

Waterlogging Stress in Maize: Analyzing Biochemical Responses and Root Trait Adaptations

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Abstract

Maize holds significant economic importance as a cereal crop on a global scale. Among several abiotic stresses, waterlogging poses a substantial challenge in attainment of potential crop yield. To recognize inbred lines that exhibit resilience to waterlogging, it is crucial to gain insights into the fundamental mechanisms and effects of waterlogging stress on various morphological, physiological and biochemical traits. The present study was carried out to identify waterlogging tolerant inbred lines using a set of 86 inbred lines for six and nine days of water logging stress at V₃₋₄ stage along with control. The results indicate that under increasing waterlogging stress, notable decrease in germination percentage, chlorophyll content and root traits viz., root length, root area, and root volume were observed. However, in the case of tolerant genotypes, the percentage reduction in these traits compared to the control was lower than in the susceptible ones. Both fresh and dry weights of roots and shoots exhibited a reduction compared to control; however, the tolerant genotypes displayed the least reduction, while the susceptible genotypes experienced a sharp reduction. Also, the chlorophyll content experienced the least reduction in tolerant genotypes as waterlogging stress increased. To validate the identified lines, a subset of 13 lines shown to be tolerant or susceptible were selected based on various experiments performed and then these lines were subjected to biochemical analysis viz., superoxide dismutase, catalase, peroxidase, ascorbic acid and tocopherol content. Tolerant genotypes viz., I 185, I 172 and SE 616 exhibited higher enzyme activity and antioxidant content, compared to susceptible genotypes.

Abbreviations

RA: Root area
RDW: Root dry weight
RFW: Root fresh weight
RL: Total root length
RV: Root volume
SDW: Shoot dry weight

SFW: Shoot fresh weight
SOD: Superoxide dismutase
T1: Treatment for 6 days
T2: Treatment for 9 days
WL: Waterlogging

Introduction

Maize cultivation faces a multitude of challenges, with susceptibility to both biotic and abiotic stresses that can significantly hamper its productivity. Among the abiotic stresses, issues such as waterlogging (WL), salt stress and extreme heat are notable threats to crop yields (Bray et al. 2000). In particular, WL which occurs due to excessive soil moisture, emerges as a critical concern for maize production in tropical Asian regions (Lone and Warsi 2009). In India, WL ranks as the second most substantial constraint in agricultural production, following closely behind drought, impacting approximately 8.5 million hectares of fertile land

due to unpredictable summer monsoon rains (Ahsan et al. 2007). This challenge is particularly pronounced in Northwestern plain zone of India, where maize is predominantly grown during the monsoon season. Irregular rainfall patterns often result in inevitable, temporary WL stress, which is further intensified by shifting climatic conditions and an increasing frequency of extreme weather events.

The effect of WL stress on maize crops is complex, as it affects various aspects of its biology such as physiology, morphology, biochemistry, anatomy and molecu-

lar processes. WL can cause an energy deficit, hamper the hydraulic conductance of roots, restrict nutrient uptake and hamper photosynthesis, ultimately resulting in substantial reductions in crop yields. The degree of damage is influenced by factors such as the growth stage, duration of stress, environmental conditions and soil characteristics. Maize is the most susceptible crop to WL during early growth stages, particularly from the V₂ (second leaf stage) to V₇ (seventh leaf stage) leading to decreased root growth and biomass (Juan *et al.* 2009). Roots bear the primary impact of WL damage and the impact on shoots can be seen later as yellowing of leaves and upon further extension of WL stress, leaves become dry which ultimately leads to loss of biomass and yield. WL stress triggers oxidative stress and the production of reactive oxygen species (ROS) within the roots, which can harm proteins, lipids and nucleic acids (Biemelt *et al.* 2000). Insufficient oxygen levels during WL stress can impede various critical processes such as seed germination, respiration, electron transport and ATP production (Hsu *et al.* 2000). Additionally, WL can modify the physical and chemical properties of the soil's rhizosphere (Zaidi *et al.* 2003). The tolerance of different maize genotypes to WL stress varies considerably and is influenced by both the severity of the stress and the genotype of the plant (Torbert *et al.* 1993).

WL conditions can lead to stomatal closure and a reduction in the photosynthetic rate (Steffens *et al.* 2005). Additionally, WL triggers the excessive production of ROS, including hydrogen peroxide (H₂O₂), hydroxyl radical (OH[•]), singlet oxygen, and superoxide radical (O^{2-•}) (Subbaiah *et al.* 2003; Jackson *et al.* 2005), disrupting normal plant metabolic processes. In response to these challenges, plants have developed intricate antioxidant defence mechanisms to safeguard cells from the harmful effects of ROS (Hussain *et al.* 2016). Key components of these defence mechanisms include enzymes and antioxidants such as catalase, superoxide dismutase, glutathione reductase, ascorbate peroxidase, peroxidase, α-tocopherol and ascorbic acid. These play a role in mitigating the oxidative stress in the case of WL by quenching reactive oxygen species (Hussain *et al.* 2016; Parveen *et al.* 2019).

Punjab, also known as the bread basket of India; maize is sown during the *kharif* season, coinciding with the monsoon period. However, erratic rainfall patterns, often resulting in flooding, pose a significant challenge to maize germination, its growth and ultimately its yield. The waterlogged conditions in specific areas are beyond human intervention, but the identification of maize genotypes capable of tolerating such saturated environments offers a viable solution to mitigate losses

associated with excessive soil moisture. Maize plants undergo an array of modifications at physiological, morphological, biochemical, anatomical and molecular levels to withstand the rigours of WL. These adaptations encompass heightened anaerobic respiration due to reduced oxygen diffusion in waterlogged soil, the development of aerenchyma tissue and the regulation of internode extension through biochemical phytohormone signalling. Given the substantial impact of WL on maize cultivation in India, there is a compelling need to identify and develop maize genotypes capable of withstanding temporary WL conditions. The aim of this research is to identify water-tolerant maize lines and gain a comprehensive understanding of the biochemical mechanisms underlying waterlogging tolerance. This knowledge is expected to significantly contribute to maize breeding programs, facilitating the development of more stress-tolerant hybrids and varieties.

Materials and methods

Plant Material

The experiments were carried out using a set of eighty-six maize inbred lines (Table S1) of different heterotic pools obtained from the Maize Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana at the maize experimental area spanning the calendar years 2021-22 and 2022-23.

Experiment I

In this experiment, five seeds from each inbred line were planted in each pot during June 2022 in three replications in three sets. All pots received optimum moisture, supplements and nutrients to ensure uniform growth. The maize plants were grown until they reached the V_{3.4} stage (2-4 inches in height with two visible leaf collars) typically within 18-20 days after sowing (Abendroth *et al.* 2011). Once over 50% of the plants in each pot reached the V_{3.4} stage, the plants were subjected to WL stress for six days (T₁) and 9 days (T₂) keeping one set as control. The pit was carefully sealed with a black plastic sheet before pot placement to prevent any seepage. Pots were categorized into three groups: one set outside the pit as control and two sets inside, experiencing varying durations of WL. The pots for treatment were placed in water-filled pit so that roots were submerged while shoots remained exposed. Daily water supply was ensured to maintain the water level. After the completion of treatments, the pots were removed from the pit for further collection of plant samples. The data recorded viz., chlorophyll content (Apogee MC-100 Chlorophyll Content Meter), shoot and root dry weight, and root traits. The shoot and root samples were harvested immediately after WL

treatment and root trait analysis was conducted using a root scanner. Images of the roots were captured with an EPSON Expression 12000XL scanner and analyzed using Biovis PSM root software (Wajahat *et al.*, 2023). To minimize root breakage, plants from both control plots and those grown under optimal moisture conditions were first soaked in water until the soil was saturated, allowing for gentle extraction. The roots were then rinsed gently with slow-running tap water and collected in Falcon tubes containing a 70% ethanol solution for storage until further analysis. The software provided measurements for projected area (cm²), total root length (in cm), and counts for root tips, forks, and segments, as well as diameter (in mm) and volume (in cm³). After root trait analysis, root and shoot underwent a subsequent drying process in a hot air oven set at 80°C for 72 hours, following which the dry weight (g) was recorded.

Experiment II

In this experiment, a sub set of thirteen including highly susceptible (five) and tolerant lines (eight) (Table S2) were further selected based on the results of an experiment I to explore changes in the biochemical activity of enzymes and antioxidants such as superoxide dismutase, peroxidase, catalase, α -tocopherol and ascorbic acid under WL conditions. These compounds play critical roles in the plant's defence mechanisms against oxidative stress and other environmental challenges. Extensive measurements and assessments were done to identify the highly susceptible and highly tolerant lines based on their change in biochemical activity.

The enzymes and antioxidants that played a significant role under WL were measured from leaf samples having 3-4 leaf stage after six days of WL treatment and enzymes viz., Superoxide dismutase (SOD), peroxidase, ascorbic acid, tocopherol, catalase were analyzed.

SOD activity was estimated as described by Marklund

and Marklund 1974. Shannon *et al.* 1966 method was used to estimate peroxidase activity. To estimate catalase activity, Chance and Machley 1955 method was followed. For ascorbic acid (vitamin C), the method by Roe and Keuther 1943 was used and for estimation of α -tocopherols (a type of vitamin E) in the leaf sample followed the Emmerie-Engel reaction as described by Rosenberg *et al.* 1992.

Statistical analysis

Statistical analyses were analyzed using R Studio version 4.3.4. The graphs were made by using Origin Pro software 2023 and MS Excel 2010.

Results and discussion

The research elucidates how various maize genotypes respond to WL stress, a condition in which excess water in the soil restricts oxygen availability to plant roots, leading to physiological and biochemical changes in plants

Experiment I: Plant growth and development

Germination

Plant survival, growth and development parameters were recorded. The impact of WL stress increases as stress duration extends as depicted by the results. After six days of WL stress, it was observed that susceptible lines began to exhibit a significantly higher mortality rate (Table 1). However, when the WL stress was prolonged to nine days, the highly susceptible lines experienced near-complete mortality. In contrast, tolerant lines displayed exceptional resilience, with almost 100 percent survival rate even after enduring 9 days of continuous WL. Inbred lines such as I 172, I 182, I 185, SE 616, PML 1231, PML 1253, and EL 1 showed upto 80% survival even after 9 days of WL. While lines such as SE571, SE544, SE565A, SE607, EML164, EML176, EML129, EML183, EML131, EML145, EML101,

Table 1 - Descriptive statistics for the morpho physiological traits

Germination %	Control			T1			T2		
	Mean	Range	F-test	Mean	Range	F-test	Mean	Range	F-test
	70.1	0-100	**	44.4	0-100	**	6.27	0-80	*
Chl	0.5	0.41-0.7	*	0.43	0.28-0.66	**	0.36	0-0.6	NS
SFW (gm)	3.5	1.23-10.45	**	3.2	1.11-9.85	**	3.04	0.55-9.21	*
RFW (gm)	3.26	1.01-8.9	*	2.9	0.76-8.7	*	2.7	0.35-7.54	**
SDW (gm)	3.15	1.01-10.17	**	2.8	0.65-9.45	*	2.8	0.72-8.81	*
RDW (gm)	2.87	0.84-8.53	**	2.6	0.62-8.12	**	2.52	0.35-7.21	*
RA (cm ²)	1823	297-3375	*	1880	248-7657	*	1714	229-7655	**
RL (cm)	1495	379-3375	*	1440	291-4012	*	1316	220-4536	*
RV (mm ³)	12921	295-72665	**	18088	314-139042	**	14237	330-97158	*

*, ** denotes 5 and 1% level of significance

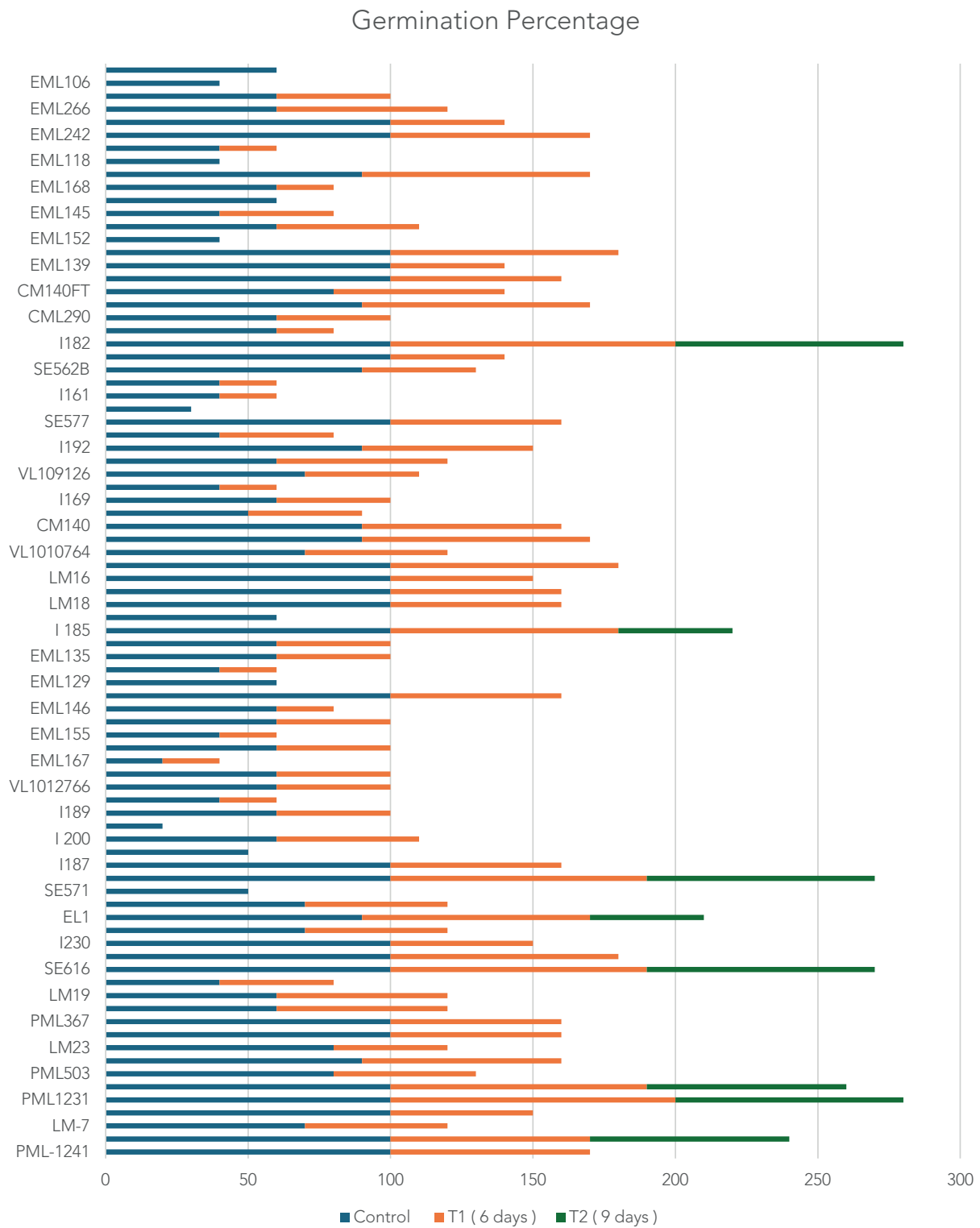


Fig. 1 - Bar graph representing germination percentages in control as well as in treatments for six days and nine days of WL

EML140, EML166, EML111 and LM5 exhibit no survival after enduring 9 days of WL stress. The differences in germination percentages only became apparent when the WL treatment was extended up to six days before that most of the lines survived the WL stress upon further extension to 6 days few lines started drying while when stress reached 9 days most of the lines died and few survived as mentioned above. Upon further extension of the WL duration, only a handful of genotypes demonstrated tolerance to the prolonged WL stress, while the majority exhibited a susceptible response (Figure 1 and Table S3).

From this study we found notable variations in the responses of different maize genotypes to WL stress, which revealed the potential mechanisms underlying their tolerance or susceptibility. When the maize inbreds from different heterotic pool were subjected to prolong WL, it became evident that highly susceptible maize lines experienced elevated mortality rates, highlighting their vulnerability to this stress. This result is indicative that there might be disruption in cellular processes, particularly root respiration, caused by oxygen deficiency. According to Alam *et al.* (2010), the negative consequences of hypoxia and anoxia, which cause delayed growth and reduced yield in many crops, result in a fall in cytoplasmic pH and cellular energy change, as well as the accumulation of hazardous metabolites and reactive oxygen species (ROS). On the other hand, tolerant genotypes exhibited remarkable resilience, maintaining higher survival rates even after enduring nine days of continuous WL. The differences observed in mortality rates underscore the genetic variation among maize genotypes and their capacity to adapt to adverse WL conditions.

Chlorophyll content

The decline in chlorophyll content was particularly observed in genotypes that were highly susceptible to WL stress whereas, tolerant genotypes displayed a remarkable capacity to preserve their chlorophyll levels, resulting in a considerably lower percentage reduction. With the extension of the WL stress duration, the reduction in chlorophyll content intensified significantly.

Inbred lines PML 1241, EL 2, EML 160, EML 159, EML 135, EML 242, EML 266, I 164, EL 2, SE 604, and VL1012766 showed severe reductions in chlorophyll content after 9 days of treatment, whereas highest reduction was observed in line VL1010764 *i.e.* 70.58%. In contrast, lines *viz.* PML 503, LM 18, EML 118, SE 533A, I 185, I 172, SE 616, EML 123 and EML 147 showed tolerance and exhibited minimal reductions in chlorophyll content even after 9 days. Inbred line I 182 exhibited the least reduction in chlorophyll content *i.e.* 16.90 %

reduction, indicating high tolerance to WL. Conversely, I 200 showed a significant reduction in chlorophyll content *i.e.* 43.54% after the same duration of WL stress indicating high susceptibility to this condition (Table S4).

Chlorophyll content, a key indicator of photosynthetic activity, exhibited significant variations across genotypes. High susceptibility genotypes displayed a substantial reduction in chlorophyll content under prolonged WL, whereas tolerant genotypes conserved their chlorophyll levels to a greater extent. This differential response indicates that susceptible genotypes are more susceptible to the detrimental effects of WL on photosynthesis. According to Zaidi *et al.* (2007), in vulnerable maize genotypes, the excess moisture stress dramatically reduced plant development but sped up senescence, and resulted in total crop failure. Severe chlorosis was the result of excessive moisture stress and was visible by the decreased chlorophyll content of the leaves. The deterioration in chlorophyll content observed in these lines can be attributed to oxygen deprivation, leading to reduced chlorophyll biosynthesis and accelerated chlorophyll degradation. In contrast, tolerant genotypes exhibit mechanisms that protect chlorophyll pigments and maintain photosynthetic efficiency under stress conditions. Also, Ren *et al.*, 2009 reported that when the WL stress lasted for nine days, the reduction in chlorophyll content became even more pronounced which corresponds with our result.

Dry Weight

Notably, the shoot dry weight (SDW) exhibited a significant reduction, akin to the trend observed in fresh weight in response to WL stress. Once again, the most severe effect was observed after T2 *i.e.* nine days as compared to T1 *i.e.* three days flooding (Table 1). Similarly, root dry weight (RDW) exhibited a parallel response to WL stress, showcasing a substantial reduction under stress conditions. The most pronounced reduction was again observed in T2. Inbred SE 616 showed the least reduction in SDW *i.e.* 9.97 %, the maximum reduction was in inbred I 188 (28.97%). For RDW maximum and least reduction was for inbreds I 200 and I 185 with 35.11 % and 10.41 %, respectively. Several lines exhibited a percentage reduction exceeding 50% in both shoot and root dry weight after T₂. Inbred lines encompassed I 200, I 188, LM 6, LM 23, LM 7, EML 152, CM 140 FT, SE 503, I 164 and EML 167 showed more than 50 % shoot and root dry weight reduction after T₂. In contrast, inbreds PML 1231, LM 18, LM 26, PML 1228, SE 604, I 185, I 182 and I 172 displayed < 15% reductions for both root and shoot dry weight (Table S5).

Assessment of the seedlings' fresh and dry weights revealed the impact of WL on growth and biomass accumulation. A consistent decrease in fresh weight

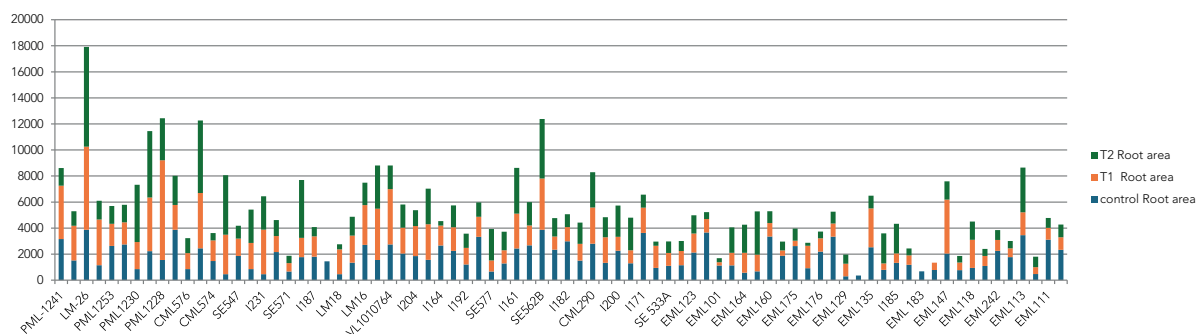


Fig. 2 a - Root area in control, T1 (6 days of waterlogging), T2 (9 days of waterlogging)

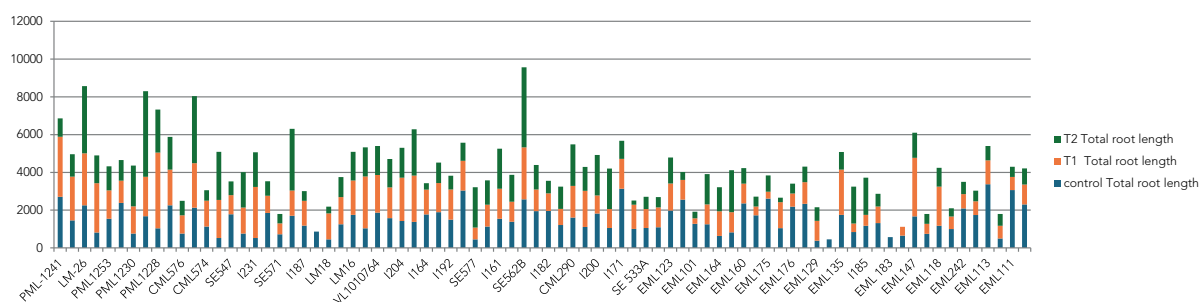


Fig. 2 b - Total Root length in control, T1 (6 days of waterlogging), T2 (9 days of waterlogging)

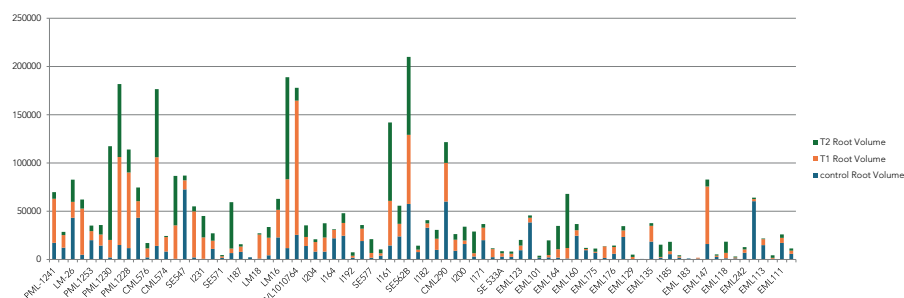


Fig. 2 c - Root volume in control, T1 (six days of waterlogging), T2 (nine days of waterlogging)

was observed in response to WL, with the most pronounced effect after nine days of stress. This reduction is a manifestation of reduced plant growth and biomass accumulation under waterlogged conditions. These variations in weight can be attributed to differences in root development, water and nutrient uptake, and photosynthesis across the genotypes (Van 2022). Huang *et al.* 2022 also revealed that there is a reduction of fresh weight as well as dry weight with increasing WL stress and a reduction in weight for tolerant lines is less compared to susceptible lines.

Root Traits

Tolerant genotypes initially displayed an increase in root length, area and volume after enduring six days of WL stress. However, this initial boost was followed by a subsequent decrease in these traits. Interestingly, in a few genotypes, there was resurgence in these traits after experiencing nine days of WL stress. In contrast,

susceptible genotypes consistently showed a reduction in all measured root traits, including length, area and volume in T₁ and T₂. These findings underscore the differential responses of tolerant and susceptible genotypes to WL stress (Figure 2 a,b,c). Tolerant genotypes initially adapt by enhancing their root traits, potentially to improve water and nutrient absorption. However, this adaptive response may not be sustained over an extended stress period, leading to subsequent reductions. Conversely, susceptible genotypes consistently demonstrate a decline in root traits under WL stress, indicating their susceptibility to unfavourable soil conditions. Inbreds showed the least reduction in root traits were LM 26, PML 1228, SE 616, I 172, I 182, I 185, SE 562B, EML 123, and EML 156 for all the root traits taken into consideration.

When exposed to WL stress, different genotypes exhibited different responses in terms of root traits like length, area, and volume. After six days of WL, tolerant

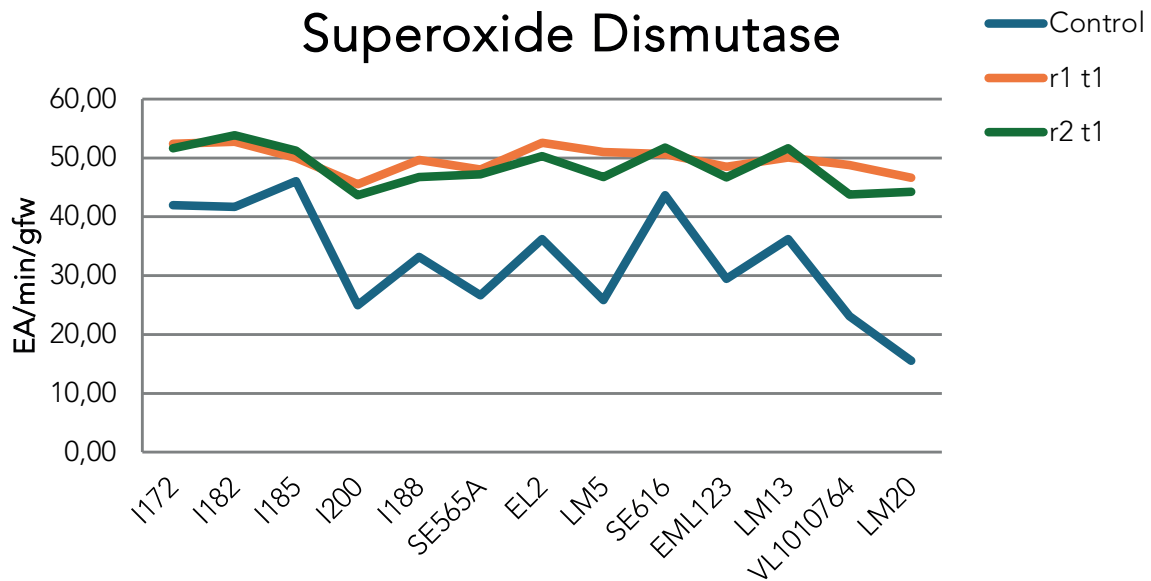


Fig. 3 a - Line graphs showing SOD enzyme activity in both Control and Treatment (6 and 9 days WL)

genotypes showed improved root traits at first, possibly as an adaptive reaction to improve the absorption of water and nutrients. Some of these improvements, though, did not hold up under prolonged stress. Susceptible genotypes, on the other hand, consistently showed a decrease in root traits, indicating their capacity to adjust to wet conditions. These results emphasize how root morphology helps maize genotypes adapt to WL. Contrary to the findings of (Grzesiak *et al.* 1999; Hamblin *et al.* 1987 and Kiel *et al.* 1992), proposed a decrease in root traits with heightened WL stress, while the research findings of root traits, it was observed that there was an increase in root traits among certain genotypes categorized as tolerant.

This discrepancy underscores the intricate nature of plant responses to WL

Experiment II: Biochemical analysis

Superoxide dismutase (SOD)

In Experiment II, a series of biochemical analyses were conducted, and the effects of enzyme activity were observed in both the control and treated environments. Through this experiment it is evident that the value of enzyme activity was higher (> 50) for I 182, I 185, SE 616, I 172, LM13 and EL2. While activity was less for the rest of the genotypes (< 50). The lowest percentage reduction for SOD activity stands for inbred I 185 while

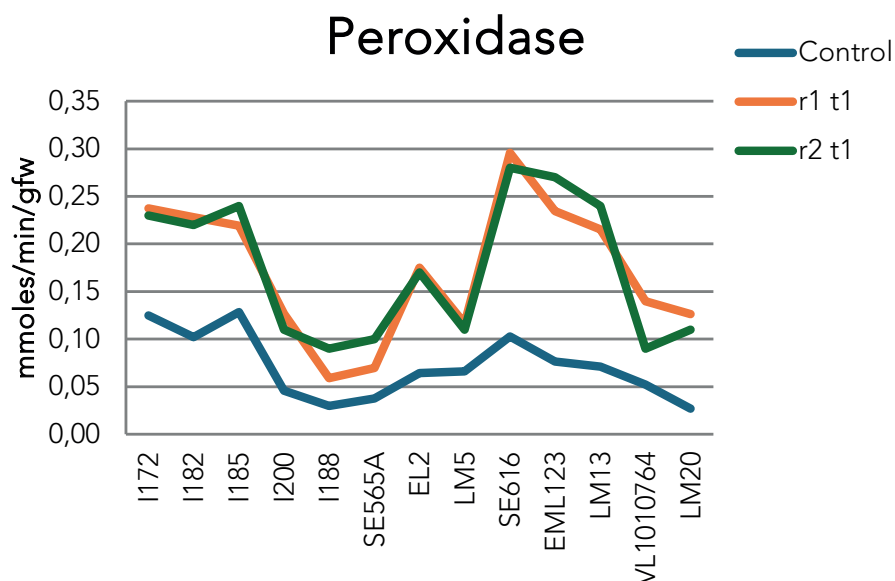


Fig. 3 b - Line graphs showing peroxidase enzyme activity in both Control and Treatment (six days and nine days WL)

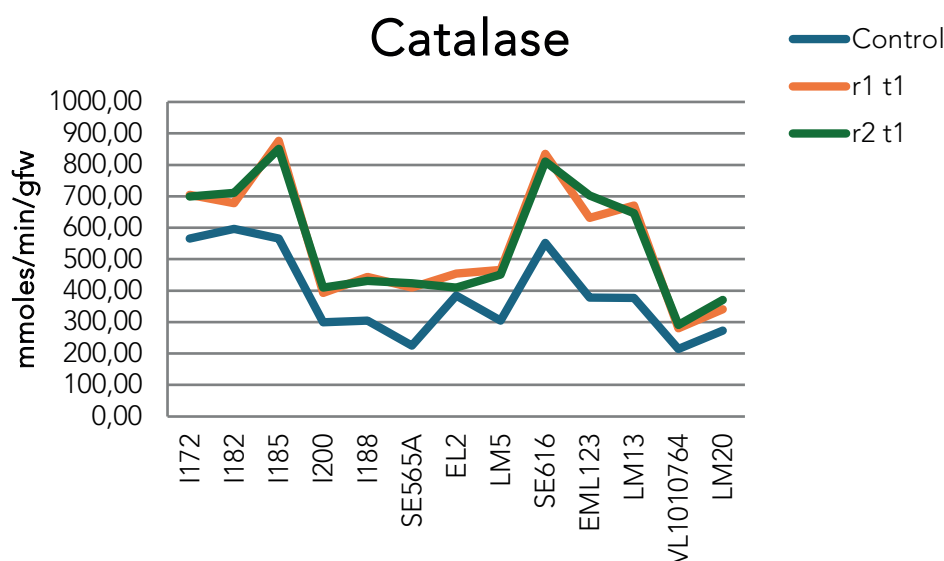


Fig. 3 c - Line graphs showing catalase enzyme activity in both Control and Treatment (six days and nine days WL)

the highest reduction is for I 200 as in Figure 3a. The activity of SOD can increase in response to WL stress, as plants try to mitigate the harmful effects of ROS accumulation. This upregulation of SOD is a part of the plant's adaptive response to waterlogged conditions and is crucial for maintaining cellular redox balance and overall plant growth in such stressful environments.

Peroxidase

In this study, it is observed that I 172, I 185, I 182, SE 616, and EML 123 had a higher enzyme activity as compared to other genotypes I 172 stands for higher enzyme activity (0.23 mmoles/min/gfw) and is 4 times higher than I188 which had the lowest enzyme activity (0.05mmoles/min/gfw) as in Figure 3b. Peroxidase is

another important enzyme in plants' response to WL stress. When plants are exposed to waterlogged or flooded conditions, their roots can experience a shortage of oxygen, leading to the formation of reactive oxygen species (ROS) within the plant tissues. These ROS, including hydrogen peroxide (H_2O_2), can cause oxidative damage to cellular components consequently; peroxidase plays a crucial role in scavenging hydrogen peroxide thereby helping the plant to adapt to water logged conditions.

Catalase

In this study (Figure 3c) it is observed that catalase activity is higher for genotypes I185 showed the highest activity (876.15 mmoles/min/gfw) as I 172, I 182, I 185,

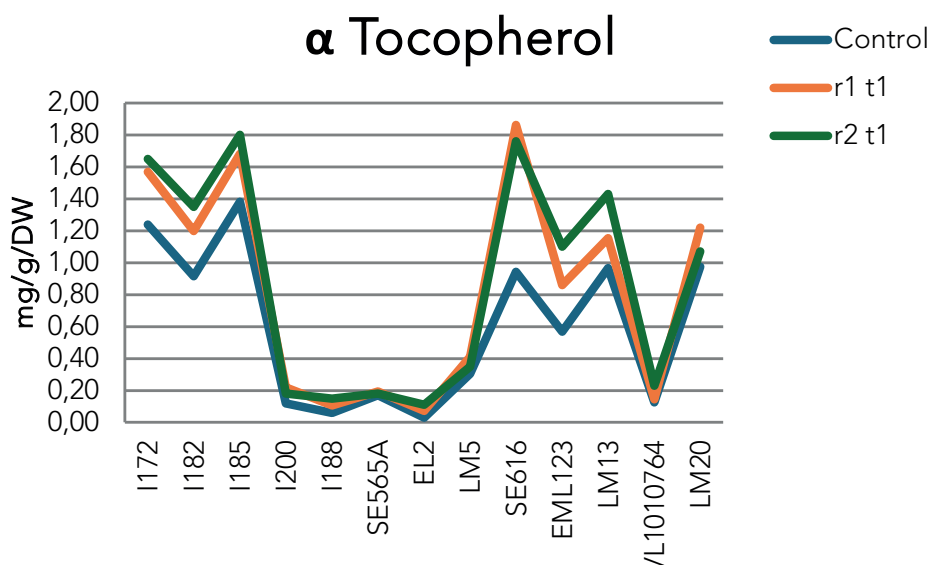


Fig. 3 d - Line graphs showing tocopherol enzyme activity in both Control and Treatment (six days and nine days WL)

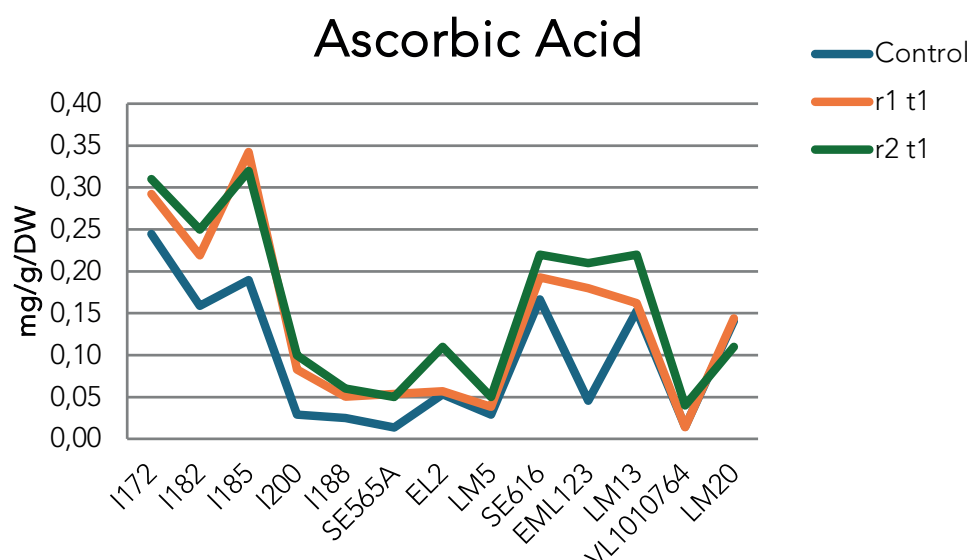


Fig. 3 e - Line graphs showing ascorbic acid enzyme activities in both Control and Treatment (six days and nine days WL)

SE 616, and LM 5. I 185 at I185 showed the highest activity (876.15) top with 876.15 which was 7 times higher than VL 1010764 123.01 mmoles/min/gfw. During WL stress, plants often increase the activity of catalase as part of their adaptive response. This upregulation of catalase activity helps maintain redox balance in plant cells and reduces the oxidative damage caused by the accumulation of hydrogen peroxide. Consequently, catalase is a key player in the plant's defence against oxidative stress under waterlogged conditions, contributing to the plant's ability to survive and adapt to this challenging environment.

α - Tocopherol

It is observed that genotypes I 172, I 185, I 182, LM 5, and LM 13 had high content. I 185 has the highest content in the case of alpha-tocopherol which was 1.68 which is 24 times higher than EL2 which was 0.07mg/g/DW (Figure 3d). α -tocopherol, a form of vitamin E, is an important antioxidant compound in plants that can play a role in mitigating oxidative stress caused by various environmental factors, including WL stress. α -tocopherol acts as an antioxidant by scavenging and neutralizing ROS, thereby protecting plant cells from oxidative damage. During WL stress, plants may increase the synthesis and accumulation of α -tocopherol as part of their defence mechanism to counteract oxidative stress. The presence of alpha-tocopherol helps maintain cellular redox balance, reduce lipid peroxidation, and protect various cellular structures, such as membranes and chloroplasts, from oxidative damage. Overall, α -tocopherol is an essential component of the plant's antioxidant defence system and can help plants cope with oxidative stress during WL or other stressful

environmental conditions.

Ascorbic Acid

The study found that I 172, I 185, I 182, LM 5, SE 616, and LM 13 had high content. The highest reading was for I 185 which was 0.34 which was 7 times higher than I 188 which was 0.050 mg/g/DW as Figure 3e. This upregulation of ascorbic acid content helps the plant cope with the challenges posed by waterlogged environments and supports its survival and recovery when oxygen availability is limited.

Thus, from our results, I 185, I 172, SE 616 and EML 123 show high enzymatic activity for all the biochemical enzymes taken into account. SOD acts as a defense mechanism against oxidative stress by catalyzing the dismutation of superoxide radicals into less harmful molecules, namely oxygen (O_2) and hydrogen peroxide (H_2O_2). By doing so, SOD helps prevent the buildup of damaging ROS in plant cells, which can otherwise cause oxidative damage to various cellular components, including proteins, lipids and DNA (Fridovich 1975). Also, stress-related peroxidase enzymes are involved in the detoxification of hydrogen peroxide. They catalyze the breakdown of hydrogen peroxide into water and oxygen, thus reducing the levels of this potentially harmful molecule in plant cells. This enzymatic activity helps protect the plant cells from oxidative stress and damage caused by the accumulation of ROS during WL stress. Upregulation of peroxidase activity helps maintain cellular redox balance and reduces the oxidative damage caused by elevated levels of hydrogen peroxide. (Lück 1965). When plants experience WL, oxygen availability to the roots is reduced, leading to a lack of oxygen in the root zone. This low-oxygen condition

can trigger oxidative stress and the formation of reactive oxygen species (ROS) within plant cells (Levshina *et al.* 1988). These ROS, including superoxide radicals and hydrogen peroxide, can cause damage to cellular components. By reducing hydrogen peroxide levels to ROS, catalase helps protect plant cells from oxidative stress and damage (Aebi 1974). Ascorbic acid functions as a potent antioxidant by scavenging and neutralizing ROS, including superoxide radicals and hydrogen peroxide. This antioxidant activity helps protect plant cells from oxidative damage caused by the accumulation of ROS during WL stress. As a result, ascorbic acid plays a vital role in maintaining cellular redox balance and mitigating the harmful effects of oxidative stress under waterlogged conditions. In response to WL stress, plants may increase the synthesis and accumulation of ascorbic acid as part of their adaptive response to combat oxidative stress (Foyer 2017). Higher antioxidant and enzyme activity levels in tolerable genotypes suggest that they can mitigate the deleterious effects of reactive oxygen species (ROS) produced during WL stress.

Conclusions

The experiments conducted to investigate the response of maize genotypes to WL stress yielded crucial insights into their varying levels of tolerance and susceptibility. One noteworthy observation was the higher mortality rates among maize lines particularly vulnerable to WL, highlighting the stress these lines experienced when subjected to extended periods of flooding. The findings underscore the genetic diversity among maize genotypes and their ability to adapt to adverse WL conditions, emphasizing the importance of understanding and harnessing these traits for crop improvement. Furthermore, the experiments explore the impact of WL on chlorophyll content, revealing it as a crucial marker of photosynthetic activity among different genotypes. Additionally, our research highlighted the role of root traits in adaptation to WL stress. Tolerant genotypes initially exhibited improved root traits, possibly as an adaptive response to enhance nutrient and water uptake. However, under extended stress, some of these improvements were lost, highlighting the dynamic nature of plant responses to changing environmental conditions. Finally, significant biochemical markers, including enzymes like alpha-tocopherol, peroxidase, catalase, ascorbic acid, and superoxide dismutase (SOD), whose activities varied among genotypes. Tolerant genotypes exhibited higher levels of antioxidant and enzyme activity, suggesting their potential to mitigate the harmful effects of reactive oxygen species (ROS) generated during WL stress. In conclusion, the research provides a comprehensive understanding of how different maize genotypes respond

to WL stress, shedding light on physiological mechanisms, genetic diversity, and root morphology in these adaptations. Further enzymatic study unveils the underlying biochemical mechanisms and offers a practical classification tool for assessing WL tolerance in maize crops. These identified lines can be used in maize breeding programs for developing WL tolerant lines or can directly be used for hybrid development.

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Conceptualization: Tosh Garg, Rumesh Ranjan; Methodology: Tosh Garg, Rumesh Ranjan and Surinder K Sandhu; Formal analysis and investigation: Prabhat Rana; Writing - original draft preparation: Prabhat Rana; Editing: Tosh Garg and Rumesh Ranjan; Resources: Prabhat Rana; Supervision: Tosh Garg, Rumesh Ranjan, Surinder K Sandhu, and Navita Ghai.

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Supplementary tables:

Table S1 - List of genotypes used in the study

SN	Genotype	SN	Genotype
1)	PML1241	44.	LM18
2)	LM13	45.	LM20
3)	LM7	46	LM16
4)	LM26	47	LM5
5)	PML1231	48	VL1010764
6)	PML1253	49	CM139
7)	PML503	50	CM140
8)	PML1230	51	I204
9)	LM23	52	I169
10)	PML1228	53	I164
11)	PML367	54	VL109126
12)	CML576	55	SE604
13)	LM19	56	I192
14)	CML574	57	EL2
15)	SE616	58	SE577
16)	SE547	59	SE565A
17)	I230	60	I161
18)	I231	61	I205
19)	EL1	62	SE562B
20)	I188	63	SE503
21)	SE571	64	I182
22)	I172	65	SE607
23)	I187	66	CML290
24)	SE544	67	SE621
25)	I 200	68	CM140FT
26)	I171	69	EML123
27)	I189	70	EML139
28)	SE533A	71	EML101
29)	VL1012766	72	EML152
30)	EML164	73	EML198
31)	EML167	74	EML131
32)	EML160	75	EML145
33)	EML155	76	EML147
34)	EML175	77	EML168
35)	EML146	78	EML140
36)	EML176	79	EML118
37)	EML159	80	EML119
38)	EML129	81	EML242
39)	EML124	82	EML275
40)	EML135	83	EML266
41)	EML156	84	EML113
42)	I 185	85	EML106
43)	EML183	86	EML111

Table S2 - List of subset genotypes for biochemical validation

SN	Genotype
1)	I172
2)	I182
3)	I185
4)	I200
5)	I188
6)	SE565A
7)	EL2
8)	LM5
9)	SE616
10)	EML123
11)	LM13
12)	VL1010764
13)	LM20

Table S3 - Genotypes with their germination percentage at different durations of waterlogging

Genotypes	Germination percentage			Genotypes	Germination percentage		
	Control	T1	T2		Control	T1	T2
PML1241	100	70	0	LM18	100	60	0
LM13	100	70	70	LM20	100	60	0
LM7	70	50	0	LM16	100	50	0
LM26	100	50	0	LM5	100	80	0
PML1231	100	100	80	VL1010764	70	50	0
PML1253	100	90	70	CM139	90	80	0
PML503	80	50	0	CM140	90	70	0
PML1230	90	70	0	I204	50	40	0
LM23	80	40	0	I169	60	40	0
PML1228	100	60	0	I164	40	20	0
PML367	100	60	0	VL109126	70	40	0
CML576	60	60	0	SE604	60	60	0
LM19	60	60	0	I192	90	60	0
CML574	40	40	0	EL2	40	40	0
SE616	100	90	80	SE577	100	60	0
SE547	100	80	0	SE565A	30	0	0
I230	100	50	0	I161	40	20	0
I231	70	50	0	I205	40	20	0
EL1	90	80	40	SE562B	90	40	0
I188	70	50	0	SE503	100	40	0
SE571	50	0	0	I182	100	100	80
I172	100	90	80	SE607	60	20	0
I187	100	60	0	CML290	60	40	0
SE544	50	0	0	SE621	90	80	0
I 200	60	50	0	CM140FT	80	60	0
I171	20	0	0	EML123	100	60	0
I189	60	40	0	EML139	100	40	0
SE533A	40	20	0	EML101	100	80	0
VL1012766	60	40	0	EML152	40	0	0
EML164	60	40	0	EML198	60	50	0
EML167	20	20	0	EML131	0	0	0
EML160	60	40	0	EML145	40	40	0
EML155	40	20	0	EML147	60	0	0
EML175	60	40	0	EML168	60	20	0
EML146	60	20	0	EML140	90	80	0
EML176	0	0	0	EML118	40	0	0
EML159	100	60	0	EML119	40	20	0
EML129	60	0	0	EML242	100	70	0
EML124	40	20	0	EML275	100	40	0
EML135	60	40	0	EML266	60	60	0
EML156	60	40	0	EML113	60	40	0
I 185	100	80	40	EML106	40	0	0
EML183	60	0	0	EML111	60	0	0

Table S4 - Chlorophyll readings for different treatments (-*= no data due to plant mortality)

Genotypes	At control	At 6 days of waterlogging	% reduction	At 9 days of waterlogging	% Reduction
PML1241	0.62	0.35	43.55	0.28	54.84
LM6	0.52	0.38	26.92	0.35	32.69
LM7	0.41	0.31	24.39	0.28	31.71
LM26	0.52	0.43	17.31	0.38	26.92
PML1231	0.6	0.44	26.67	0.38	36.67
PML1253	0.64	0.53	17.19	0.49	23.44
PML503	0.45	0.37	17.78	0.32	28.89
PML1230	0.42	0.3	28.57	0.26	38.10
LM23	0.42	0.28	33.33	0.26	38.10
PML1228	0.55	0.48	12.73	0.4	27.27
PML367	0.58	0.48	17.24	0.36	37.93
CML576	0.43	0.38	11.63	0.32	25.58
LM19	0.52	0.42	19.23	0.35	32.69
CML574	0.42	0.36	14.29	0.33	21.43
SE616	0.49	0.42	14.61	0.38	22.86
SE547	0.48	0.38	20.83	0.33	31.25
I230	0.54	0.43	20.37	0.4	25.93
I231	0.52	0.45	13.46	0.38	26.92
EL1	0.44	0.38	13.64	0.33	25.00
I188	0.54	0.48	11.11	0.44	18.52
SE571	0.48	_*	_*	_*	_*
I172	0.62	0.54	12.90	0.48	22.58
I185	0.54	0.5	7.41	0.46	14.81
SE544	0.48	_*	_*	_*	_*
LM18	0.52	0.45	13.46	0.4	23.08
LM20	0.43	0.36	16.28	0.28	34.88
LM16	0.52	0.45	13.46	0.38	26.92
LM5	0.56	0.44	21.43	0.36	35.71
VL1010764	0.46	0.32	30.43	0.3	34.78
CM139	0.52	0.44	15.38	0.34	34.62
CM140	0.52	0.43	17.31	0.34	34.62
I204	0.52	0.42	19.23	0.38	26.92
I169	0.51	0.44	13.73	0.36	29.41
I164	0.57	0.42	26.32	0.33	42.11
VL109126	0.5	0.46	8.00	0.32	36.00
SE604	0.52	0.4	23.08	0.31	40.38
I192	0.54	0.3	44.44	0.36	33.33
EL2	0.7	0.3	57.14	0.28	60.00
SE577	0.52	0.44	15.38	0.38	26.92
SE565A	0.66	_*	_*	_*	_*
I161	0.54	0.47	12.96	0.43	20.37
I205	0.57	0.45	21.05	0.40	29.82
SE562B	0.6	0.48	20.00	_*	_*
SE503	0.53	0.44	16.98	0.38	28.30
I182	0.58	0.53	8.62	0.44	24.14

SE607	0.6	0.44	26.67	_*	_*
CML290	0.55	0.4	27.27	0.36	34.55
SE621	0.55	0.4	27.27	0.36	34.55
I200	0.59	0.48	18.64	0.36	38.98
CM140FT	0.44	0.4	9.09	0.33	25.00
I171	0.68	_*	_*	_*	_*
I189	0.52	0.44	15.38	0.40	23.08
SE533A	0.48	0.44	8.33	0.40	16.67
VL1012766	0.68	0.41	39.71	0.20	70.59
EML123	0.65	0.58	11.54	0.52	20.85
EML139	0.48	0.4	16.67	0.33	31.25
EML101	0.68	0.66	2.94	0.60	11.76
EML152	0.65	0.48	26.15	0.40	38.46
EML164	0.55	0.42	23.64	_*	_*
EML167	0.5	0.38	24.00	0.30	40.00
EML160	0.65	0.45	30.77	0.38	41.54
EML155	0.55	0.44	20.00	0.37	32.73
EML175	0.54	0.4	25.93	0.33	38.89
EML146	0.52	0.44	15.38	0.38	26.92
EML159	0.59	0.4	32.20	0.35	40.68
EML124	0.67	0.57	14.93	0.50	25.37
EML135	0.54	0.31	42.59	0.28	48.15
EML156	0.53	0.48	9.43	0.45	15.09
I 185	0.58	0.4	31.03	0.38	34.48
EML198	0.57	0.45	21.05	0.38	33.33
EML145	0.6	0.51	15.00	_*	_*
EML147	0.57	0.45	21.05	0.38	33.33
EML168	0.52	0.47	9.62	0.33	36.54
EML140	0.52	0.42	19.23	_*	_*
EML118	0.59	0.52	11.86	0.48	18.64
EML119	0.61	0.44	27.87	0.38	37.70
EML242	0.69	0.54	21.74	0.35	49.28
EML275	0.66	0.52	21.21	0.00	0.00
EML266	0.52	0.48	7.69	0.28	46.15
EML113	0.54	0.4	25.93	0.33	38.89
EML106	0.53	0.42	20.75	_*	_*
EML111	0.63	_*	_*	_*	_*

Table S5 - Seedlings dry weight (grams) as observed in different intervals of waterlogging (-*= No data due to plant mortality)

Genotypes	Control		T1		T2	
	Shoot	Root	Shoot	Root	Shoot	Root
PML1241	3.31	2.18	2.97	2.45	2.74	2.47
LM6	1.88	0.98	0.97	0.84	0.77	0.78
LM7	1.56	1.45	1.45	1.32	1.09	0.97
LM26	8.14	7.81	7.54	7.26	7.12	7.01
PML1231	4.87	4.31	4.51	4.21	4.03	3.96
PML1253	2.52	2.41	2.23	2.09	2.21	2.09
PML503	10.17	8.53	9.45	8.12	8.81	7.21
PML1230	5.14	4.49	4.98	4.42	4.78	4.21
LM23	2.94	2.46	2.33	2.15	1.97	1.56
PML1228	6.65	5.75	6.12	5.23	6.01	5.11
PML367	3.14	2.86	2.36	2.29	2.16	2.26
CML576	3.47	3.01	3.12	3.01	2.96	2.65
LM19	5.94	5.67	5.44	5.09	5.23	5.01
CML574	2.54	2.17	2.01	1.87	1.78	1.65
SE616	5.26	4.69	4.35	4.19	4.25	4.01
SE547	2.73	2.45	2.42	2.32	2.27	2.14
I230	4.56	4.78	4.32	4.21	4.12	3.99
I231	3.04	2.58	2.48	2.36	2.28	2.34
EL1	3.29	2.96	3.05	2.81	2.97	2.74
I188	4.97	4.81	4.23	4.12	4.09	4.01
SE571	2.36	2.09	-*	-*	-*	-*
I172	4.95	4.71	4.41	4.28	4.21	4.05
I187	2.32	2.11	2.14	1.98	1.97	1.78
SE544	1.23	1.14	-*	-*	-*	-*
LM18	2.35	2.12	2.14	1.89	1.85	1.65
LM20	2.76	2.58	2.48	2.23	2.14	2.25
LM16	2.71	2.54	2.45	2.22	2.34	2.21
LM5	5.51	5.27	5.23	5.04	5.12	4.98
VL1010764	2.82	2.74	2.34	2.17	2.25	1.98
CM139	2.56	2.19	2.03	1.89	1.98	1.75
CM140	2.37	2.17	1.95	1.87	1.74	1.65
I204	2.98	2.84	2.45	2.36	2.39	2.21
I169	3.59	3.37	3.21	3.04	3.06	2.97
I164	1.85	1.79	1.45	1.12	1.45	1.01
VL109126	3.01	2.84	2.54	2.12	2.34	1.98
SE604	3.98	3.64	3.56	3.42	3.36	3.24
I192	3.71	3.59	3.26	3.31	3.27	3.02
EL2	3.56	3.21	3.012	2.87	2.98	2.78
SE577	2.97	2.71	2.45	2.35	2.28	2.18
SE565A	2.28	1.95	-*	-*	-*	-*
I161	5.07	4.93	4.54	4.21	4.29	3.94
I205	3.01	2.91	2.65	2.21	2.45	2.014
SE562B	2.71	2.54	2.21	2.01	-*	-*
SE503	1.76	1.54	1.24	0.99	1.01	0.88
I182	5.29	5.07	4.34	4.012	4.23	3.75

SE607	3.95	3.87	3.45	3.12	_*	_*
CML290	3.49	3.06	3.12	2.95	2.91	2.65
SE621	1.94	1.74	1.67	1.43	1.56	1.32
I200	3.74	3.32	3.45	3.18	3.24	3.09
CM140FT	1.91	1.75	1.48	1.35	1.05	0.35
I171	1.47	1.29	_*	_*	_*	_*
I189	5.91	5.79	5.41	5.12	5.34	4.96
SE533A	1.91	1.67	1.44	1.27	1.36	1.21
VL1012766	2.54	2.36	2.45	2.36	2.35	2.01
EML123	1.74	1.41	1.29	1.15	1.26	1.09
EML139	2.79	2.57	2.24	2.05	2.14	1.93
EML101	4.91	4.34	4.41	4.12	4.26	3.87
EML152	1.76	1.58	1.44	0.77	1.32	0.67
EML164	2.38	2.16	1.88	1.76	_*	_*
EML167	1.56	1.37	0.97	0.78	0.72	0.65
EML160	1.09	0.84	1.42	1.37	1.41	1.27
EML155	1.25	1.17	0.98	0.85	0.81	0.75
EML175	2.91	2.69	2.39	2.15	2.21	2.01
EML146	3.47	3.29	3.05	2.91	2.84	2.74
EML159	2.54	2.41	2.21	2.05	1.93	1.85
EML129	1.89	1.74	_*	_*	_*	_*
EML124	1.91	1.74	1.45	1.12	1.36	0.99
EML135	3.91	3.74	3.74	3.42	3.54	3.31
EML156	2.36	2.16	2.32	2.01	2.19	1.97
I185	1.45	1.29	1.34	1.27	1.23	1.19
EML183	1.96	1.74	_*	_*	_*	_*
EML198	1.74	1.56	1.42	1.33	1.24	0.89
EML131	_*	_*	_*	_*	_*	_*
EML145	1.78	1.54	1.34	1.21	_*	_*
EML147	4.93	4.67	4.38	4.01	4.25	3.98
EML168	2.99	2.61	2.45	2.28	2.36	2.19
EML140	1.94	1.68	1.45	1.21	_*	_*
EML118	2.57	2.21	2.06	1.96	1.98	1.65
EML119	3.56	3.14	3.12	3.08	2.94	2.78
EML242	1.64	1.37	1.04	1.01	0.94	0.85
EML275	1.99	1.71	1.48	1.34	1.25	1.09
EML266	5.95	5.78	5.39	5.28	5.16	5.09
EML113	2.54	2.39	2.18	1.85	1.96	1.75
EML106	1.01	0.91	0.65	0.62	_*	_*
EML111	1.37	1.28	_*	_*	_*	_*