*Indian J. Plant Physiol.*, Vol. 12, No. 1, (N.S.) pp. 28-33 (Jan.-Mar., 2007)

# WATERLOGGING RESISTANCE IN MAIZE IN RELATION TO GROWTH, MINERAL COMPOSITIONS AND SOME BIOCHEMICAL PARAMETERS

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Received on 23 Jan., 2006, Revised on 12 Feb., 2007

## SUMMARY

**Nine genotypes of maize were grown in polythene bags containing 1.50 kg of garden soil. Plants were subjected to waterlogging stress after 14 days of sowing. Observations pertaining to dry matter accumulation, leaf area, leaf rolling, leaf conductance, chlorophyll content, soluble protein content and superoxide dismutase (SOD) activity in leaves, and nitrogen, phosphorus, and potassium contents in various plant parts were determined in normal and waterlogged plants between 22 to 25 days of waterlogging treatment. Genotype CML-49 registered minimum reduction in dry matter accumulation and CML-80 the maximum. Accordingly they were classified as resistant and susceptible to waterlogging stress, respectively. The resistant genotypes registered lesser reduction in leaf area, chlorophyll content and leaf conductance under waterlogged condition compared to susceptible ones. SOD activity, nitrogen and potassium contents decreased but phosphorus and sodium contents increased under waterlogging condition. Resistant genotype maintained lesser reduction in SOD, N and K contents. It is suggested that high level of K, in resistant genotype is advantageous in maintaining plant water relation and leaf conductance, while relatively higher level of SOD prevents oxidative damages. It is observed that genotype which require low level of N for normal growth, tolerates waterlogging stress.**

***Key words*: Leaf conductance, maize, mineral content, SOD, waterlogging**

## INTRODUCTION

In India, maize is grown in 6.45 m ha area with a total production of 11.11 m tonnes and average productivity of 1723 kg $ha^{-1}$. It is estimated that India may have to produce 20 m tonnes of maize by 2020 to meet its requirements (Singhal 1999). Waterlogging is a serious problem for maize and nearly 2.5 m ha land under maize is affected by waterlogging, resulting in an average loss of 25-30% maize production every year. Several studies have been undertaken to examine the impact of waterlogging on maize and to understand the basic mechanism behind resistant and susceptible reactions (Scholowing and Techming 1997, Rathore *et al.* 1997, 1998, Rai *et al.* 2004), but the promising morpho-physiological and biochemical traits associated with excess moisture resistance are yet to be identified. This investigation was carried out with these objectives in view.

## MATERIALS AND METHODS

Experiments were carried out during *kharif* 2004 and summer 2005 in the Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. Seeds of nine genotypes of maize, viz. CML-49, CML-56, CML-142, CML-150, CML-80, CML-204, KHI-536, CM-211 and CML-85 were

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procured from the Department of Genetics and Plant Breeding of the same Institute. Seeds were sown in plastic bags containing 1.5 kg of garden soil. After 7 days of germination thinning was done to maintain three healthy and uniform seedlings in each bag. Waterlogging stress was imposed from 14 days after sowing (DAS) by placing one set of bags (three bags of each genotype) in water filled containers and the water level was kept 2.0 to 2.5 cm above the soil surfaces of the bags. This water level was maintained by adding tap water twice a day. Remaining bags were maintained at normal moisture as control. Observations were recorded on waterlogged and normal plants between 22 to 25 days after treatment.

Dry weight of plant parts was recorded after carefully removing them and drying at 104°C for one hour, followed by at 65°C till constant weight. Leaf area was measured by leaf area meter (Systronics 211). Extent of leaf rolling was scored on 1 (minimum rolling) to 9 (maximum rolling) scale. Chlorophyll content was measured by chlorophyll meter (Minolta) and expressed as SPAD unit. Conductance from adaxial ($g_{ad}$) and abaxial ($g_{ab}$) surfaces of leaf was measured by diffusion porometer (Dalta T-Device, ADC, UK) between 10-11 h and leaf conductance ($g_s$) was calculated as:

$$g_s = g_{ad} + g_{ab}$$

Soluble protein content in leaf and superoxide dismutase (SOD) activity were measured as described by Lowery *et al.* (1951) and Dhindsa *et al.* (1981), respectively. All observations were made on first fully expanded leaf from top. Nitrogen, phosphorus, potassium and sodium contents in root, stem and leaf of a waterlogging resistant (CML-49) and a susceptible (CML-80) genotype, were determined after digestion with $H_2SO_4$ : $HClO_4$ (10 :1). Nitrogen was determined by Nessler's reagent (Snell and Snell 1959), phosphorus by ammonium vandate reagent (Jackson 1967), and Na and K by flame photometer (Tandon 1995). Data were collected in triplicates and analyzed by CRD.

## RESULTS AND DISCUSSION

Perusals of data indicate that waterlogging reduced plant dry matter (Table 1).The minimum reduction in plant weight was observed in the genotype CML-49 (31%) and the maximum in CML-80 (76%). Genotype CML-49 recorded higher total biomass and minimum was in CML80 under waterlogging. Genotypes with high biomass have been reported to sustain better growth and development after release of stress (Setter *et al.* 1989, Singh *et al.* 1997).

In general, excess soil moisture reduces leaf area in maize irrespective of genotype (Grineva *et al.* 1988). In this investigation the maximum reduction in leaf area was noted in genotype CML-150 (146%) and minimum in KHI-536 (60%) (Table 1). Number of green leaves per plant also reduced (Table 1). The reduction in green leaf area was mainly due to death of existing leaves as the total leaf number (green + dead) did not change (Table 1). Water logging induced leaf rolling (Table 1). Leaf rolling is associated with water saving avoidance strategy in plants under drought condition (Levitt 1972), but in the present investigation leaf rolling has been recorded minimum in CML-49, which maintained high dry matter under waterlogging and the maximum in CML-80, in which waterlogging resulted in maximum dry matter reduction.

Genotypic differences were evident with respect to plant weight, green leaf number and leaf area per plant under the influence of waterlogging condition. Genotype CML-49 generally exhibited minimum reduction in these parameters and CML-80 the maximum. Accordingly, they were classified as relatively resistant and susceptible to waterlogging stress, respectively.

Waterlogging decreased chlorophyll content of leaves (Table 2) in all the genotypes, except in genotypes CML-85 and KHI-536. Such variable results have been reported earlier also (Sinha *et al*. 1995). The reduction in chlorophyll content was observed to be the minimum in CML-49 (10%) and maximum in CML-80 (27%) under waterlogged condition. In rice, decrease in chlorophyll content due to waterlogging has been linked with reduction in dry matter (Aleman *et al.* 1984), while in the present investigation reduction in chlorophyll content appears to be poorly associated with decrease in plant dry matter under waterlogging.

Decreased protein content and significant change in protein profile pattern under prolonged waterlogging has

**Table 1.** Root, shoot, plant dry weight, leaf number, leaf area and extent of leaf rolling (visual score) in two genotypes of maize under normal and waterlogged conditions.

| Genotype | Parameter | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Root dry weight (mg plant$^{-1}$) | | Shoot dry weight (mg plant$^{-1}$) | | Plant dry weight (mg plant$^{-1}$), | | Leaf number (plant$^{-1}$) | | Leaf area (cm$^2$ plant$^{-1}$) | | Leaf rolling** |
| | N# | W# | N | W | N | W | N | W | N | W | |
| CML-49 | 352.82 | 354.47 | 1056.37 | 613.27 | 1409.19 | 967.74 | 5 (2)* | 4 (3) | 128.9 | 51.3 | 1 |
| CML-56 | 434.10 | 157.78 | 715.09 | 570.67 | 1150.00 | 728.45 | 3 (2) | 3 (3) | 152.0 | 53.1 | 2 |
| CML-142 | 379.03 | 96.18 | 934.37 | 356.42 | 1313.04 | 452.60 | 4 (3) | 3 (4) | 145.7 | 29.8 | 6 |
| CML-150 | 446.95 | 183.05 | 885.55 | 429.13 | 1332.50 | 612.18 | 6 (2) | 4 (4) | 199.0 | 53.5 | 4 |
| CML-80 | 360.28 | 43.88 | 1126.45 | 242.00 | 1486.73 | 285.88 | 5 (2) | 2 (4) | 149.0 | 20.0 | 9 |
| CML-204 | 422.73 | 140.70 | 1072.95 | 427.85 | 1495.68 | 568.55 | 5 (3) | 5 (3) | 145.1 | 69.4 | 3 |
| KHI-536 | 471.60 | 112.28 | 867.08 | 441.17 | 1338.68 | 553.45 | 5 (2) | 3 (5) | 111.2 | 48.2 | 7 |
| CM-211 | 378.00 | 171.23 | 998.70 | 597.80 | 1376.70 | 769.03 | 5 (3) | 4 (4) | 143.3 | 58.4 | 5 |
| CML-85 | 345.03 | 223.08 | 1109.17 | 744.67 | 1454.20 | 967.75 | 5 (4) | 4 (5) | 164.8 | 78.8 | 8 |
| Mean | 454.50 | 164.74 | 974.06 | 491.44 | 1373.03 | 656.18 | 4.8 (2.6) | 3.6 (3.9) | 148.8 | 51.4 | |
| CD at 5% for Treatment (T) | 37.40 | | 117.80 | | 116.08 | | 0.31 (0.21) | | 45.4 | | |
| Genotype (G) | 79.34 | | 249.89 | | 246.00 | | 0.65 (0.45) | | 59.9 | | |
| T x G | 112.20 | | 353.39 | | 348.23 | | 0.92 (0.64) | | 84.7 | | |

#N= Normal, W= Waterlogged. *Values in ( ) indicate the number of dead leaves. **1 indicates minimum rolling and 9 the maximum

been documented (Rai *et al.* 2004, Ho and Sachs 1989). Under anaerobic condition new proteins are synthesized in roots (Sachs *et al.* 1996), however, prolonged waterlogging reduces total protein content on account of general reduction in protein synthesis (Sayed 1998). Significant reduction in protein content of leaf was recorded under waterlogging condition (Table 2). Though the maximum reduction in protein content (60%) was recorded in susceptible genotype, CML-80, its pattern could not be linked with susceptible and resistance reactions of the genotypes as CML-49, which exhibited resistance also registered 50% reduction in protein content.

Superoxide radicals are ($O^-_2$) involved in causing cellular injury in maize under waterlogging. It is suggested that superoxide radicals induce lipid peroxidation causing membrane damage, and excess accumulation of $O^-_2$ is due to reduced activity of super oxide dismutase (SOD) under waterlogging condition (Yan Bin *et al.* 1996). In the present investigation, under waterlogging SOD activity decreased (Table 2). The reduction in enzyme activity was observed to be minimum in relatively resistant genotype, CML-49 and the maximum in the susceptible one, i.e. CML-80.

Leaf conductance was found to be higher from abaxial surface (Table 3). On an average, waterlogging condition decreased leaf conductance. The reduction has been more in abaxial leaf conductance. In some of the genotypes (CML-142 and KHI-536) adaxial leaf conductance rather increased marginally under waterlogging condition, which might be due to increased humidity in the interior of rolled leaf as in this genotype

**Table 2.** Chlorophyll content, protein content and superoxide dismutase activity in maize genotypes under normal and waterlogged conditions.

| Genotype | Parameter | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Chlorophyll (SPAD units) | | Protein (mg $g^{-1}$ fresh weight) | | Superoxide dismutase activity (units)* $g^{-1}$ fresh weight | | $mg^{-1}$ protein | |
| | N# | W# | N | W | N | W | N | W |
| CML-49 | 21.30 | 19.47 | 0.37 | 0.19 | 14 | 10 | 5.32 | 1.90 |
| CML-56 | 20.40 | 16.67 | 0.30 | 0.20 | 16 | 8 | 4.80 | 1.60 |
| CML-142 | 22.30 | 19.33 | 0.29 | 0.21 | 6 | 1 | 1.74 | 0.21 |
| CML-150 | 18.90 | 17.87 | 0.33 | 0.22 | 11 | 7 | 3.62 | 1.54 |
| CML-80 | 22.60 | 16.23 | 0.51 | 0.21 | 13 | 3 | 6.76 | 0.63 |
| CML-204 | 16.90 | 16.37 | 0.35 | 0,17 | 10 | 7 | 3.51 | 1.19 |
| KHI-536 | 15,60 | 16.80 | 0.37 | 0.29 | 6 | 3 | 2.22 | 0.87 |
| CM-211 | 18.73 | 16.27 | 0.39 | 0.20 | 10 | 8 | 3.90 | 1.60 |
| CML-85 | 15.53 | 21.00 | 0.40 | 0.31 | 7 | 4 | 2.80 | 1.25 |
| Mean | 19.14 | 17.78 | 0.37 | 0.22 | 10.33 | 5.66 | 3.85 | 1.20 |
| CD at 5% for | | | | | | | | |
| Treatment (T) | 1.64 | | 0.01 | | 0.20 | | 0.02 | |
| Genotype (G) | 3.47 | | 0.03 | | 0.41 | | 0.04 | |
| T x G | 4.91 | | 0.04 | | 0.58 | | 0.05 | |

#N= Normal, #W= Waterlogged, *calculated as described by Dhindsa *et al.* (1981)

**Table 3**: Abaxial, adaxial and total leaf conductance in maize genotypes under normal and waterlogged conditions.

| Genotype | Leaf conductance(mmol $m^{-2}s^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | Abaxial | | Adaxial | | Total | |
| | N# | W# | N | W | N | W |
| CML-49 | 228.00 | 199.33 | 159.33 | 86.67 | 387.33 | 286.00 |
| CML-56 | 408.67 | 113.33 | 84.67 | 115.00 | 493.34 | 228.33 |
| CML-142 | 203.00 | 159.67 | 80.33 | 143.00 | 283.33 | 302.67 |
| CML-150 | 636.67 | 178.67 | 215.67 | 113.33 | 854.34 | 292.00 |
| CML-80 | 736.67 | 138.67 | 151.00 | 48.33 | 887.67 | 187.00 |
| CML-204 | 184.67 | 108.67 | 39.83 | 141.33 | 224.50 | 250.00 |
| KHI-536 | 195.00 | 115.00 | 116.67 | 88.00 | 311.67 | 203.00 |
| CM-211 | 175.83 | 107.67 | 76.00 | 89.67 | 215.83 | 197.34 |
| CML-85 | 478.67 | 502.78 | 135.00 | 23.33 | 613.67 | 526.11 |
| Mean | 360.80 | 180.42 | 117.61 | 94.30 | 474.52 | 274.72 |
| CD at 5% for | | | | | | |
| Treatment (T) | 48.99 | | 19.70 | | 60.00 | |
| Genotype (G) | 103.92 | | 41.79 | | 130.00 | |
| T x G | 146.97 | | 59.10 | | 180.00 | |

#N= Normal, #W= Waterlogged.

leaf rolling was pronounced. As far as genotypic differences are concerned, the percentage reduction in total leaf conductance was maximum in genotype CML-80 (79%). In genotype CML-142 and KHI-536, it increased marginally, while in CML-49 though the leaf conductance decreased on account of waterlogging condition but to a lesser extent (26%). Reduced leaf conductance is a common feature in stressed plants (Srivastava and Chaturvedi 1989), which results in reduced transpiration as well as photosynthetic rate, and the latter leads to reduced dry matter accumulation. It is also evident that the genotype CML-80, identified as most susceptible to waterlogging condition, also registered maximum reduction in leaf conductance. This genotype also exhibited maximum reduction in chlorophyll content (Table 2). It is, therefore, evident that reduced influx of $CO_2$ as well as reduced photosynthetic activity (in terms of decreased chlorophyll content) leads to reduced dry matter accumulation under water logging in maize, and these parameters are affected most in susceptible genotypes.

About 1% of dry matter of crop plants is represented by minerals. Derangements in mineral nutrients acquisition also affect plant growth and development. Reports are available to indicate that water logging alters mineral nutrient status of plants (Thomson *et al.* 1989, Atwell and Steer 1990). On an average, N and K contents in various parts of maize plants decreased under waterlogging, while P and Na contents increased (Fig. 1). It is evident that waterlogging resistant genotype of maize had lower requirements for N, as under normal condition CML-49 contained significantly lower amount of N (Fig. 1a). But this genotype has higher requirements for K (Fig. 1c). Though waterlogging reduces N and K contents, but the reduction has been lesser in resistant genotype. Resistant genotypes also maintained higher levels of P in various plant parts (Fig. 1b). Role of K is well known in osmoregulation of plants. Higher K content in waterlogging resistant genotype of maize, especially in roots, may help in maintenance of pace of water absorption and turgor of cells in this genotype. The susceptible genotype, CML-80, accumulated exceptionally high amount of Na in roots (Fig. 1d). It is given that low concentration of $O_2$ in rooting medium due to waterlogging decreases the selectivity of $K^+/Na^+$ uptake by roots in favour of $Na^+$ and retards the transport of $K^+$ to shoots (Thomson *et al.* 1989)

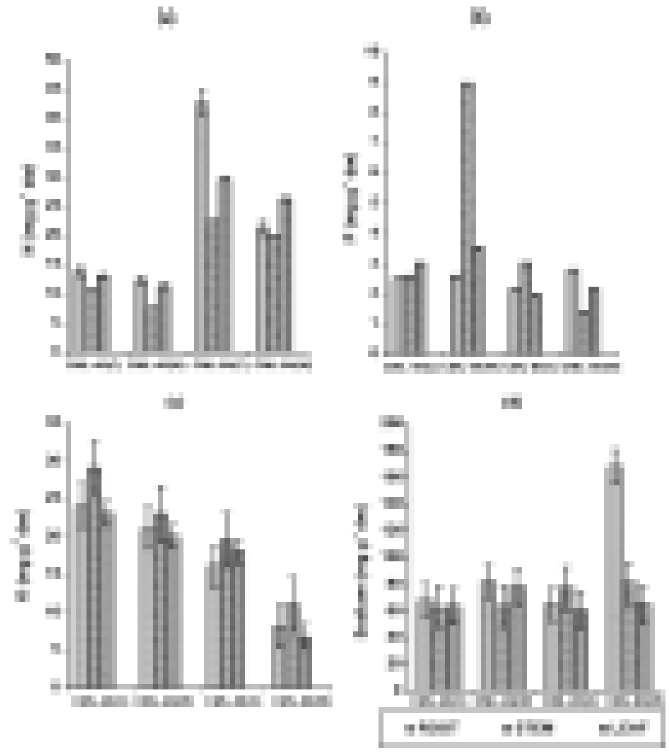

**Fig. 1. Nitrogen (a), phosphorus (b), potassium (c) and sodium (d) contents in different plant parts of two genotypes of maize under normal (C) and waterlogged (W) conditions**

Present investigation indicates that waterlogging resistant genotypes exhibit lesser reduction in photosynthetic area, chlorophyll content, and leaf diffusion resistance under waterlogged condition. Biochemical markers such as higher activity of SOD and higher K and lower Na contents (especially of roots) under waterlogged condition might be taken to screen waterlogging resistant genotypes of maize. In the present investigation, genotype with lower N requirement under normal condition performed better under waterlogging, and it can be taken as an screening criterion.

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