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Assessment of waterlogging-induced changes in enzymatic antioxidants and carbohydrate metabolism in peanuts genotypes

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ABSTRACT

Soil flooding, manifesting as submergence or waterlogging stress, significantly impacts plant species composition and agricultural productivity, particularly in regions with low rainfall. This study investigates the biochemical responses of two peanut (*Arachis hypogaea* L.) genotypes, DH-86 and GJG-32, under waterlogging stress. The experiment involved in-vivo pot trials where peanut plants were subjected to continuous waterlogging for 12 days at the flowering stage. Biochemical analyses of leaves conducted and revealed significant alterations in enzyme activities and metabolite concentrations. Key findings include variations in superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPOD), α-amylase, invertase, acid phosphomonoesterase activities, and changes in starch, proline, reducing sugars, and chlorophyll content. SOD, CAT, and GPOD activities exhibited differential responses between genotypes, highlighting DH-86's quicker recovery post-waterlogging. Notably, DH-86 demonstrated higher resilience, reflected in its rapid normalization of biochemical parameters, while GJG-32 showed prolonged stress effects. These findings underscore the importance of antioxidative enzyme systems in mitigating oxidative damage induced by waterlogging. This study enhances our understanding of the biochemical adaptations of peanut genotypes to waterlogging stress, offering valuable insights for breeding programs focused on improving flood tolerance in crops.

1. Introduction

Soil flooding imposes complex stress on plants, categorized as either submergence or waterlogging stress, depending on the water table's depth. These stresses significantly influence species composition within ecosystems [1]. Waterlogging stress is estimated to impact 12 % of cultivated areas worldwide [2]. Particularly problematic in regions with low rainfall, waterlogging primarily damages crops through oxygen deficiency. This leads to plant wilting, despite the excess water, which adversely affects nutrient and water uptake [3]. Abiotic stressors, such as poor drainage in heavy soils, can lead to waterlogging [4,5]. These stressors often arise from significant rainfall during certain seasons or improper irrigation practices. Excess water reduces the oxygen concentration around plant roots, leading to lower cellular oxygen levels, a condition known as hypoxia [5,6]. Furthermore, the increasing frequency of waterlogging due to global warming poses a serious threat to global food security [7].

In Heilongjiang, the largest agricultural province in northeastern China, there is a significant risk of soil waterlogging. Preliminary statistics indicate that waterlogging-prone areas in the Sanjiang Plain and the Songnen Plain constitute approximately one-third of the total arable land in Heilongjiang Province [8]. Many changes in plant metabolism occur to cope with hypoxia and waterlogging stress. Chlorophyll, a key component of the photosynthetic machinery, plays a crucial role in these processes. Photosynthesis and chlorophyll content are closely connected. When plants are waterlogged, their chlorophyll levels often decrease, leading to reduced photosynthesis. This reduction has been observed in various crops, including mung beans [9], soybeans [10], corn [11], barley [12], pigeon pea [13].

Reduction in photosynthesis limits plant metabolism of carbohydrates, negatively impacting sugar availability [14]. For instance, while starch accumulation was observed in Luffa leaves under waterlogged conditions [15], maize exhibited a reduction in sugar concentration [16]. Under hypoxic and anoxic conditions, the generation of reactive oxygen species (ROS) is induced by a low energy supply and altered redox state of cells. Elevated ROS levels are associated with oxidative

Abbreviations: CAT, catalase; POD, guaiacol peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; A. hypogaea, Arachis hypogaea.

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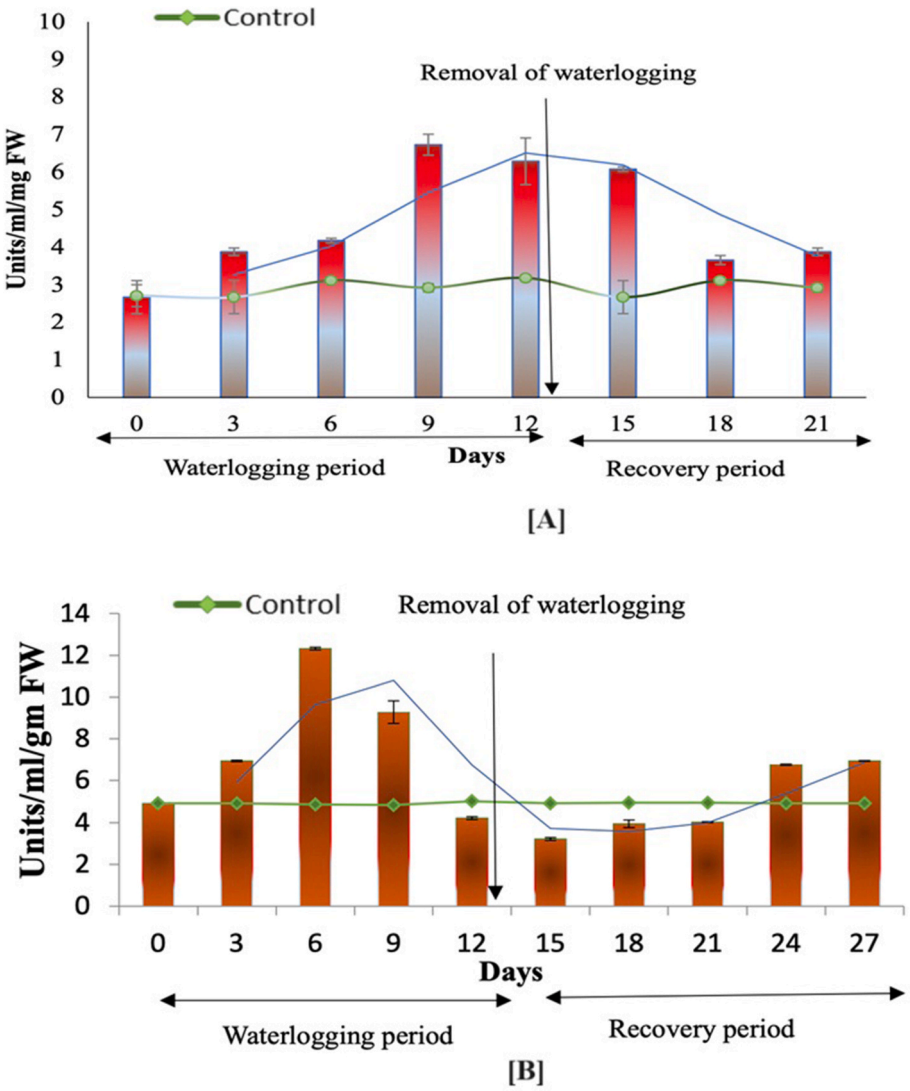

Fig. 1. Superoxide dismutase activity in the leaves of *A. hypogaea* [A] DH-86 [B] GJG-32 during waterlogging and recovery periods.

damage to DNA, lipid peroxidation, and enzyme inactivation. Lipid peroxidation, resulting from oxidative damage due to waterlogging, leads to membrane disintegration. Studies have reported that waterlogging reduces membrane stability in barley [17], maize [11], and winter rape [18]. The role of the root antioxidant enzyme system in various crops' resistance to waterlogging has been extensively examined [13]. When aerobic organisms experience a partial reduction in oxygen under anaerobic conditions, it can result from either normal or abnormal metabolic processes. ROS are known to oxidize biological components, leading to oxidative damage in living tissues [17,18]. Various enzymatic and non-enzymatic processes can mitigate the harmful effects of ROS by converting them into harmless molecules. The initial cellular defence mechanism involves superoxide dismutase (SOD), which is followed by catalase (CAT) and guaiacol peroxidase (POD) [17,18]. SOD catalyzes the dismutation of superoxide radicals into oxygen and hydrogen peroxide, which are then broken down by CAT into water and oxygen, thereby mitigating oxidative stress. POD further aids in decomposing hydrogen peroxide by using phenolic compounds as electron donors, thus preventing the build-up of harmful peroxides within cells [19,20]. Collectively, these antioxidant enzymes form a crucial defence network that protects plant cells from oxidative damage induced by environmental stresses such as waterlogging. Currently, various farmland management strategies are employed to mitigate waterlogging damage and improve crop growth conditions. These measures include constructing drainage facilities, enhancing farming practices, selecting waterlogging-resistant crop varieties, timely and appropriate fertilization, chemical treatments, and replanting [21].

Peanut plants (*Arachis hypogaea* L.), a major global source of protein and oil, are especially susceptible to waterlogging. Originating from the ancient hybridization of two diploid ancestors, the cultivated tetraploid peanut suffers significantly under waterlogged conditions, leading to reduced pod yield and kernel quality [22,21]. Excessive precipitation causes soil waterlogging, impacting various physiological processes such as photosynthesis [23,24], energy metabolism, and the antioxidant system [25,26]. It also affects root respiration [26,27], nutrient absorption [26,27], plant morphology [28], ultimately results in a decline in pod yield [29,30]. The factors that determine the activity include things like the localization and synthesis of antioxidants, the ability to induce antioxidant defences, the cooperation between enzymes, and the compartmentalization of ROS formation or antioxidants [17].

The goal of the current study was to investigate the impact of waterlogging stress on biochemical parameters in different peanut genotypes, highlighting the crop's high sensitivity to this stressor. Additionally, we aimed to compare the levels of tolerance by examining the recovery of both genotypes. Peanut is a crucial crop, making it essential to address the adverse effects of excess water on its growth. It's also important to explore ways to enhance the crop's resistance to water stress.

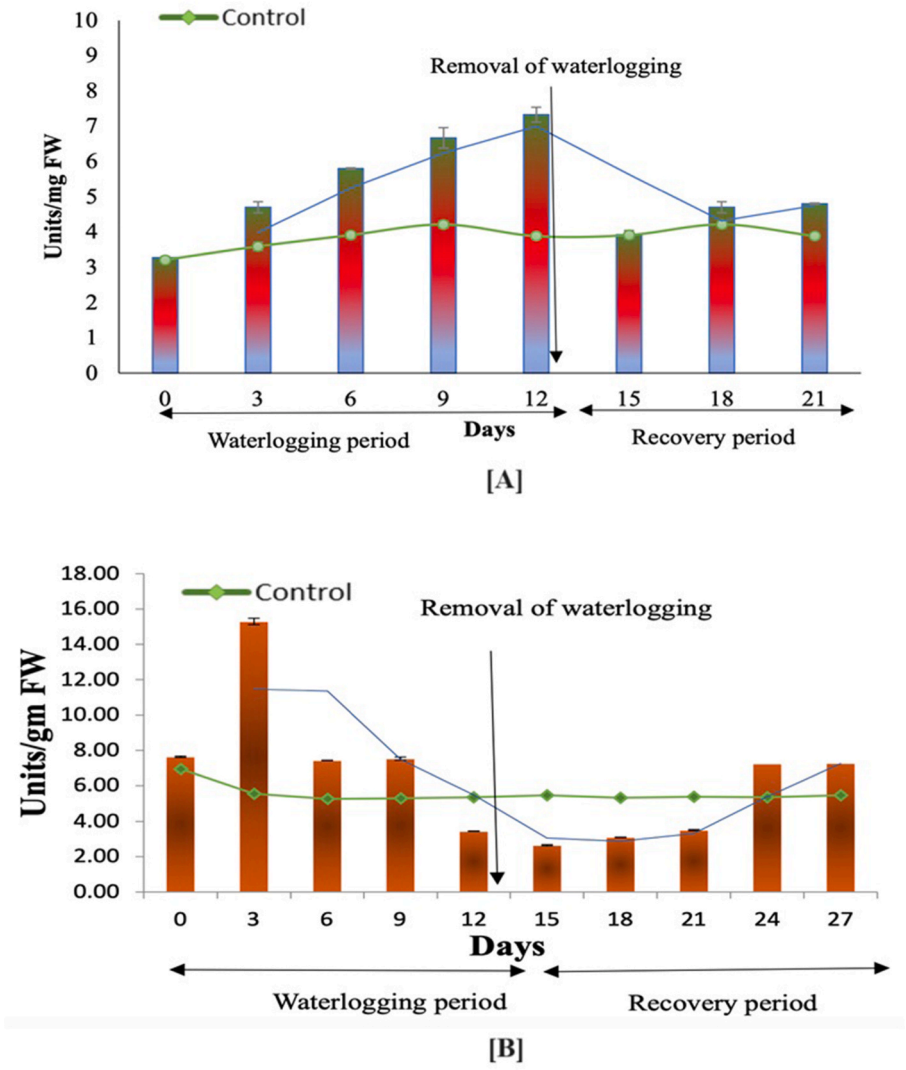

Fig. 2. Catalase activity in the leaves of *A. hypogaea* [A] DH-86 [B] GJG-32 during waterlogging and recovery periods.

2. Material and methods

2.1. Plant material and growth conditions-

The authentic seeds of various genotypes/varieties [DH-86 and GJG-32] of *A. hypogaea* were procured from the Maharana Pratap University of Agriculture and Technology, Udaipur Rajasthan, India [24°35′14.97″N, 73°42′38.75″E]. *In-vivo* pot experiments were carried out to evaluate the effect of waterlogging on the biochemical characteristics of different peanut genotypes. To determine the waterlogging-induced changes, the peanut seeds were first surface sterilized by 0.1 % $HgCl_2$ and then sowed in pots containing 70 % clay and 48 % slit, under greenhouse conditions at 35 ± 5 °C temperature and 12 h photoperiod and 60 % relative humidity. The seedlings were irrigated daily with water.

2.2. Waterlogging treatment-

The germinated seedling was regularly watered till the flowering stage. After that, at the stage of 4–5 fully developed flowers, the peanut plants were divided into two sets. Each set contains 100 plants replicates. Out of two sets, one set was subjected to waterlogging stress by continuous water supply and the remaining one set served as control and watered as per field capacity. The waterlogging treatment was continued for 12 days after the flowering of peanuts. The biochemical analysis was done in leaves of control and waterlogged plants at 3-day intervals during the waterlogging and recovery periods.

2.3. Sample preparation for biochemical analysis

The peanut leaves of control and treated plants were homogenized in pre-chilled mortar and pestle with appropriate phosphate buffers. The homogenate was centrifuged in a high-speed centrifuge at 10,000 rpm for 20 min. The supernatant was collected and used for enzyme assay and protein estimation. The results of the study are averages of three replicates.

2.3.1 SOD activity was determined spectrophotometrically by measuring its ability to inhibit the photochemical reduction of Nitro Blue Tetrazolium (NBT) at 560 nm. The reaction mixture consisted of 100 μl L-methionine, 100 μl NBT, 10 μl riboflavin, and 100 μl enzyme extract, with the volume made up to 3 ml using 0.05 M Na_2CO_3.

2.3.2 CAT activity was measured by the consumption of H_2O_2 at 240 nm. The reaction mixture included 120 μl enzyme extract and 80 μl H_2O_2 (500 mM), with the final volume adjusted to 3 ml by adding 2.8 ml potassium phosphate buffer (50 mM).

2.3.3 GPOD activity was determined spectrophotometrically by measuring changes in absorbance at 436 nm for 15 s up to 5 min.

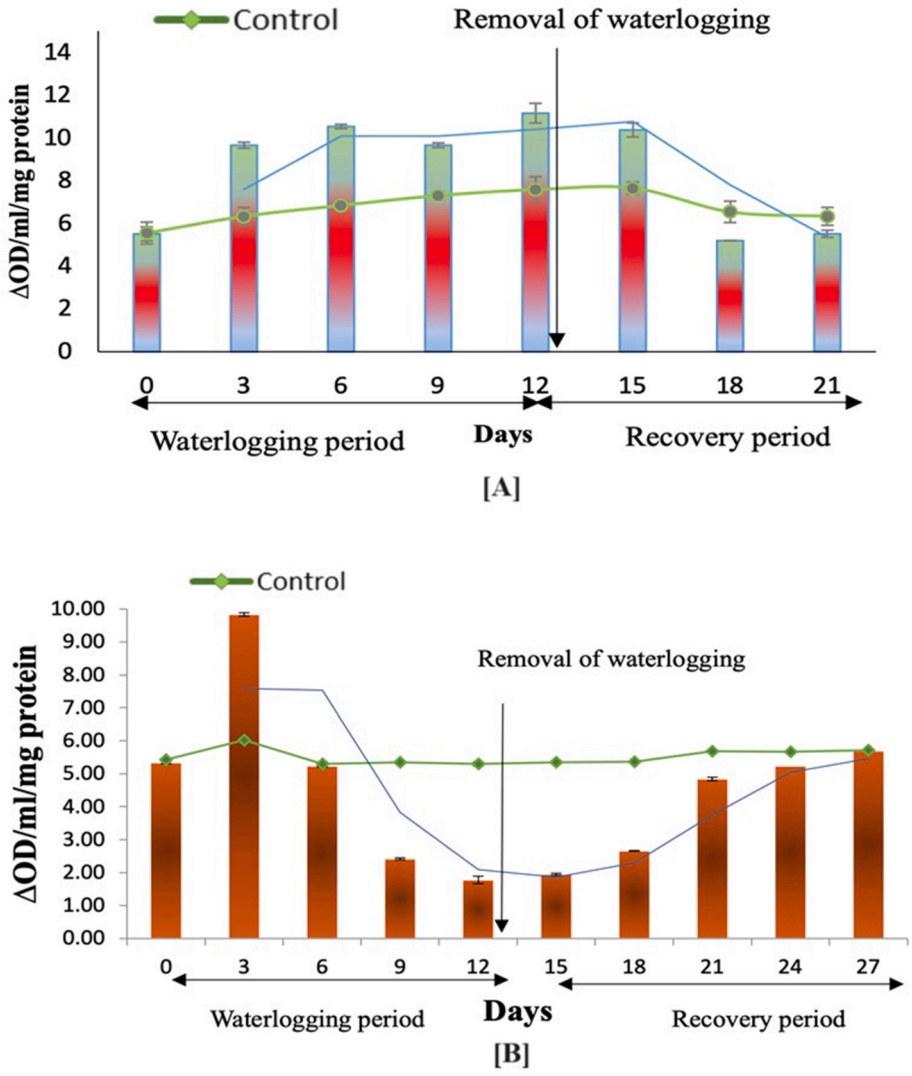

Fig. 3. Guaiacol peroxidase activity in the leaves of *A. hypogaea* [A] DH-86, [B] GJG-32 during waterlogging and recovery periods.

The reaction mixture contained 1 ml guaiacol (1 %) and 1.7 ml phosphate buffer (0.05 M, pH 7.0). The reaction was initiated by adding H_2O_2.

2.3.4 α-amylase assay [31] method was used for assaying the activity of α-amylase.

2.3.5 β-fructofuranosidase assay done by using modified method acid invertase given by Ref. [32]. The procedure involved in enzymatic breakdown of sucrose into glucose and fructose and estimating the quantity of glucose [32].

2.3.6 Acid phosphatase assay, the acid phosphatase activity was assayed by using *p*-nitrophenyl phosphate as substrate [33].

2.3.7 Starch estimation **t**he starch was measured by the method described by Ref. [34].

2.3.8 Proline estimation proline content was measured by the method given by Ref. [35].

2.3.9 Reducing sugar estimation**,** reducing sugars were calculated according to the method of [36].

2.3.10 Chlorophyll content measurement**,** the fresh leaves of all treated and control plants (300 mg) were collected. The chlorophyll content was measured using the given formula-

Chlorophyll *a* (in mg/g) = $[12.7xA_{663} - 2.69xA_{645}] \times V/1000 \times W$

Chlorophyll *b* (in mg/g) = $[22.9xA_{645} - 4.86xA_{663}] \times V/1000 \times W$

Chlorophyll $a + b$ (in mg/g) = $[8.02 \times A_{663} + 20.20xA_{645}] \times V/1000 \times W$

Where V = volume of the extract (ml); W = Weight of fresh leaves (g) [34].

In this study, statistical analysis was conducted to assess the significance of measurements using ANOVA, followed by a Tukey HSD test ($p = 0.05$), utilizing SPSS software (version 22.0). The figures presented only include measurements that had a significant value of $p \leq 0.05$. To create an unbiased color code, the values were normalized and scaled between 1 and 100, with a color scheme of blue indicating high values (100 %), yellow indicating medium values (50 %), and green indicating low values (1 %) used to generate the heat map. The correlation between the parameters was analyzed using a grid correlation matrix and expressed using a color code between +1 and −1 by using python software.

3. Results

3.1. Enzyme activities

3.1.1. Superoxide dismutase

In DH-86, the SOD enzyme activity showed a little elevation during the first phase of waterlogging and continued until the sixth day of the waterlogged period. On the ninth day, there was a noticeable increase in

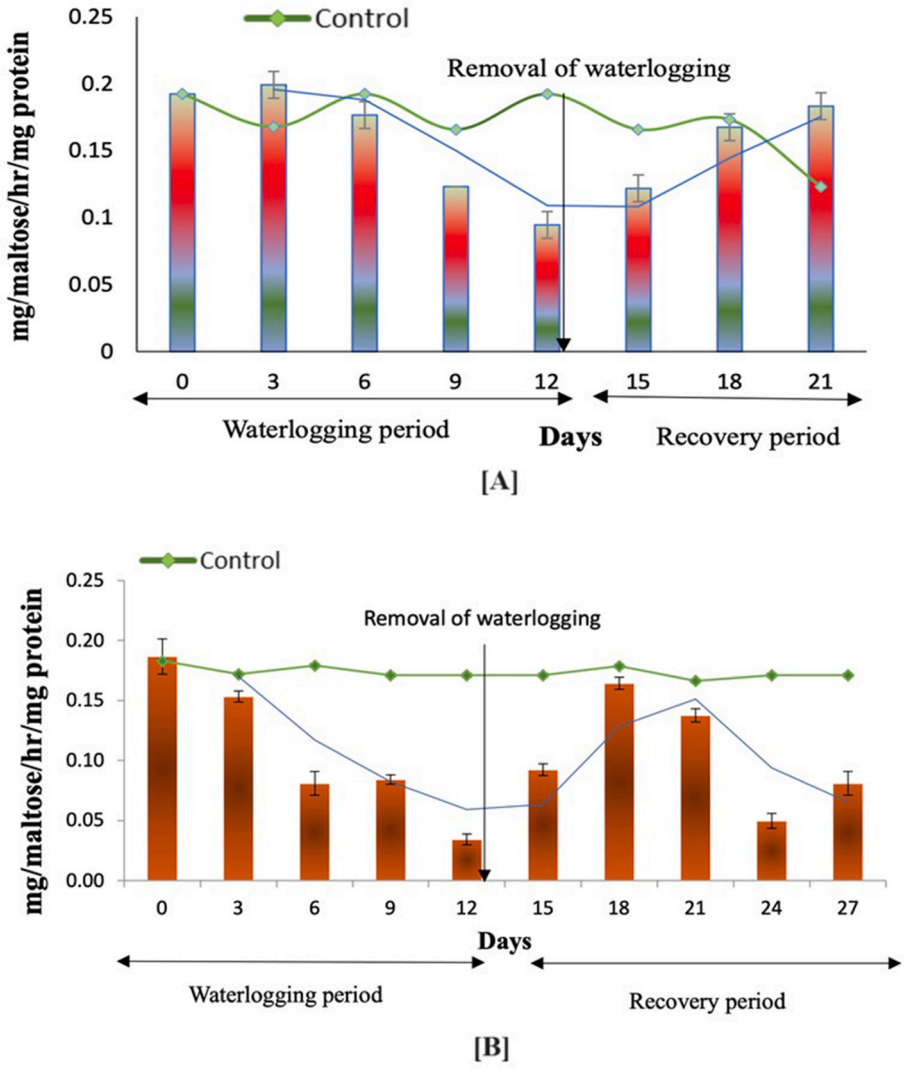

Fig. 4. α-amylase activity in the leaves of *A. hypogaea* [A] DH-86, [B] GJG-32 during waterlogging and recovery periods.

SOD enzyme activity, nevertheless, and this peak was maintained until the twelfth day of waterlogging. In GJG-32, the SOD gene expression peaked after six days of waterlogging and then started to decline until the twelfth day. Upon the termination of the waterlogging stress, the SOD enzyme activity demonstrated no significant alteration within the first 03 days in DH-86. Notably, on the 18th day following the removal of waterlogging, the enzyme activity underwent a complete recovery, attaining levels comparable to the control group in DH-86. But, in GJG-32 upon the removal of waterlogging treatment, there was no significant change in SOD enzyme activity up to 21 days in GJG-32. After subsequently, there was a slight increase in SOD expression that continued in GJG-32 until day 27. The plant's capacity to modify its antioxidant systems is demonstrated by the observed recovery in SOD activity throughout the post-waterlogging phase which demonstrates in Fig. 1.

3.1.2. Catalase

CAT activity in *A. hypogaea* DH-86 leaves showed a gradual enhancement during chronic waterlogging, peaking on day 12. CAT activity in GJG-32, however, showed a roughly twofold rise in response to waterlogging therapy after three days, and by the sixth day, it had returned to normal. On the twelfth day of waterlogging in GJG-32, however, a further decline in enzyme activity was noted. Unexpectedly, after the waterlogging stress was removed, the enzyme expression returned to baseline in just three days. The CAT enzyme expression did not change during this recovery phase, indicating a quick and effective return of DH-86's normal enzymatic activity denoted in Fig. 2 [A]. Up to 21 days following the removal of the waterlogging stress in GJG-32, CAT activity showed no discernible modifications denoted in Fig. 2 [B]. After three more days, there was a noticeable increase in CAT activity, and this elevated level persisted until the 27th day.

3.1.3. Guaiacol peroxidase

In the DH-86 genotype, GPOD activity increased quickly and modestly within the first three days of the waterlogging treatment and continued until the twelfth day. The leaves of the treated plants in GJG-32 showed significant alterations following the commencement of the waterlogging treatment. On the other hand, after the waterlogging ended, the enzyme activity was reasonably constant for the first three days. Additionally, a significant and substantial drop in GPOD activity was identified in DH-86 on the 18th day post-removal of waterlogging stress. Despite this, the enzyme activity continuing to rise steadily and gradually, peaking in GJG-32 on day 27 as shown in Fig. 3.

3.1.4. α-amylase

In the early days after waterlogging, α-amylase expression showed consistent stability at the control level, remaining constant until the 6th day in DH-86. The expression of α-amylase was thereafter gradually down regulated, and this continued until the twelfth day of constant

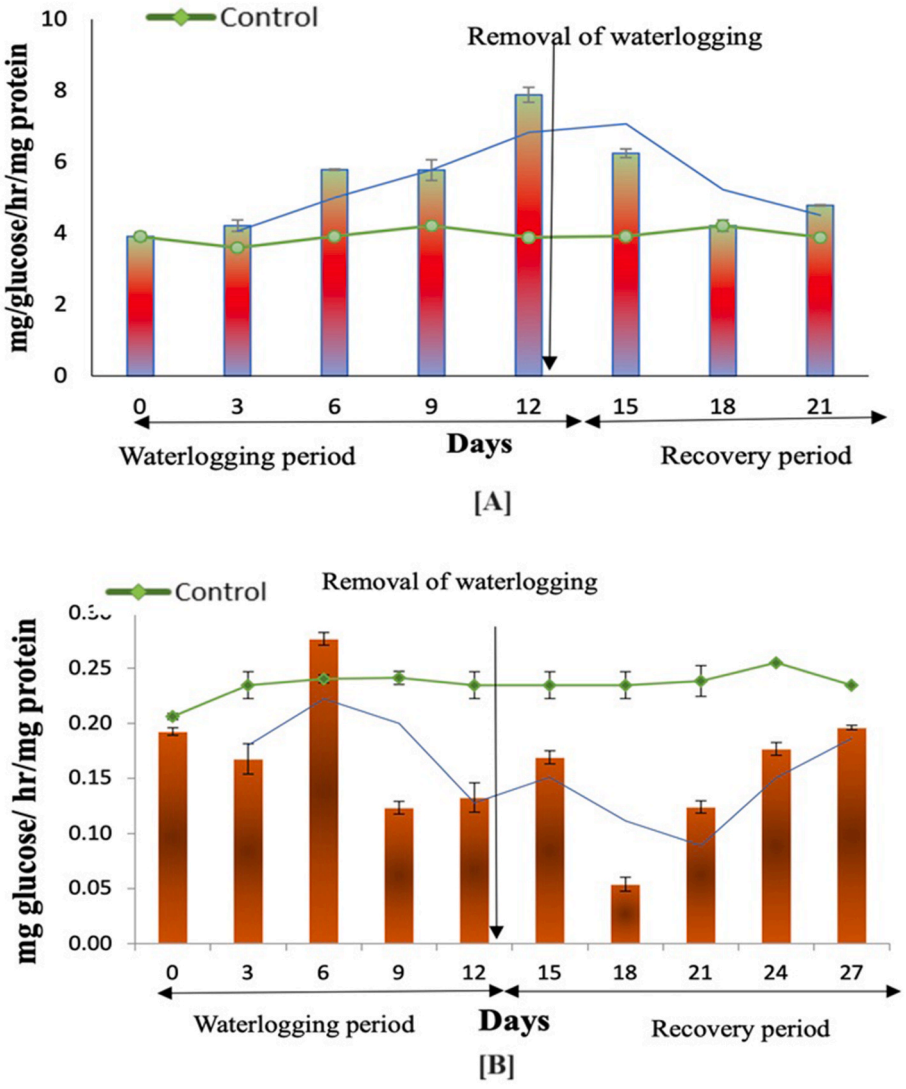

Fig. 5. Invertase activity in the leaves of *A. hypogaea* [A] DH-86, [B] GJG-32 during waterlogging and recovery periods.

waterlogging.

During the waterlogging treatment, there was a consistent decrease in the α-amylase enzyme activity in GJG-32, which peaked on the 12th day. After the waterlogging stress was removed, the α-amylase activity showed a steady increase that required 21 days in DH-86 to reach levels similar to the control group. On the other hand, there was a noticeable recovery in α-amylase activity, which peaked on day 18 in GJG-32. Subsequently, the downregulation of enzyme expression indicates a complicated regulatory response to waterlogging, as it continues even beyond the 15-day recovery period both genotype DH-86 and GJG-32 activity shown in Fig. 4A and B, respectively.

3.1.5. Invertase

During the first 12 days of waterlogging, there were only slight variations in the invertase activity. However, after the 12th day, there was a significant increase in the invertase activity in DH-86, indicating a change in dynamics. During the initial six days of the recovery period, there was a slight reduction in invertase activity, which suggested a progressive adaptation during the early stages of recovery. Interestingly, on day 21, there was a noticeable increase in invertase activity, approaching levels similar to those in the control group. In contrast, in GJG-32, the first stage of waterlogging stress results in a slight decrease in invertase activity on day three. After six days of continuous waterlogging, there is a noticeable increase in enzyme activity that reaches its peak. After that, on day 12, when the waterlogging was more intense, there was a noticeable drop in enzyme activity. Up to the 27th day, GJG 32 post-stress recovery was typified by an ongoing rise in enzyme activity. Even with this increasing trend, the enzyme activity did not reach the control levels, indicating that the plant's physiological functions were still being affected by the waterlogging stress DH-86 and GJG-32 activity shown in Fig. 5A and B, respectively.

3.1.6. Acid phosphomonoesterase

After three days of waterlogging in DH-86, there was a noticeable increase in acid phosphomonoesterase activity, which peaked on the ninth day shown in Fig. 6 [A]. However, after 12 days of constant waterlogging, there was a noticeable decrease in the production of the enzymes. The decreased activity of acid phosphomonoesterase did not change during the recovery phase following the end of waterlogging, which further raises the possibility of long-term regulatory changes in DH-86 shown in Fig. 6 [B]. During waterlogging treatment, a systematic decrease in enzyme activity was noticed in *A. hypogaea* genotype GJG-32. The lowest enzymatic activity was reached on the twelfth day of continuous waterlogging stress. The expression of the enzymes was gradually upregulated over the course of the recovery phase. Although it did not approach the control level, the peak of enzyme activity was

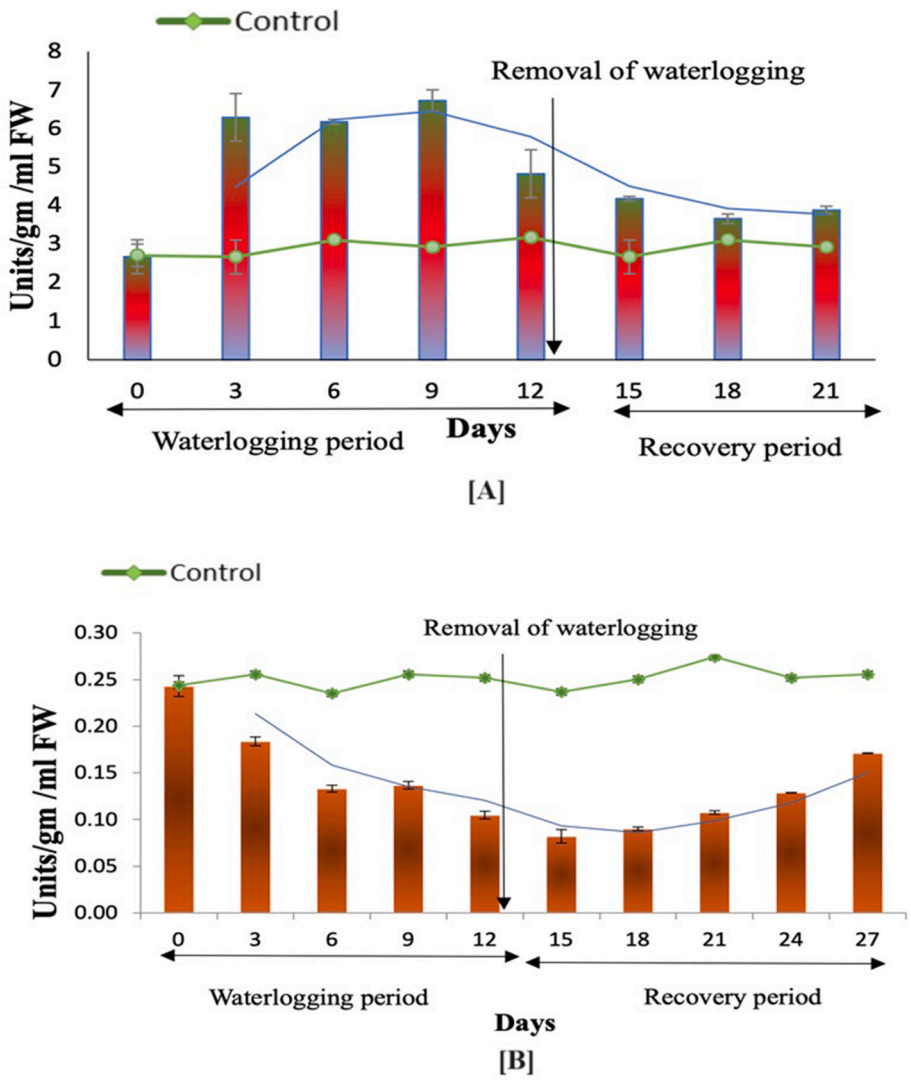

Fig. 6. Acid phosphomonoasterase activity in the leaves of *A. hypogaea* [A] DH-86, [B] GJG-32 during waterlogging and recovery periods.

reached on the 27th day.

3.2. Metabolites concentrations

3.2.1. Starch content

The analysis of the starch content in *A. hypogaea*, DH-86 leaves showed no discernible variations during the periods of waterlogging or post waterlogging periods present in Fig. 7 [A]. On the ninth day of waterlogging, the highest concentration of starch was seen in the DH-86, where a clear pattern had developed. Even after this high, further examination revealed little changes in starch content, which ultimately decreased and return to control levels. The starch content in the GJG-32 genotype decreased gradually, and achieved minimal level during 12 days of continuous waterlogging shown in Fig. 7 [B]. The leaves had very low starch content when the waterlogging ceased, but after 21 days, they showed a remarkable recovery, with their starch levels rising to their peak.

3.2.2. Proline content

Proline content in the DH-86 genotype displayed only minor alterations until the ninth day after waterlogging treatment. On the 12th day, however, there was a noticeable increase in the proline concentration, indicating a strong reaction to extreme waterlogging stress. After being waterlogged for 12 days, the leaves' proline concentration showed an initial increase, rapidly decreased after 18 days, and then stabilised at a steady level upon being returned to normal conditions. However, even in severe conditions, the imposition of waterlogging stress in GJG-32 did not result in a significant change in the proline content of the plants. After, the waterlogging stress was removed for six days; there was a noticeable drop in the proline content. This decrease remained constant for the next twelve days both genotype DH-86 and GJG-32 graphically expressed the proline level in Fig. 8A and B, respectively.

3.2.3. Reducing sugar

A. hypogaea DH-86 leaves showed a significant reduction in sugar content when subjected to waterlogging stress. Before the first nine days of the waterlogging treatment, there were only minor changes in the decreasing sugar content. On the other hand, DH-86 showed a marked decrease in its lowering sugar content on day twelve. This decrease continued for the next three days even after the waterlogging therapy was stopped. After this first decline, the sugar content was gradually increased, reaching high levels by the 21st day after waterlogging in DH-86. A little reduction in the amount of reducing sugar was seen in GJG-32 following three days of waterlogging treatment. This decrease accelerated, reaching about half of the starting levels after 06 days, and then stayed at that level. For the full 27 days of the experiments, when the waterlogging was removed, no appreciable changes in the decreasing sugar content were seen. Graphically present in Fig. 9A and

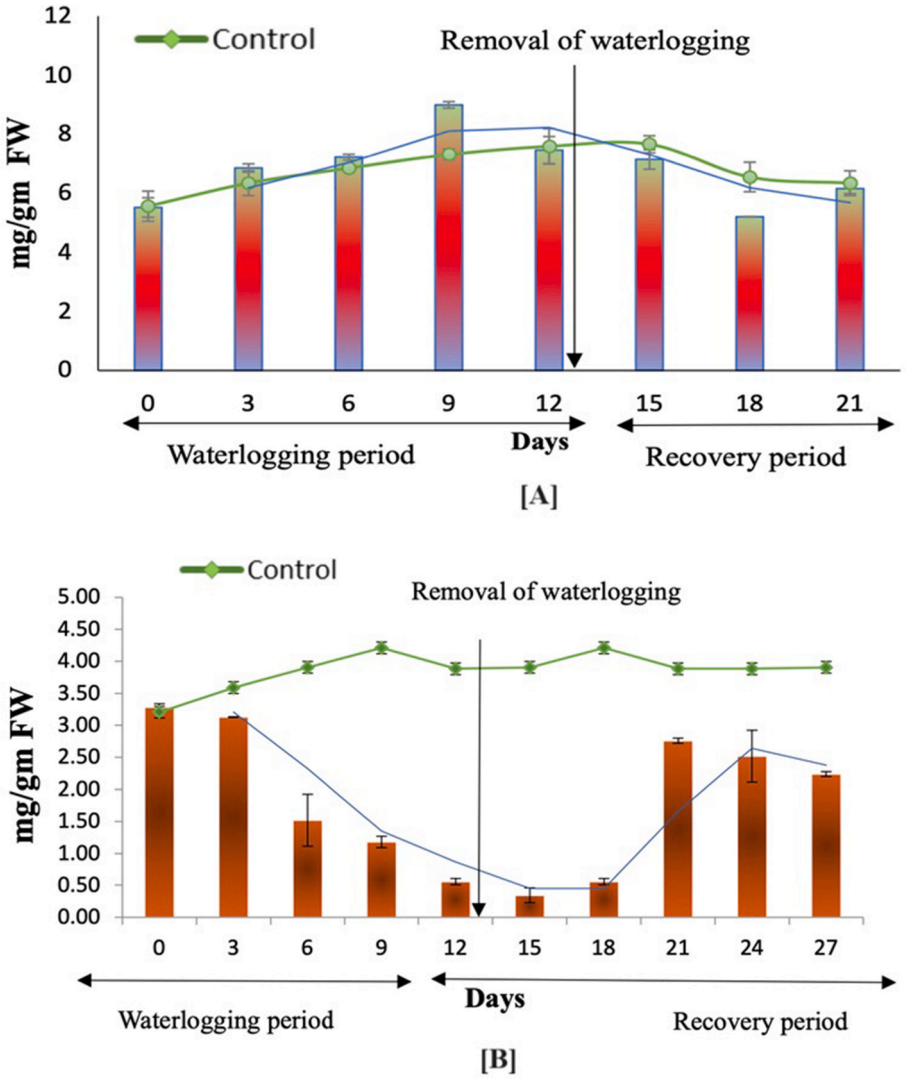

Fig. 7. Starch content in the leaves of *A. hypogaea* [A] DH-86, [B] GJG-32 during waterlogging and recovery periods.

B, for both genotype DH-86 and GJG-32 respectively.

3.2.4. Chlorophyll content

A. hypogaea genotypes DH-86 and GJG-32 exhibit negative effects on chlorophyll concentration on their leaves when subjected to waterlogging stress, shown in Fig. 10 A&B, respectively. The amount of chlorophyll was gradually declining, and this trend continued until the twelfth day of the waterlogging treatment. But in recovery phase a rise in chlorophyll content through day 21 was reported, eventually reaching levels similar to the control in DH-86.

All the parameters have inter relation which present in correlation matrix graph in Fig. 11.

4. Discussion

Waterlogging is a major problem that impacts agricultural production and growth in low-lying rainfed environments. Biochemical reaction of ROS scavenging enzyme has been present in Fig. 12. Due to oxygen's low solubility and slow diffusion rate in water, plants are subject to a reduction in their oxygen supply during waterlogging or submergence [37]. The waterlogging induces a lack of oxygen, which causes the energy metabolism to switch from aerobic to anaerobic. Plant development and productivity are significantly reduced when there is either an oxygen deficit (hypoxia) or an oxygen shortage (anoxia) brought on by waterlogging. Prolonged waterlogging causes a change in the soil's microbial ecology that favours anaerobic microorganisms that utilized other electron acceptors besides oxygen. Extended periods of water accumulation cause the soil accumulate more reduced and phytotoxic forms of mineral ions, such as ferrous (from ferric) and nitrite (from nitrate) ions, to which few plants are adapted for growth [35]. Different plant species and even cultivars react differently to waterlogging. Consequently, further research is needed to determine the molecular, biochemical, and physiological processes that offer plants enhanced floods and anaerobic tolerance.

The present investigation revealed the changes in the expression of various antioxidative enzymes (superoxide dis mutase, catalase and guaiacol peroxidase), cellular enzymes (α-amylase, invertase, and acid phosphomonoasterase) and cellular metabolites (proline, reducing sugars, starch, and chlorophyll contents) under waterlogging. The roles of these enzymes in plant defence were also described by interaction of between the enzymes and other cellular metabolites.

Plants can rely on antioxidant enzyme systems and other active antioxidants to maintain the dynamic equilibrium of ROS under waterlogging stress, hence minimising the degree of oxidative damage.

Comparing DH-86, a waterlogged sensitive variety of *A.hypogaea*, GJG-32, revealed a notable increase in SOD activity. As the waterlogging stress was removed, on the other hand, DH-86 recovered rapidly as compared to GJG-32. Plant's ability to effectively scavenge reactive

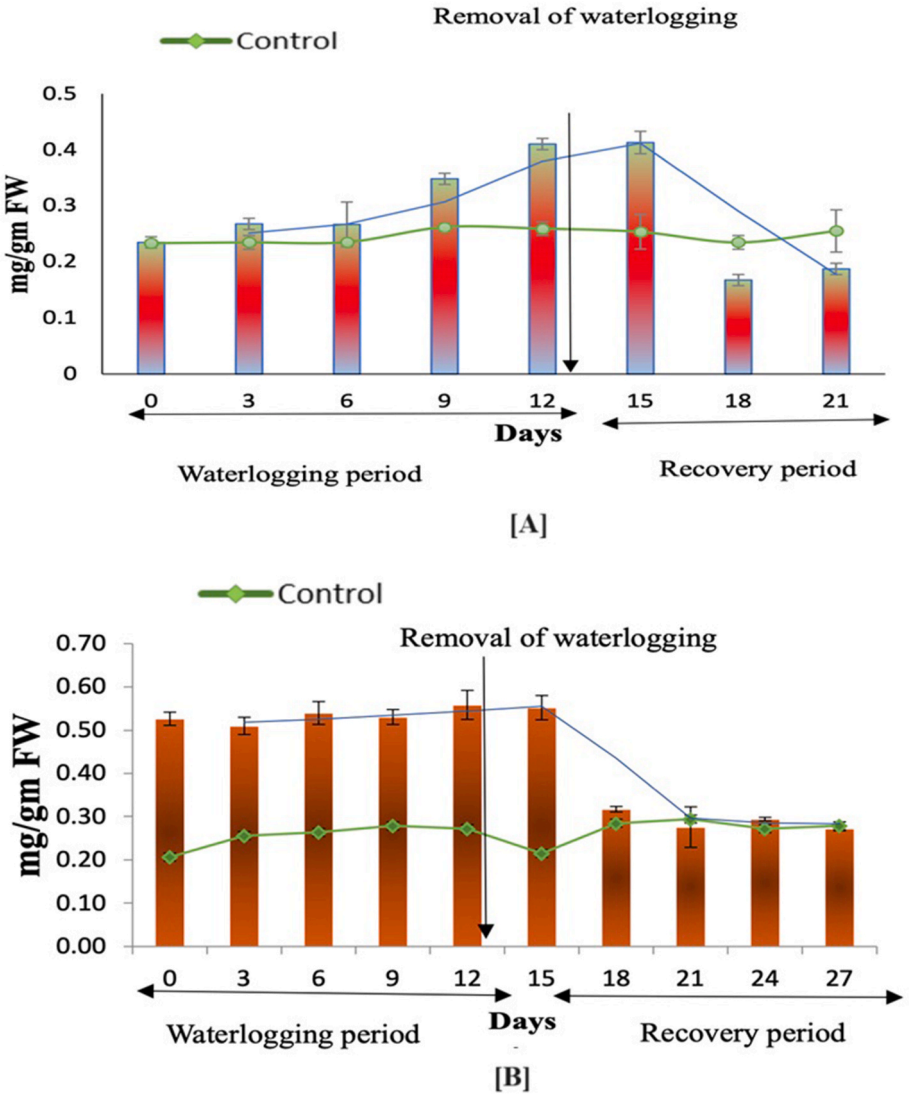

Fig. 8. Proline content in the leaves of *A. hypogaea* [A] DH-86, [B] GJG-32 during waterlogging and recovery periods.

oxygen species is demonstrated by the production of the SOD enzyme both under and after stressful situations. Tobacco, lucerne, potato, and cotton transgenics have all been shown to exhibit SOD-mediated stress tolerance [41–45]. Similar results with lucerne were studied by the researcher [42,44]. *A. hypogaea* plants with the DH-86 genotype exhibit SOD-mediated waterlogging tolerance, as evidenced by the over expression and restoration of SOD activity throughout the waterlogging and recovery phases, respectively.

Two antioxidant enzymes, CAT and GPOD, assist in the detoxification of H2O2. During the waterlogging phase, DH-86 showed an increase in CAT and GPOD activities. This increased enzyme activity allows peanut plants to manage the accumulated H_2O_2 under waterlogging conditions. Similarly, after the waterlogging stress was removed, DH-86 quickly restored its enzymatic activity in comparison to GJG-32. The overexpression of CAT and GPOD indicates the existence of an effective metabolic detoxification mechanism for H_2O_2 and validates the occurrence of waterlogging tolerance in *A. hypogaea* genotype DH-86. Similarly, maize genotypes that could withstand waterlogging stress showed increased SOD, POD, and CAT activities [36]. Similarly, it was shown that the *Sorghum bicolor* waterlogging-tolerant lines over expressed SOD and CAT activities [37].

Exposure to various environmental conditions greatly alters the biochemistry linked to the metabolism of carbohydrates in plants. Many plant species have undergone extensive research on the biochemistry of glucose metabolism in hypoxic or anaerobic environments developed by waterlogging. Additional adaptive features include the provision of energy through alcoholic fermentation and the breakdown of stored carbohydrates during waterlogging-induced anoxia, which is generated by α-amylase activity [48,49]. In the current research, the DH-86 genotype of peanut plant showed an initial upregulation of α-amylase, which was subsequently lowered under waterlogging stress. This suggests that the genotypes were capable of breaking down stored starch under waterlogged conditions. In DH-86, a rise or minor fall in α-amylase activity signifies the ability to withstand waterlogging. Waterlogging tolerance capacity in *A. hypogaea* DH-86 genotype is also indicated by the quick recovery of enzyme activity during the post-waterlogging period.

According to [50], acidic/alkaline invertases catalyse the hydrolysis of sucrose to produce glucose and fructose for ATP production. The ability of the DH-86 variety of peanuts to produce precursors for ATP biosynthesis under waterlogged conditions was demonstrated by their progressive elevation of invertase activity throughout the waterlogging stress. Invertase enzyme expression in GJG-32 was decreased by extreme waterlogging. To counteract the negative effects of the waterlogging stress in DH-86, glucose and fructose are produced, which is reflected in the induction of invertase activity.

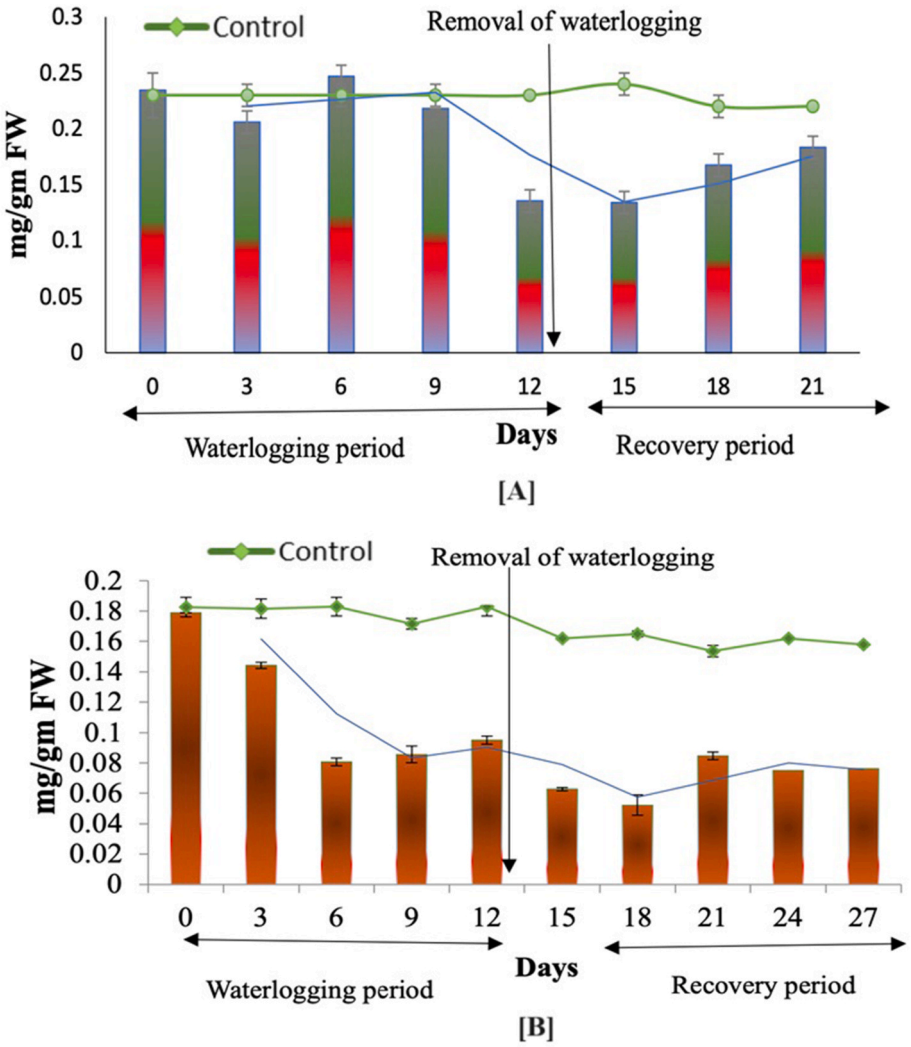

Fig. 9. Reducing sugar content in the leaves of *A. hypogaea* [A] DH-86, [B] GJG-32 during waterlogging and recovery periods.

Maintaining intracellular phosphate (Pi) equilibrium and activity is crucial for cell development and metabolism. Acid phosphomonoesterase is known to respond to stress by ensuring that plant cells maintain a specific concentration of inorganic phosphate [38–40]. According to multiple studies, genes encoding phosphatases play a key role in a variety of cellular signalling pathways that control the regulation of ion channels in guard cells, abiotic stress responses, abscisic acid functions, morphogenesis, and light-responsive transcription [54–58].

One of the most important markers of the plant's response to Pi deprivation is the induction of ATPase activity [59–61]. In the current investigations, waterlogging of the DH-86 and GJG-32 plants resulted in the activation of acid phosphomonoesterase. The findings are in line with earlier studies [62–65]. that showed that when plants are exposed to abiotic stressors, their acquisition and utilisation of Pi are reduced and their acid phosphomonoesterase activity is increased.

Plants that accumulate soluble sugar are able to withstand waterlogging. It is commonly recognized that starch has negative associations with both α-amylase activity and soluble sugars, while soluble sugars have high positive connections with both. Even if α-amylase enzyme expression is sufficiently high, the increase in starch content indicates that *A. hypogaea* DH-86 cultivars are resistant to waterlogging. The GJG-32 genotype of peanut plants is sensitive to waterlogging, as seen by the steady decrease in starch concentration that occurs as the duration of waterlogging increases.

Proline is an imino amino acid, act as an osmolyte and signalling molecule in plants exposed to environmental stressors [66–67]. Proline content in the roots of rice, bananas, and peaches increased dramatically under waterlogging conditions [68–69]. Proline also accumulated in the waterlogged plants of DH-86 and GJG-32 in the current investigations. Three factors—loss of feedback inhibition, inhibition of xylem transfer, and stimulation of synthesis—all contribute to the synthesis of proline in response to waterlogging [69–70]. The primary pigments that capture light, chlorophylls, are thought to be among the key biological indicators of environmental stressors. When plants are exposed to any kind of biotic or abiotic stress, the concentration of chlorophyll molecules is significantly changed [71–78]. Waterlogging in the current investigations resulted in a minor decrease in chlorophyll in DH-86 and a significant reduction in GJG-32. The preservation of chlorophyll homeostasis in DH-86 versus GJG-32 indicates that DH-86 peanut plant cultivators are resistant to waterlogging.

5. Conclusion

The study investigated the impact of waterlogging stress on biochemical parameters in different genotypes of peanuts, specifically DH-86 and GJG-32. Waterlogging, an abiotic stressor, significantly affects various physiological processes, including photosynthesis, energy metabolism, antioxidant systems, root respiration, nutrient absorption, plant morphology, and ultimately, pod yield. The results demonstrated distinct responses in the two genotypes under waterlogging conditions.

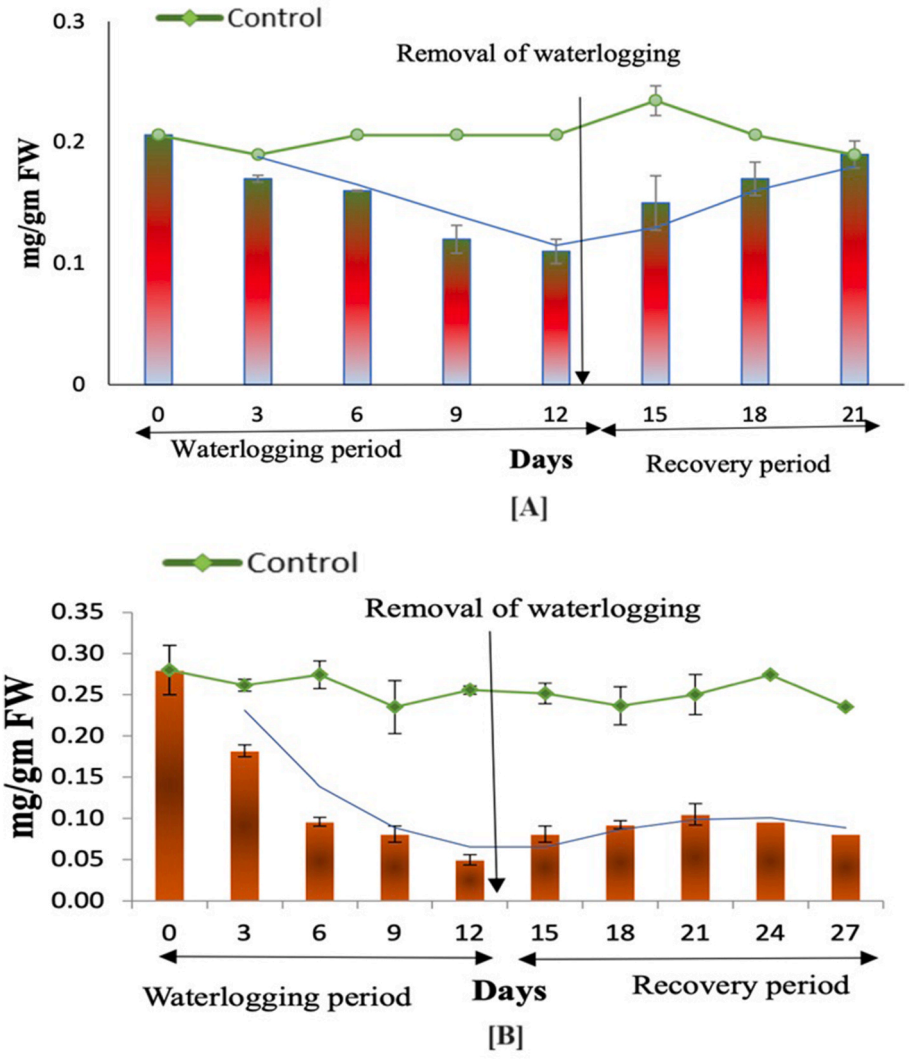

Fig. 10. Chlorophyll content in the leaves of *A. hypogaea* [A] DH-86, [B] GJG-32 during waterlogging and recovery periods.

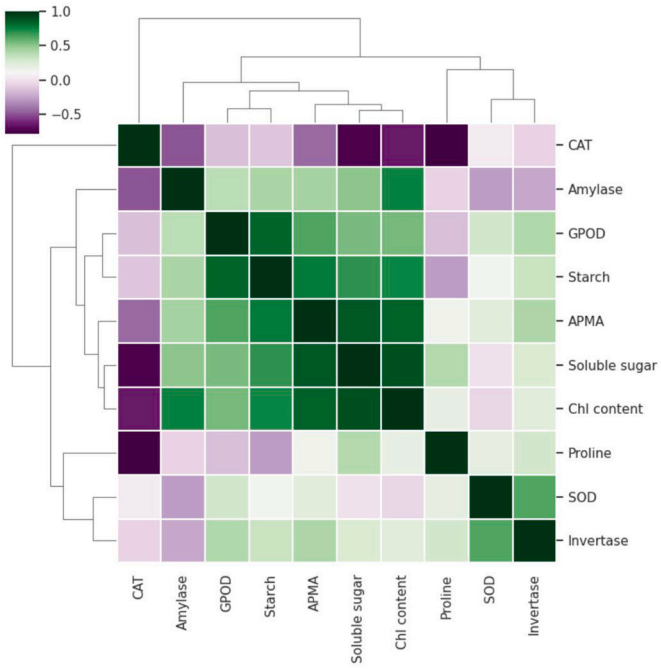

Fig. 11. Heatmap is showing correlation between the biochemical parameters of *A. hypogaea* in [A] DH-86, [B] GJG-32 during waterlogging and recovery periods.

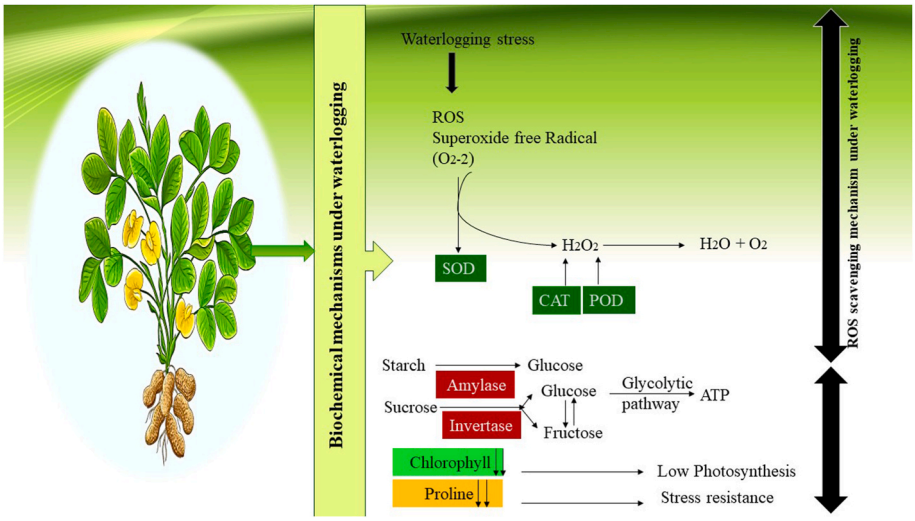

Fig. 12. Enzyme activity present under waterlogging condition.

DH-86 exhibited a more robust ability to cope with waterlogging stress, as evidenced by the modulation of various enzymes and metabolites. The recovery phase in DH-86 showed a rapid return to normal enzymatic activities, highlighting its resilience. Furthermore, DH-86 exhibited adaptive changes in carbohydrate metabolism, with increased activities of α-amylase and invertase under waterlogging stress. The accumulation of proline in both genotypes and the maintenance of chlorophyll content in DH-86 further underscored its adaptive responses to waterlogging stress. In contrast, GJG-32 showed a less efficient response, with variable enzyme activities and metabolite levels. DH-86 demonstrated greater resilience and adaptive mechanisms, indicating its potential as a waterlogging-tolerant cultivar. These findings contribute to our understanding of the complex interactions between genotypes and environmental stressors, paving the way for the development of more resilient peanut varieties with improved waterlogging tolerance for sustainable agriculture. Furthermore, the negative impacts intensified as the duration of the waterlogging stress increased.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors are agreeing for publication.

The datasets generated during the current study are available from the corresponding author on reasonable request.

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CRediT authorship contribution statement

Shubhangani Sharma: Writing – original draft, Methodology, Investigation. **Upma Bhatt:** Writing – review & editing, Investigation. **Garishma shah:** Writing – review & editing, Software. **Vineet Soni:** Validation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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