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PHYSIOLOGICAL AND MOLECULAR ADAPTATION RESPONSE OF SOYBEAN SEEDLINGS UNDER OSMOTIC STRESS

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Abstract

Water is essential for plant growth and development; however, an excessive and lower amount of water negatively affects crop productivity and survival. In natural ecosystems, flash floods may cause the complete submergence of plants in water, which results in the induction of multiple stress tolerance mechanisms. The conditions underwater and the reaction of plants to these conditions are low oxygen, low light, and nutrient deficiency come under the former category of drought stress and are conditions that the plant faces underwater. Production of endogenous hormones and activation of signaling molecules of the glutamate family are the plant responses to the above stress conditions. A high risk of infection is a consequence of being immersed in water. In this study, we aimed to explore soybean's tolerance mechanisms and acclimatization responses to partial and complete submergence and drought at the physiological and molecular levels, which will provide insights into the regulatory networks eliciting tolerance during water stress. The results suggested that upon exposure to the osmotic stress, there is an increase in the concentration of histidine, arginine, proline, and glutamate contents in the complete submergence and drought stress group as compared to the control group. Moreover, the results also suggested that the SA level increases in its 12 hours and then decreases in the next 120 hours. Interestingly the regulation of ABA is the opposite. It increases as it increases with time. An increased width leaf was observed in all study groups except the control group.

Key words: Physio-molecular, ROS, Soybean, Water stress.

Introduction

Climate change is the biggest challenge of the century, which negatively affects all the components of an ecological system (Houghton, 1992; Sala *et al.*, 2000). Moreover, anthropogenic climate change causes various types of stresses, affecting plants' quality and abundance yield. Plants are sessile organisms; therefore, they are always in contact with their surrounding environment and must respond to it to thrive. Globally, various parameters, such as temporal rainfall distribution, evapotranspiration, and sun exposure, directly and indirectly determine the extent of stress (Chalker-Scott, 1999; Ahuja *et al.*, 2010; Blum, 2011). Additionally, frequent weather changes pose a great challenge for the agriculture industry and food security. Abiotic stress is a persistent threat towards the agriculture industry. Among the various abiotic stresses, water stress constitutes an important abiotic stress, which arrests agricultural productivity (Khan *et al.*, 2021; Ozturk *et al.*, 2021). A decline in crop yield and quality is caused by either an abundance of water or a lack of water. Although flooding and drought are contrasting abiotic stresses, both adversely affect agricultural productivity (Kramer, 1963; Eck *et al.*, 1987).

Drought is an important destructive stress which arrests the agricultural yield progressively and threatens food security (Palmer, 1965). Drought stress arises when the rate of water loss becomes greater than that of absorbance by the roots. It mostly occurs in regions of low rainfall. By 2050, it has been estimated that drought will affect more than 50% of the plants. Drought is a multidimensional stress that affects the physiological, morphological, biochemical, and omics status of the plant. It also alters the microbial interactions of the plants and results in compartment-specific restructuring (Bakkenes *et al.*, 2002). The physiological effects of drought stress include reduced leaf area, shoot length, root elongation, and dry and fresh biomass concentrations. Moreover, plants show restricted growth and development under stress. The signs of drought stress include yellowing of leaves, leaf scorch, defoliation of trees, the appearance of bark cracks, and wilting (Keyantash & Dracup, 2002; Panu & Sharma, 2002; Tsakiris *et al.*, 2007; Van Loon, 2015). In general, drought stress causes agronomical yield loss. It has a detrimental effect on the plant at every step, from the germination process all the way to harvesting (Shaffique *et al.*, 2022).

Flooding is a natural stress that adversely affects agricultural productivity. The depth, turbidity, and height of the water column determine the level of stress. Flooding can be categorized into three types (Jackson & Colmer, 2005) water logging, that is, when the water covers only the roots and the plant shifts to anaerobic metabolism (Steffens *et al.*, 2005); partial submergence, that is when water covers the roots and all parts of shoots; complete submergence, that is when the whole plant is immersed in water (Blom *et al.*, 1994; Setter *et al.*, 1997). Many physiological, anatomical, and biochemical responses are triggered in plants in response to the stress of flooding. At the physiological level, it disturbs the water relation, carbon fixation, and stomatal closure and decreases transpiration. Biochemically, submergence stress causes hypoxia, ethylene formation, and a shift toward anaerobic metabolism. Anatomically, it induces the formation of the aerenchyma lacunae (Sullivan & Eastin, 1975; Myers, 1988; Maimaitiyiming *et al.*, 2017). Furthermore, flooding promotes the development of adventitious roots, induction of a new root system, and re-orientation of the root system (Posso *et al.*, 2020; Zhou *et al.*, 2020; Samanta *et al.*, 2021).

The soybean (*Glycine max*) is a widely consumed stable food that is used for its protein, vitamin, and polyphenol content. Moreover, it is the cheapest source of vegetarian protein (Pedersen *et al.*, 2004; Medic *et al.*, 2014), and the consumption of soybeans reduces the risk of various diseases. From the time of germination until it becomes a seedling, it requires a steady supply of water due to its extreme sensitivity to drought. (Wilcox, 2004; Egli & Crafts-Brandner, 2017; Xiong *et al*., 2021). The present study was conducted to evaluate the physiological and molecular response of the soybean under osmotic stress.

Material and Methods

Plant growth under flooding and drought stress: The present study was conducted in the Department of Applied Biosciences, Kyungpook National University, Daegu, Republic of Korea. The soybean seeds (Pungsanamul) were scattered in horticulture soil. The soil was air dried, and soil composition was recorded (Table 1). A polyvinyl chloride (PVC) tube (40 cm long and 2 cm wide) was attached to a funnel and placed in a pot. The tube was placed 7 cm below the seeds, and the funnel was placed above that. Thereafter, uniform irrigation was performed to reduce surface evaporation. After 1 week of germination, uniformly sized and strong shoots were harvested from the soil and put into containers (height: 34 cm, depth: 25 cm). The experiment comprised four groups (NS; no stress, PSS; plants under partial submergence stress, CSS; plants under complete submergence stress, DS; plants under drought stress). The drought stress is caused by holding the water for five days and then plant samples roots, and shoots were collected for further analysis. To evaluate the capacity for reprogramming plant development, we generated flooding for 5 days and then halted the overflowing. The procedure was followed as described by (Liao & Lin, 2001; Loreti *et al*., 2016). The plant samples were collected for the physio morphological characteristics such as root shoot length and biomass as described by (Shaffique *et al*., 2022).

Determination of chlorophyll content: The chlorophyll content of leaves was determined using a CCM-300 Chlorophyll Content Meter (Opti-Sciences, Inc., Hudson, NH, USA) before and after stress as described previously (Gholizadeh *et al*., 2017).

Nutritional assays: The contents of essential macronutrient elements like phosphorus, iron, potassium, and calcium were measured using inductively coupled plasma mass spectrometry (ICP-MS), and those of oxygen, carbon, hydrogen, and nitrogen in the shoots and roots were determined using the elemental analyzer as described by (Polatajko *et al*., 2007; Trujillo-Reyes *et al*., 2014).

Abscisic acid (ABA) content determination: For the determination of ABA content, 0.5 g of dried nitrogen freeze-plant sample was mixed with 50 mL of extract solution (glacial acetic acid: 10%, isopropanol: 95%) and with standard solution of ABA. A rotary evaporator method was used to filter out the solution. The filtrate was mixed with sodium hydroxide solution and washed thrice with methylene chloride (5 mL) to remove lipophilic traces. The pH of the aqueous phase was reduced by using HCl. The extract was then vaporized and re-suspended in a phosphate buffer solution through a PVC column. The buffer solution was once again separated thrice with ethyl acetate. All extractions were combined and put into a rotary evaporator. The sample was methylated with diazomethane to quantify ABA content using GC-MS/SIM apparatus (6890N network gas chromatograph, Agilent Technologies). The entire procedure was followed with slight modification as described by (Xiong *et al*., 2006; Tian *et al*., 2015).

Salicylic acid (SA) content determination: To quantify the SA content, freeze-dried powder of the plant sample was mixed with 90% ethanol, and then centrifuged for 3 min at 15,000 rpm. The obtained pellets were mixed with 5% trichloroacetic acid. The resulting solution was divided into layers using isopropanol/ cyclopentane /acetate (1: 49.5: 49.5, v/v). The outer layer, free of SA, was transferred to a new vial, and the remaining layers were dried with nitrogen gas and mixed with 1 mL of 70% methanol. High-performance liquid chromatography was performed to measure the SA level. Shimadzu fluorescence detector (Shimadzu, Japan), namely, 10 AXL, was used for the measurement of SA content at a flow rate of 1.0 $mL\cdot min^{-1}$. The excitation and emission were detected at 305 nm and 365 nm, respectively. The salicylic acid determination protocol was followed by (Khan *et al*., 2013; Khan *et al*., 2015).

Amino acid quantification: For the quantification of amino acids, the procedure was followed as described by (Khan *et al*., 2020). The amino acids were analyzed using an atomic amino acid analyzer (L-8900, Hitachi, Japan).

Statistical Analysis

The experiments were performed in a randomized fashion and subjected to statistical analysis. Three replicates were considered, with 20 plants in each replicate. GraphPad Prism software (Version 6.01, San Diego, CA, USA) was used for plotting the graphs. The mean and standard error were comparatively analyzed using the Duncan's multiple range test in SAS (V9.1, Cary, NC, USA).

Results

Physiochemical properties of soil: Monitoring soil properties is crucial because the soil contains varying amounts of water, minerals, and organic matter. The fertility of soil depends on the total mineral content, available minerals, and organic matter. The fertility data showed that the soil contained 1.43 $g\cdot kg^{-1}$ total nitrogen, 0.63 $g\cdot kg^{-1}$ total phosphorus, 54.22 $g\cdot kg^{-1}$ total potassium, 25.02 $g\cdot kg^{-1}$ organic matter, and 12.0 $mg\cdot kg^{-1}$ available phosphorus as shown in (Table 1).

Table 1. Characteristics of soil.

Total nitrogen $g\cdot kg^{-1}$	Total phosphorus $g\cdot kg^{-1}$	Total potassium $g\cdot kg^{-1}$	Organic matter $g\cdot kg^{-1}$	Available phosphorus $mg\cdot kg^{-1}$	Available potassium $mg\cdot kg^{-1}$	NO_3^--N $mg\cdot kg^{-1}$	NH_4^+-N $mg\cdot kg^{-1}$
1.43	0.63	54.22	25.02	12.0	149.90	45.22	33.30

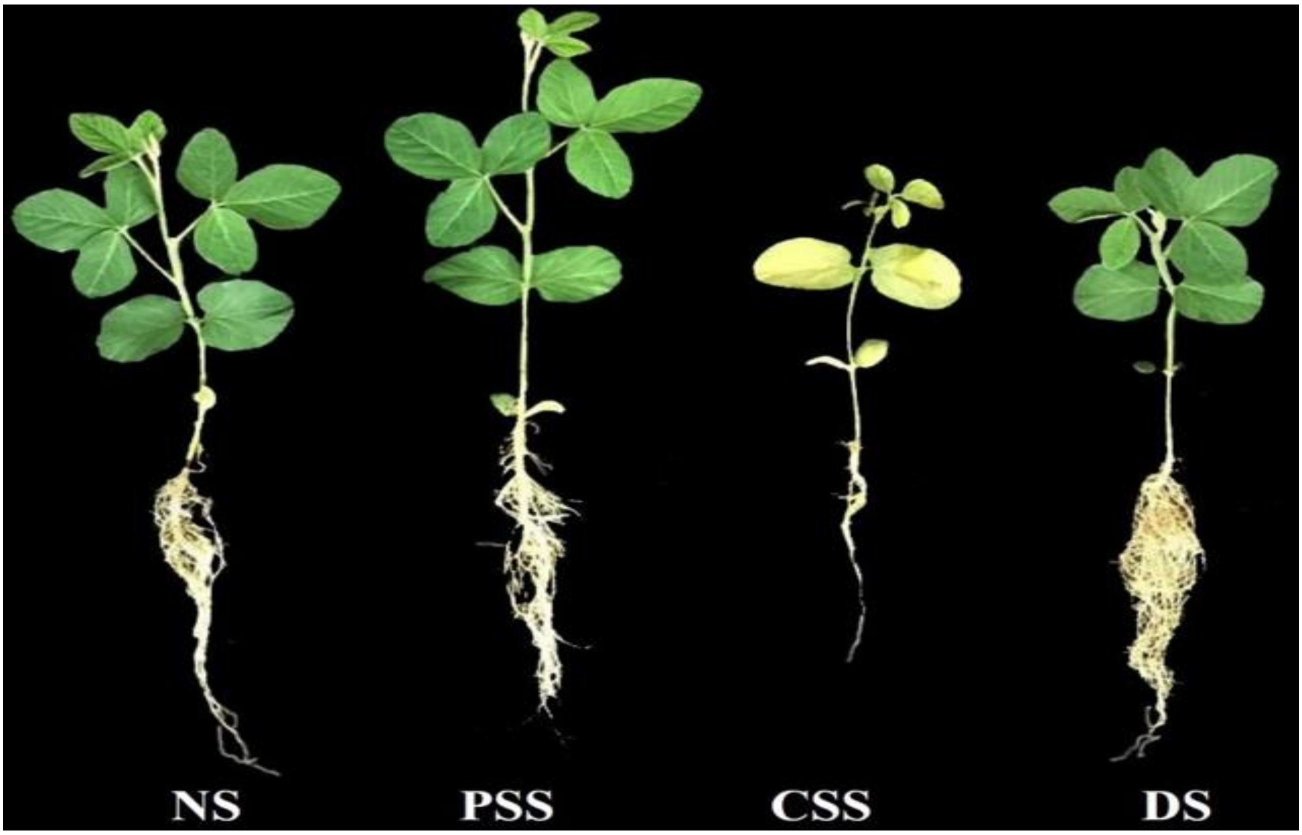

Fig. 1. Physiological characteristics of soybeans under osmotic stress.

Effect of drought and flooding stress on physiological biomarkers, photosynthesis, and chlorophyll content: The shoots respond to partial submergence stress by elongation until they reach above the apparent water level (Voesenek *et al*., 2003; Vriezen *et al*., 2003). However, the shoots of most submerged plants did not extend above the water surface. In this study, the shoots elongated rapidly after submergence from 16.3 ± 0.32 cm to 17.9 ± 2.48 cm, which aggregately increased the fresh shoot biomass from 4.2 ± 0.46 g to 4.4 ± 0.90 g.

The submergence tolerance is determined by measuring the ratio of leaf area to leaf width. The increase in leaf area was observed after 120 h of stress. Plants show submergence tolerance by quiescence and through leaf elongation under all stress conditions partial and complete submergence and drought. The results showed an increase in leaf width (2.4250–4.2250 cm) of plants under all stress conditions as shown in (Fig. 1). In partially submerged plants, the chlorophyll innards of the leaves increased at 120 h; however, the increase was not as high as in a non-stressed plant. The chlorophyll content increased from 501.1 to 506.1 mg/m^{2,} but it decreased from 500.5 to 219.8 mg/m^2 when plants underwent complete submergence. The chlorophyll content increased in plants under drought stress (585.6 mg/m^2) compared to that in non-stressed plants (533.6 mg/m^2). The results are given in (Figs. 2 and 3).

Effect of ABA contents on osmotic stress plants: The result shows a significant increase in ABA levels from 640.9437 to 894.0403 ng/g after 24 h of water logging stress. However, the ABA level decreased in the next 48 h (from 698.3388 to 687.3796 ng/g), which implies that the plants may die or may not be able to cope with the stress. Under drought stress, the maximum level of ABA was observed after 48 h, which suggests that ABA was transported from the old leaves to the young leaves. The results are shown in (Fig. 4).

Effect of salicylic acid contents on osmotic stress plants: SA is an important helpful hormone that is secreted under stress in plants and cross-talks with other hormones. SA boosts the glutathione cycle under stress and helps in scavenging free radicals. In this study, under submergence stress, SA levels increased for the first 12 h and subsequently decreased up to 120 h, which suggests that the plants may not survive after 120 h of stress. Under drought stress, SA levels increased drastically and reached a maximum value at 120 h, thus implying maximum resistance against drought stress at 120 h. The results are described in (Fig. 5).

Effects of Glutamate family (arginine, histidine, proline, and glutamate) content on osmotic stress in plants: In the present study, compared with that in the non-stressed plants, there was a significant increase in the amount of signaling molecules to combat the submergence stress (Fig. 6). The analysis of the glutamate family in plants revealed that glutamate, histidine, arginine, and proline levels were 8.8 ± 0.02 mg/g, 4.3 ± 0.01 mg/g, 10.9 ± 0.12 mg/g, and 6.7 ± 0.01 mg/g, respectively, under complete submergence, which are greater than those observed under partial submergence (2.4 ± 0.02, 1.0 ± 0.01, 2.9 ± 0.04, and 3.4 ± 0.01, respectively). Under drought stress, the glutamate (5.7 ± 0.02), histidine (2.5 ± 0.03), arginine (5.8 ± 0.02), and proline (5.2 ± 0.01) levels were significantly increased in shoots.

Effect on nutritional status of soybean under osmotic stress: During the 5 days of water stress, the nutrient uptake was greatly influenced as shown in (Tables 2 and 3) and three. Water stress (drought and submergence) caused a nutritional deficiency in plants, and the levels of carbon, oxygen, and hydrogen were reduced in roots, shoots, and dry biomass. However, the levels of nitrogen and phosphorus were increased, which implies that plant tolerance to stress is related to the increase in nitrogen and phosphorus contents. Thus, there is a strong intermediate coefficient correlation among plants under water stress.

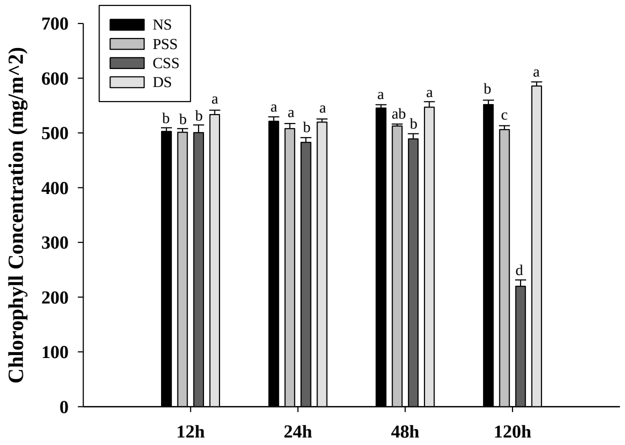

Fig. 2. Chlorophyll contents of the plant under osmotic stress. Each data point is the mean of five replicates. Error bars represent the standard error osmotic stress. Each data point is the mean of five replicates. Error bars represent the standard error.

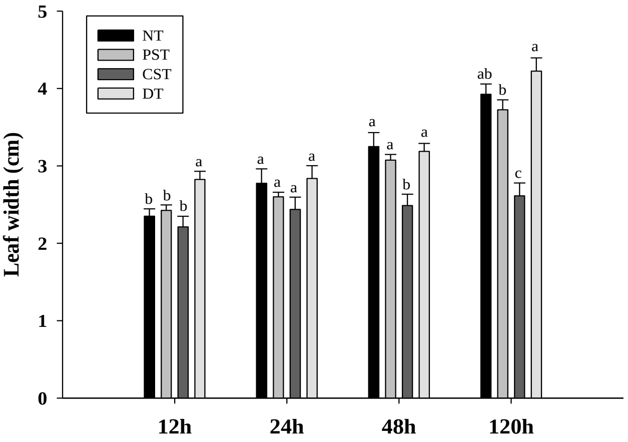

Fig. 3. Leaf width of soybean under osmotic stress. Each data point is the mean of five replicates. Error bars represent the standard error osmotic stress. Each data point is the mean of five replicates. Error bars represent the standard error

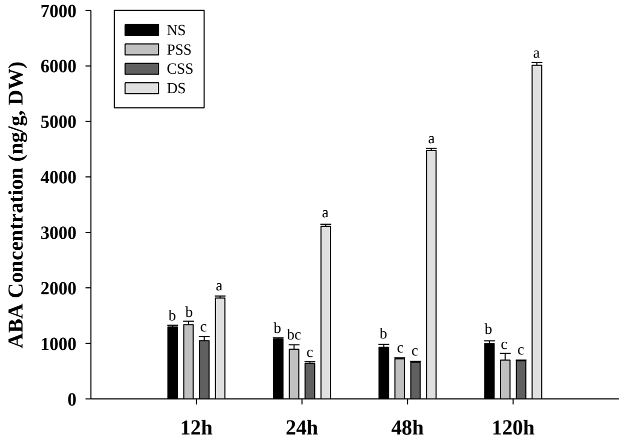

Fig. 4. Abscisic acid concentrations in soybean under osmotic stress. Each data point is the mean of five replicates. Error bars represent the standard error.

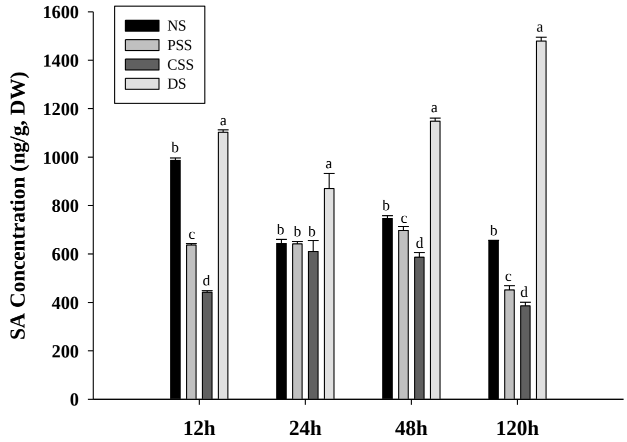

Fig. 5. Salicylic acid concentration in soybeans under osmotic stress. Each data point is the mean of five replicates. Error bars represent the standard error.

Table 2. Effect of osmotic stress on the assimilation of essential macronutrients in soybeans.

Essential macronutrient (%)	NS		PSS		CSS		DS	
	Shoot (cm)	Root (cm)	Shoot (cm)	Root (cm)	Shoot (cm)	Root (cm)	Shoot (cm)	Root (cm)
C	41.6 ± 0.01b	38.2 ± 0.04 a	41.4 ± 0.01 b	38.8 ± 0.02 b	40.2 ± 0.02 a	38.1 ± 0.01a	43.5 ± 0.01 a	41.6 ± 0.02 a
O	43.5 ± 0.01a	40.5 ± 0.01 b	43.9 ± 0.04 a	39.3 ± 0.02 a	37.2 ± 0.01b	37.4 ± 0.03 b	41.7 ± 0.01b	39.5 ± 0.01 b
H	6.0 ± 0.01c	5.3 ± 0.03 c	5.9 ± 0.01 c	5.3 ± 0.01 c	5.6 ± 0.01 c	5.3 ± 0.01 c	6.1 ± 0.02 c	5.5 ± 0.01 c
N	2.4 ± 0.01d	3.0 ± 0.05 d	2.9 ± 0.02 d	2.9 ± 0.01 d	5.5 ± 0.03 d	5.0 ± 0.02 d	3.8 ± 0.01 d	5.0 ± 0.02 d

Each data point is the mean of five replicates. Error bars represent the standard error

Table 3. Effect of osmotic stress on the content of essential micronutrients in soybean shoots and roots. Each data point is the mean of five replicates. Error bars represent the standard error.

Nutrients (mg/g, DW)	NS		PSS		CSS		DS	
	Shoot (cm)	Root (cm)	Shoot (cm)	Root (cm)	Shoot (cm)	Root (cm)	Shoot (cm)	Root (cm)
P	3.5 ± 0.02 b	5.0 ± 0.02 b	4.5 ± 0.02 b	7.5 ± 0.01 a	6.8 ± 0.02 b	7.2 ± 0.01a	4.3 ± 0.01 b	4.0 ± 0.02 b
Ca	6.8 ± 0.02 a	4.3 ± 0.01 c	5.8 ± 0.02 a	4.8 ± 0.01b	11.6 ± 0.04 a	4.8 ± 0.01 c	7.3 ± 0.01a	6.5 ± 0.01 a
Fe	0.5 ± 0.01 d	0.4 ± 0.01d	0.2 ± 0.01d	0.7 ± 0.01 d	0.9 ± 0.01 d	0.5 ± 0.01 d	1.2 ± 0.01d	0.6 ± 0.01 d
Mg	3.1 ± 0.02 c	5.6 ± 0.02 a	2.7 ± 0.01 c	4.5 ± 0.01 c	4.2 ± 0.01 c	5.7 ± 0.01 b	3.7 ± 0.01c	3.8 ± 0.02 c

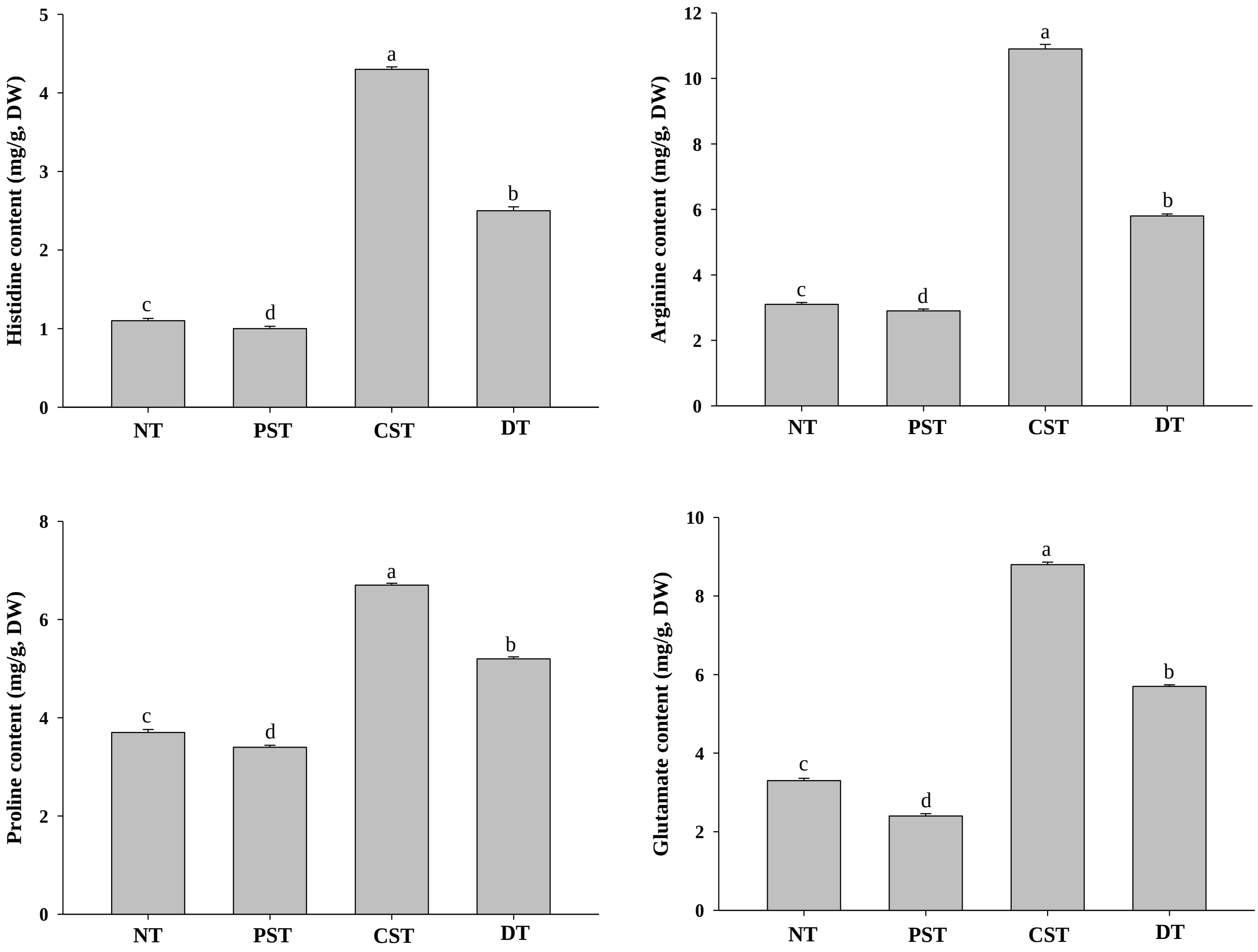

Fig. 6. Response of glutamate family under osmotic stress. Each data point is the mean of five replicates. Error bars represent the standard error.

Discussion

Water is the molecule that is very important for the growth and development of plants, and it plays an important role in agriculture food security. The agriculture industry had faced a dual burden, one is the environmental stress such as flooding, climate change, drought stress etc. and the other one is the overpopulation. It is estimated the world population will increase over 10 billion in 2050 with particular impact on agronomy (Escalona *et al.*, 2002; Zlatev & Lidon, 2012). Water is an important molecule that regulates thermotolerance, photosynthesis as well as acting as a solvent in the transportation of the osmolytes. Water molecules regulate the stomatal opening, and it maintains the photosynthetic process. Photosynthesis is a physiochemical process by which plants utilize the water in the presence of sunlight and transfer it into chemical energy i.e., glucose, starch, proteins etc. (Chartzoulakis *et al.*, 1999; Egli & Bruening, 2004).

Photosynthesis is restricted under water stress due to numerous factors, such as turbidity of water, which greatly condenses sunlight emission intensity, stomatal closure, low absolute absorption, and distribution of carbon dioxide in water (Bohnert & Jensen, 1996; Chaitanya *et al.*, 2003). This might trigger an imbalance between photochemical disruption at PSII and electron supply for photosynthesis, thus causing over-excitation and subsequently inactivating the PSII reaction center (Tezara *et al.*, 1999; Chaves *et al.*, 2002; Ghannoum, 2009). In the present study, no drastic increase in the content of chlorophyll was observed in non-stressed plants. In partially submerged plants, the amount of chlorophyll went up a little bit, showing that photosynthesis is restricted, whereas in completely submerged plants and drought-subjected plants, the chlorophyll content reduced with time till 120 h, which clearly shows the reticence of photosynthesis.

During submergence, photosynthesis is inhibited (Laan & Blom, 1990; Summers *et al.*, 2000) and ethylene production is increased that augments shoot elongation and triggers leaf senescence. Because ethylene gas dispersion is 104-fold slower in solution than in air, the soybean shoots elongate quickly to contact air above the water surface, which causes the exhaustion of carbon dioxide (Naing *et al.*, 2022; Sehar *et al.*, 2022).

The Glutamate family is crucial because it is composed of signaling molecules. Glutamate is a predecessor of various proteins and polypeptides (including glutamine, proline, arginine, and histidine), non-protein amino acid (γ-aminobutyric acid, GABA), antioxidant tripeptide (glutathione, GSH), and chlorophyll (Davenport, 2002; Forde, 2014; Okumoto *et al.*, 2016). The Glutamate family is imperative because of their chemical constancy and metabolic generation (Lam *et al*., 1998; Qiu *et al*., 2020). Consequently, it consists of multifunctional signaling molecules in plants. The high levels of these amino acids suggest that they protect the plants and provide resistance under stress. These molecules also play roles in germination and adaptation (Grenzi *et al*., 2022; Yu *et al*., 2022).

Plants rely heavily on phytohormones to help them endure stressful environments and thrive despite their difficulties. Various recent studies supported that the ABA and salicylic acids are important phytohormones in tolerance of stress and enhancing the defense of the plant (Ma *et al*., 2019; Amaresan *et al*., 2020). The results of this study showed that there is a lot of overlap between the physiological and biochemical responses prompted by flood and drought stress. The main physiological reactions to submergence were low oxygen levels, which were related with alterations in root respiration, stomatal conductance, photosynthesis, and changed metabolic pathways. Water scarcity, on the other hand, causes higher ABA concentrations, which causes stomatal closure and a decrease in photosynthetic rates. ABA is a phytohormone that affects water balance under diverse stress circumstances (Daszkowska-Golec, 2022).

Under water logging stress, ABA content briefly increases in leaves and roots and then decreases gradually. Plants face hypoxia during water logging stress that induces the formation of ethylene, which promotes the production of ABA (Cutler & Krochko, 1999; Raghavendra *et al*., 2010). ABA levels differ in different plant species. When plants are subjected to drought conditions, ABA triggers the closure of their stomata by causing the outflow of potassium ions, decreasing the turgor pressure, and prompting stomatal closure. Closure of stomata alters the status of hydrogen peroxide (H_2O_2) in cells, and H_2O_2 stimulates the release of antioxidant enzymes. These hormones interact under both types of stress. During submergence, the hypoxia in plants increases ethylene production, which downregulates ABA production by impeding the rate-limiting enzymes in ABA biosynthesis and catabolizing ABA into phaseic acid (Daszkowska-Golec and Szarejko, 2013; Arve *et al*., 2014). Roots respond to drought stress by activating a signaling pathway that transmits information from the roots to the shoots through the xylem (and which may or may not be ABA-dependent) (Mukherjee *et al*., 2023; Yang *et al*., 2023).

Recent research suggests that when a plant detects a shortage of water, it responds by reducing leaf development. The buildup of ethylene may counteract the effects of water limitation and ABA accumulation on the regulation of gas exchange and leaf development (Mukherjee *et al*., 2023). Salicylic acid is also an important phytohormone that provides stress tolerance. It improves the plant immunity when under various ecological stress. When a plant is stressed, there is a rise in the formation of reactive oxygen species, which increases the oxidative stress (Jia & Jiang, 2023; Rezayian *et al*., 2023). This hormone is very important as it has anti-inflammatory effects, which minimize the load of the oxidative stress and transducing the defense mechanism (Bagautdinova *et al*., 2022; Lukan & Coll, 2022; Marash *et al*., 2022).

Conclusion

Plants are sessile in nature, so they have evolved mechanistic adaptation to combat the stress during growth and developmental phase. Osmotic stress negatively affects the plant the physiological process specially photosynthesis process. The plant growth is restricted in severe osmotic stress due to impaired stomatal conductance ultimately restricted photosynthesis. In our present study, we assessed how plants react to water deficits throughout their developmental stages. In addition, we identify the factors involved in adaptation and tolerance. In the process of adapting to stress, phytohormones including abscisic acid and salicylic acid, as well as members of the glutamate family, are regarded to be very significant. The stress signaling molecule abscisic acid (ABA) rises in concentration in response to osmotic stress, allowing the plant's intrinsic antioxidant defense mechanism to be better mobilized. ABA and SA act as antagonistic in stress tolerance.

Future prospective

The present study gave us the entire mechanism of the stress tolerance in the model plant soybean. This study also directs how plants cope with stress situations via activation of endogenous phytohormones and signaling molecules (glutamate family).

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