKHAR, B. AHMAD, 2009 Catalogue of Indian maize inbred lines, Vol. 2, Directorate of Maize Research, New Delhi, India. Technical Bulletin No. 2009/7, pp. 1-62.

KEMPTHORNE O., 1957 An introduction to genetic statistics John. Wiley and sons, Inc. New York.

KENNEDY R.A., M.E. RUMPHO, T.C. FOX, 1992 Anaerobic metabolism in plants. Plant Physiol. **100:** 1-6.

KHERA A.S., B.S. DHILLON, V.K. SAXENA, H.S. BARAR, N.S. MALHI, 1990 Genetics and physiological studies in maize on tolerance to stress caused by water-logged conditions. Project Final Report, Ad-hoc project, ICAR, New Delhi, India.

MANO Y., M. MURAKI, M. FUJIMORI, T. TAKAMIZO, B. KINDIGER, 2005 AFLP-SSR maps of maize × teosinte and maize × maize: comparison of map length and segregation distortion. Plant Breed. **124:** 432-439.

MANO Y., O. FUMIE, H.L. CARLOS, M.B. ROBERT, 2009 QTL mapping of the ability to form adventitious roots at the soil surface during flooding in a maize × teosinte "*Zea nicaraguensis*" backcross population. Plant Root **3:** 3-9.

MUKHTAR S., J.L. BAKLER, R.S. KANWAR, 1990 Maize growth as affected by excess soil water. Trans. ASAE **33:** 437-442.

OTT L., 1988 An Introduction to Statistical Methods and Data Analysis, 3rd ed., PWS-Kent, Boston.

PATTERSON H.D., E.R. WILLIAM, 1976 A new class of resolvable incomplete block designs. Biometrika **63:** 83-89.

PRESTERL T., G. SEITZ, W. SCHMIDT, H.H. GEIGER, 2002 Improving nitrogen-use efficiency in European maize - comparison between line per se and test cross performance under high and low soil nitrogen. Maydica **47:** 83-91.

RATHORE T.R., M.Z.K. WARSI, J.E. LOTHROP, N.N. SINGH, 1996 Production of maize under excess soil moisture (water-logging) conditions. pp. 56-63. *In:* 1st Asian Regional Maize Workshop, 10-12 Feb 1996, P.A.U., Ludhiana.

RATHORE T.R., M.Z.K. WARSI, P.H. ZAIDI, N.N. SINGH, 1997 Water-logging problem for maize production in Asian region. TAM-NET News Letter **4:** 13-14.

RATHORE T.R., M.Z.K. WARSI, N.N. SINGH, S.K. VASAL, 1998 Production of maize under excess soil moisture (water-logging) conditions. *In:* 2nd Asian Regional Maize Workshop PCCARD, Los Banos, Philippines, Feb 23-27, 1998.

SHORT K.E., G.O. EDMEADES, 1991 Maize improvement for water and nitrogen deficient environments pp. 63-68. *In.* J.F. MacRobert (Ed.), Proc. Crop Sci. Society Zimbabwe, 21st Anniversary of Crop Production Congress.

SUBBAIAH C., M.M. SACH, 2003 Molecular and cellular adaptations of maize to flooding stress. Ann. Botany **90:** 119-127.

TORBERT H.A., R.G. HOEFT, R.M. VANDEN-HEUVEL, R.L. MULVANEY, S.E. HOLLINGER, 1993 Short-term excess water impact on corn yield and nitrogen recovery. J. Prod. Agric. **6:** 337-344.

YAN B., D. QIUJIE, D.J. DAI, Q.J., 1995 Ameliorative effects of exogenous active oxygen scavengers of water-logging injury in maize. Acta Agric. Boreali Sinica **10:** 51-55.

ZAIDI P.H., N.N. SINGH, 2001 Effect of water-logging on growth, biochemical compositions and reproduction in maize. J. Plant Biol. **28:** 61-69.

ZAIDI P.H., N.N. SINGH, 2002 Identification of morpho-physiological traits for excess soil moisture tolerance in maize. pp. 172-183. *In:* K.K. Bora, K. Singh, A. Kumar (Eds.), Stress and Environmental Physiology. Scientific publishers, Jodhpur, India.

ZAIDI P.H., S. RAFIQUE, N.N. SINGH, G. SRINIVASAN, 2002 Excess moisture tolerance in maize - progress and challenges. pp. 398-412. *In:* Proc. 8th Asian Regional Maize Workshop, 5-9 August 2002, Bangkok, Thailand.

ZAIDI P.H., S. RAFIQUE, N.N. SINGH, 2003 Response of maize (*Zea mays* L.) genotypes to excess moisture stress: morpho - physiological effects and basis of tolerance. Eur. J. Agron. **19:** 383-399.

ZAIDI P.H., S. RAFIQUE, P.K. RAI, N.N. SINGH, G. SRINIVASAN, 2004 Tolerance to excess moisture in maize (*Zea mays* L.): Susceptible crop stages and identification of tolerant genotypes. Field Crop Res. **90:** 189-202.

ZAIDI P.H., P. MANISELVAN, A. SRIVASTAVA, R.P. SINGH, N.N. SINGH, G. SRINIVASAN, 2007a Association between line *per se* and hybrid performance under excessive soil moisture stress in tropical maize (*Zea mays* L.). Field Crop Res. **101:** 117-126.

ZAIDI P.H., P. MANISELVAN, P. YADAV, A.K. SINGH A.K., P. DUREJA, N.N. SINGH, and SRINIVASAN G., 2007b Stress-adaptive changes in tropical maize (*Zea mays* l.) under excessive soil moisture stress. Maydica **52:** 159-173.

ZAIDI P.H., P. MANISELVAN, M. YADAV, R.P. SINGH, S.B. SINGH, S. DASS, G. SRINIVASAN, 2007c Importance of secondary traits in improvement of maize (*Zea mays* L.) for improving tolerance to excessive soil moisture stress. Cereal Res. Comm. **35:** 1427-1435.

ZAIDI P.H., MEHRAJUDDIN, M.L. JAT, K. PIXLEY, R.P. SINGH, S. DASS, 2009 Resilient maize for improved and stable productivity of rain-fed environment of South and South-East Asia. *Maize for Asia - Emerging Trends and Technologies.* Proc. 10th Asian Regional Maize Workshop, 20-23 October, 2008, Makassar, Indonesia (in press).

ZHOU M.X., L. HONGBIN, M. NEVILLE, S. SALTER, 2004 Inheritance of water-logging tolerance of barley (*Hordeum vulgare* L.). *In:* Proc. 4th Intl. Crop Science Congress, 26 September - 1 October 2004, Brisbane, Queensland, www.regional.org.au. ISBN 1 920842 20 9 (2004) [Refereed Conference].