RESEARCH ARTICLE

Comparative assessment on physiological and biochemical response mechanism in some commercial banana cultivars of Assam under abiotic stresses

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Abstract

Banana cultivation is in great constrain due to wide variety of biotic and abiotic stresses, although abiotic stresses significantly impact banana growth and yield. Assam recorded extraordinary diversity of wild and cultivated banana germplasm. Under stressful environmental conditions, plants altered their physiological and biochemical machinery to adopt themselves to the changing environment. In the present study, six banana cultivars (*Malbhog, Seni, Amritsagar, Kaskol, Jahaji, Grand Nine (G9)*) were subjected to five different types of abiotic stresses (cold, heat, drought, water submergence and acid soil stresses) and their influences in physiological and biochemical parameters were investigated. The studies revealed significant changes in physiological parameters such as reduced RWC and chlorophyll contents in all the six cultivars of bananas that were subjected to different types of abiotic stresses when compared with control. The biochemical constituents such as total flavonoid content (TFC), total carbohydrate content (TCC), total phenolic content (TPC), lipid peroxidation, and hydrogen peroxide content were found significantly increased. A significant increase in flavonoid and carbohydrate content were recorded in the cultivars that were subjected to heat and water submergence stresses. Furthermore, a significant increase in ROS (H_2O_2) were observed while moderate to significant increase in antioxidant compounds (TFC, TPC and TCC) were also recorded in response to counter ROS production in all five types of abiotic stresses induced in banana cultivars when compared with control groups.

Keywords: Abiotic Stresses; Banana Cultivars; Physiobiochemical Changes; Reactive Oxidative Species; Oxidative Stress

1. Introduction

Banana (*Musa × paradisiacal* L.) is one of the largest monocot plants belonging to Musaceae family with high commercial and nutritional potential which is found popularly cultivated in more than 130 countries across the world but reported to be native to Malaya and Phillipines (Govaerts, 2004). It is subsistence food crop of the tropical and subtropical world and ranked fourth after cereal crops in global production scale (FAO, 2015). The Banana cultivars (cv) are proven as potential energy source endowed with high content of carbohydrate, iron, potassium and other essential minerals that ensures food, health and livelihood security for millions of people around the world (Sreedharan et al., 2015). Edible bananas are reported to be originated in South East Asia and found distributed mainly on the margin of tropical rain forest however, it had spread rapidly in all six continents simultaneously. The edible bananas of today are reported to be originated from intra- or inter-specific crosses between the two wild diploid species *Musa acuminata* and *Musa balbisiana* which is now technically represented as *M. acumita x M. balbisiana* (Valmayor et al., 1991) and it was first described by Linnaeus (1753) as *Musa × paradisiaca* L. The cultivated banana cultivars (cv) of today are different from the wild species by being seedless and parthenocarpic (Simmonds and Shepherd, 1955; POWO: https://powo.science.kew.org/). The genome constitution of *M. acuminata* is reported to be AA, whereas, genome constituents of *M. balbisiana* is reported to be BB. These wild *Musa* species are seeded with little starch and the flash pith amount is less making it non-consumable for the human (Perrier et al., 2019). With the changing global climate scenario, abiotic stresses tend to diminish plant growth development and yield. Abiotic stresses have the potential to cause a huge production loss (Nahar et al., 2018).

Abiotic stresses are reported to be caused by various contributing factors such as, light (high light, UV, darkness), temperature (frost, low, heat), nutrients (nutrient imbalance), salt, water (deficit, desiccation, flooding), oxidative stress, hypoxia and physical factors, and these environmental factors caused disorders are non-infectious in nature (Ravi and Vaganan, 2016). The extremes of water, temperature, salts, nutrients and light normally cause stress to the plants, which triggers production of reactive oxygen species (ROS) in plants to mitigate the harmful ROS molecules (Saikia et al., 2021) by instigating its inherent defense physiochemical and biochemical machinery (Pradhan et al., 2020). Literature evidences suggested only few reports available to date on the banana cultivars of India and the Northeast Region of India with special reference to abiotic stress responses which confer scopes for detail investigation (Ravi and Vaganan, 2016). The North Eastern part of the India is known for its variegated topography with wide range of edaphic and climatic conditions which supports luxuriant growth, rapid radiation and proliferation of banana genetical diversity (Borborah et al., 2016a; 2016b; 2016c). Although morphometric and markers studies have been reported on banana diversity in recent decades (Borborah et al., 2020a; 2020b), however, assessment based on physiochemical and biochemical entities have been found poorly evaluated. The state of Assam in the Northeast India is reported to be rich in banana diversity, however, research focused on commercially as well as traditionally important cultivars of bananas are found scanty to date. Keeping that in mind, the concept of present study was mooted and evaluated the biochemical and physiological parameters and behavior of six banana cultivars of Assam that were subjected to different types of abiotic stresses.

2. Materials and methods

2.1. Plant materials and abiotic stress treatments

Uniform growth of six commercially available and traditionally important banana cultivars (cv) of one month old and three leaf condition plantlets (sword suckers) were collected and maintained in green house condition prior to abiotic stress treatments. The plantlets were planted in 20 cm long polybags containing mixture of soil and cow dung in the ratio 2:1. The plantlets were then exposed to different types of abiotic stresses (cold, heat, drought, water submergence, acid soil). For control group, three plantlets for each cultivar were maintained for each abiotic stress in normal environmental conditions without stress. For heat stress experimental group, 16 h light/8 h dark cycle; 200 µmolm⁻²s⁻¹ light intensity; 42/36 °C and 70% relative humidity were maintained (Vidhya et al., 2018). For cold stress experimental group, plantlets were maintained at 4 °C for 48 h (Feng et al., 2015). For drought stress experimental group, a drought stress was caused by withdrawing of watering (Ochola et al., 2015); For water submergence stress experimental group, the plantlets were submerged in concrete tank filled with water (Panda and Barik, 2020). For acid soil stress experimental group, the plantlets were transferred in acidic soil of pH range between 4-5. The development of stress symptoms in each plantlet in each experimental groups were observed and recorded in regular interval of time after onset of the experiments. Visual assessment of the plant condition was the initial evaluation approach used in the experiment. Color of leaves, wilting symptoms after every stress was surveyed and categorized them in grade scale. Sampling was done on plants showing the extreme symptoms (González and González-Vilar, 2003). Three biological and experimental replicas were taken for each sample.

2.2. Determination of relative water content (RWC)

To determine the relative water content, the fresh weight was measured initially, and then kept on deionised water for 4 h at room temperature in dark. After 4 h when shoot become fully turgid, it was reweighed. Final weight was taken after drying for 72 h. The shoot RWC was calculated by using the formula suggested previously by Smart and Bingham (1974).

2.3. Determination of chlorophyll content

The total chlorophyll content was determined by using the method suggested by Kapoor and Pande (2015). Fresh leaves of 300 mg were crushed in mortar and pestle along with 5 ml of 80% acetone and then it was filtered. The absorbance of the filtrate was taken at 663 nm and 645 nm (UV-Vis spectrophotometer 119, Systronics).

2.4. Determination of total flavonoid content

The total flavonoid content was determined by following the protocol suggested previously (Tohidi et al., 2017). An aliquot of 125 ml of the extract solution was added to 75 ml of 5% NaNO2 solution. The mixture was then allowed to stand for 5 min before adding 150 ml of aluminum chloride (10%) solution. Then, 750 ml of NaOH solution (1 M) was added and the final volume of the mixture was adjusted to 2500 ml by adding distilled water. After that the reaction mixture was incubated for 15 min and then the absorbance was recorded at 510 nm (UV-Vis spectrophotometer 119, Systronics).

2.5. Determination of total phenolic content (TPC)

The total phenolic content was estimated with a Folin–Ciocalteu assay (Ainsworth and Gillespie, 2007) with minor modifications. Samples were taken with 85% methyl alcohol and subsequently centrifuged at 6000×g for 10 min. Then 300 µL of plant extract was mixed with 10% Folin–Ciocalteu reagent. Then, after allowing the solution to react for about 5 min, 700 mM Na_2CO_3 was added to the mixture with agitation for 120 min at room temperature. Gallic acid was taken as blank and the absorbance was recorded at 765 nm (UV-Vis spectrophotometer 119, Systronics), expressed as mg/g FW.

2.6. Determination of total soluble carbohydrates

The total soluble carbohydrates content was recorded following the protocol of Yemm and Willis (1954). In 0.50g fresh leaves 5 mL of ethanol (80%) was added and then centrifuged at 1500×g for 15 min and the supernatant was collected. To the 100 µL of the extract 3 mL (0.2%) anthrone reagent was added. The solution mixture was boiled for 10 min at 95 °C and then, the reaction was terminated quickly in an ice bath. The absorbance of the samples was recorded at 620 nm (UV-Vis spectrophotometer 119, Systronics) against glucose as the standard and expressed as mg soluble carbohydrates g⁻¹ fresh weight.

2.7. Determination of H_2O_2 content

Hydrogen peroxide (H_2O_2) content was measured according to Sagisaka (1976). 0.2 g of the leaf and root tissue was homogenized in 5% TCA and the homogenate was centrifuged at 17,000×g (Eppendorf 5430R) at 0°C for 10 min. The reaction mixture contained 1.6 ml of the supernatant leaf extract, 0.4 ml TCA (50%), 0.4 ml ferrous ammonium sulphate and 0.2 ml potassium thiocyanate. The absorbance was then recorded at 480 nm (UV-Vis spectrophotometer 119, Systronics).

2.8. Determination of MDA value

Lipid peroxidation was measured by the amount of malondialdehyde (MDA) which is determined by the thiobarbituric acid (TBA) reaction. Following Heath and Packer (1968) method, 0.2 g of fresh leaf and root samples were homogenized with 5 ml of 0.25% TBA containing 10% TCA (tri-chloro acetic acid). The homogenate was boiled 30 min in a water bath and centrifuged at 10,000×g for 10 min. Absorbance values were recorded at 532 nm and 600 nm (UV-Vis spectrophotometer 119, Systronics).

2.9. Statistical analysis

The data were analyzed by two-way analysis of variance (ANOVA) and sample means were compared by Tukey's test. Three significant P-values were considered. Significant code $p \leq 0.05$"*", $p \leq 0.01$"**", $p \leq 0.001$"***".

3. Results

3.1. Physiological responses, chlorophyll content and relative water content

Chlorophyll a, b and total chlorophyll contents were observed to be degrading in all the plantlets that were exposed to five different types of abiotic stresses with increasing duration of stress exposure time. In cold stress group, the total chlorophyll content as well as relative water content (RWC) were found drastically reduced in *Amritsagar* (76.75%), *Kaskol* (89.88%) and *Grand Nine* (85.83%) which showed earlier cold stress symptoms in 7th days when compared with control group. Whereas, cultivars (cv) *Malbhog, Seni* and *Jahaji* showed some tolerant traits when compared with other cultivars. As these three cultivars survived the extreme stress conditions, they showed their stress symptoms little late in 21st days resulting in very low chlorophyll content when compared with control group (Figure 1 and 2).
In heat stress group, lower chlorophyll content and RWC were observed in Seni (6.01 mg/l, 78.06%) and *Grand Nine* (G9) (9.5284 mg/l, 70.37%) cultivars on 14th days when compared with control group. However, the *Amritsagar, Kaskol* and *Malbhog* cultivars took 28th days to show their extreme stress symptoms. In drought stress group, *Kaskol* cultivars showed tolerant characters and the cv *Kaskol* showed stagnant chlorophyll content whereas, the cultivar *Seni* showed stress symptoms and demonstrated significant drop in chlorophyll content (1.85 mg/l). In water submergence stress group, a lower chlorophyll contents were found in the experimental cultivars *Seni* (19.12 mg/l) and *Grand Nine* (6.56 mg/l) on 7th days of stress treatment. However, *Jahaji* cultivar showed significant drop in chlorophyll (9.08mg/l) and RWC (70.91%) content in 7th days and it took twenty-eight days to show its full symptom by significant reduction in RWC (70.29%) and chlorophyll (13.53 mg/l). In acid soil stress cv *Seni* showed the tolerance characters and on 21st day the RWC is 73.18 % and chlorophyll content is 0.53 mg/l which is presented in Figure 1 and 2.
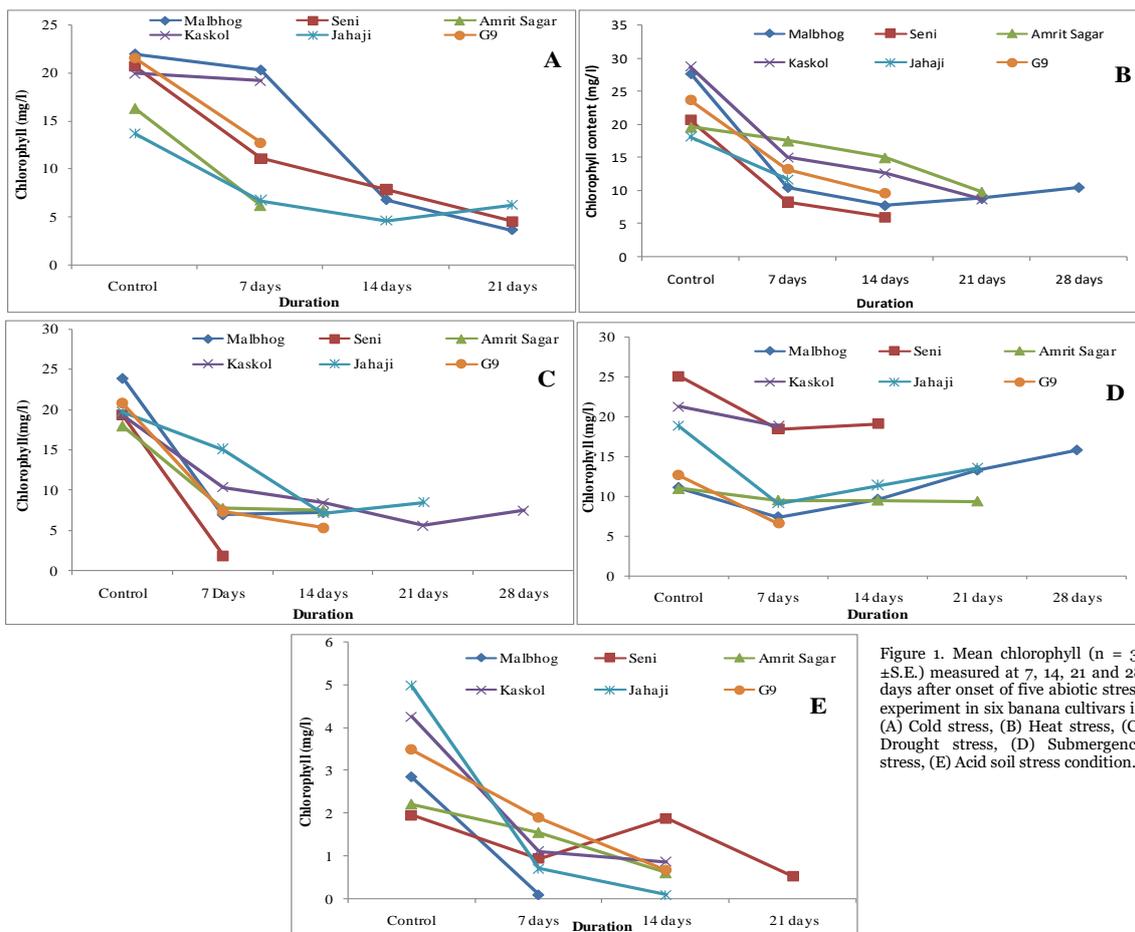
Figure 1. Mean chlorophyll (n = 3, ±S.E.) measured at 7, 14, 21 and 28 days after onset of five abiotic stress experiment in six banana cultivars in (A) Cold stress, (B) Heat stress, (C) Drought stress, (D) Submergence stress, (E) Acid soil stress condition.
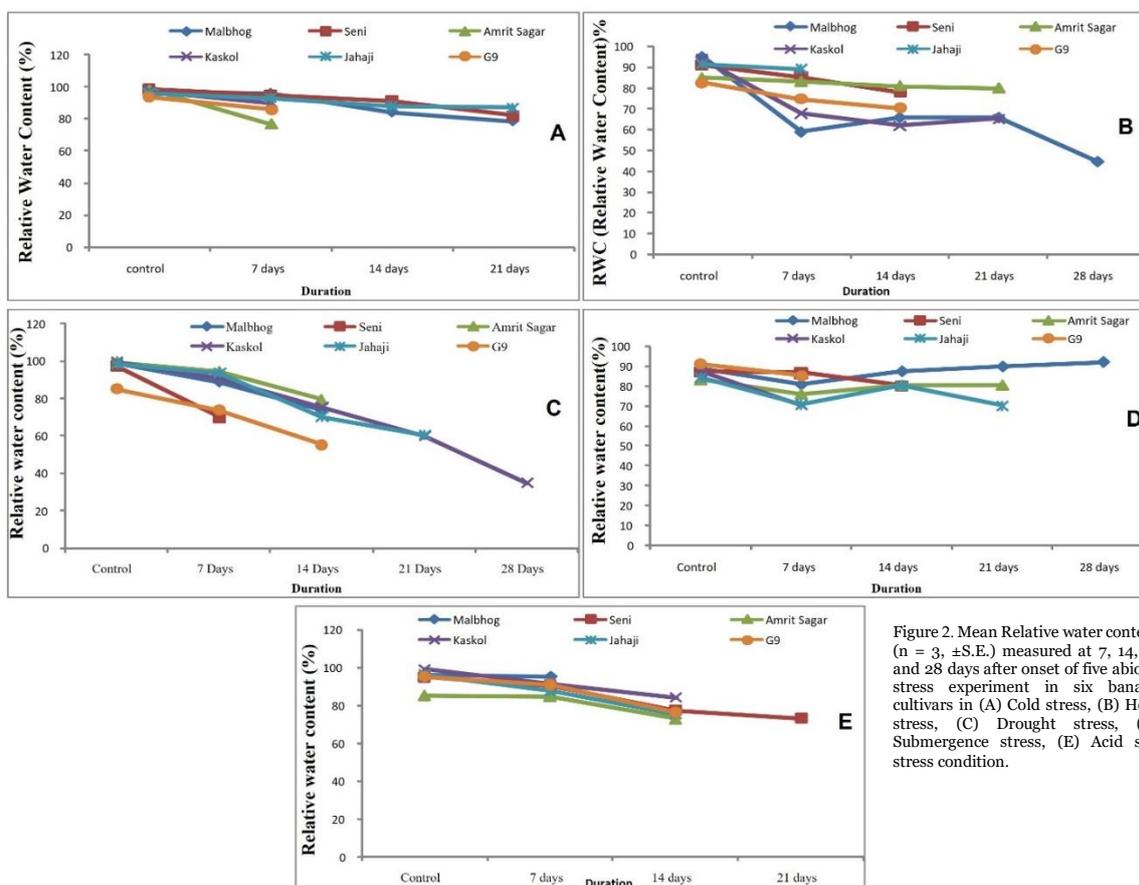

Figure 2. Mean Relative water content (n = 3, ±S.E.) measured at 7, 14, 21 and 28 days after onset of five abiotic stress experiment in six banana cultivars in (A) Cold stress, (B) Heat stress, (C) Drought stress, (D) Submergence stress, (E) Acid soil stress condition.

3.2. Total flavonoid content (TFC)

In cold stress cultivar group, a significant increase in total flavonoid content were observed only in *Kaskol* (0.0702 mg/g Fw) whereas, all the other cultivars showed insignificant flavonoid contents (Fig. 3). In heat stress group, a significant elevation in total flavonoid contents were observed in all the experimental cultivars when compared with control group. In the drought stress groups, flavonoid content was found between the range of 0.049-0.086 mg/g Fw. However, increased flavonoid contents were noted in *Jahaji* (0.086 mg/g Fw), *Kaskol* (0.076 mg/g Fw) and Amrit Sagar (0.058 mg/g Fw) whereas the cultivars Grand Nine (G9), Seni and *Malbhog* showed insignificant increase in flavonoid content. In water submergence stress group, flavonoid contents were found increased in all the experimental cultivars when compared with control group. In acid soil stress group, *Seni* (0.111 mg/g Fw), *Jahaji* (0.117 mg/g Fw), *Kaskol* (0.158 mg/g Fw), *Amrit Sagar* (0.258 mg/g Fw) and *Grand Nine* (0.259 mg/g Fw) have showed significant increase in the total flavonoid content, however, an insignificant change in flavonoid content was observed in *Malbhog* cultivar (Figure 3).

3.3. Total phenolic content (TPC)

The increased total phenolic contents were observed in the cold stress cultivars which falls within the range of 0.388-1.143 mg GA/gm. The highest increase in TPC was observed in *Grand Nine* (4.321 mgGA/g). *Amrit Sagar* showed no significant change in TPC content when compared with control. In the heat stress group, all the experimental cultivars were noted with significant increase in their phenolic content within the range of 0.608-0.177 mgGA/gm. However, except in the cultivar *Seni*, a significant increase in TPC were observed in all the cultivars that were exposed to drought stress condition. In submergence and acid soil stress groups, increased total phenolic content were observed high among all the six cultivars when compared with control group (Figure 4).

3.4. Total carbohydrate content (TCC)

The cultivars exposed to different abiotic stress conditions demonstrated different accumulation pattern of carbohydrate content (Fig.5). The increased total carbohydrate content in the range of 11.401-6.642 mg/g Fw were recorded in all the cultivars under cold stress group. Whereas, in heat stress group, increased carbohydrate accumulation was recorded in all the cultivars except in *Amrit Sagar*. In drought stress group, only *Jahaji* cultivar showed significant increase in carbohydrate content (2.684 mg/g Fw) whereas Total carbohydrate content of *Amrit Sagar* (1.667 mg/g Fw) and *Grand Nine* (1.634 mg/g Fw) were also found affected due to water submergence stress. In acid soil stress group, increased carbohydrate content was recorded in cultivar *Seni* (1.669 mg/g Fw) while, other cultivars did not show any change in TCC when compared with control group (Figure 5).

3.5. Hydrogen peroxide content (HPC)

The hydrogen peroxide content was found highest in *Amrit Sagar* (5005.00 μmol/g) in cool stress group which is presented in Figure 6, followed by *Grand Nine* (3705.00 μmol/g) and *Seni* (830.667 μmol/g). However, no significant difference was observed in hydrogen peroxide content in other cultivars. In heat stress group, a significant increase in hydrogen peroxide was observed in all the cultivars although; *Jahaji* cultivar showed highest increase in hydrogen peroxide when compared with control. Hydrogen peroxide content was found highest in the cultivar *Seni* when compared with other drought stressed cultivars groups. The HPC was recorded between the range of 4095-6860 μmol/g in *Jahaji* cultivars in water submergence stress group whereas, it was found increased in all the other cultivars. Furthermore, in acidic soil stress group, *Malbhog, Kaskol, Jahaji* and *Grand Nine* showed significant increase in HPC whereas, Seni, Amrit Sagar did not show any change when compared with control groups (Figure 6).

3.6. Total lipid peroxidation value (MDA Content)

In cold stress group of cultivars, the MDA content were found between the range of 1.286-24.073 μmol/g Fw (Fig.7).

A significant increase in MDA content was recorded in four cultivars, namely, Seni (23.63 μmol/g Fw), *Amrit Sagar* (24.07 μmol/g Fw), *Grand Nine* (13.45 μmol/g Fw) and *Malbhog* (13.52 μmol/g Fw) whereas it was found insignificant in *Jahaji* and *Kaskol*. In heat stress group, significant increase in MDA content was recorded in *Kaskol* (21.26 μmol/g Fw), Seni (13.64 μmol/g Fw), *Jahaji* (12.28 μmol/g Fw) and *Grand Nine* (6.77 μmol/g Fw) cultivars, whereas a significant decrease in MDA content was noted in *Amrit Sagar* when compared with control. In drought stress group, the MDA content were recorded between the range of 4.830-13.643 μ mol/g. Elevated MDA content were observed in the four experimental cultivars, namely, *Malbhog* (3.111 μmol/g Fw), Kaskol (3.062 μmol/g Fw), *Jahaji* (2.650 μmol/g Fw) and *Grand Nine* (3.475 μmol/g Fw) when compared with control group (Figure 7).

4. Discussion

Abiotic stresses are the major constraint for banana growth and yield. In response to the abiotic stresses, plants restructured its whole growth cycle as well as it's physio-biochemical attributes. Physiological attributes including reduction of light harvesting photosynthetic pigments through distortion of the chloroplast structure and ultimately diminish the chlorophyll content (Anjum et al., 2017). Chlorophyll content assessments are considered as fundamental tool to identify the stress-induced damages in the plants. Reduction in chlorophyll content greatly alters plant's yield and decreases growth and development (Joshi et al., 2009; Kalaji et al 2018; Raja et al., 2020). Chlorophyll fluorescence has been reported as nonfatal index to assess the impact of different abiotic stresses on plants. Evaluation of chlorophyll activity contributes vital information on severity of the stresses (Hanachi et al., 2014; Murchie and Lawson, 2013). In another study, four eggplant cultivars exposed to drought and heat stresses had demonstrated reduction in chlorophyll content (Hanachi, et al., 2022). In our present studies, a significant reduction in chlorophyll content were recorded in the plantlets of six banana cultivars that were exposed to five different types of abiotic stresses which is in agreement with the previous findings of Hanachi et al. (2022).

Relative water content (RWC) has been considered as remarkable stress marker in abiotic stress condition in plant. Under stressed environment, plants tend to keep-up the equilibrium via higher RWC especially when the transpiration rate is increased (Tabassum, 2018). In our study, it was observed that different stresses influenced the RWC to different extend. It was observed through RWC and chlorophyll data that the cultivars *Jahaji*, *Seni* and *Malbhog* showed water submergence stress tolerant activity. Reduction in RWC leads to reduction in photosynthetic activities in plants (Slatyer, 1955). In our present studies, a moderate to significant changes in phyio-biochemical parameters were observed in the six cultivars of bananas that were exposed to five different types of abiotic stresses. It was also observed that abiotic stress activates ROS production, imbalanced MDA content, stimulated excess production of ROS (hydrogen peroxide content). However, to protect the cells from oxidative stress via scavenging excess ROS (H_2O_2) production in the cells, these banana cultivars enhanced the production of anti-oxidant compounds, namely, total flavonoid content (TFC), and total phenol content (TPC) and total carbohydrate content (TCC) in response to the onset of oxidative stresses caused by abiotic stresses.

Flavonoid is a group of secondary metabolites responsible for scavenging ROS molecules and act as an antioxidant when plants are exposed to different types of abiotic stresses. Hichem et al. (2009) reported significant increase in total phenolic and flavonoids compounds in maize under stressful environment which concordat with our study. Over the past several decades, researchers have been consistently working on the relationship between abiotic stresses and ROS mechanisms; however, even in the absence of stress, ROS were produced which were then scavenged naturally by antioxidant molecules synthesized in plants (Laxa et al., 2019; Said et al., 2015; Alhaithloul et al., 2020). Flavonoids directly scavenge the ROS by donating a hydrogen atom and return back to phenoxyl radical thus, play a critical role in resistance mechanism (Amic et al., 2003).
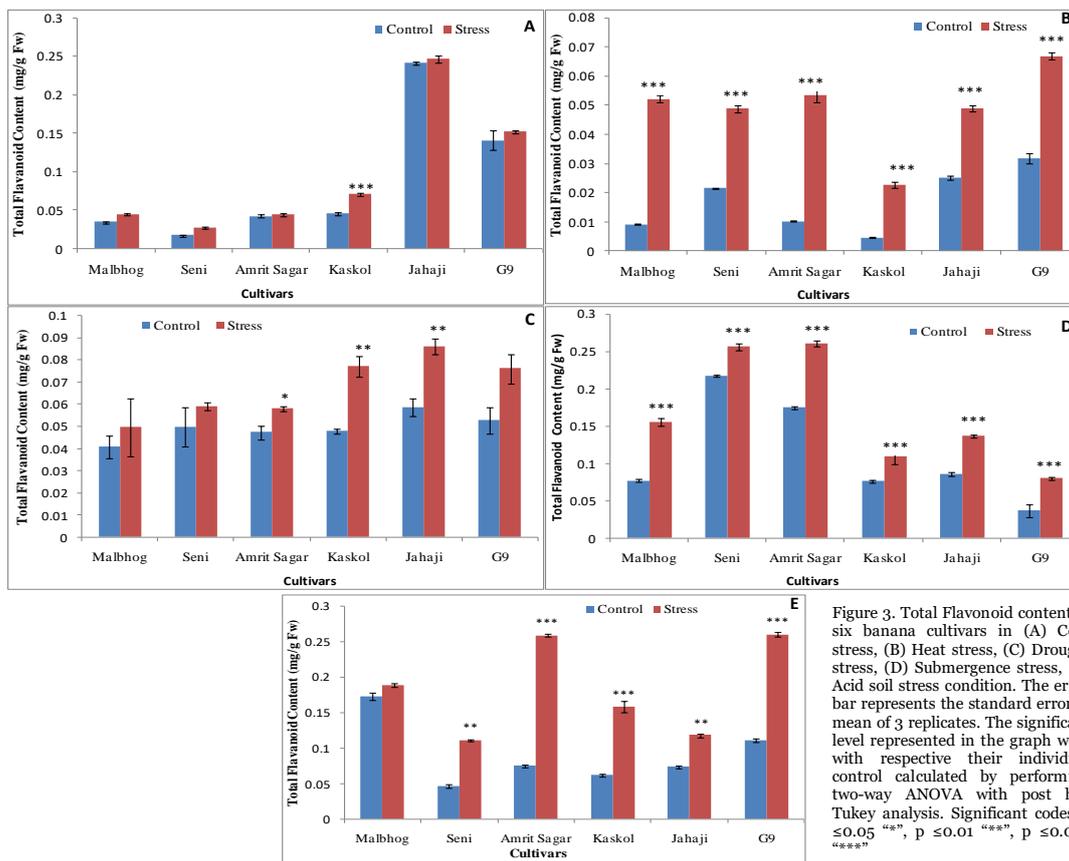
Figure 3. Total Flavonoid content of six banana cultivars in (A) Cold stress, (B) Heat stress, (C) Drought stress, (D) Submergence stress, (E) Acid soil stress condition. The error bar represents the standard error of mean of 3 replicates. The significant level represented in the graph were with respective their individual control calculated by performing two-way ANOVA with post hoc Tukey analysis. Significant codes p ≤0.05 "*", p ≤0.01 "**", p ≤0.001 "***"
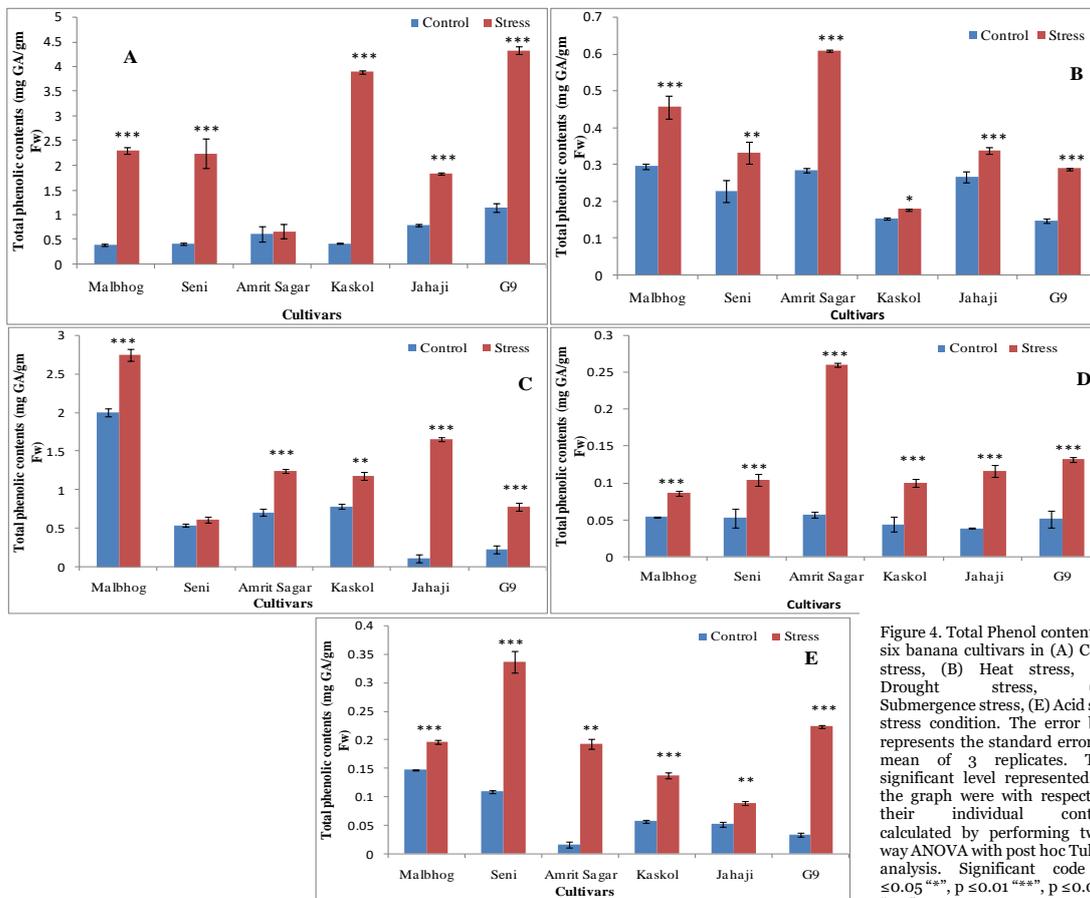

Figure 4. Total Phenol content of six banana cultivars in (A) Cold stress, (B) Heat stress, (C) Drought stress, (D) Submergence stress, (E) Acid soil stress condition. The error bar represents the standard error of mean of 3 replicates. The significant level represented in the graph were with respective their individual control calculated by performing two-way ANOVA with post hoc Tukey analysis. Significant code p ≤0.05 "*", p ≤0.01 "**", p ≤0.001 "***"

It has been reported that high phenolic content in plants are directly responsible for antioxidant potential and their ability to scavenge free radicals via donating hydrogen atom or electrons (Amarowicz et al., 2004). In the present studies, when the experimental cultivars were exposed to five abiotic stress conditions, significant increase in phenolic contents were observed. Sugars plays dual role in plants and they are reported to regulate genes involved in photosynthesis, sucrose metabolism as well as production of osmoprotectants, and the carbohydrates are also reported to be involved in various metabolic events (Gupta and Kaur, 2005). Phenolic compounds play a crucial role in maintaining redox-homeostasis; help in improving stress tolerance in plants (Šamec, 2021). In the present studies, heat and submergence stresses groups, *cv Amrit sagar* showed highest phenolic content when compared with control group. This has clearly indicated heat and water submergence stress tolerance of the *cv Amrit sagar*.

A study conducted by Rejsˇkova´ et al. (2005) indicated a significant increase in carbohydrate accumulation of olive seedlings under low- temperature and salinity stress. Increase in starch and its derivatives were mainly observed in response to cold stress (Kanai et al., 2007). Previous studies also suggested the accumulation of carbohydrate under cold stress (Guy, 1990; Castonguay et al., 1995; Guy et al., 2008), osmotic stress (Wang et al., 2000) and under drought stress (Whittaker et al., 2001). Study conducted on transgenic rice plant also demonstrated accumulation of trehaloes under salt, drought and cold stress (Garg et al., 2002; Saswati and Anirban, 2020). Similarly, in our study, cv *Jahaji* demonstrated higher accumulation of total carbohydrate content under cold, heat and water submergence stress. However, under drought stress condition, only *Jahaji* cultivar showed significant increase in carbohydrate content, and in acid soil stress *cv Seni* showed a significant increase in carbohydrate content.

Scarcity of H_2O_2 acts as secondary messenger for initiating scavenging mechanism (Pandey et al., 2017). Increase in H_2O_2 level indicates stress in plants while H_2O_2 triggers plant responses and induces production of H_2O_2 under drought and light stress (Sharma and Dubey, 2005; Mullineaux et al., 2006) and salinity stress (Al-Sammarraie et al., 2020). In the present study, accumulation of H_2O_2 was observed in banana cultivars that were exposed to different levels abiotic stress conditions. In another studies, positive correlation between the H_2O_2 concentration and stress intensity were reported (Asaeda et al., 2017; De Silva and Asaeda, 2017a; Parveen et al., 2017). In Acid soil, cold and water submergence stress, *Malbhog cv* showed highest H_2O_2 accumulation which indicated that the *Malbhog* cv is susceptible to these stresses.

Malondialdehyde (MDA) is widely considered as a marker for lipid injury in plants under various abiotic stress conditions. Increase in MDA content has been reported earlier by different researchers working on abiotic stress (Hasanuzzaman et al., 2020). Similarly in our study, exposure of the six banana cultivars to five different abiotic stresses (cold, heat, drought, submergence and acid soil) significantly increases the MDA activity in a different ratio as compared to the control. Accumulation of MDA result of oxidative damage to membrane layer under stress is in agreement with other reports in different plants (Yin et al., 2005; Ozkur et al., 2009; Sachdev et al., 2021).

5. Conclusion

The present studies have unveiled significant physio-biochemical changes in six banana cultivars that were exposed to five different types of abiotic stresses. A significant reduction in total chlorophyll and relative water contents were observed in all the six cultivars exposed to cold, heat, drought, water submergence and acid soil stresses while significant increase in H_2O_2 contents were also observed in all the six cultivars but found highest in the *Amrit Sagar, Grand Nine* and Seni cultivars under cold stress condition which clearly showed stress symptoms in the banana plantlets investigated. The *Seni* and *Amrit Sagar* cv did not show elevation of H_2O_2 under acidic soil stress condition which clearly indicating their acid tolerant characters. However, to counter the oxidative stress physiology due to excess production of ROS (H_2O_2)

molecules, an increased Total Flavonoid Content (TFC), Total Phenolic Content (TPC) and Total Carbohydrate Content (TCC) were observed in all the six cultivars. However, TCC was found significantly high in *Jahaji* (2.684 mg/g Fw) in heat and drought stress experimental groups. A significant increase in TFC was observed in *Kaskol* cv while highest increase in TPC were observed in *Grand Nine* (4.321 mgGA/g) under cold stress condition. A moderate to significant increase in TPC were observed in all six cultivars that were exposed to heat, drought, water submergence and acidic soil stress conditions. Total Lipid Peroxidation Value (MDA Content) were also found moderate to significantly high in four cultivars namely, *Malbhog Kaskol, Jahaji, Amritsagar, Seni* and *Grand Nine* that were exposed to five types of abiotic stresses. However, highest MDA contents were observed in *Seni* (23.63 μmol/g Fw), *Amrit Sagar* (24.07 μmol/g Fw) under cold stress conditions which indicating severe damages in lipid and cell membrane caused by the oxidative stress. The physiological and biochemical changes observed in six banana cultivars under abiotic stresses in present studies would be useful for mapping tolerant and susceptible cultivars which could be further utilized for future gene editing and crop improvement programs to develop climate smart and stress resilient banana cultivars.

Acknowledgements

Authors' contributions

DS conducted the experiments, analyzed the data and prepared the manuscript; BT designed the experiments, supervised the work and finally edited the manuscript.

Conflict of interests

The authors declare no conflict of interest.

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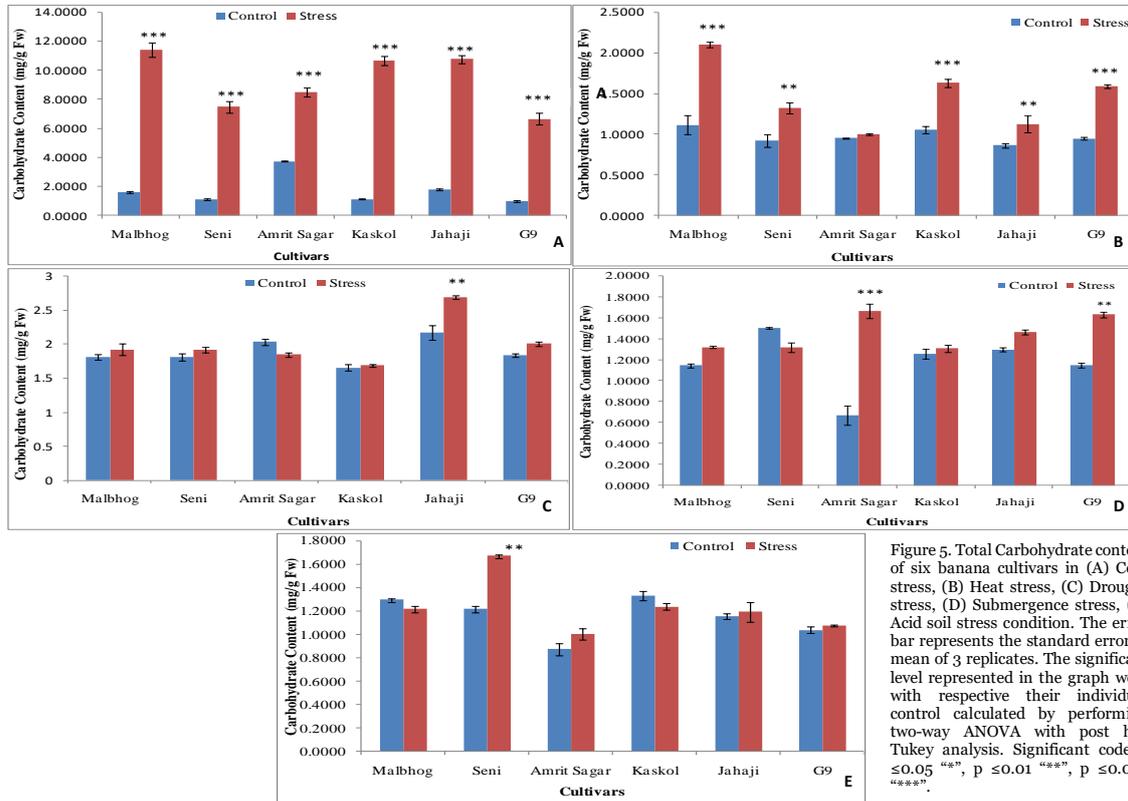
Figure 5. Total Carbohydrate content of six banana cultivars in (A) Cold stress, (B) Heat stress, (C) Drought stress, (D) Submergence stress, (E) Acid soil stress condition. The error bar represents the standard error of mean of 3 replicates. The significant level represented in the graph were with respective their individual control calculated by performing two-way ANOVA with post hoc Tukey analysis. Significant code p ≤0.05 "*", p ≤0.01 "**", p ≤0.001 "***".
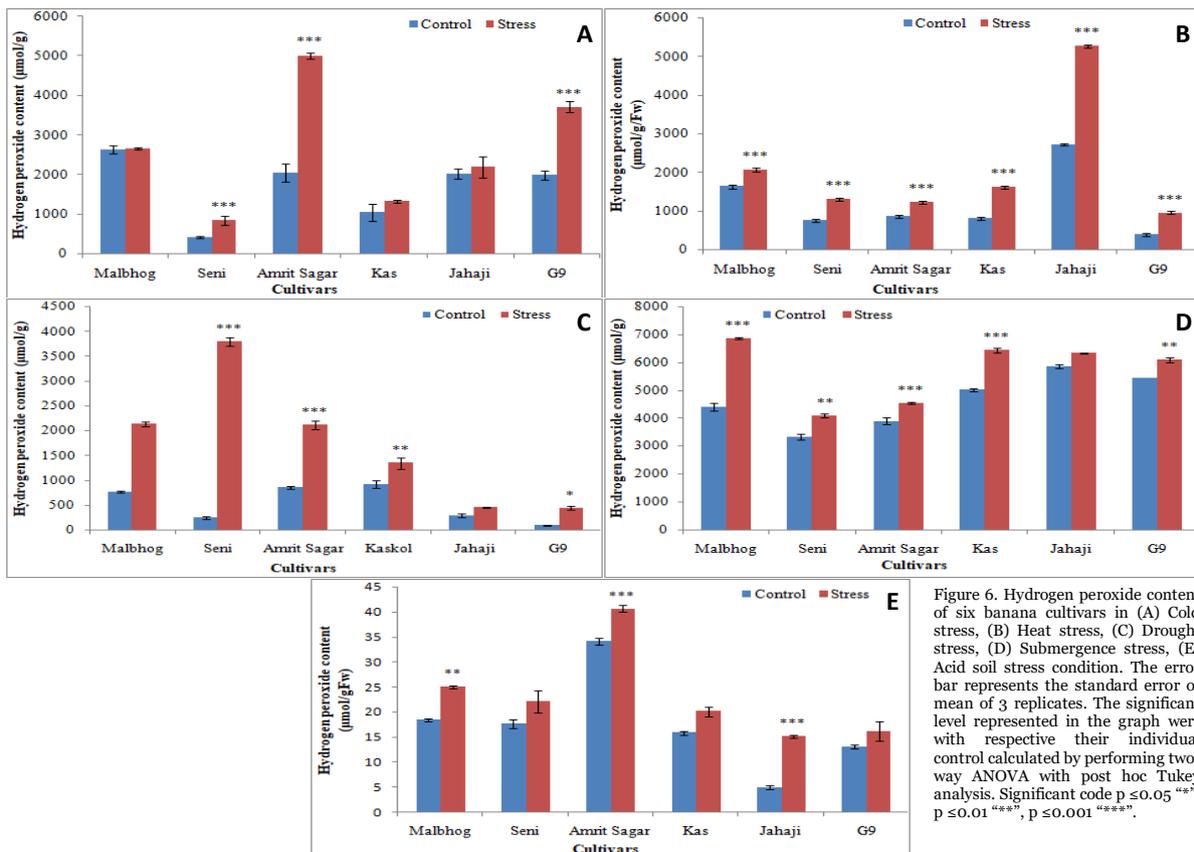

Figure 6. Hydrogen peroxide content of six banana cultivars in (A) Cold stress, (B) Heat stress, (C) Drought stress, (D) Submergence stress, (E) Acid soil stress condition. The error bar represents the standard error of mean of 3 replicates. The significant level represented in the graph were with respective their individual control calculated by performing two-way ANOVA with post hoc Tukey analysis. Significant code p ≤0.05 "*", p ≤0.01 "**", p ≤0.001 "***".
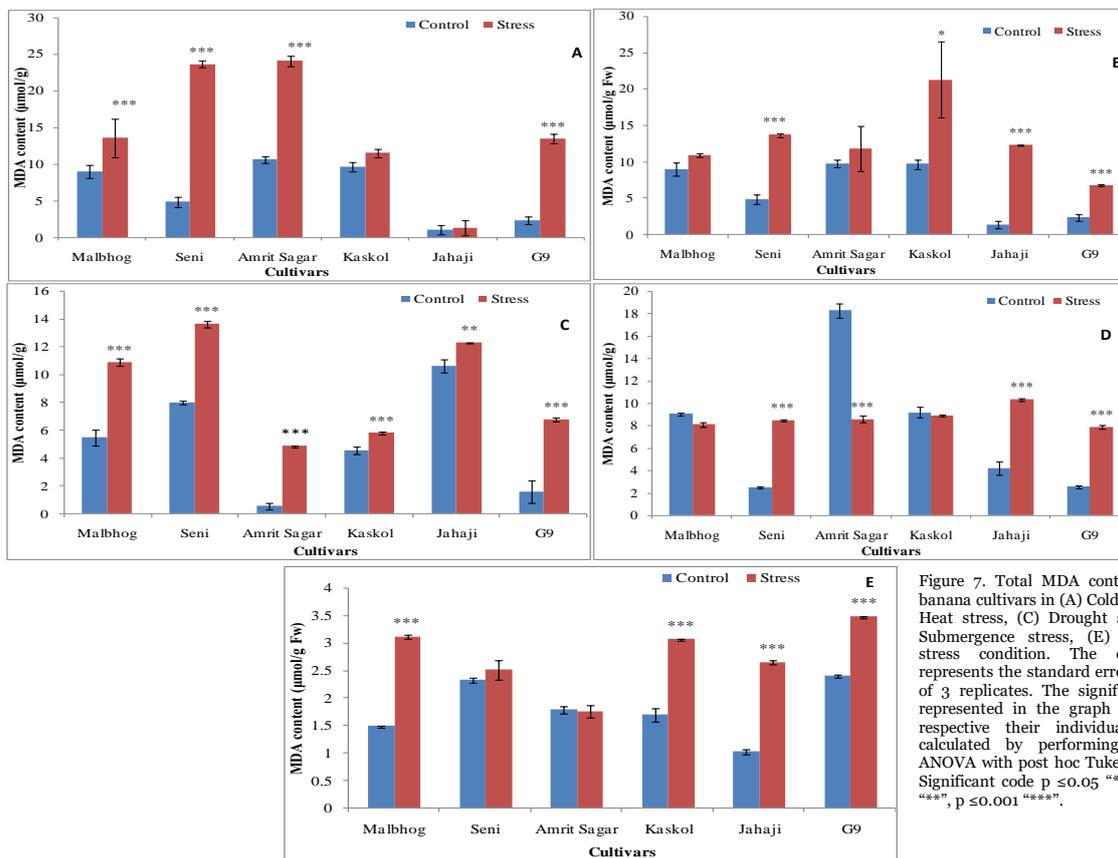
Figure 7. Total MDA content of six banana cultivars in (A) Cold stress, (B) Heat stress, (C) Drought stress, (D) Submergence stress, (E) Acid soil stress condition. The error bar represents the standard error of mean of 3 replicates. The significant level represented in the graph were with respective their individual control calculated by performing two-way ANOVA with post hoc Tukey analysis. Significant code $p \leq 0.05$ "*", $p \leq 0.01$ "**", $p \leq 0.001$ "***".

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