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# STRESS-ADAPTIVE CHANGES IN TROPICAL MAIZE (*ZEA MAYS* L.) UNDER EXCESSIVE SOIL MOISTURE STRESS

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**ABSTRACT** - Response of maize plants to excessive soil moisture (EM) has been studied extensively. However, systematic information on the stress-adaptive changes and cascade of events conferring the EM-tolerance is yet to be established. We attempted to assess the stress-adaptive physiological changes associated with EM-induced anoxia stress, and to establish mechanism of EM-tolerance in tropical maize. Tropical/sub-tropical elite maize inbred lines with known reaction to EM-stress were used in this study. Germplasm were exposed to EM-stress at knee-high stage ($V_{7-8}$ growth stage) by flooding the plots continuously for seven days. EM-induced changes in root geotropism (surface rooting) and increased brace roots development were identified as stress-responsive traits; however, the later one was found to be a stress-adaptive trait resulting in improved stress tolerance. Anatomical studies showed drastic changes in cortical region of root tissues in tolerant genotypes in terms of development of large aerenchymatous spaces. In terms of stress-induced metabolic adjustments, increased $NAD^+$-alcohol dehydrogenase (ADH) activity was prevalent in all the genotypes under EM-conditions. Though, the enzyme activity was slightly higher in tolerant entries but not high enough to justify the significant genotypic variability. However, the product of ADH-activity (ethanol) was relatively much higher in root and leaf tissues of susceptible genotypes. Analysis of ethanol concentration in shoot, root and inundated water showed that the level of ethanol was relatively much higher in the water present in rhizosphere of relatively tolerant genotypes. The finding suggested that EM-tolerant maize genotypes were able to extrude out the toxic level of ethanol from root tissues to rhizosphere. Our results suggest that mechanism of EM-tolerance in maize germplasm involves morphological and anatomical adaptation through development of brace roots and aerenchyma formation, and metabolic adjustment through regulatory induction of alcohol dehydrogenase (ADH) and extrusion of ethanol out of root tissues.

KEY WORDS: Maize; Excessive soil moisture; Waterlogging; Tolerance mechanism.

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## INTRODUCTION

Inability of non-wetland crop species, including maize, to withstand low oxygen conditions in rhizosphere, caused by excessive soil moisture or any other factor, resulted in substantial yield losses. In tropics severe crop losses due to excessive soil moisture conditions due to contingent flooding, waterlogging, continuous rainfall coupled with inadequate drainage or high water table is a common problem. Maize crops grown during *Kharif* (summer-rainy) season in tropics occasionally face extreme climatic conditions and various biotic and abiotic pressures that limits yield potential. Among various abiotic stresses, excessive soil moisture caused by contingent/intermittent flooding or waterlogging is one of the most important constraints for maize production in Asian region and many other parts of the world. In South and South-East Asia alone, over 15% of the total maize growing areas are frequently affected by floods and waterlogging problems (RATHORE *et al.*, 1997). In India out of total 6.6 million ha area of maize over 2.5 million ha is prone to face excessive soil moisture/waterlogging conditions, which causes on average 25-30% loss of national maize production almost every year (DMR, 2001).

Excessive moisture or submergence lead to reduced gas exchange between the plant tissues and the atmosphere, because gases (specially oxygen) diffuse 10,000 times more slowly in water than in air (ARMSTRONG, 1979). There is no ventilation system in maize plants for gaseous exchange between aboveground plant parts and inundated roots. Therefore, plant roots suffer with progressive decline of oxygen, hypoxia (low oxygen) followed by anoxia (no oxygen), whenever it faces prolonged (>3 days) excess soil moisture situation (ZAIDI and SINGH, 2002). Extent of damage due to excess mois-

ture stress varies significantly with developmental stage. Previous studies have shown that maize crop is comparatively more susceptible to excess moisture stress before tasseling stage (PALVADI and LAL, 1976; MUKHTAR *et al.*, 1990; EVANS *et al.*, 1990; RATHORE *et al.*, 1998; ZAIDI *et al.*, 2003). At later growth stages, the genotypes with inbuilt capacity to produce brace roots and morphological adaptation like air space (aerenchyma) formation in cortical region of brace roots on exposure to the stress, can tolerate excess water situation in rhizosphere up to some extent (DREW *et al.*, 1979; RATHORE *et al.*, 1996; ZAIDI *et al.*, 2003). However, considerable genetic variability has been observed in maize with respect to excessive moisture tolerance (TORBERT *et al.*, 1993; RATHORE *et al.*, 1996, 1998; ZAIDI and SINGH, 2001; ZAIDI *et al.*, 2002, 2004). Such responses inevitably raise the question whether there are any fundamental differences between genotypes in their responses to imposed anaerobiosis. Systematic information on such differences may be exploited in developing maize cultivars that can tolerate excessive moisture-induced hypoxia/anoxia conditions. In past, response of maize plants to excessive moisture stress has been studied extensively. At physiological level, anoxia affects phytohormone homeostasis, plant morphology and anatomy (JACKSON, 1990), resulted in stunted growth, considerably reduced dry matter production, leaf area development, transpiration, prolonged anthesis-silking interval (ASI) and eventually resulted in poor grain yields (RATHORE *et al.*, 1997; ZAIDI *et al.*, 2002, 2003). However, systematic information on the cascade of events conferring the stress tolerance in maize is not yet established which is essentially required for genetic enhancement of tropical maize germplasm for improved tolerance to excessive moisture situation. Large volume of information is available on the responses of excessive moisture/waterlogging stress on maize; however, the major challenge is to identify the stress-adaptive traits among the various effects/changes under the stress on different stress-responsive traits. Several traits have been proposed as secondary traits, putatively related to improved survival or tolerance of maize genotypes under excessive moisture, mainly on the basis of phenotypic correlations between those traits and grain yield (LIU *et al.,* 1991; RATHORE *et al.,* 1996; ZAIDI *et al.,* 2003). Much research has supported the benefits of stress-adaptive traits for waterlogging, including stress-induced early brace root development, increases in aerenchyma and root porosity, root suberisation, ethanolic fermentation, carbohydrate reserves, tolerance to post anoxic shock and recovery mechanisms (SETTER and WATERS, 2003). However, not all of these are clearly shown to contribute to waterlogging tolerance, and sometime conflicting reports have occurred where different varieties or conditions have been used. In the present study, we attempted to identify the stress-adaptive changes in the relatively tolerant maize genotypes and their contribution in improving tolerance to excessive moisture stress in maize.

## MATERIALS AND METHODS

### *Germplasm*

A total 25 elite maize inbred lines were selected from the line evaluation trials conducted on tropical/subtropical lines ($S_4$-$S_n$) during past five years (1998-2003) to identify the tolerant sources of germplasm for EM-stress. Germplasm screened in the line evaluation trials include CM-lines of All India Coordinated Maize Improvement Project (AICMIP), advance generation/released elite lines from Regional Research Station, Haryana Agriculture University, Karnal, India, and CML-lines from tropical and sub-tropical maize program, International Maize and Wheat Improvement Center (CIMMYT), Mexico, which includes the lines from population 21, 22, 26, 28, 31, 33, 42, 43, 44, 45, 501 and 502. From breeding nurseries of different programs germplasm were selected for line evaluation trial on the basis of morphological traits, such as - seedling and plant vigor, brace root development, absence of plant-logging and good yield potential. Beginning from *Kharif* 1998, on an average 100 lines were screened under EM-stress every year and top-ranking 10-15% entries were selected on the basis of their superior performance under EM-stress as well as under normal moisture regime. Since the lines have never been exposed and selected for excessive moisture stress, at the beginning none of the lines as such have shown good tolerance to EM-stress. However, significant genotypic variability was found in terms of their relative performance under stress. Individual plants from relatively superior entries were tagged, maintained through plant-to-plant sibbing within entry, and planted ear-to-row in next season. The same procedure was followed next year as well and susceptible fraction was discarded. The procedure was continued till the line became uniform in its performance under stress condition. On the basis of their consistent performance in the line evaluation trials for excessive moisture tolerance a total 25 inbred lines were identified for the present study, including 10 highly susceptible, 7 moderately tolerant and 8 lines (CML-327-4-2-1-3, WL-7-*-*-1, WL14-*-*-1, WL15-*-*-2, WL28-*-*-2, WL29-*-*-2, CML-311-2-1-3-B, CML-425-3) were highly tolerant to EM-stress (ZAIDI *et al.*, 2003).

### *Experimental site, cultural practices and stress treatment*

The experiment was conducted during *Kharif* (summer-rainy season) of 2003 and 2004 at maize research farm, Indian Agricultural Research Institute, New Delhi, India (28.4°N, 77.1°E, 228.2 masl). Soil of the experimental station is characterized as sandy loam with a pH of 7.8. Entries were planted under excessive moisture conditions using 'cup method' and grown under the EM-stress from planting till 20 days (ZAIDI *et al.*, 2003). One set

of all the entries was grown under normal moisture. Simultaneously, two sets of all the entries, one for normal and another for EM-stress, were planted in field using alpha (0, 1) lattice design (PATTERSON and WILLIAMS, 1976) with three replications. In both the years, planting was done during last week of June. All the entries were over sown and thinned to one plant per hill at $V_2$ growth stage to give a population density of 54000 plants $ha^{-1}$. Each entry was planted in two rows, each 3.0 m long, with 0.25 m spacing within rows and 0.75 m between rows. Before planting 60 kg nitrogen (N) $ha^{-1}$ in from of urea, 60 kg phosphorous $ha^{-1}$ as single super phosphate, 40 kg potassium $ha^{-1}$ as muriat of potash and 10 kg zinc as zinc sulfate were applied before planting as basal dose; second and third dose of N both @30 kg N $ha^{-1}$ was side-dressed at knee-high and tasseling stage. Pre-emergence application of pendimethalin and atrazine (both 0.75 kg $ha^{-1}$ a.i.) was sprayed to keep the crop weed free. Experiments were kept free from insect-pests, weeds and diseases using recommended post-emergence chemical measures. Experiments were managed under optimal agronomic practices so as to provide an ideal growing environment to the crop. The EM-trial was also grown under normal moisture till the stress treatment was applied. Waterlogging treatment was applied at knee-high stage ($V_{7-8}$ growth stage) continuously for seven days with an average ponding depth of 10.0 ± 0.5cm. After completion of the stress treatment field was drained out; though excessive moisture situation was continued till 12$^{th}$ day.

***Measurements***

Plant height was recorded after completion of 50% male flowering, as the distance between ground surface and node bearing flag leaf, on ten plants and averaged. Leaf senescence was scored two times, one - immediately after and another at one week after EM-treatment (WAE) using a 1-10 scale (1 = 10% and 10 = 100% dead leaf area). *In vivo* chlorophyll concentration in the ear leaf was determined two times similarly as in case of senescence score, using a Minolta SPAD-502 chlorophyll meter in each plot on 10 plants per plot and averaged. Plant logging was calculated on the basis of counting of total number logged plants in each plot immediately after completion of EM-treatment and total number of plants in the respective plot, and percent logging was computed. Surface rooting was scored on the basis of visible root tips (white tips) around the stem base at one WAE using 1-5 scale (1 = absent or nominal and 5 = extensive). Data on brace root was recorded at 50% male flowering on 10 plants and averaged by counting the aboveground nodes bearing brace roots. Days from planting to anthesis and silking, indicated when 50% of plants had extruded anther or produced silk, was recorded by daily visual observations during the flowering period. Anthesis-silking interval (ASI) was calculated as the difference between number of days to 50% silking and 50% anthesis. Under EM-stress the highly susceptible lines failed to reach to 50% silking, which resulted in barren plants. In such cases maximum days to 50% silking of the trial was considered as days to 50% silking of those genotypes, and the same was used for calculation of ASI. However, complete barrenness was considered as such, and final grain yield were taken as zero for those genotypes. At maturity, ears were harvested, excluding two plants close to alley from both ends of the rows. Ears were oven dried to a constant moisture level and grain yield was recorded on a shelled grain basis at 15% grain moisture.

Root porosity was measured using the pycnometer method (NOORDWIJK and BROUWER, 1988), which is based on a comparison of the density of intact root tissues including air-filled pores, and that of root homogenate without air spaces. Sampling for ADH-activity and leaf/root ethanol concentrations was done on alternate day, starting from first day of waterlogging and continued till 8 days after the stress treatment. $NAD^+$ - alcohol dehydrogenase activity (E.C.1.1.1.1) was assayed in fresh root samples (underground adventitious roots) collected from the field trial. Enzyme activity was determined in the direction of $NAD^+$ reduction as described by GOMES *et al.* (1982). One unit of ADH-activity was defined as the amount of enzyme necessary to produce a change of 0.001 $min^{-1}$ at $A_{340}$. Protein content in the samples was determined by Lowry method (LOWRY, 1951) and the specific activity of ADH was calculated on the basis of $mg^{-1}$ protein $min^{-1}$. Sampling for ethanol concentration was also done on alternate days on the plants exposed to EM-stress in 'cup screening' and in water sample from the trays, starting from 5$^{th}$ day of planting and continued till 21$^{st}$ day. Ethanol concentration was determined enzymatically in neutralized perchloric acid extracts (RIVOAL *et al.*, 1989).

***Statistical analysis***

Analysis of variance was computed using MSTATc with completely randomized block design (2-factors) for the cup experiment and randomized complete block design (2-factors) for the finding of field experiments. For all experiments, data from two years findings were pooled on the basis of test for homogeneity of error variance for the two-year datasets using Hartley's $F_{max}$ test (OTT, 1988). Correlation coefficient and linear regression between secondary traits and grain yield was computed using MSTATc.

Percent superiority in grain yield for each tolerant entry over the average yield of susceptible ones under excessive moisture stress was computed and the contribution of the three distinct mechanisms, i.e. morphological (surface rooting and brace root development), anatomical (root porosity) and metabolic (ADH activity and ethanol concentration) adaptations were worked out on the basis of changes in these traits under stress condition and their correlation with grain yield. The data was used for genotype x trait (GT-biplot) analysis, which is an application of the GGE biplot technique to study of the genotype by trait data, and to examine its usefulness in visualizing trait relationships, and its application in cultivar evaluation, comparison and selection (YAN and RAJCAN, 2002).

## RESULTS

### *Genotypic variability under excessive moisture stress*

The group of genotypes, i.e.- highly tolerant, moderately tolerant and highly susceptible lines, showed distinct genotypic variability under excessive moisture condition (Table 1), though, the variation in most of the traits under normal moisture regime was nominal and statistically non-significant. In susceptible genotypes the EM-stress significantly suppressed vertical growth of plant, accelerated senescence and causes severe plant logging. However, the tolerant genotypes were able to continue

TABLE 1 - *Means and standard deviations (±) for various physiological and phenological traits in sub-tropical maize inbred lines grown under normal and excessive moisture stress. Standard deviations were computed from mean values of the traits obtained from the trial.*

| Traits | Tolerant lines | | Moderately tolerant lines | | Susceptible lines | | Significant differences | | |
|---|---|---|---|---|---|---|---|---|---|
| | NMa | EM | NM | EM | NM | EM | G | E | G x E |
| Plant height (cm) at 50% anthesis | 131.9 (10.4) | 101.7 (18.3) | 124.6 (12.4) | 69.1 (17.9) | 127.4 (18.2) | 58.9 (9.9) | * | ** | * |
| Logging (%) | 0.0 | 5.9 (1.4) | 1.3 (0.6) | 13.1 (3.2) | 0.8 (0.5) | 27.3 (10.2) | * | * | ns |
| Surface rooting (1-5) | 0.0 | 1.06 (0.14) | 0.0 | 2.03 (0.25) | 0.0 | 3.04 (0.31) | ns | * | * |
| Nodes with brace roots | 0.69 (0.13) | 3.21 (0.63) | 0.71 (0.31) | 2.10 (1.01) | 0.81 (0.36) | 1.01 (0.43) | * | ** | * |
| Root porosity (%) | 3.9 (0.8) | 32.3 (3.7) | 3.7 (1.1) | 21.4 (2.9) | 3.6 (1.0) | 6.9 (1.6) | * | ** | ** |
| Chlorophyll (SPAD unit) | 44.04 (4.06) | 36.89 (6.49) | 43.24 (4.92) | 34.94 (7.69) | 43.15 (4.86) | 25.34 (6.49) | * | ** | ns |
| Senescence score (1-10) | 0.11 (0.08) | 1.77 (0.49) | 0.26 (0.18) | 3.12 (0.77) | 0.17 (0.09) | 3.18 (0.84) | ns | * | * |
| Anthesis-silking interval (d) | 1.87 (1.12) | 2.03 (1.89) | 1.94 (1.03) | 4.03 (2.01) | 1.84 (1.21) | 7.64 (3.85) | * | ** | ** |
| Alcohol dehydrogenase activity (unit $mg^{-1}$ protein) | 16.70 (3.21) | 238.54 (9.87) | 16.82 (4.12) | 232.49 (11.36) | 17.13 (3.16) | 230.12 (9.28) | * | ** | * |
| Leaf ethanol[b] | 0.18 | 3.56 (0.89) | 0.13 | 15.26 (2.23) | 0.21 | 23.04 (3.16) | ns | ** | * |
| Root ethanol[b] | 2.56 | 18.02 (1.69) | 3.12 | 26.17 (2.21) | 2.96 | 39.42 (4.01) | ns | ** | * |
| Grain yield (t/ha) | 2.89 (1.13) | 2.10 (0.69) | 2.91 (1.26) | 1.13 (0.72) | 2.86 (1.38) | 0.54 (0.32) | * | ** | * |

*, ** Significant at P <0.01 and 0.05, respectively. ns indicates genotypic variability for the trait within the trial was non-significant at P<0.05.
[a] NM = normal condition, EM = excessive moisture, G = genotype, E = environment.
[b] observed on 3rd day of excessive moisture treatment.

plant growth and comparatively less suffered with leaf senescence and logging. EM-stress caused severe chlorosis, which was apparent in term of reduced leaf chlorophyll content in leaves. Loss of chlorophyll was comparatively severe in case of susceptible group of genotypes (41.3%). However, moderately tolerant and tolerant genotypes were able to maintain the leaf chlorophyll under stress condition with a nominal loss of 19.2 and 16.2%, respectively.

Excessive moisture resulted in stress-induced changes in plant morphology, including surface rooting, brace root development and increased root porosity, and biochemical traits, including $NAD^+$-alcohol dehydrogenase (ADH) activity and ethanol content in root and leaf tissues (Table 1). Across the germplasm, surface rooting was completely absent under normal moisture regime, whereas under EM-condition extensive surface rooting was observed in case of susceptible genotypes and also in moderately tolerant entries; however the surface rooting was comparatively less in tolerant group of entries. Brace roots at the nodes present above ground surface was observed under normal moisture as well, however, under EM-stress there was remarkable increase in brace root development, particularly in tolerant group of genotypes (4.6 times) and also in moderately tolerant (3.0 times). The newly emerged brace root was comparatively less in susceptible entries (1.24 times). Similarly, the root porosity was nominal under normal moisture, which increased drastically with exposure to EM-conditions. The stress-induced increase in root porosity was most remarkable in tolerant genotypes (8.3 times), fol-

TABLE 2 - *Analysis of variance for important secondary traits and grain yield of maize inbred lines grown under normal moisture and excessive moisture stress at V7 growth stage.*

| Source | d.f. | Sum of squares | Mean squares | % of total SS |
|---|---|---|---|---|
| **Brace root** | | | | |
| Environment | 1 | 98.091 | 98.091** | 48.29 |
| Error | 2 | 0.540 | 0.270 | 0.13 |
| Genotype | 24 | 53.828 | 2.243* | 1.10 |
| G x E interaction | 24 | 47.977 | 1.999* | 0.98 |
| Error | 96 | 1.285 | 0.013 | 0.01 |
| Total | 149 | 203.123 | | |
| CV (%) | 7.39 | | | |
| **Root porosity** | | | | |
| Environment | 1 | 18.689.653 | 18689.653** | 96.03 |
| Error | 2 | 6.470 | 3.235 | 0.023 |
| Genotype | 24 | 189.567 | 7.898* | 0.04 |
| G x E interaction | 24 | 542.047 | 22.585** | 0.12 |
| Error | 96 | 17.265 | 0.179 | 0.001 |
| Total | 149 | 19.461.350 | | |
| CV (%) | 13.26 | | | |
| **Anthesis-silking interval** | | | | |
| Environment | 1 | 270.950 | 270.950** | 35.87 |
| Error | 2 | 0.026 | 0.013 | 0.002 |
| Genotype | 24 | 238.025 | 9.918* | 1.31 |
| G x E interaction | 24 | 238.833 | 9.951* | 1.32 |
| Error | 96 | 3.067 | 0.032 | 0.004 |
| Total | 149 | 755.415 | | |
| CV (%) | 29.94 | | | |
| **ADH-activity** | | | | |
| Environment | 1 | 178.642.537 | 178642.53** | 97.68 |
| Error | 2 | 29.889 | 14.944 | 0.008 |
| Genotype | 24 | 1.672.645 | 69.694* | 0.04 |
| G x E interaction | 24 | 2.409.039 | 100.377* | 0.05 |
| Error | 96 | 46.328 | 0.483 | 0.0003 |
| Total | 149 | 182.880.300 | | |
| CV (%) | 42.96 | | | |
| **Grain yield** | | | | |
| Environment | 1 | 84.525 | 84.525** | 63.79 |
| Error | 2 | 0.058 | 0.029 | 0.022 |
| Genotype | 24 | 23.854 | 0.994* | 0.75 |
| G x E interaction | 24 | 22.732 | 0.947* | 0.71 |
| Error | 96 | 0.450 | 0.005 | 0.0038 |
| Total | 149 | 132.498 | | |
| CV (%) | 4.12 | | | |

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

lowed by moderately tolerant entries (5.8 times) and least in case of susceptible genotypes (1.91 times). Stress-induced anoxia condition resulted in anaerobic respiration in maize roots, which was apparent with drastic increase in ADH activity (13-14 fold on 3rd day of waterlogging) in root tissues (Table 1). Increase in the enzyme activity was observed in all the entries, irrespective of their reaction to EM-stress. Though, the activity was slightly higher in case of tolerant group of genotypes. As a result of enhanced ADH-activity, there was many fold increase in ethanol concentration in both root and leaf tissues. However, the extent of increase in ADH-activity in different group of genotypes was

not related to ethanol concentration in leaf and root tissues. Across the group of genotypes, variation in ADH-activity was nominal, however, increase in ethanol concentration in both leaf and root tissues were relatively much higher in susceptible genotypes followed by moderately tolerant entries, and least in tolerant genotypes. In general, the amount of ethanol was comparatively higher in root tissues, irrespective of their reaction to EM-stress.

Overall impact of EM-stress on various morphological, physiological and biochemical traits was finally expressed on reproductive phase and grain yield (Table 1). Anthesis-silking interval was significantly delayed under EM-conditions. The effect was comparatively more pronounced on susceptible genotypes, where the stress condition delayed A.S.I. by 5.8 days, while other two groups of genotypes were able to maintain the A.S.I. below 5.0 days under EM-stress. Under EM-stress the susceptible group of genotypes faced severe yield penalty (81.1%), followed by moderately tolerant entries (61.2%). However, the tolerant genotypes, by virtue of possessing stress-adaptive traits and functions, were able to maintain the grain yield and faced comparatively least penalty (27.3%) under EM-stress.

Analyses of variance of key secondary traits and grain yield indicate that genotypic variability was more apparent under stress conditions (Table 2). Genotypic variance was comparatively more pronounced with secondary traits than grain yield. Impact of environment was comparatively much stronger than genotype and genotype x environment (G x E), which accounted for 97.68, 96.03, 63.79, 48.29 and 35.87% of the total sums of squares for ADH-activity, root porosity, grain yield, brace root and ASI, respectively. Next to environment the contribution of G x E interaction was comparatively higher than genotype, except in case of brace root. Variance due to environment for all the traits, including grain yield was significant at $P<0.01$, while variance due to genotype and G x E interaction was significant at $P<0.05$, except in case of root porosity where G x E was significant at $P<0.01$. Contribution of G x E was relatively higher than environment in the error variance. Coefficient of variation was relatively higher with ADH-activity and root porosity traits, which was due to strong variation for the traits between tolerant and susceptible genotypes.

### *Anaerobic metabolism under EM-stress*

Observations recorded on root ADH-activity and ethanol concentration in root and leaf tissues on alternate days during stress treatment till seven days after the stress treatment are reported in Fig. 1. Data on ADH activity revealed that the enzyme activity was nominal under normal moisture in case of both tolerant and susceptible germplasm. However, under EM-conditions, irrespective of reaction of genotypes to the stress, many-fold increase in root ADH-activity was observed, which was on its peak by 3rd day of waterlogging treatment. On 5th day onward a down-

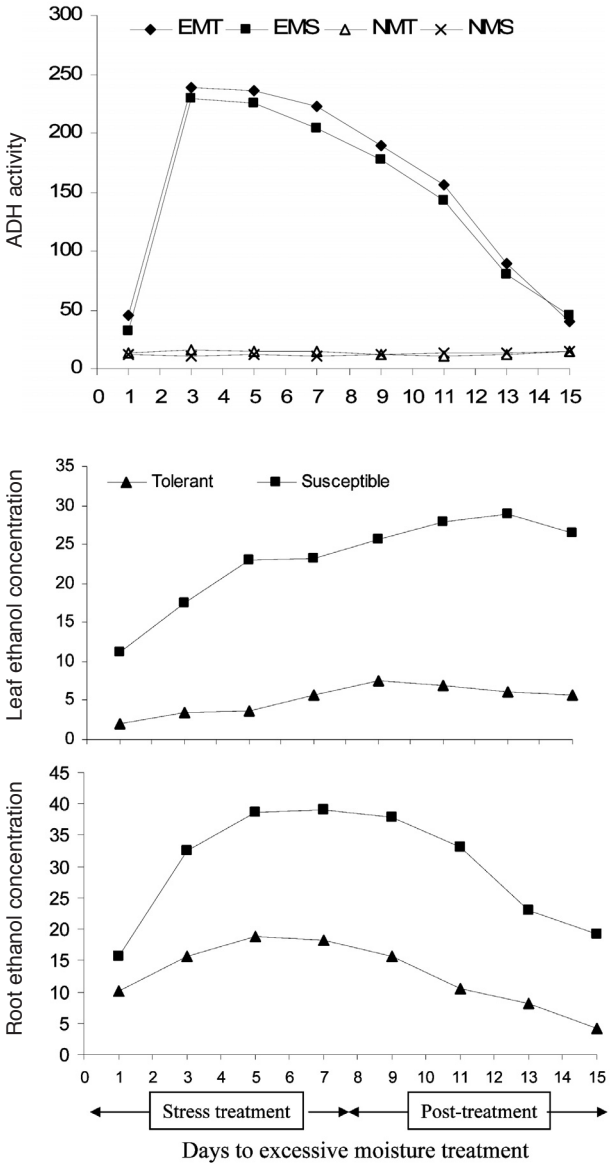


FIGURE 1 - NAD+-alcohol dehydrogenase activity (unit-1mg protein min-1) and ethanol concentration (μ mol -1) in leaf and root tissues of maize inbred lines during excessive moisture stress applied at V7 stage.

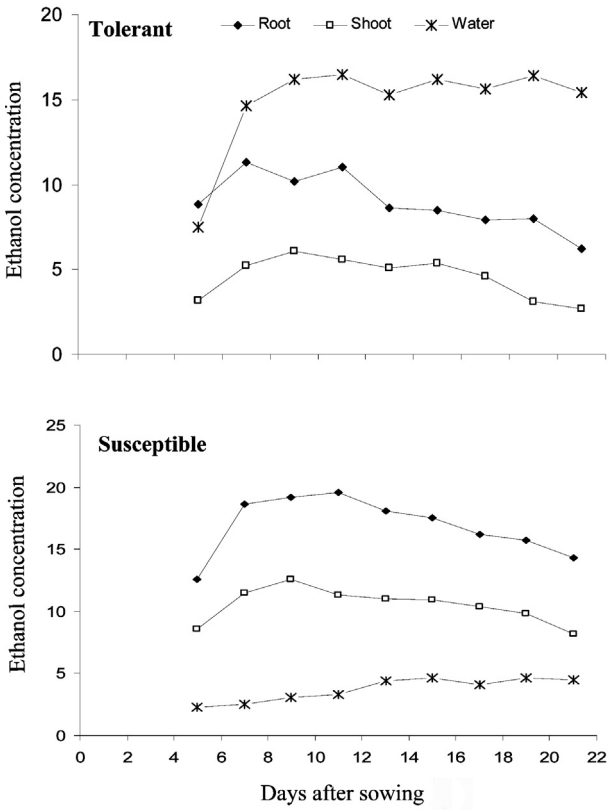


FIGURE 2 - Ethanol concentration (μ mol $g^{-1}$) in shoot, root and water in rhizosphere of maize inbred lines exposed to excessive soil moisture stress at early growth stage using 'cup method'.

trend in the enzyme activity was observed, which was continued till the enzyme activity came down to the level of normal moisture (about eight days after completion of EM-treatment). Though, the enzyme activity was slightly higher in case of tolerant lines as compared to susceptible genotypes but the difference was nominal and statistically non-significant. However, there was quite large difference in the ethanol concentration (micro mol/gram fresh wt.) in both leaf and root tissues of tolerant and susceptible genotypes (Fig. 1). Ethanol accumulation was comparatively much higher (3-5 times) in susceptible genotypes. The difference was pronounced at 3rd day of EM-treatment and continued till eight days after the stress treatment. Starting from 5th days after sowing, regular monitoring of ethanol in root, shoot and inundated water in cup screening showed similar trend of comparatively high ethanol concentration in root and shoot tissues of susceptible genotypes. However, the ethanol concentration in inundated water was comparatively much higher with tolerant entries in comparison to susceptible ones (Fig. 2). The finding suggest that ethanol was extruded from the root tissues to growth medium in case of tolerant genotypes, which explains the low ethanol concentration in root as well as in leaf tissues of tolerant entries, in spite of slightly higher ADH activity in these genotypes.

### *Relationship between grain yield and secondary traits under EM-stress*

Correlation analysis between various morphological traits and grain yield indicate that the relationship varied significantly with moisture regime (Table 3). Plant height had positive correlation with final grain yield under both normal and excessive moisture. However, the relationship was statistically non-significant under normal moisture, while under EM-stress it relatively much stronger and significant at P<0.01 (r = 0.52**). Similarly, plant logging had negative correlation with yield under both normal and excessive moisture; however, the relationship was strong and statistically significant (r = -0.41*) under excessive moisture, while under normal moisture it was week and statistically non-significant (r = -0.19). Similar trend of relatively weak correlation under normal and strong under stress was observed with other traits, including chlorophyll, anthesis-silking interval, ADH activity, root and leaf ethanol concentration as well (Table 3). In few traits, such as – brace root, root porosity, leaf senescence, and surface rooting, there was drastic change in the relationship under normal and EM-stress (Table 3). The relationship of grain yield with brace

TABLE 3 - *Phenotypic correlation (r) between grain yield and different morpho-physiological traits in maize inbred lines under normal and excess moisture stress applied at V7 growth stage.*

| Traits | Normal moisture | Excessive moisture |
|---|---|---|
| Plant height | 0.22 ns | 0.52** |
| Plant logging | -0.19 ns | -0.41* |
| Brace roots | -0.18 ns | 0.59** |
| Root porosity | -0.11 ns | 0.72** |
| Chlorophyll | 0.33* | 0.56** |
| Senescence | 0.13ns | -0.44* |
| Anthesis-silking interval | -0.20 ns | -0.62** |
| ADH- activity | 0.09 ns | 0.21 ns |
| Leaf ethanol | -0.13 ns | -0.63** |
| Root ethanol | -0.19 ns | -0.51** |
| Surface rooting | 0.08 ns | -0.23ns |

**, ** indicate significance at P<0.05 and 0.01, respectively, ns indicates that the relationship was statistically non-significant.*

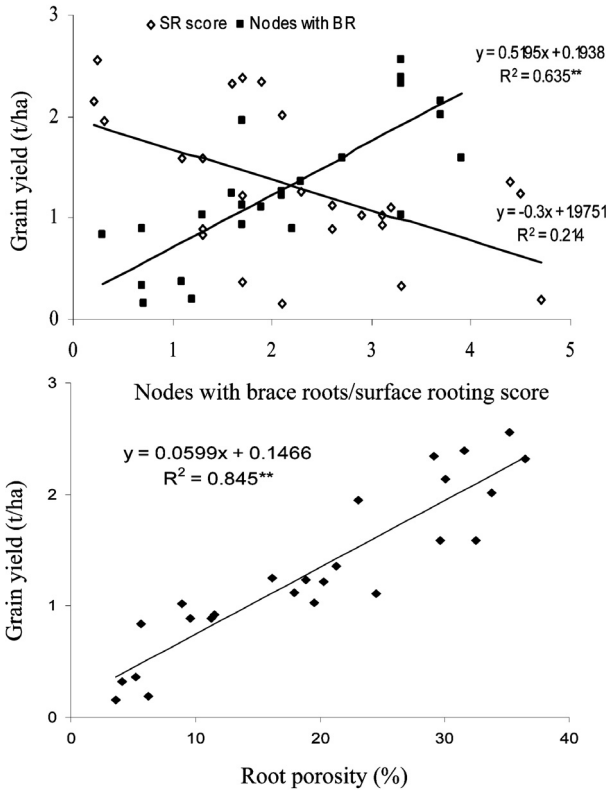


FIGURE 3 - Grain yield as a function of mean surface rooting score, nodes with brace roots and root porosity in maize inbred lines exposed to excess moisture stress under field conditions at V7 growth stage.
** indicates statistical significance at P<0.01.

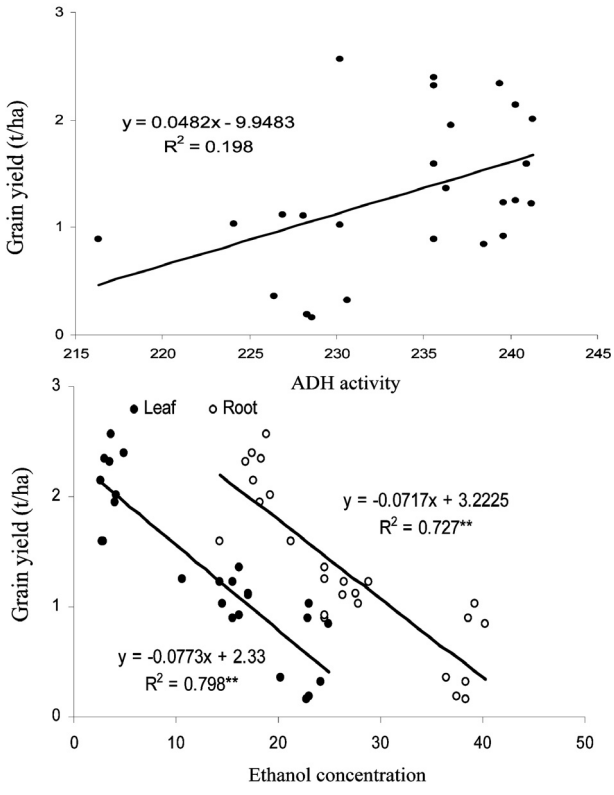


FIGURE 4 - Grain yield as a function of NAD⁺-alcohol dehydrogenase activity (unit-1mg protein $min^{-1}$) and ethanol concentration (µ mol $g^{-1}$) in leaf and root tissues of maize inbred lines exposed to excess moisture stress under field conditions at V7 growth stage.
** indicates statistical significance at P<0.01.

root and root porosity was negative under normal moisture (r = -0.18$^{ns}$ and -0.11$^{ns}$, respectively), while a strong positive correlation was observed under EM-stress (r = 0.59** and 0.72**, respectively). Similarly, in case of leaf senescence and surface rooting the correlation was weak with positive sign under normal moisture, while under EM-stress it was strong with negative sign (r = -0.44* and -0.23$^{ns}$, respectively); though, statistically non-significant in case of surface rooting.

Linear regression analysis of key secondary traits on grain yield under EM-stress (Fig. 3) indicates strong dependence of grain yield on root porosity ($R^2$ = 0.845**) and brace root ($R^2$ = 0.635**), while stress-induced surface rooting does not seems to be contribute much to final grain yield under stress ($R^2$ = 0.214$^{ns}$). Low ethanol accumulation in both root and leaf tissues under excessive moisture was found to be closely related to improved yield under EM-stress (Fig. 4). However, yield under stress was poorly related on root ADH-activity ($R^2$ = 0.198$^{ns}$).

***Contribution of different mechanism towards EM-stress tolerance***

Analysis of the contribution of various morphological (surface rooting and brace root development), anatomical (root porosity) and metabolic traits (ADH activity and ethanol concentration) indicated that all these changes are stress-adaptive and contribute towards improved performance under stress (Fig. 5). However, the contribution of the individual mechanism varied significantly among the 15 tolerant lines and none of them alone seems to be able to protect a genotype from the penalties due to stress. Contribution of anatomical adaptation (increased root porosity) in the stress tolerance found to be fairly high and significant in all the 15 tolerant genotypes, ranging from 29 - 52%. However, on the basis of the contributions of morphological and metabolic mechanism of adaptations the genotypes can be grouped into three categories.

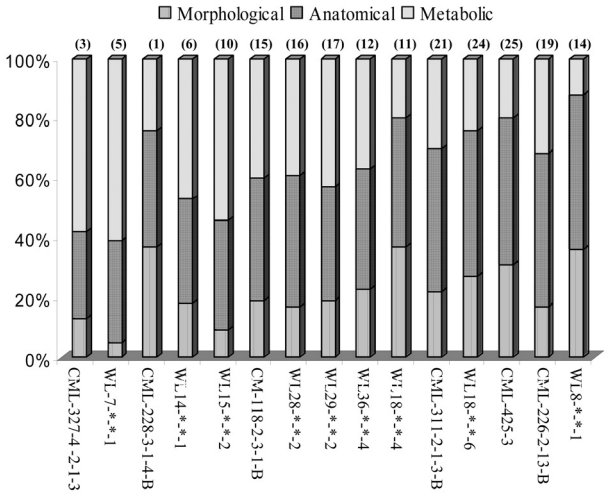


FIGURE 5 - Relative contribution of different mechanisms towards excessive moisture tolerance in the selected maize inbred lines. Figures in parenthesis above the bars indicate the entry number.

Most of the genotypes (entry 3, 5, 6, 10, 15, 16, 17 and 19) belong to the first category, in which the contribution of metabolic adaptation was comparatively much higher than stress-induced morphological changes (Fig. 5). In another category of genotypes (entry 12, 21, 24 and 25) both morphological and metabolic adaptations have similar contributions in the stress tolerance. In third category of genotypes the stress tolerance was largely based on morphological adaptation (entry 1, 11 and 14) and the contribution of metabolic adjustments under stress showed least contribution.

GT-biplot analysis of the 1st and 2nd principal components for stress-adaptive responses indicate the direction of increasing dependence of EM-stress tolerance on various mechanism of tolerance (Fig. 6). Metabolic and anatomical responses to EM-stress seem to be the best traits in discriminating the genotypic response to the stress tolerance. However, morphological traits showed poor efficiency for genotype discrimination. Genotypes with negative PC1 and PC2 scores indicate negative interaction with morphological adaptation, whereas, the lines with negative PC1 and positive PC2 value indicate strong negative interaction with stress-induced anatomical and metabolic changes under EM-stress. All the highly susceptible entries were clustered in the opposite direction of anatomical and metabolic adaptation, and the encircled entries (broken line) turned out to be the highly susceptible to EM-stress. The entries with higher PC1 and lower PC2 scores were identified as the best entries with relatively high mean yield under EM-stress (entries encircled with solid line).

## DISCUSSION

Excessive soil moisture in rhizosphere, in general, affected most of the morpho-physiological, biochemical and reproductive traits of maize plants. However, the effect was comparatively more aggressive on susceptible group of entries, followed by moderately tolerant genotypes (Table 1). Our previous studies (ZAIDI and SINGH, 2001; ZAIDI *et al.*, 2002, 2004) and other workers have also reported significant genotypic variability to EM-stress in maize (TORBERT *et al.*, 1993; RATHORE *et al.*, 1996, 1997). In some genotypes a drastic change in root geotropism with exposure to excessive moisture was observed. Within 2-3 days of waterlogging in field large number of root tips (white tips) was visible around the stem. In general, the white tips were relatively more pronounced in case of susceptible genotypes. Surface rooting has been identified as an adaptive strategy for coping with flooding in a number of wetland species (JUSTIN and ARMSTRONG,

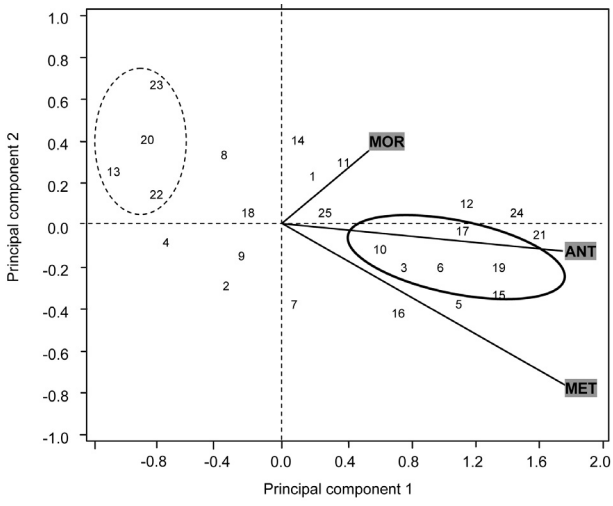


FIGURE 6 - AMMI-analysis of the 1st and 2nd principal components for stress-adaptive responses in sub-tropical maize inbred lines exposed to excessive moisture stress at V7-growth stage. Lines drawn towards mechanism of tolerance (MOR = morphological adaptation, ANT = anatomical changes, MET = metabolic changes) indicate the direction of increasing dependence on that mechanism of tolerance. Line encircles genotypes indicate the highly susceptible (broken line) and tolerant (solid line) inbred lines for excessive moisture stress.

1987). However, for non-wetland species, like maize, the EM-conditions are highly temporary that comes as intermittent stress for a limited period, and after release of the stress those surface roots has no role to play. Surface rooting might have some temporary role to cope up with EM-stress because the visible root tips and shallow roots are placed under hypoxic rather than anoxic condition, and therefore, might sustain partial aerobic respiration. After release of the stress, however, such changes in root geotropism resulted in highly inefficient root system to support further growth and development of plants (data not shown), and probably, therefore, it has poor relationship with yield under EM-stress (Fig. 3). In case of tolerant genotypes surface rooting was negligible; rather those genotypes responded with initiation of aboveground nodal roots (Table 1). EM-induced nodal roots at nodes above and/or below the ground surface has been reported to have large air spaces in cortical region (RATHORE *et al*., 1996; ZAIDI *et al.,* 2004), which increases root porosity that might help in improving oxygen supply, nutrients and water, and improve anchorage, where severe damage of primary roots under excessive moisture has occurred.

EM-stress resulted in many-fold increase in root porosity in tolerant and moderately tolerant genotypes (Table 1). Though, the increase was comparatively less in case of susceptible entries. Root porosity was found to be strongly related to final grain yield under EM-stress ($R^2$ = 0.845**), as observed in the present study (Fig. 3) and also reported by other workers (KUO, 1993; ZAIDI *et al.*, 2003). Under EM-conditions the submerged roots, especially stress-induced nodal roots, produces large air spaces that eventually increase the root porosity (ZAIDI *et al.*, 2005). In maize plant, aerenchyma in roots is not a constitutive trait; rather it seems to be a stress-adaptive trait that develops with exposure to EM-stress. Aerenchyma tissues develop in the root cortex of maize by lyses (lysigenous) of cortical cells (KUO, 1993). VARTAPETIAN and JACKSON (1997) reported that ethylene is the principal mediator in promoting the development of aerenchyma in maize roots. CAMPBELL and DREW (1983) observed that ethylene-induced cell lyses leading to aerenchyma formation is a process of progressive cell deterioration or precocious senescence, beginning about 10 mm behind the root tip in intact roots of maize, in cells initiated somewhere 12-18 hrs earlier, and first detectable by the collapse of cells in the mid-cortex. Dissolution of protoplasm and much of the cell wall is completed within next 12-24 hrs, leaving gas-filled spaces (lacunae) that interconnect to gas space system in the shoot. Aerenchyma provides a low resistance diffusion path for the transport of $O_2$ from aerial parts of the newly developed brace root to the roots present under severe anoxic conditions (KAWASE and WHITMOYER, 1980; LAAN *et al.*, 1989). It also provides a path for diffusion for toxic compound such as ethylene, methane, $CO_2$, ethanol and acetaldehyde (VISSER *et al*., 1997; VARTAPETIAN and JACKSON, 1997).

Under EM-stress plants face not only the problem of oxygen deficit but also the problem of nutrients availability, especially nitrogen, due to severe leaching/runoff of the soluble nutrient from rhizosphere (RATHORE *et al.*, 1996) and poor nutrient uptake due to energy starvation caused by anaerobiosis (DAVIES, 1980). Accelerated senescence and reduced chlorophyll concentrations in susceptible genotypes to EM-stress (Table 1), in spite of well-fertilized conditions, clearly indicate comparatively low nitrogen availability in plant tissues under EM-stress. However, the loss in chlorophyll content and plant senescence was comparatively less in tolerant genotypes. Improvement of germplasm with consideration of these traits during selection for EM-tolerance at early seedling and knee-high stage might have improved nutrient uptake/use efficiency in EM-tolerant genotypes. In a similar approach of multi-trait selection BANZIGER *et al*. (2002) suggested that selection and improvement of maize germplasm with a focus on mid-season drought tolerance improved nutrient uptake/use efficiency.

ADH activity was nominal under normal moisture in case of both tolerant and susceptible germplasm. However, under excessive moisture conditions there was many-fold increase in ADH activity in all the genotypes (Table 1 and Fig. 1). However, the ADH activity was slightly higher in case of tolerant genotypes in comparison to susceptible lines, but not higher enough that can explain the EM-tolerance. High levels of ADH activity and ethanol production during anaerobiosis have been reported for flood-tolerant plants (AVADHANI *et al*., 1978; TRIPEPI and MITCHELL, 1984). In other studies, activity of ADH was found to be positively correlated with the magnitude of flood injury in different genotypes (LIAO and LIN, 1995; ZAIDI *et al.*, 2003). Increased ADH-activity appears to be one of the general responses to anoxia, not necessarily related to stress tolerance, as observed in the present study

(Fig. 4) and also reported in our previous studies (ZAIDI *et al.*, 2002, 2004). LIU *et al.* (1991) suggested that increased alcoholic fermentation was a temporary adaptation and a major cause of root injury during flooding, and that flooding tolerance was related to low ethanol fermentation. In the present study we also found that ethanol concentration, both in root and leaf tissues, was comparatively much higher in susceptible genotypes than tolerant ones (Table 1 and Fig. 1). Daily monitoring of ethanol in root, shoot and inundated water in cup screening showed that amount of ethanol in water was comparatively much higher with tolerant entries in comparison to susceptible ones (Fig. 2). The finding indicate that ethanol was extruded out through diffusion and/or venting of the produced ethanol in the root tissues of tolerant genotypes to surrounding medium (LIAO and LIN, 2001), which explains the low ethanol concentration in root and particularly in leaf tissues of tolerant entries, in spite of slightly higher ADH activity in these genotypes (Fig. 1). Ethanol concentration in root and shoot tissues showed strong negative correlation with EM-stress tolerance (Fig. 4). Negative relationship between higher ethanol productions with flooding tolerance has been observed in other studies as well (CRAWFORD, 1978; BARTA, 1984). It has been proposed that the accumulated ethanol may have a "self-poisoning role" in susceptible genotypes. Our results clearly indicate that in tolerant genotypes the poisoning due to excessive accumulation of ethanol was avoided because roots of those genotypes were able to excrete the ethanol produced by anaerobic metabolism (Fig. 2).

The overall impact of EM-stress was apparent on reproductive behavior of maize genotypes. Female flowering was comparatively more susceptible to EM-stress than male flowering (data not shown). Delayed silking resulted in a long ASI and severe barrenness in susceptible genotypes (Table 1). However, in tolerant and moderately tolerant genotypes ASI was maintained at around five days or less. The process of female flowering, reflected in term of silk emergence, was found to be largely dependent on the availability of current photosynthetic (BOLANOS and EDMEADES, 1996). Reduced current photosynthesis in susceptible genotypes under excessive moisture stress (HUANG *et al.*, 1994) apparently reduces assimilates for silk growth and cob development. Our earlier studies showed a strong relationship between ASI and grain yield under EM-stress (ZAIDI and SINGH, 2001; ZAIDI *et al.*, 2002, 2003). Prolonged ASI has also been reported under other abiotic stresses, such as drought (AGRAMA and MOUSSA, 1996; BOLANOS and EDMEADES, 1996), high population density (EDMEADES, 1976; DOW *et al.,* 1984), and low-N fertility (BANZIGER and LAFITTE, 1997). It appears to be an ubiquitous response of the maize plant to any stress that reduces photosynthesis per plant at flowering.

GT-biplot analysis indicate that contribution of the individual mechanism varied significantly among the tolerant lines and none of the them alone seems to be able to protect a genotype from the penalties due to EM-stress (Fig. 5). Metabolic and anatomical adaptations to EM-stress were found to be the best traits in discriminating the tolerant and susceptible maize genotypes to EM-stress (Fig. 6) that might add stability in the performance across locations (ZAIDI *et al.*, unpublished data from All India Coordinated trials). Several reports has supported the benefits of adaptive traits for waterlogging including increases in aerenchyma and root porosity, adaptive changes in plant phenology, alcoholic fermentation etc. (SETTER and WATERS, 2003). However, not all of these are clearly shown to contribute in waterlogging tolerance and sometime conflicting reports have occurred where different varieties or conditions have been used. In native wetland species the main strategies of waterlogging and flooding tolerance involve maintenance of high internal aeration through constitutive aerenchyma and creation of an oxidized zone around root tips through radial $O_2$ loss (ARMSTRONG *et al.*, 1994), metabolic adaptation that maintains energy production under hypoxia (BRÄNDLE and CRAWFORD, 1987) and substantial pre-stress storage of carbohydrates for fermentation under conditions of low $O_2$ (BRÄNDLE, 1991). SETTER and WATERS (2003) argued that similar strategies present in the tolerant genotypes of those cereals that are not adapted to waterlogging. Our studies suggest that mechanism of excessive moisture tolerance in maize involves metabolic adjustment through regulatory induction of ADH activity coupled with venting of toxic level of ethanol out of root tissues into surrounding medium and anatomical adaptation through aerenchyma formation in cortical region of roots, and to some extent the morphological adaptation through development of brace roots with high root porosity (Fig. 5). However, the tolerance largely based on the first two strategies was relatively superior (Fig. 6) and, hopefully, stable across locations in target environment.

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