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Comparative analysis of physiological and anti-oxidative enzyme responses of maize genotypes under waterlogging stress and screening of tolerant genotypes

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Abstract

Waterlogging is one of the most concerning environmental challenges affecting crops worldwide. In this study, the anti-oxidative defense against oxidative stress under waterlogging condition was observed. Also, screening of tolerant genotypes surviving in the stress was conducted. Ten-days-old maize seedlings of six genotypes (CML 54×CML 487, CML 40, CML 54, CML486×CML487, CML487 and CML 486) were exposed to waterlogging stress (anoxia) in hydroponic condition. The physiological changes (chlorophyll and leaf canopy cover) and the anti-oxidative enzymes activities of the maize seedlings were observed in the 2nd, 4th and 6th day of waterlogging stress considering 0 day as control. The lowest reduction in canopy cover and chlorophyll content was observed in CML 54×CML 487 and CML 40, respectively. Additionally, cell damage and lipid peroxidation in root of CML 54 and CML 487 by histochemical staining suggested higher membrane damage by producing malondialdehyde due to oxidative stress. Also, highest catalase (CAT) activity was found in CML 40. In case of peroxidase (POD), the activity was highest in CML 54×CML 487. In addition, the highest ascorbate peroxidase (APX) and glutathione peroxidase (GPX) activity was observed in CML 40 and CML 487, respectively. The expression of isoenzymic activities in SDS-PAGE supported the results of the activities. Conclusively, the investigation revealed that, three genotypes (CML 54×CML 487, CML 40 and CML 487) have shown increased anti-oxidative enzyme activities and less physiological damage under waterlogging stress.

Keywords: Canopy cover; Glutathione peroxidase; Reactive oxygen species; Soil plant analysis development, Waterlogging.
Abbreviations: ROS_ reactive oxygen species, Chl_ chlorophyll, CAT_ catalase, POD_ peroxidase, APX_ ascorbate peroxidase, GPX_ glutathione peroxidase, SPAD_ soil plant analysis development.

Introduction

Almost every plant requires water and oxygen for their survival as well as normal growth and yield. However, excess water is injurious to plant, because, if a plant's root is submerged in excess water more than its requirement, the plant's root faces hypoxia (deficiency of O_2) /anoxia (absence of O_2) resulting in even death of the plant (Nishiuchi et al., 2012). Besides, waterlogging is one of the most serious abiotic stresses which is caused by excess precipitation, faulty irrigation, poor drainage, unpredicted rainfall, lack of properly labeled soil areas and so on. Waterlogging causes destruction of plants in various morphological, physiological, biochemical and anatomical as well as metabolic changes such as reduction of chlorophyll (chl) content, nitrogen deficiency, disruption of cell membrane and most importantly oxidative stress which ultimately cause the damage of protein, lipid, nucleic acid even DNA of plant cell (Bansal and Srivastava, 2011). However, plants possess different self-defense mechanisms to cope with the waterlogging stress condition for their survival. The most effective adaptive mechanism is the production of enzymatic (catalase, peroxidase, ascorbate peroxidase, glutathione peroxidase etc.) as well as non-enzymatic antioxidants (reduced glutathione, ascorbic acid, tocopherols, carotenoids etc.) that scavenges reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$) (Tian et al., 2019). For maize plants, anti-oxidative defense system due to waterlogging stress is an utmost need because they possess a highly up-regulated gene called *Rboh* (Respiratory Burst Oxygen Homolog) that is a major ROS producer under the waterlogging stress (Bailey-Serres et al., 2012; Petrov et al., 2015; Phukan et al., 2016; Rajhi et al., 2011). Different studies have also demonstrated the effect of waterlogging stress on various plant species including wheat (Cheng et al., 2015b), tomato (Horchani et al., 2010), citrus (Arbona et al., 2008), cotton (Christianson et al., 2010) etc. Hereby, we studied the effect of waterlogging stress on maize highlighting the chlorophyll content, SPAD reading, canopy cover measurement and ROS scavenging enzymatic effects. The aim of this study was to demonstrate the effects of waterlogging stress on the chlorophyll content as well as anti-oxidative enzymes with their isozyme analysis to

understand how they are being induced or affected by waterlogging stress in scavenging ROS. The comprehensive analysis of six maize genotypes in this aspect will also help to screen the waterlogging sensitive and tolerant genotypes so that farmers can be benefited.

Result

Canopy cover, soil plant analysis development (SPAD) value and photosynthetic pigments

In CML 54 × CML 487, canopy cover was decreased by 8%-23% with the progress of treatment of 2^{nd}, 4^{th} and 6^{th} days of waterlogging compared to the control (0 day of treatment) indicating the decrease of chlorophyll pigments due to oxygen deprivation in waterlogging stress. Accordingly, in CML 40, the decrease of canopy cover was 10%-43%; in CML 54, 14%-41%; in CML 486 × CML487, 5%-30%; in CML 487, 26%-47% and in CML 486, 26%-51%, respectively, compared to control (Table 1).

In case of SPAD, compared to control, the decrease of SPAD value in CML 54 × CML 487 was 12%-31%; in CML 40, 10%-30%; in CML 54, 8%-27%; in CML 486 × CML487, 25%-41%; in CML 487, 15%-28% and in CML 486 12%-48% as waterlogging progressed (Table 2). Therefore, the lowest SPAD values were obtained in CML 486. Chlorophyll *a* and *b* contents were decreased due to waterlogging stress. The lowest and highest reduction of Chl *a* content was found in CML 54 × CML 487 and CML 486, respectively, at 2^{nd}-6^{th} day of waterlogging stress compared to control. Whereas, the lowest and highest reduction of Chl *b* content was found in CML 54 and CML 486, respectively, compared to control with the increased stress duration (Table 3).

Lipid peroxidation and membrane damage

The oxidative stress-induced lipid membrane damage was identified by intense pink-red color by histochemical staining of the roots using Schiff's reagent. CML 54 and CML 487 showed the most intensive pink-red color (Fig 1) among the six genotypes. Plasma membrane disintegration was also identified by staining the roots with Evan's blue dye showing intense dark blue color. CML 54, CML486 × CML487 and CML 487 showed the most intensive blue color (Fig 2).

Activity of Antioxidant Enzymes

Catalase (CAT) enzyme activity

In waterlogging, the CAT activity increased by 24% and 18% in CML 54 × CML 487 at 2^{nd} and 6^{th} day, respectively, compared to control, but slightly decreased at 4^{th} day by 16% (Fig. 3A). In CML 40, the CAT activity increased significantly by 10%, 16% and 24% at 2^{nd}, 4^{th} and 6^{th} day, respectively. In CML 54, the CAT activity gradually increased by 2%-32% with the progress of stress duration. On the other hand, in CML486 × CML487, the activity increased 7% and 15% at 2^{nd} and 6^{th} day, but decreased at 4^{th} day by 16%. In CML 487, the activity increased significantly by 13%, 48% and 56% at 2^{nd}, $4^{th}$and 6^{th} day of treatment. In case of CML 486, the value increased 38% and 35% at 4^{th} and 6^{th} day with the slight decrease at 2^{nd} day by 4%. The intensification in CAT isozymes also revealed similar result (Fig. 3B).

Peroxidase (POD) enzyme activity

Waterlogging condition had increased result of POD activity in some genotypes while some had decreased values (Fig. 4A). CML 54 × CML 487 showed increased POD activity by 31%-36% of POD at 2^{nd}-6^{th} day compared to control. In CML 40, the value increased 9% and 19% at 2^{nd} and 4^{th} day, respectively, but the value had decreased by 10% at 6^{th} day. In CML 54, the activity increased by 20% at day 2 and then it gradually decreased by 10% and 9%, respectively, at 4^{th} and 6^{th} day. In CML486 × CML487, 45% and 63% increased activities were obtained at 2^{nd} and 4^{th} day, respectively, while it decreased at 6^{th} day by 42%. In CML 487, the activity increased by 38% at 2^{nd} day, but gradually decreased by 30% and 28% at 4^{th} and 6^{th} day, respectively. In CML 486, the activity significantly increased by 19%-35% with the increase of stress duration. The in-gel activity also supported the specific activity (Fig. 4B).

Ascorbate peroxidase (APX) enzyme activity

The APX activity of CML 54 × CML 487 was increased by waterlogging by 3%-5% at 2^{nd}-6^{th} day compared to control (Fig. 5A). In CML 40, the activity increased 6%, 2% and 6% at 2^{nd}, 4^{th} and 6^{th} day, respectively. In case of CML 54, the activity increased 26% at 2^{nd} day then it decreased by 5% and 8% at 4^{th} and 6^{th} day, respectively. In CML486 × CML487, the activity increased 2% and 40% at 2^{nd} and 4^{th} day of treatment, respectively, but decreased thereafter. In CML 487, the value increased by 14% at 2^{nd} day, but decreased with stress duration. Similar activity pattern was observed in CML 486. Similar changes were also found in in-gel activity (Fig. 5B).

Glutathione peroxidase (GPX) enzyme activity

As compared to control, in CML 54 × CML 487, the GPX activity increased by waterlogging at 2^{nd} day by 20%, but the activity decreased at 4^{th} and 6^{th} day by 19% and 18%, respectively (Fig. 6A). In CML 40, the activity increased by 1%-12% with stress progress. In case of CML 54, the activity increased by 1%, 20% and 19% at 2^{nd}, 4^{th} and 6^{th} day, respectively, while the activity increased in CML486 × CML487 by 11%-30% and in CML 486 by 24%-28% depending on stress duration. In case of CML 487, the activity increased by 17% and 7% at 2^{nd} and 6^{th} day, respectively, but decreased 3% at 4^{th} day of treatment. The in-gel activity also indicated similar trend in changing GPX activity (Fig. 6B)

Discussion

It is well-known that waterlogging causes reduced uptake of essential nutrients resulting the reduction of leaf canopy cover and reduced photosynthesis followed by reduced chlorophyll content (Hasanuzzaman et al., 2017). In our study, leaf canopy cover reduced in almost all the genotypes in waterlogged condition (Table 1). Reduction of SPAD value and chlorophyll content decreased significantly in a time-dependent manner compared to the normal condition (Table 2 and 3). These reductions may be resulted due to the plants being unable to uptake nitrogen, subsequent decline in photosynthetic enzymes, and disruption of fine structure of chloroplast or efficiency damage of photosystem II (Ashraf, 2012; Rohman et al., 2016). These results are corroborated with the findings in maize (Wang et al., 2011) and in pigeon pea genotypes (Bansal and Srivastava, 2015).

Maize roots are the first to be damaged during waterlogging. Overproduction of ROS under stress causes lipid peroxidation, protein oxidation, enzyme inhibition and eventually leads to cell death (Gill and Tuteja, 2010). The H_2O_2 content was increased when seedlings were imposed to stress because of the production of ROS and resulted

Table 1. Changes of canopy cover under waterlogging stress at 0, 2nd, 4th and 6th day of stress treatment. Values represent the mean ± SE from three independent experiments. Different letters within a column are statistically significant at P≤0.05.

Genotype	Treatment duration			
	Day 0	Day 2	Day 4	Day 6
CML54 × CML 487	0.53±0.00^{a}	0.49±0.01^{ab}	0.44±0.00^{a-d}	0.39±0.03^{b-e}
CML 40	0.40±0.06^{b-e}	0.37±0.04^{c-f}	0.30±0.02^{e-h}	0.24±0.01^{gh}
CML54	0.37±0.02^{c-f}	0.31±0.02^{e-h}	0.29±0.01^{e-h}	0.22±0.01^{h}
CML 486 × CML 487	0.39±0.01^{b-e}	0.37±0.02^{c-f}	0.33±0.01^{d-h}	0.27±0.01^{f-h}
CML 487	0.46±0.02^{a-c}	0.34±0.02^{d-g}	0.31±0.02^{e-h}	0.24±0.01^{gh}
CML 486	0.49±0.01^{ab}	0.36±0.03^{c-f}	0.32±0.03^{e-h}	0.24±0.00^{gh}

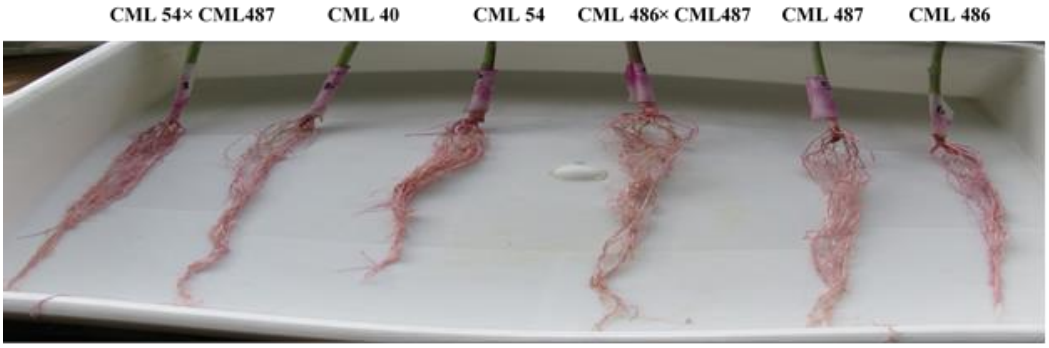

Fig 1. Histochemical detection of malondialdehyde (MDA) of roots of maize seedling

Table 2. SPAD readingunderwaterloggingstressconditionat0, 2nd, 4th and 6th day of stress treatment. Values represent the mean ± SE from three independent experiments. Different letters within a column are statistically significant at P≤0.05.

Genotypes	Treatment Duration			
	Day 0	Day 2	Day 4	Day 6
CML 54 × CML487	32.60±0.58^{b-e}	28.69±0.31^{d-g}	25.85±0.20^{f-i}	22.30±0.98^{hi}
CML 40	40.18±0.71^{a}	36.53±0.59^{ab}	34.33±1.37^{bc}	27.53±0.25^{e-h}
CML 54	36.55±0.46^{ab}	34.15± 0.20^{b-d}	29.98±0.25^{c-g}	26.08±1.11^{f-i}
CML 486 × CML 487	36.47±3.07^{ab}	27.45±0.78^{e-h}	25.30±0.20^{g-i}	21.88±0.91^{i}
CML 487	32.10±0.12^{b-e}	27.75±0.43^{e-h}	27.43±0.36^{e-h}	23.03±1.95^{hi}
CML 486	31.10±0.84^{b-f}	27.43±0.51^{e-h}	25.48±0.13^{g-i}	15.83±1.72^{j}

Fig 2. Histochemical detection of cell damage of maize roots using Evan's blue

Table 3. Changes of Chl *a* and *b* contents during different levels of waterlogging stress condition. day of stress treatment. Values represent the mean ± SE from three independent experiments. Different letters within a column are statistically significant at P≤0.05.

Genotypes	Chl *a*			
	Day 0	Day 2	Day 4	Day 6
CML 54 × CML 487	0.36±0.11^{a-c}	0.24±0.07^{a-c}	0.14±0.05^{bc}	0.08±0.05^{c}
CML 40	0.50±0.03^{a}	0.43±0.01^{ab}	0.33±0.03^{a-c}	0.22±0.06^{a-c}
CML 54	0.22±0.05^{c}	0.15±0.04^{bc}	0.09±0.03^{c}	0.07±0.03^{c}
CML 486 ×CML 487	0.25±0.08^{a-c}	0.17±0.08^{bc}	0.14±0.08^{b}	0.07±0.07^{a}
CML 487	0.26±0.04^{b}	0.20±0.01^{ab}	0.15±0.01^{a}	0.10±0.03^{b}
CML 486	0.42±0.03^{ab}	0.33±0.06^{a-c}	0.25±0.09^{a-c}	0.09±0.03^{c}
Genotypes	**Chl *b***			
	Day 0	**Day 2**	**Day 4**	**Day 6**
CML 54 × CML 487	0.25±0.04^{a-d}	0.19±0.04^{b-d}	0.15±0.03^{cd}	0.09±0.03^{d}
CML 40	0.49±0.13^{a-c}	0.42±0.16^{a-d}	0.28±0.13^{a-d}	0.18±0.08^{b-d}
CML 54	0.24±0.06^{a-d}	0.17±0.03^{b-d}	0.14±0.03^{cd}	0.09±0.03^{d}
CML 486 × CML 487	0.60±0.07^{a}	0.53±0.06^{ab}	0.37±0.04^{a-d}	0.21±0.03^{b-d}
CML 487	0.37±0.07^{a-d}	0.29±0.09^{a-d}	0.19±0.05^{b-d}	0.11±0.06^{d}
CML 486	0.43±0.03^{a-d}	0.34±0.05^{a-d}	0.23±0.06^{a-d}	0.13±0.01^{cd}

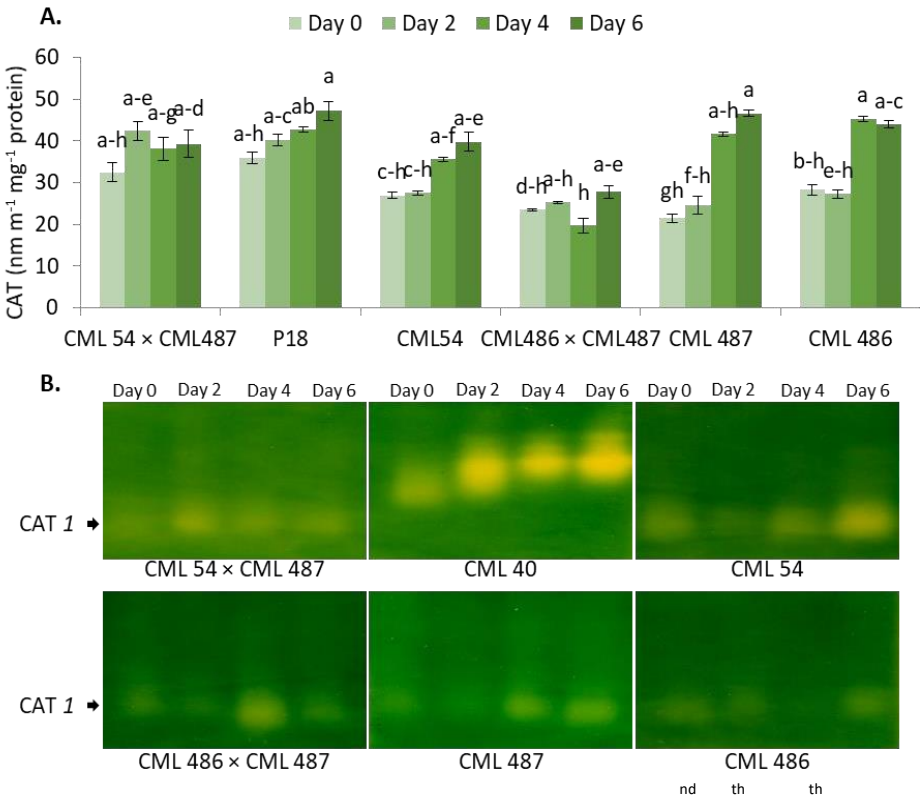

Fig. 3 A. CAT enzyme activity of 6 maize genotypes under waterlogging stress in 0, 2nd, 4th and 6th day. **B.** CAT isozyme in gel staining. Values represent the mean ± SE from three independent experiments. Bars with same letters are not significantly different at P≤0.05.

increased lipid peroxidation and cell membrane damage. Root staining with Schiff's reagent showed concentrated pink red color in CML 54 and CML 487 genotype compared to other genotypes suggesting increased malondialdehyde content resulting serious lipid membrane damage. Root staining with Evan's blue showed intensive blue color in CML 54, CML 486 × CML 487 and CML 487 suggesting more cell membrane damage compared to other genotypes in waterlogging stress. The extent of damage is negatively correlated with the synthesis of anti-oxidative enzymes and positively correlated with the synthesis of ROS. These findings are supported by previous studies in maize (Hasanuzzaman et al., 2017; Jaiswal and Srivasta, 2016; Tang et al., 2010). The lipid peroxidation and cell membrane damage indicated increased electrolytes leakage and ROS production (especially, H_2O_2). Immediately after waterlogging an increased CAT and POD activity was found with efficient detoxification of H_2O_2 conferring waterlogging resistance in maize. The role of APX lies in converting H_2O_2 into water along with the regeneration of $NADP^+$ that suggests ROS scavenging activities (Bansal and Srivastava, 2015; Tian et al., 2019). Most of the genotypes of this study showed an increased activity of CAT in waterlogging stress except two hybrids CML 54 × CML 487 and CML 486 × CML 487 (Fig 3A).

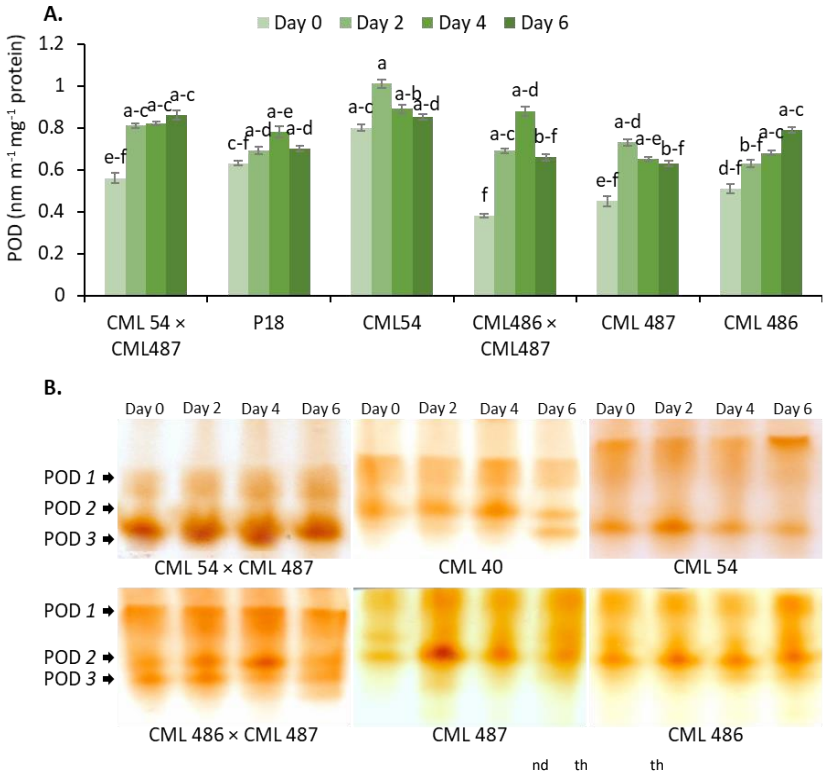

Fig 4 A. POD activity of 6 maize genotypes under waterlogging stress at 0, 2nd, 4th and 6th day. **B.** POD isozymes in gel staining. Values represent the mean ± SE from three independent experiments. Bars with same letters are not significantly different at P≤0.05.

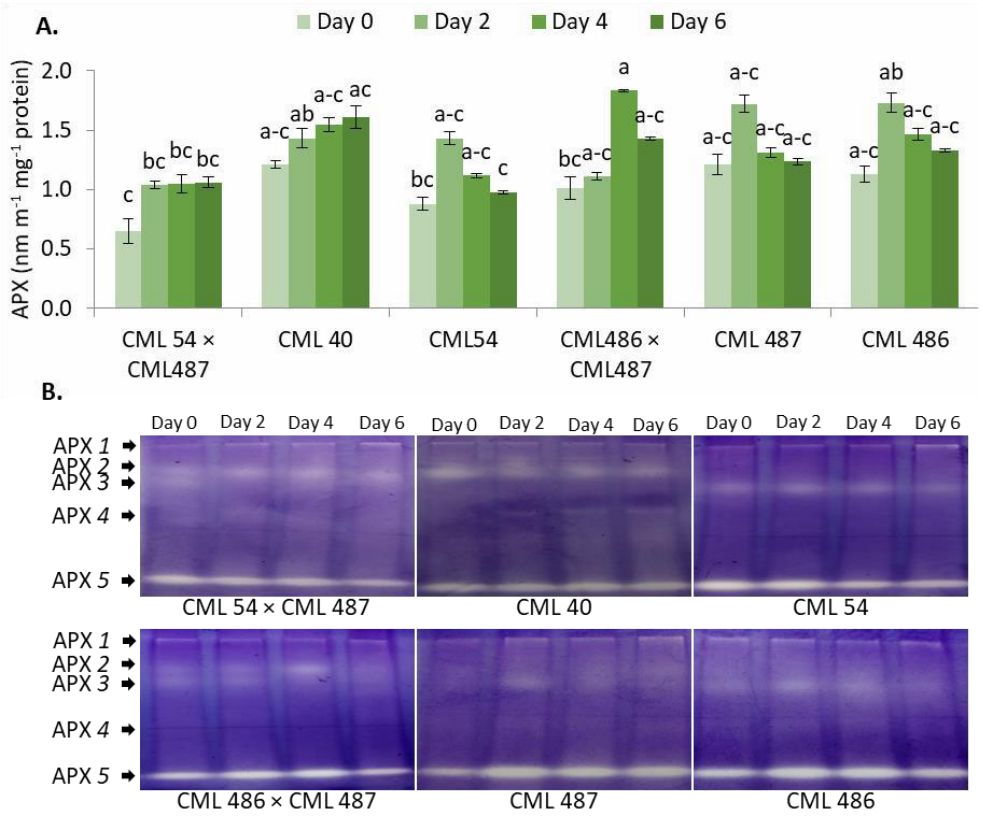

Fig 5. A. APX activity of 6 maize genotypes under waterlogging stress in 0, 2nd, 4th and 6th day. **B.** APX isozymes in gel staining. Values represent the mean ± SE from three independent experiments. Bars with same letters are not significantly different at P≤0.05.

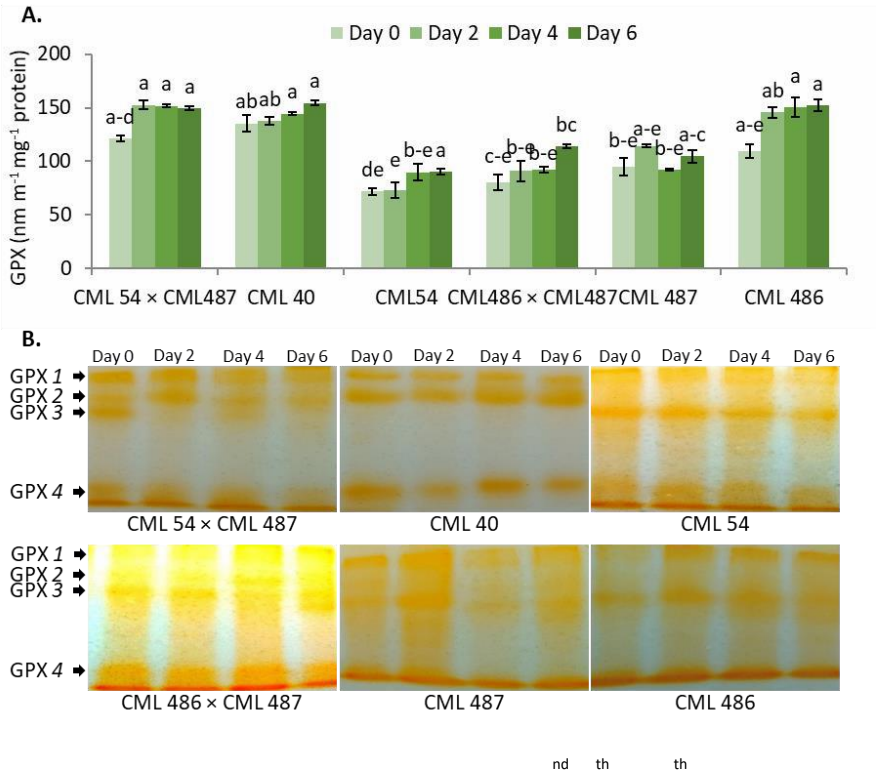

Fig. 6 A. GPX activity of 6 maize genotypes under waterlogging stress in 0, 2nd, 4th and 6th day. **B.** GPX isozymes in gel staining. Values represent the mean ± SE from three independent experiments. Bars with same letters are not significantly different at P≤0.05.

In these two genotypes, the CAT activity slightly decreased at 4th day and again increased at 6th day. These results are consistent with the previous findings in barley (Mubeen et al., 2017; Phukan et al., 2016; Sairam et al., 2009; Zhang et al., 2007) and in maize (Tian et al., 2019). In in-gel activity, one isoform of CAT was found in all the six genotypes that supported the graphical representation (Fig 3B). Also, the POD activity of the maize seedlings showed higher activity during stressed condition in CML 54 × CML 487 and CML 486 compared to the rest of the genotypes while in other genotypes, there was irregular up and down-regulation of the activity (Fig 4A), although the POD activity increased immediately after inducing waterlogging stress in all the genotypes compared to control. Similar result was found in maize (Tang et al., 2010). In gel staining, three isoforms of POD were found in support of the findings (Fig 4B). Moreover, APX activity was increased in CML 54 × CML 487, CML 40 and in CML 486 × CML 487 conferring tolerance in adverse effect of H_2O_2 under waterlogging. However, in case of CML 54, CML 486 and CML 487, the APX activity was increased only at 2nd day which decreased thereafter, but the activity remained more that the control in these three genotypes (Fig. 5A). The underlying reason in this case is genotype dependent but, the higher activity compared to control have important role in H_2O_2 metabolism under waterlogging. The increased APX activity with increased stress has also been found in pigeon pea (Sairam et al., 2009) and in maize (Chugh et al., 2011; Sairam et al., 2009). In gel staining, five isoforms of APX were found which supported the result (Fig 5B). Glutathione peroxidase is another enzymes with important role in H_2O_2 metabolism (Gill and Tujeta, 2010). In this study, we determined the activity of GPX in maize seedlings under waterlogging stress (Fig 6). Previous studies are hardly found to study the GPX activity under waterlogging stress in maize. The binding of different xenobiotic and their electrophilic metabolites are known to produce less toxic and water-soluble conjugates to protect plants from oxidative stress which is aided by the enzymes GPX and GST together (Gill and Tuteja, 2010). In this study, we found the increased activity of GPX in almost all the genotypes except CML 487 (Fig 6A). Those genotypes with higher GPX activity had better H_2O_2 scavenging capacity in waterlogging condition than the genotype with lower GPX activity. Similar results are found in sesame (Anee et al., 2019). In gel activity, three isoforms of GPX has been found in all six genotypes that helped to confirm the result (Fig 6B). The whole discussion collectively suggests that among the six genotypes, CML 54 × CML 487, CML 40 and CML 486 showed the better ROS metabolism capacity during the period of oxygen deprivation and an increased survival rate. Other three genotypes (CML 54, CML 487 and CML 486 × CML 487) showed less effective result collectively against ROS, because in some cases, there was irregular up and down-regulation of the ROS scavenging enzymatic activities. However, the physiological explanation of the decreased enzyme activity may be that the antioxidant enzymes are more effective at the early stage of waterlogging treatment. In the long term of the stress, the genotypes become less effective to take part in scavenging the ROS elements. SDS-PAGE was performed to confirm the results through band intesity of isoforms found in the gels. The higher intense isoforms indicates higher anti-oxidative enzyme activity resulting enhanced ROS scavanging following reduced lipid peroxidation and membrane damage as well.

Materials and Methods

Plant materials and stress treatment

Six maize genotypes, CML 54 × CML 487, CML 40, CML 54, CML486 × CML 487, CML 487 and CML 486, collected from

the Plant Breeding Division of Bangladesh Agricultural Research Institute (BARI) were selected for the study of physiological (chlorophyll content) and anti-oxidative enzyme activity against ROS metabolism under waterlogging stress. The study was started in the glass house where the seeds of genotype of interest were sown in the winter season (Rabi) and the analyses were performed at Molecular Breeding Laboratory of BARI. Seeds were sown on a mixture of coarse sand and rock grindings in trays which were previously autoclaved and kept in glass house at 22°C for germination. After 6 days, seeds were germinated uniformly. Then 8-day-old young seedlings were transferred to hydroponic condition using Hoagland solution (pH 6.2) and kept for two days for hardening. Since waterlogging causes oxygen deprivation to the plants (hypoxia/anoxia), the treatment imposed to the seedlings was the absence of oxygen (anoxia). During this period, the seedlings were maintained in the glass house at 22°C and 12 hours of light. The leaf samples were collected at 0 (10^{th} day of transplanting), 2^{nd} (12^{th}), 4^{th} (14^{th}) and 6^{th} (16^{th}) day of stress implementation for chlorophyll and anti-oxidative enzymes analysis in the laboratory.

Determination of canopy cover, SPAD value, chl content and membrane damage

The N_2 status was measured by measuring the canopy cover with the aid of Green seeker (Handheld Crop Sensor, Trimble) (Hunt et al., 2013; Rambo et al., 2010). The SPAD readings were captured by using a SPAD meter (SPAD-502 plus, Konica Minolta) following the method of (Widjaja Putra and Soni, 2017) and chlorophyll was measured spectrophotometrically (UV-1800, Shimadzu, Japan). Chl *a* and *b* were determined by measuring the absorbance of the homogenized leaf samples in 80% acetone with the spectrophotometer at 663, 645 and 470 nm (Lichtenthaler, 1987) and calculated using the equations proposed by (Arnon, 1949).

Lipid peroxidation in the roots was determined by histochemical staining using Schiff's reagent with slight modification of (Srivastava et al., 2014). The loss of plasma membrane integrity in the roots was measured by histochemical staining using Evan's blue solution with slight modification of (Schützendübel et al., 2001).

Protein extraction, quantification and gel electrophoresis

Protein in the crude extract was determined following the Coomassie Brilliant Blue (G-250) dye binding method (Bradford, 1976). The absorbance was recorded at 595 nm. Protein concentration was calculated with the help of standard curve using BSA (Bovine Serum Albumin).

Enzyme extract and determination of enzymatic activities

For the extraction of enzymes protocol of (Rohman et al., 2017) was followed and assay activities of catalase (CAT, EC 1.11.1.6), peroxidase (POD, EC: 1.11.1.7), ascorbate peroxidase (APX, EC: 1.11.1.11) and glutathione peroxidase (GPX, EC: 1.11.1.9) were done as per procedures described in Rahman et al. (2019).

SDS-PAGE and activity staining

The comprehensive methods stated by (Islam et al., 2020) have been used to visualize the activities of CAT, POD, APX and GPX enzymes using SDS-PAGE (8% gel).

Statistical Analysis

Data generated from this study were analyzed using STATISTIX software (version 10.0) where needed. Data were analyzed following completely randomized design with three replications. Means were separated by Duncan's Multiple Range Test and $P \leq 0.05$ was considered to be the significance level. The graphs were prepared in MS Excel, 2010. Mean values ± standard errors (SEs) were presented in tables and graphs from three independent experiments.

Conclusion

It can be concluded from the study that, waterlogging tolerant maize seedlings possess the higher ROS scavanging activities due to higher activities of anti-oxidative enzymes. In this study, CML 54 × CML 487, CML 40 and CML 486 genotypes are noted to be the most effective genotypes under waterlogging stress condition as they showed less reduction of chlorophyll content and high activity of anti-oxidative enzymes under waterlogging condition while CML 54, CML486 × CML 487 and CML 487 were less effective under the streesed condition. So, these higher ROS scavanging genotypes under waterlogging stress may be the target for the farmers in this era of climatic change and disaster. The study on the canopy cover and GPX anti-oxidative enzyme activity in maize seedlings under waterlogging stress is hardly available. So, further investigation is needed to examine the effect of canopy cover and GPX enzyme on maize genotypes under waterlogging stress condition.

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