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RESPONSES OF SEVEN MAIZE GENOTYPES DURING FLOODING STRESS AND IDENTIFICATION OF CULTIVARS MOST TOLERANT TO FLOODING CONDITIONS

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Abstract

Among the various environmental stresses, flooding stress is one of the major challenges crop plants have to face all across the world which negatively effects the growth, development and productivity of the plant. Seven maize (Zea mays) genotypes- Bharat Nath 101 (BN-101), Bharat Nath 1133 (BN-1133), Dhanya 849 (DMH-849), Swarna, Pioneer 30V92, Kaveri Super 244(KS-244) and Kaveri Super 244+ (KS-244+) were subjected to flooding stress in pot culture. They were grown in the earthen pots and were subjected to flooding stress by holding water in the pots. The test plants were then sampled on the 3rd, 5th and 7th day along with the control plants which were sampled on the 0-day of stress. Antioxidative enzyme activity like POX (peroxidise), APOX (ascorbate peroxidise), CAT (catalase), SOD (superoxide dismutase) and GR (glutathione reductase) showed an increasing trend in the activity in the initial days of flooding stress but gradually with prolonged stress the activity decreased in all the test cultivars. The total antioxidant activity in the leaves of the test plants was determined with respect to percent of DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) inhibition. In flooding stress KS 244, KS 244+, 30V92 and BN 101 increased during the period of stress but showed a slight decrease in the DPPH inhibition on the 7th day of stress. The other cultivars showed a significant decrease in the DPPH inhibition during the period of. Lipid peroxidation increased during the period of stress in 30V92, BN 1133, Swarna and Dhanya but the increase was very insignificant in KS 244+ and BN10.1Cell membrane injury in the leaves of seven cultivars of maize during flooding stress showed very less electrolyte leakage in KS 244, KS 244+ and BN 101 indicating that these cultivars are more tolerant to flooding stress than the rest of the cultivars. . Flooding stress also led to a significant increase in chlorophyll breakdown in all seven cultivars of test plant. Maximum decrease during flooding stress was observed in Dhanya, Swarna and BN 1133.Thus results suggest that KS 244, KS 244+ and BN 101 are the tolerant cultivars and 30V92 BN 1133, Swarna and Dhanya are the susceptible cultivars of flooding stress.
Keywords : Maize, Flooding, Antioxidative defense, Lipid peroxidation, Electrolyte leakage.

Introduction

Environmental conditions that reduce growth and yield below optimum levels are defined as abiotic stress by (Skirycz & Inzé, 2010; Kumar and Janeja, 2018; Sharma *et al*., 2019; Singh *et al*., 2020) (CRAMER, 2010). Among them, flooding stress is a wide spread phenomenon (Voesenek, Colmer, Pierik, Millenaar, & Peeters, 2006; Saini *et al*., 2014). This occurs when the soil water content of surface layer exceeds at least 20% higher than field's carrying capacity (Voesenek *et al*., 2006; Sudhakar *et al*., 2015; Vyas 2017; 2019).

Maize is one of the important cereals cultivated in the world. Maize plants are not able to endure low oxygen availability in the rhizosphere, caused by flooding, resulting in substantial losses in productivity (Singh *et al*., 2016; 2019; Wailare and Kesarwani, 2017). In South Asia, 15% of maize acreage is affected by flooding, whereas in India, flooding has already affected 8.5 million hectares of maize cultivation (Pervez Haider Zaidi, P. Maniselvan, P. J. Yadav, Amit Kumar Singh, Rafat Sultana, Prem Dureja, Rajan Pratap Singh, 2007).

Water logging causes a condition of hypoxia (low oxygen concentrations) in soil, because of the low solubility of oxygen in water (Barrett-Lennard, 2003; Kumar and Kumar, 2019; Sharma *et al*., 2019). Excessive generation of reactive oxygen species (ROS) i.e. under oxidative stress is an integral part of many stress situations, including hypoxia (BLOKHINA, 2003; Malik *et al*., 2013; Jassal *et al*., 2016). When ROS level exceeds the capacity of the plant to scavenge, lipid peroxidation in biological membranes increases, thereby effecting the physiological processes of the cell. Malondialdehyde (MDA) is one of the final products of oxidative modifications of lipids, and is responsible for cell membrane damage (Sharma, Jha, Dubey, & Pessarakli, 2012; Kumar and Kumar, 2018; Kaith and Bakshi, 2018). Increase in the rate of O2−. and H_2O_2 generation under soil hypoxia was shown in barley and maize leaves to be correlated with intensification of LPO (Sowa, Duff, Guy, & Hill, 1998; Singh *et al*., 2016; 2019). Protection of plants from oxidative damage is associated with functioning of low molecular antioxidants (carotenoids, ascorbic acid, glutathione etc.) and antioxidant enzymes such as SOD, catalase, the enzymes of ascorbate-glutathione cycle and other pathways neutralizing ROS (Kumutha *et al*., 2009; Prabhakar *et al*., 2013; 2014; 2020) reported increase in the activity of antioxidant enzymes during water logging is Pigeonpea plants. (Yiu, Liu, Yi-Tan Fang, & Lai, 2009; Nankar *et al*., 2017; Sharma *et al*., 2019) reported a rapid increase in MDA content in flooded plants of (*Allium fistulosum* L.). In flooding experiments performed by Hetherington and Stewart (1991) in eight winter barely cultivars the most sensitive cultivar (Maris Otter) showed maximum electrolyte leakage during flooding stress. Water stress causes stomatal closure, which reduces the CO_2/O_2 ratio in leaves and inhibits photosynthesis (Lawlor, 2002; Mishra, 2019a, 2019b). Chlorophyll is one of the major chloroplast components for photosynthesis, and relative chlorophyll content has a positive relationship with photosynthetic rate. The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation (Kuamr *et al*., 2018; Kumar *et al*., 2019; Kaur *et al*., 2020) . Photosynthetic pigments are important to plants

mainly for harvesting light and production of reducing powers. Both the chlorophyll a and b are prone to soil dehydration (Farooq, Wahid, Kobayashi, Fujita, & Basra, 2009Farooq 2019a, 2019b). In experiments performed by (González, Gallardo, Hilal, Rosa, & Prado, 2009; Chauhan *et al*., 2017), on Quinoa (*Chenopodium quinoa* Willd.) total chlorophyll and chlorophyll a and b content was lower in plants under water logged condition.

Materials and Methods

Plant material: Seven cultivars of maize Bharat Nath 101 (BN-101), Bharat Nath 1133 (BN-1133), Dhanya 849 (DMH-849), Swarna, Pioneer 30V92, Kaveri Super 244(KS-244) and Kaveri Super 244+ (KS-244+) were used for the experiments. The seeds were soaked overnight and surface sterilized with 0.1% $HgCl_2$. Next day seeds were transferred to autoclaved petriplates in laminar air flow. The seeds were allowed to germinate in the petriplates. Then the young seedlings were transferred in pots and were grown at a temperature range of 30-34^{0}C, RH 65-70% and 16 h photoperiod.

Extraction and assay of enzyme activities

Preparation of enzyme extract: The leaves collected from treated and control plants were ground to fine powder with a mortar and pestle under liquid nitrogen in cold 50 mmol sodium phosphate buffer, pH 7.5, containing 1% (w/v) polyvinylpolypyrrolidone. The homogenate was then centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was directly used as crude extract for enzyme assays

Assay of activities

Peroxidase (POX: EC. 1.11.17): Peroxidase activity was assayed spectrophotometrically in UV VIS spectrophotometer (Model 118 SYSTRONICS) at 460 nm by monitoring the oxidation of O-dianisidine in presence of H_2O_2 (González *et al*., 2009). Specific activity was expressed mmol 0-dianisidine oxidised mg protein^{-1} min-^{1}.

Ascorbate peroxidase (APOX: EC.1.11.1.11): Activity was assayed as decrease in absorbance by monitoring the oxidation of ascorbate at 290 nm as described by the method of (Asada, 2006) with some modification. Enzyme activity was mmol ascorbate oxidised mg protein^{-1} min^{-1}.

Catalase (CAT: EC.1.11.1.6): Catalase activity was assayed by estimating the breakdown of H_2O_2 which was measured at 240 nm in a spectrophotometer (MAEHLY & CHANCE, 1954). The enzyme activity was expressed as µmol H_2O_2 degraded mg protein $^{-1}$ min^{-1}.

Superoxide dismutase (SOD: EC 1.15.1.1): SOD activity was assayed by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT). The absorbance of samples was measured at 560nm and 1 unit of activity was defined as the amount of enzyme required to inhibit 50% of the NBT reduction rate in the controls containing no enzymes given by (DHINDSA, PLUMB-DHINDSA, & THORPE, 1981). The enzyme activity was expressed as EU mg protein^{-1}.

Glutathione reductase (GR: EC 1.6.4.2): Glutathione reductase activity was determined by the oxidation of NADPH at 340 nm as described by (Lee & Lee, 2000). Enzyme activity was expressed as µmol NADPH oxidized mg protein^{-1} min^{-1}.

Estimation of total antioxidant activity: The estimation of total antioxidant activity was done by the method of (Blois, 1958). Initial absorbance was taken in 0-min (AT_0) and then the reaction mixture was kept in dark and after 30-min(AT_{30})another absorbance was taken at 517snm in UV-VIS spectrophotometer (Model 118 systronics).The inhibition percentage of the absorbance of DPPH solution was calculated using the following equation:

$$\text{Inhibition } (\%) = (AT_0\text{-}AT_{30})/AT_0) \times 100.$$

Electrolyte leakage (%): Electrolyte leakage was measured by the process given by (LUTTS, 1996) with few modifications. Electrolytic leakage was recorded in a conductivity meter (Labindia) with K=0.946, cell constant=1, solution condition=84 µS, coefficient-1 at 25°C.

The EL was defined as follows:

$$EL\ (\%) = (L1/L2) \times 100$$

Lipid peroxidation (µM MDA/ g tissue): Lipid peroxidation was measured as MDA determined by the thiobarbituric acid (TBA) reaction. Cells (0.25 g) were homogenized in 2 ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm for 10 min. To 0.5 ml of the aliquot of the supernatant, 2ml of 20% TCA containing 0.5% (w/v) TBA were added. The mixture was heated at 95 °C for 30 min and then quickly cooled on ice. The absorbance was measured at 532 nm and 600. The concentration of MDA was calculated using an extinction coefficient of 155 mmol^{-1} as given by (Heath & Packer, 1968).

Chlorophyll content: Chlorophyll was estimated according to the method of (Harborne, 1984) by homogenizing 0.250g of leaf tissue in 80% acetone and filtered through Whatman No.1 filter paper. 80% acetone was repeatedly added from the top till the residue became completely colourless. Then the filtrate was collected and total volume was measured. Estimation of chlorophyll was done by measuring the OD at 663nm and 645nm respectively in a UV-VIS spectrophotometer (UV-VIS Spectrophotometer 118 systronics) against a blank of 80% acetone and calculated using the formula as given by (Arnon, 1949).

Results and Discussion

Antioxidative enzyme activities: During flooding stress antioxidative enzymes like POX, CAT, APOX, SOD and GR showed different trends in their activity in the leaves of different test cultivars (Table 1). POX activity showed an increasing trend in the activity in the initial days of flooding stress. Similar reports were given by (Yordanova, Christov, & Popova, 2004) in barely where POX activity significantly increased and 120 h after flooding. But gradually with prolonged stress all activity decreased in all the test cultivars of maize. In KS 244, KS 244+ and BN 101 the activity decreased on the 7th day of stress and in BN1133, 30V92, Swarna and Dhanya the activity decreased from the 5th day of stress.CAT activity showed a gradual, significant increase in KS 244, KS 244+ and BN 101.But in BN 1133, 30V92, Swarna and Dhanya the catalase activity decreased during the period of stress. APOX activity and SOD activity in BN 101, KS 244, KS 244+ showed an increase in activity till the 5th day of flooding stress but a decrease in the activity was seen on the 7th day of stress, a different trend was seen in case of BN 1133, Swarna, Dhanya and 30V92 where the APOX activity showed an initial increase and then decreased in activity after the 3rd day stress. The GR activity in KS 244, KS 244+ and BN 101 showed a significant increase in

activity during the period of flooding stress. But like APOX and SOD, the GR activity showed same trend in BN 1133, Swarna, Dhanya and 30V92 during flooding stress. (Arbona, Hossain, López-Climent, Pérez-Clemente, & Gómez-Cadenas, 2008) also reported in their experiments performed on citrus during water logged condition there was an enhanced activated oxygen species' scavenging capacity in terms of an increased antioxidant enzyme activity and higher content in polar antioxidant compounds. Studies done by (Kumutha *et al*., 2009) also showed an increase in the antioxidative enzymes during flooding stress in of pigeonpea (Cajanus cajan L. Halls).

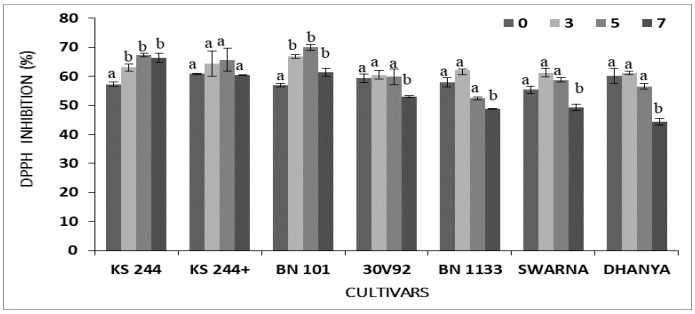
Table 1: Antioxidative enzyme activity in the leaves of seven cultivars of maize during flooding stress.

Cultivars	No. of Days	POX	CAT	APOX	GR	SOD
KS244	0	1.14±0.046[a]	0.80±0.076[a]	0.61±0.040[a]	0.19±0.020[a]	0.44±0.004[a]
	3	2.49±0.080[b]	1.03±0.094[a]	0.76±0.017[a]	0.21±0.015[a]	0.68±0.006[b]
	5	4.60±0.014[b]	1.20±0.029[b]	1.04±0.015[b]	0.30±0.012[b]	0.83±0.001[b]
	7	2.79±0.029[b]	1.25±0.020[b]	0.99±0.032[b]	0.35±0.015[b]	0.66±0.006[b]
KS244+	0	1.23±0.106[a]	0.85±0.020[a]	0.60±0.076[a]	0.20±0.004[a]	0.48±0.007[a]
	3	2.33±0.101[b]	0.95±0.086[a]	0.68±0.028[a]	0.21±0.008[a]	0.71±0.003[b]
	5	3.95±0.155[b]	1.12±0.007[b]	0.91±0.036[b]	0.30±0.012[b]	0.86±0.002[b]
	7	2.60±0.369[b]	1.19±0.023[b]	0.83±0.034[b]	0.32±0.010[b]	0.62±0.004[b]
BN 101	0	1.00±0.165[a]	0.75±0.012[a]	0.61±0.025[a]	0.18±0.004[a]	0.56±0.007[a]
	3	2.96±0.009[b]	0.84±0.018[b]	0.82±0.009[b]	0.23±0.006[b]	0.78±0.003[b]
	5	3.69±0.036[b]	0.90±0.039[b]	1.07±0.070[b]	0.33±0.013[b]	0.86±0.001[b]
	7	2.48±0.046[b]	1.06±0.018[b]	0.86±0.033[b]	0.33±0.011[b]	0.62±0.003[b]
30V92	0	0.82±0.017[a]	0.69±0.046[a]	0.57±0.052[a]	0.20±0.028[a]	0.42±0.068[a]
	3	2.69±0.125[b]	0.66±0.012[a]	0.63±0.025[a]	0.25±0.004[a]	0.70±0.004[b]
	5	2.13±0.059[b]	0.57±0.072[a]	0.52±0.021[a]	0.20±0.005[a]	0.68±0.005[b]
	7	1.17±0.029[b]	0.35±0.021[b]	0. 35±0.021[b]	0.18±0.018[a]	0.33±0.031[b]
BN1133	0	0.94±0.024[a]	0.55±0.055[a]	0.55±0.027[a]	0.15±0.022[a]	0.34±0.015[a]
	3	2.27±0.109[b]	0.48±0.024[a]	0.68±0.036[a]	0.22±0.007[a]	0.49±0.004[b]
	5	1.25±0.028[a]	0.37±0.009[a]	0.65±0.009[b]	0.18±0.003[a]	0.42±0.038[b]
	7	0.74±0.116[a]	0.23±0.046[b]	0.37±0.023[b]	0.15±0.019[a]	0.32±0.069[b]
SWARNA	0	1.19±0.107[a]	0.68±0.024[a]	0.56±0.071[a]	0.16±0.022[a]	0.47±0.011[a]
	3	2.18±0.218[b]	0.65±0.022[a]	0.59±0.011[a]	0.20±0.018[a]	0.60±0.012[b]
	5	1.18±0.041[a]	0.44±0.018[b]	0.45±0.089[a]	0.16±0.014[a]	0.59±0.004[b]
	7	0.99±0.110[a]	0.31±0.067[b]	0.40±0.085[a]	0.13±0.009[a]	0.33±0.007[a]
DHANYA	0	1.02±0.094[a]	0.63±0.038[a]	0.47±0.043[a]	0.16±0.023[a]	0.32±0.010[a]
	3	2.33±0.027[b]	0.51±0.027[b]	0.64±0.030[a]	0.21±0.016[a]	0.63±0.062[b]
	5	1.29±0.111[a]	0.35±0.032[b]	0.44±0.020[a]	0.16±0.002[a]	0.56±0.015[b]
	7	0.76±0.039[a]	0.22±0.022[b]	0.36±0.033[a]	0.14±0.018[a]	0.29±0.002[a]

Results are expressed as mean of three replicates. Different letters indicate significant differences in respect to control ($P < 0.01$), ± = Standard error. Enzyme activities are expressed as POX: mmole 0-dianisidine mg protein-1 min-1, APOX: m mole ascorbate mg protein-1 min-1, CAT: µmole H2O2 mg protein -1 min-1, Enzyme activities is expressed as SOD: EU mg protein-1, GR: µmoles NADPH oxidized mg protein -1 min-1.

The total antioxidant activity: The total antioxidant activity in the leaves of the test plants was determined with respect to percent of DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) inhibition. In flooding stress KS 244, KS 244+, 30V92 and BN 101 increased on the 3rd and 5th day of stress but showed a slight decrease in the DPPH inhibition on the 7th day of stress. The other cultivars- BN 1133, Swarna and Dhanaya showed a significant decrease in the DPPH inhibition on the 5th and the 7th day of stress indicating greater negative influence of flooding stress in these cultivars (Fig 1). Suaeda maritime is a halophytic plant which showed an increase in DPPH activity during water logged condition in experiments performed by (Alhdad, Seal, Al-Azzawi, & Flowers, 2013). Similar results were given by (Ali, Ashraf, & Anwar, 2010) where there was decrease in DPPH scavenging activity due to water stress along with phenolics and carotenoid content.

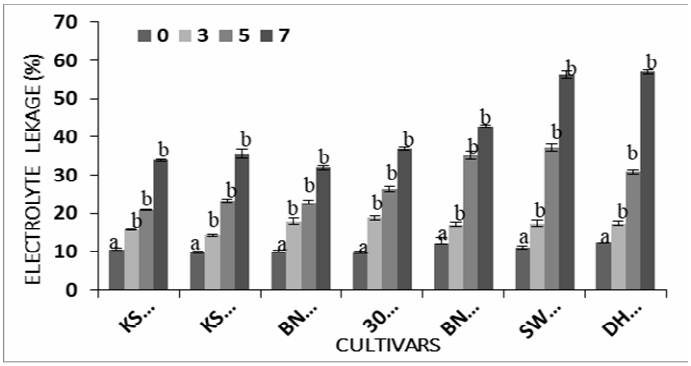
Fig 1: Total antioxidant activity in the leaves of seven maize cultivars during flooding stress



Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at $P \leq 0.05$ in Students 't' test within each cultivar.

Electrolyte leakage: Cell membrane injury in the leaves of seven cultivars of maize during flooding stress showed very less electrolyte leakage in KS 244, KS 244+ and BN 101 indicating that these cultivars are more tolerant to flooding stress than the rest of the cultivars (Fig 2).

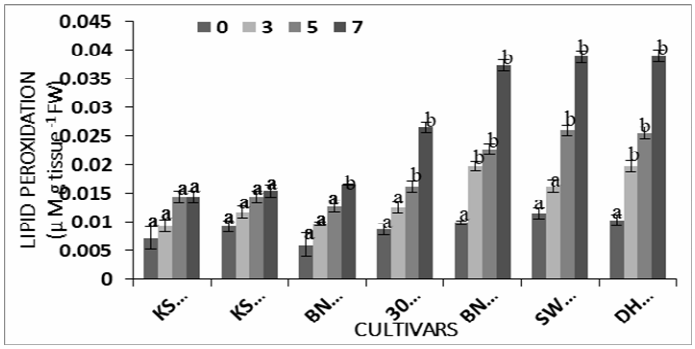
Fig 2: Electrolyte leakage in the leaves of seven cultivars of maize during flooding stress



Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in Students 't' test within each cultivar

Lipid peroxidation: An increase in lipid peroxidation during the period of stress was observed in the leaves of different test cultivars (Fig 3). 30V92, BN 1133, Swarna and Dhanya showed maximum increase in lipid peroxidation and on the 7th day the increase was double of that of the other cultivars. In KS 244+ and BN101 the increase was very insignificant. Intensification of lipid peroxidation under soil flooding was shown both in the heterotrophic root tissues subjected directly to the action of soil hypoxia and in the photosynthesizing organs surviving an indirect effect of this stress factor (Balakhnina & Borkowska, 2013). In experiment performed by (Tang, Xu, Zou, Zheng, & Qiu, 2010) in two maize genotypes, lipid peroxidation was enhanced significantly only in K12 (sensitive) and there was no difference in HZ32 (tolerant) up to 6 d after waterlogging stress (Fig. 3).

Fig 3: Lipid peroxidation in the leaves of seven cultivars of maize during flooding stress



Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in Students 't' test within each cultivar

Chlorophyll content: Flooding stress led to a significant decrease in the chlorophyll content of in the leaves of seven cultivars of test plant. Maximum decrease during flooding stress was observed in Dhanya, Swarna and BN 1133 (Table 2).Similar results on flooding stress in maize leaves were reported by (Yan, Dai, Liu, Huang, & Wang, 1996).

Table 2: Total chlorophyll, chlorophyll-a and chlorophyll-b content in the leaves of maize cultivars during flooding stress.

Cultivars	Treatment No. of Days	Total chlorophyll (mg/g tissue FW)	Chlorophyll-a (mg/g tissue FW)	Chlorophyll-b (mg/g tissue FW)
KS 244	0	1.88±0.002[a]	1.14±0.017[a]	0.74±0.018[a]
	7	1.10±0.018[b]	0.66±0.009[b]	0.44±0.022[b]
KS 244+	0	1.85±0.029[a]	1.11±0.030[a]	0.74±0.037[a]
	7	1.06±0.016[b]	0.62±0.029[b]	0.44±0.033[b]
BN 101	0	1.82±0.031[a]	1.09±0.016[a]	0.73±0.023[a]
	7	1.11±0.062[b]	0.80±0.017[b]	0.31±0.044[b]
30V92	0	1.50±0.020[a]	0.86±0.007[a]	0.64±0.013[a]
	7	0.62±0.024[b]	0.37±0.006[b]	0.25±0.023[b]
BN 1133	0	1.95±0.012[a]	1.02±0.005[a]	0.93±0.017[a]
	7	0.62±0.024[b]	0.37±0.006[b]	0.25±0.023[b]
DHANYA	0	1.74±0.027[a]	0.94±0.007[a]	0.80±0.033[b]
	7	0.55±0.014[b]	0.37±0.016[b]	0.18±0.030[b]
SWARNA	0	1.78±0.028[a]	0.98±0.008[a]	0.79±0.033[b]
	7	0.67±0.018[b]	0.40 ±0.015[b]	0.27±0.010[b]

Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in Students 't' test within each cultivar.

Conclusion

Thus in our study there is a marked significant change in antioxidative enzyme activities of seven cultivars of maize during water logged condition, along with chlorophyll breakdown in all cultivars. Maximum breakdown was observed in BN 1133, Swarna and Dhanya. These cultivars also showed a significant increase in peroxidation and electrolyte leakage during flooding stress indicating higher oxidative damage in these cultivars marking them more sensitive cultivars to water logging.

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