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Seed priming with 28-homo brassinolide attenuates sodium fluoride induced phyto-toxicity in *Pisum sativum* L.

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ABSTRACT

Purpose: Anthropogenic factors have become the dominant cause of environmental pollution particularly salinity in soils resulting in alternation in plant's physiological and biochemical responses. Salinity-induced stress deleteriously affects crop productivity and has damaged about 6 million hectares in Pakistan. Sodium fluoride (NaF) has emerged as a prominent source of salinity stress and its accumulation in crops has become a major concern worldwide. Seed priming has marked its position as a beneficial technique for salt stress attenuation in various crops.

Methods: The current research work was intended to scrutinize the valuable aspect of seed priming with 28-homobrassinolide (HBR) on morphological and biochemical attributes of *Pisum sativum* L. (pea) plants under NaF stress. *P. sativum* seeds were subjected to seed priming with three different concentrations of HBR (1µM, 5µM, and 10µM L^{-1}).

Results: Sodium fluoride-induced toxicity exhibited a significant decline in the growth of pea plants. The results showed that NaF negatively affected total chlorophyll content (25%), stomatal conductance (28%), and rate of transpiration (46%) and photosynthetic rate (28%) as compared to Control. Nevertheless, seed priming with HBR2 enhanced carotenoid content (65%) and consequent improvement in shoot length (64%), and root's dry biomass (59%) of plants as compared to Control plants were ascertained. All three treatments improved the above-mentioned traits but a maximum increment was observed in response to 5µM L^{-1}HBR treatment as compared to the Control. Furthermore, HBR2 improved the gas exchange parameters and proline content (74%) under stress conditions.

Conclusions: Although, plants under saline stress demonstrated a decline in morpho-physiological attributes of pea plants but these deleterious effects of salinity were alleviated by HBR priming of the pea seeds.

Key-words: *28-homobrassinolide, Salt stress, P. sativum, Proline.*

INTRODUCTION

Environmental limitations cause a threat to the productivity of crops and disturb the plant's physiology. Different stresses including salt stress, ionic stress as well as osmotic stress damage the plant's morphology and plant's physiology, thus responsible for alteration in biochemical responses in plants[1]. Salinity is an environmental stress of grave concern and lowers the plant's yield. It has caused a loss of one-third of food output and a perturbation of 20% of irrigated land[2]. The amount of agricultural land damaged by salinity is amplifying globally owing to both natural occurrences and agricultural processes[3]. Salinity has deleteriously impacted the 6.67 Mha area in Pakistan.

Fluoride belongs to the halogen family and is regarded as the most phyto-toxic environmental contaminant which can persevere in the environment for long period even at extremely meager concentrations[4]. Fluoride interacts remarkably with plants and negatively influences their development, productivity, and several physical-biochemical attributes[5]. Weathering of minerals, volcanic ash emissions as well as marine aerosols release fluoride into the environment. Several anthropogenic activities like the application of phosphate fertilizers, detergents, ceramics, and pesticides as well as pollution through industrial operations and fossil burning are considered major contributors to fluoride pollution[6]. One of the major sources of fluoride is biomass burning which releases fine particles spreading over vast areas away from the emitter. This element is absorbed by plant leaves in polluted areas as well as incorporated into plants from polluted soil, particularly one that is contaminated with phosphate fertilizers[7]. Fluoride can accumulate in soil up to a level of 20-1000μg^{-1} and depends on various factors including soil adsorption ability, fluoride portability in soil, pH, and absorbent types present. In soil, the holding of fluoride depends upon natural carbon content as well as pH[8].

Fluoride ion adversely impacts photosynthesis, amino acid, and protein metabolism as well as result in the lowering of chlorophyll levels and other physio-biochemical disorders[9]. Fluoride accumulation in tissues of plants minimizes growth and affects plant development with evident damage signs like leaf tip burn, chlorophyll breakdown, declined biomass, and plant height[10]. Fluoride toxicity causes oxidative stress in plants leading to the production of ROS (reactive oxygen species) namely hydroxide and hydrogen peroxide[11] which disrupts lipid membranes and increases the electrolyte leakage from cells[12].

To achieve stress tolerance as well as mitigation of salt-induced stress, various approaches are adapted from time to time such as genetic engineering, mutation breeding, and polyploidy breeding as well but traditional breeding methods have been limited because of manpower requirements and energy obligations. So, a cost-effective and simple method was required for effective stress mitigation[13]. Priming has marked its position as an important aspect in this regard. This treatment is applied before seed sowing, and involves seed hydration that is adequate for metabolic events to progress before germination onset but arrests radicle emergence. Seeds are treated with different solutions under controlled conditions for a particular duration of time. Different metabolic functions are stimulated improving germination, and seed emergence especially vegetable seeds, small-seeded grasses as well as ornamental species[14]. Seed priming can play a major role in successful crop production. This technique, suggested by Heydecker in 1937, is a cost-effective approach to achieve high seed vigour and uniform emergence leading to improved crop yield[15]. Seed priming is a propitious method for stimulating the germination of seeds and enhancing metabolic processes. This feasible and economically important technique includes beneficial effects such as reduction in fertilizers application, synchronizing seed germination leading to increased crop yield as well as inducing resistance in crops and has been found favorable for many crops including mungbean, wheat, barley, sweet corn, cucumber, and lentil. However, the type of plant species, priming time duration, priming media concentrations, and conditions including temperature affects its performance[16].

Salinity stress can be alleviated via the application of antioxidants or phytoprotectants as priming agents and a number of antioxidants are used in this regard but brassinosteroids are the most promising ones for alleviating salinity-induced stress. Brassinosteroids have contributed to plant development as well as germination of seed, photosynthesis, protein production, cell elongation, photomorphogenesis etc[17]. Brassinosteroids constitute 6th group of hormones that are plant-specific and are acknowledged for improving plant growth as well as plant development. Three major brassinosteroids, 28-homobrassinolide, brassinolide and 24-Epibrassinolide have roles in various aspects. Brassinosteroids have been secluded from *Brassica napus* (rape plant) pollens and 70 various types of correlated compounds have been isolated from plants[18]. Treatment of tomato plant under chromium-induced stress with brassinosteroids showed marked improvement in molecular and physiological pathways[19].28-homobrassinolide administration mitigated the stress induced by salinity and temperature in *Brassica juncea* and inhibited the production of ROS which was elevated in case of stress[20].

Legumes have a great contribution to human nutrition by providing proteins, vitamins, minerals, and calories to consumers. Pea (*Pisum sativum* L.) is well known for its different properties such as being cheap, a major protein source as well as a source of vitamins, minerals, and carbohydrates, and appreciable nutrient density[21]. It shows various anticancer, antifungal, antibacterial, anti-diabetic, anti-inflammatory, and antioxidant activities. It marks 4th position in world food production of legumes after soybean, peanut, and dry bean. A high concentration of phenolic compounds is present in peas which act as strong antioxidants[22]. Salt stress has displayed negative effects on pea plants in numerous study reports[23, 24]. Therefore, the present study aimed to attenuate the detrimental effects induced by Sodium fluoride (NaF) stress via the application of 28-homobrassinolide (HBR) through the seed priming approach. Additionally, the goal of this work was to assess the ability of HBR to positively influence the morphological growth, biomass, and biochemical attributes of pea plants.

MATERIAL AND METHODS

Seed priming and experimental setup

Pea seeds (*Pisum sativum*) were purchased from the local trade market and were subjected to sterilization with 0.5% NaOCl solution for the time duration of 5 minutes and then washed thoroughly with distilled water three times. After that, the sterilized seeds were subjected to seed priming. They were immersed in various concentrations of 28-homobrassinolide (HBR) viz. 1µM, 5µM, and 10µM at room temperature for 4 hours. These treatments were designated as HBR1, HBR2, and HBR3 respectively following the primed seeds were dried on bloating paper for 2 hours. Sandy loamy soil was placed in each of 24 pots organized in Randomized Complete Block Design (RCBD) at the Botanical Garden, University of the Punjab, Lahore, Pakistan. There were three replicates of each treatment, Control, NaF, HBR1, HBR2, HBR3, HBR1+NaF, HBR2+NaF, and HBR3+NaF. Primed seeds were sown in pots labeled HBR1, HBR2, HBR3, HBR1+NaF, HBR2+NaF, and HBR3+NaF. 5 seeds were sowed in every pot at equal distances. Twenty days after sowing, thinning of seedlings was practiced and only one was permitted to grow in the assigned pot. Then, 100mL of 200ppm NaF solution was given to plants labeled NaF two times a week via the soil drench method till harvest. The Control plants were only provided with tap water. Harvest was collected after two months and plants were taken to the laboratory for analyzing different growth and biomass attributes.

Evaluation of morphological growth characteristics

The plants were carefully separated from the soil and washed to remove all sorts of particles from their surface. After bringing them to the laboratory in polythene bags, their different growth features like root, shoot, and leaf length, no. of leaves, area of leaves, and the number of tendrils, nodules, and branches were analyzed.

Estimation of biomass attributes

Roots were segregated from shoots and their fresh weights were recorded with the help of electrical balance (Sartorius GMBH, type 1216MP 6E, Gottingen, Germany). Plants of pea were kept in clean paper bags and then dried in an oven (Wiseven, Model WOF-105, Korea) for a few hours at 70ºC as well as air dried for the evaluation of their dry weights.

Determination of plant photosynthetic pigments

The concentrations of photosynthetic pigments of plants were evaluated including Chlorophyll *a*, Chlorophyll *b*, and total chlorophyll content by the method of Arnon, (1949), and the concentration of carotenoids was determined through the method given by Davis, (1976). Fresh leaves of *Pisum sativum* L. after crushing with pestle and mortar and extraction with 10ml of 80% solution of acetone were stored at low temperatures. Then, at 4ºC, the extract was subjected to centrifugation for five minutes at 10,000 rpm. The optical density was measured with Spectrophotometer (Shimadzu UV-1800) and measurements were taken at 480nm, 645nm, and 663nm.

Estimation of gas exchange parameters

The rate of photosynthesis (A), rate of transpiration (E), and stomatal conductivity (gs) were evaluated by use of IRGA (Infrared gas analyzer) LCA-4 system (ADC, Ltd) device. The reading values for these variables were recorded from completely extended leaves between 10:00 - 11:00 am.

Assessment of proline concentration

Proline concentration was calculated via the use of Bates *et al.* (1973) method. 0.25g of ice chilled leaves samples were vortexed carefully with the addition of 3% sulphosalicylic acid (10mL). After filtration of the mixture via Whatman's no.2 filter paper, 2mL filtrate was combined with glacial acetic acid (2mL) and 2mL of ninhydrin (2.8g of ninhydrin, 72.8mL of acetic acid and 48.16mL of 85% H_3PO_4). The homogenized mixture was kept in a water bath (N.S Engineering Concern, Model XMTG-9000) at 100 ºC. Then, the mixture was cooled after 1h. The mixture was then combined with 4mL of toluene and placed to vortex for 30sec. Chromophore containing toluene was used for aspiration purposes. The solution was then allowed to set for half an hour at 25 ºC. The absorbance was measured at 520nm on a spectrophotometer (Shimadzu UV-1800) and was contrasted with the L-proline standard curve.

Statistical analysis

During the growing season of pea, mean and standard error were calculated using the data from the pot experiment. Means of various treatments were compared by ANOVA through software (IBM-SPSS Statistics version 20) by Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$ significant level. To quantify relationships between the variables, Pearson's Correlation Analysis was used. Rstudio was used to determine the Pearson correlation coefficients and Principal Component Analysis (PCA) between the pea-measured variables.

RESULTS

Morphological growth attributes: The results representing the effect of HBR under NaF stress on different growth attributes of pea are represented in Table 1. Minimum root and shoot length were seen in plants treated with NaF with a decline of 17% and 16%, respectively compared to Control. The highest length of root and shoot was visualized in HBR2 (5µM) with an increase of 49% and 64%, respectively compared to Control. The increment was also seen in leaf length and number in HBR-treated plants with a maximum in HBR2. The area of leaves was significantly decreased by 12% in NaF but the stress-induced reduction was maximally alleviated via HBR priming by 30% in treatment HBR2+NaF compared to NaF-treated pea plants. The number of branches, tendrils, and nodules was also found to be highest at HBR-5µM concentration with an increase of 78%, 80%, and 37% respectively compared to the Control.

Biomass assessment: Table 2 represents the effect of NaF and HBR on the biomass features of pea plants. Fresh weight and dry weight of the shoot was declined by 19% and 10% respectively in NaF-treated pea plants. The HBR2 treatment showed the highest shoot fresh and dry weight with an increase of 86% and 51%, respectively compared to Control. Treatments, NaF+HBR1, NaF+HBR2, and NaF+HBR3 recorded a decline of 28%, 29%, and 31% as compared to HBR1, HBR2, and HBR3 respectively in shoot fresh weight. Roots also showed a decrease in fresh and dry weights by 19% and 29% respectively in NaF treatment. However, these deleterious effects were mitigated via HBR priming and maximum attenuation of stress was achieved in HBR2+NaF with a rise of 70% and 85% in the root's fresh and dry weights respectively as compared to NaF.

Plant photosynthetic pigments: Photosynthetic pigments showed a marked reduction in pea plants via NaF treatment with a decline of 18%, 29%, 25%, and 20% in Chl *a*, Chl *b*, total chlorophyll, and carotenoids, respectively. Chl *a* displayed prominent elevation in treatments with antioxidant HBR with a maximum increase of 75% in HBR2 compared to Control. A maximum increase in Chl *b* and total chlorophyll was also recorded in HBR2 with an increase of 98% and 89%, respectively. HBR increased the carotenoid content in all treatments but maximum increase was recorded in HBR2 with a rise of 65% compared to Control. Treatment with HBR1+NaF recorded a decrease of 30%, 31% in HBR2+NaF and 29% in HBR3+NaF as compared to HBR1, HBR2 and HBR3, respectively.

Gas exchange parameters: The results specify a decline in stomatal conductivity (gs), rate of transpiration (E), and rate of photosynthesis (A) by 28%, 46%, and 28%, respectively under NaF exposure compared to Control plants. Seeds primed with HBR noticeably increased the gas exchange parameters with a maximum increase in HBR2 treatment with an increase of 94%, 83%, and 92% in stomatal conductivity, rate of transpiration, and photosynthesis respectively in comparison with the Control pea plants.

Proline estimation: A marked increase was recorded in NaF treatment with an increase of 29% compared to Control plants. Priming with HBR increased the proline content by 31% in HBR1, 58% in HBR2, and 44% in HBR3 as compared to Control. Other treatments viz. HBR1+NaF, HBR2+NaF and HBR3+NaF observed an increase of 5%, 10% and 4%as compared to HBR1, HBR2 and HBR3, respectively. An increase of 38%, 74%, and 49% was recorded in HBR1+NaF, HBR2+NaF, and HBR3+NaF, respectively as compared to Control.

Correlation between various growth parameters: Pearson correlation demonstrated that the Total chlorophyll content of the plant has a positive correlation with root length, shoot length, number of branches, number of tendrils, number of leaves, area of leaves, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, carotenoids, no. of nodules, stomatal conductivity, rate of photosynthetic activity and rate of transpiration and with proline content in *P. sativum* plants. Figure 4 displays that all morphological and biochemical parameters are in positive correlation with each other.

Principal component analysis: Principal component analysis (PCA) illustrated an interrelation between various growth parameters of *P. sativum* under NaF stress as depicted in figure 4. The total dataset is 95.5 % and data was divided into two portions with Dim1 contributing 89.4% and Dim2 having the contribution of 6.1%. The data mainly showed an interconnection with Dim1 depicting positive values. PC1 variables showed a positive correlation with root dry weight, root fresh weight, shoots fresh weight, transpiration rate, number of branches, no. of nodules, carotenoids concentration, no. of leaves, and proline content in pea plants.

Table 1: Impact of 28-homobrassinolide on morphological parameters of pea under NaF stress

Treatments	Morphological Traits						
	Shoot length (cm)	Root length (cm)	No. of leaves	Area of leaves (cm^2)	No. of Nodules	No. of Branches	No. of Tendrils
Control	31.13±0.67b	11.07±0.34b	43.33±1.45b	8.10±0.23ab	13.33±0.66b	6.33±0.66b	21.33±0.88b
NaF	26.06±1.05a	9.17±0.24a	33±2.31a	7.10±0.79a	8.66±0.88a	4±0.57a	16.33±0.88a
HBR1	36.66±0.56c	13.17±0.17d	57.67±4.09cd	8.94±0.28b	14.67±0.88b	8.33±0.88bc	34.33±1.76e
HBR2	51.33±0.98e	16.6±0.60f	73.67±3.28f	11.96±0.54d	18.33±0.88c	11.33±0.88d	40±1.15f
HBR3	40.83±0.78d	15±0.40e	66.67±2.91ef	10.60±0.50c	16±1.15bc	9.33±0.33c	36.67±1.20ef
HBR1+NaF	30.63±0.48b	11.9±0.20bc	55.67±2.61c	8.52±0.27b	13.66±0.88b	6.67±0.33b	23.33±1.20b
HBR2+NaF	38.2±0.87cd	13.1±0.32d	64.67±0.88d	9.3±0.26b	15.67±0.88bc	7.33±0.33b	30.66±0.88d
HBR3+NaF	33.07±0.97b	12.43±0.29cd	59.67±1.20cd	8.7±0.18b	14.66±0.88b	7±0.57b	27±1.15c

Each treatment is an average of three replicates and ± indicates standard error (SE). Significant differences in the treatments are indicated by non-identical letters at P≤ 0.05. C=Control; NaF= 200ppm NaF; HBR1=1 µM HBR; HBR2=5 µM HBR; HBR3=10µM HBR.

Table 2: Impact of 28-homobrassinolide on biomass assessment of pea under NaF stress.

Treatments	Biomass Attributes			
	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Control	7.82±0.07b	0.15±0.003b	1.05±0.08a	0.06±0.01ab
NaF	6.32±0.02a	0.12±0.009a	0.94±0.03a	0.04±0.009a
HBR1	11.31±0.18e	0.17±0.009b	1.55±0.04d	0.08±0.006bc
HBR2	14.61±0.32g	0.23±0.009d	2.003±0.05f	0.11±0.009c
HBR3	13.12±0.10f	0.2±0.009c	1.84±0.02e	0.09±0.006bc
HBR1+NaF	8.05±0.08b	0.16±0.006b	1.21±0.01b	0.07±0.003b
HBR2+NaF	10.4±0.22d	0.22±0.009cd	1.43±0.01cd	0.08±0.009bc
HBR3+NaF	9.05±0.09c	0.18±0.009b	1.38±0.01c	0.08±0.010bc

Each treatment is an average of three replicates and ± indicates standard error (SE). Significant differences in the treatments are indicated by non-identical letters at P≤ 0.05. C=Control; NaF= 200ppm NaF; HBR1=1 µM HBR; HBR2=5 µM HBR; HBR3=10µM HBR

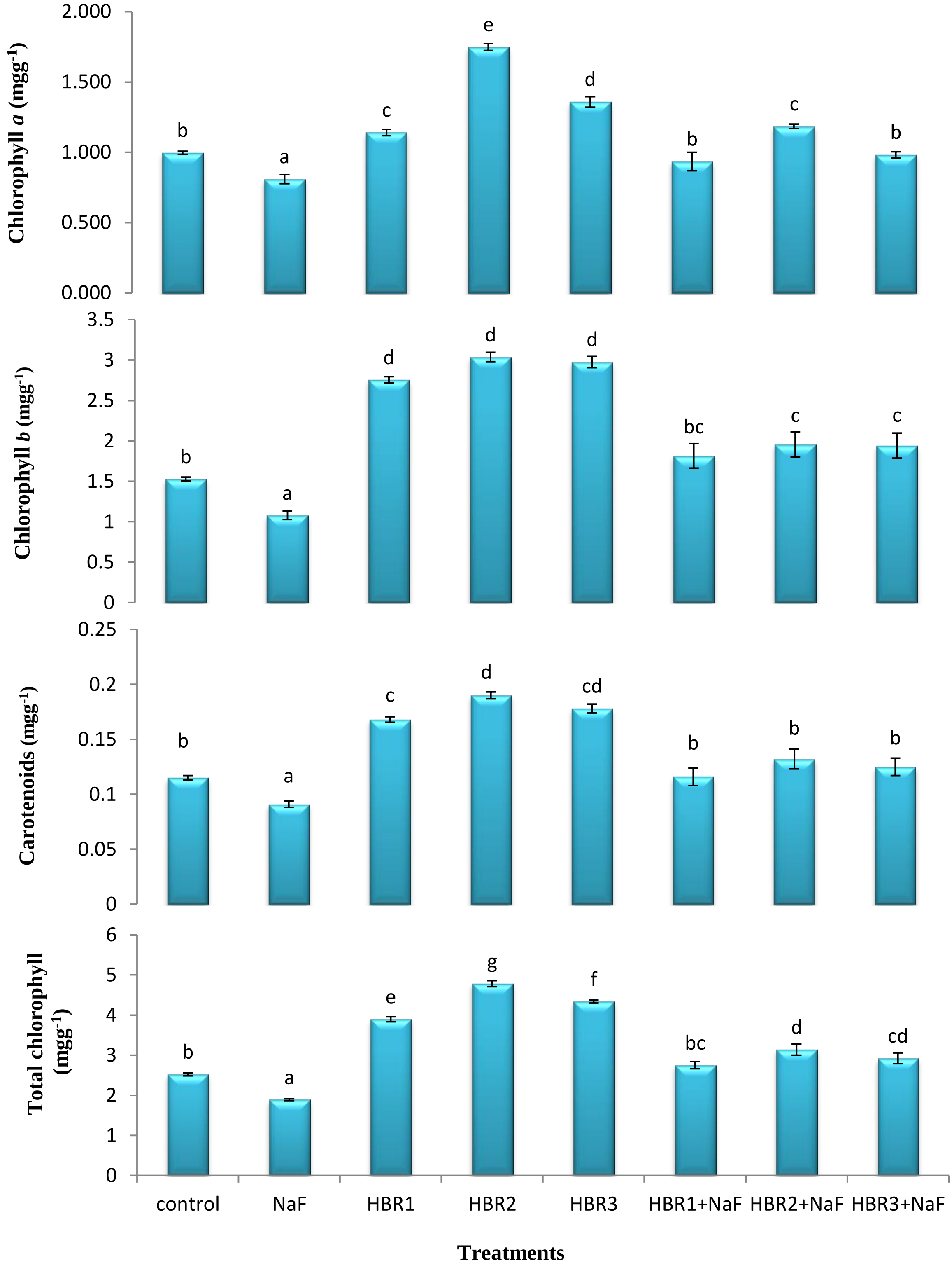


Figure 1: Effect of 28-homobrassinolide and NaF on photosynthetic pigments of pea. Data displays average ± SE of three replicates. Significant differences in the treatments are indicated by non-identical letters at P≤ 0.05. C=Control; NaF= 200ppm NaF; HBR1=1 µM HBR; HBR2=5 µM HBR; HBR3=10µM HBR

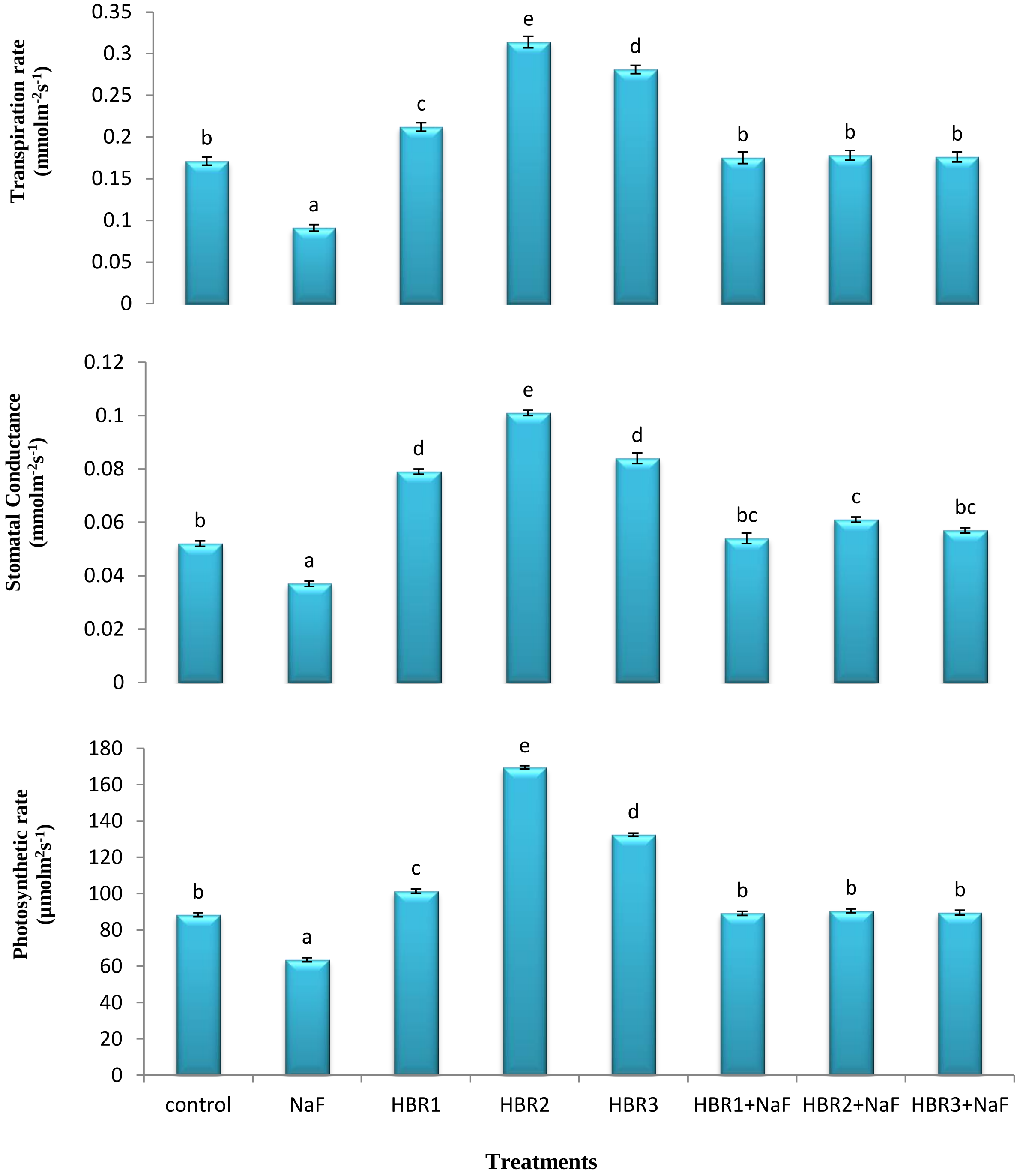


Figure 2: Effect of 28-homobrassinolide and NaF on gas exchange parameters of pea. Data displays average ± SE of three replicates. Significant differences in the treatments are indicated by non-identical letters at P≤ 0.05. C=Control; NaF= 200ppm NaF; HBR1=1 µM HBR; HBR2=5 µM HBR; HBR3=10µM HBR

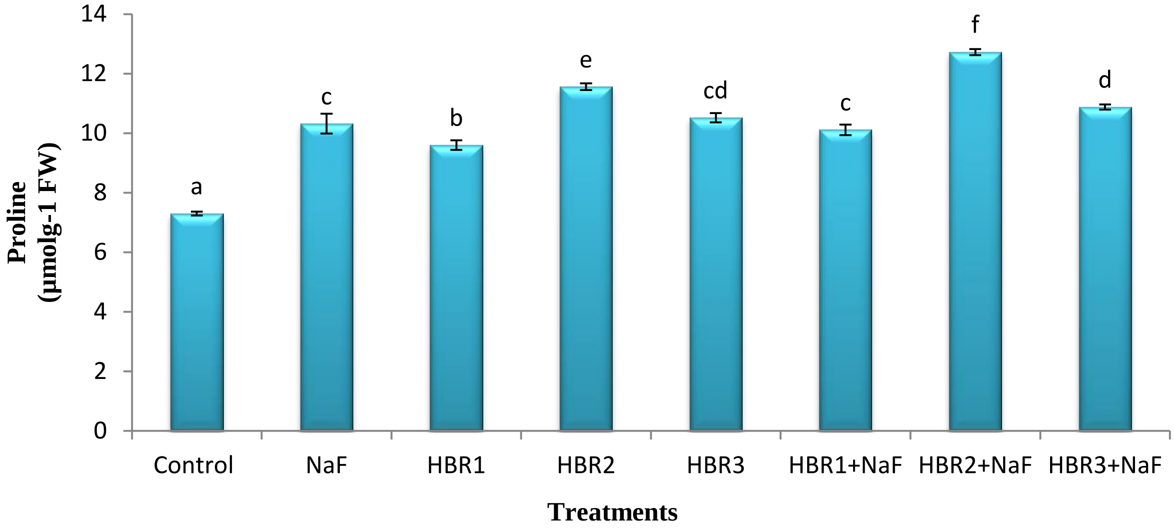
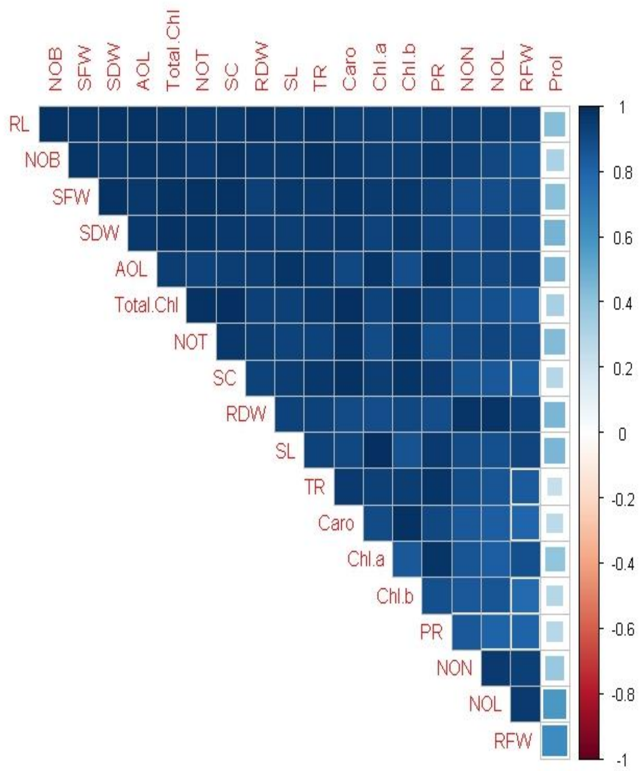


Figure 3: Effect of 28-homobrassinolide and NaF on Proline content of pea. Data displays average ± SE of three replicates. Significant differences in the treatments are indicated by non-identical letters at P≤ 0.05. C=Control; NaF= 200ppm NaF; HBR1=1 µM HBR; HBR2=5 µM HBR; HBR3=10µM HBR.



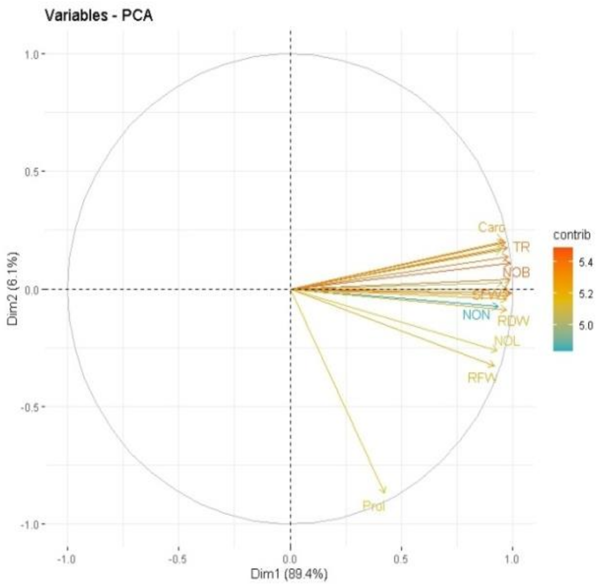


Figure 4: Correlation and principal component analysis of different attributes of *Pisum sativum* L. grown under NaF stress. The abbreviations shown in his figure represent the following: RL (root length), NOB (no. of branches), SFW (shoot fresh weight), SDW (shoot dry weight), AOL (area of leaves), Total.Chl (total chlorophyll), NOT (no. of tendrils), SC (stomatal conductivity), RDW (root dry weight), SL (shoot length), TR (transpiration rate), Caro (carotenoids), Chl.*a*

DISCUSSION

Fluorine present in the earth's crust is marked as the thirteenth most plentiful element. Accumulation of fluorine compounds has contaminated the natural environment and it has arisen as a main factor adversely affecting the growth and development of the plant. The existence of fluorides of various kinds has become a leading cause of stress for plants. Pea (*Pisum sativum* L.) is marked as an important fresh pea as well as dry pulse and has been grown economically for the canning industry[9]. The effect of sodium fluoride-induced stress on pea plant productivity has been evaluated by applying a 200ppm concentration of NaF.

Fluoride has become an area of serious concern owing to its toxicity affecting plant growth[25]. Retardation in seedling growth was recorded in this study which is in accordant with the research work of Dadrwal[26]. Sodium fluoride concentration visualized a prominent reduction in plant's morphological as well as biochemical attributes which is in accordant with the work of Verma[24] who found that plant growth, photosynthetic capacity, biomass productivity, and other physiological as well as biochemical attributes were adversely affected by fluoride application. Similar results were also reported in *Hordeum vulgare* L. and *Cicer arietinum* L.[6] Shankar[27] also studied the negative effects of NaF on *Rauvolfia tetraphylla* L. and found a prominent reduction in shoot and root length as well as biomass attributes. Similar outcomes have also been described in three winter wheat cultivars where sodium fluoride showed negative effects on germination and early plant growth[28].

Mitigation of stress was achieved in the present study with the application of antioxidant which shows consistency with the findings of Sardar[29] who ameliorated the stress in *Coriandrum sativum* L. via the application of karrikinolide. Moreover, seed priming has also proved beneficial in the attenuation of Cd stress in coriander[30]. Priming of seeds with antioxidant enhanced the photosynthesis, growth, and biomass production of developed seedlings. Similar findings were also reported in *Cajanus cajan*[31], *Praecitrullus fistulosus*[32], and *Calotropis procera*[33].

The current study was focused on the application of a brassinosteroid, 28-homobrassinloide which has become center of attraction recently due to particular positive aspects like the stimulation of seed germination, quick seedling vigor, and increase in photosynthetic activity and enzymes level and contribution in stress resistance[34]. The outcome of the present work was found similar to Sridhara[34] and Hayat[35] where plant growth was enhanced in treated conditions. The explanation for the advancement in plant development might be related to increased cell division as well as cell elongation driven by HBR's higher functional metabolism.

Root and shoot length showed a significant reduction due to NaF-induced stress which is in accordance with the results of Chakrabarti[36] who described a significant decline in average root and shoot length with fluoride application. Similar results have also been reported in barley where the deleterious influence of fluoride reduced the overall plant height[37]. This could be due to the imbalanced uptake of nutrients by plants. A decrease in fresh and dry weight was also visualized with NaF-200ppm application. Similar reductions have also been described in cluster bean[38], paddy[39], and mustard[40]. Reduction in metabolic activity is responsible for this decline because fluoride is a metabolic inhibitor. Roots were more responsive to salt stress compared to shoots in terms of dry weight. Dry weight of the root was markedly decreased by salinity as compared to the shoot which was in contrast with the findings of Essa[41] where the shoot dry weight showed a significant reduction than the root dry weight in soybean cultivars due to salinity-induced stress.

Brassinosteroids improved plant resistance to stress and mitigated the harmful effects on growth and development. In current research work, the deleterious impact of NaF on the root, shoot length, and biomass were relieved by priming with HBR. Similar conclusions have also been described by Mehmood[42] where BRs have attenuated the negative repercussions of salinity. Implementation of HBR enhanced the dry and fresh weight of the pea plant. The maximum increase was recorded in 5 µM. Treatments with HBR showed improved fresh and dry weight. These findings are accordant with Shahana[17] who mitigated the drought-induced stress in pigeon pea by application of 28-homobrassinolide and 24-Epibrassinolide. Particular enhancement of growth features in HBR-treated plants could be allocated to the prominent effect of HBR on cell elongation and cell cycle progression.

The current study showed a reduction in the number of leaves which is compatible with the study work of Jangra[43] who found a decline in the leaf number of *Sorghum bicolor* L. due to salt-induced stress. The leaves under salt stress were smaller and the leaf surface area showed a decline as compared to the Control. These findings show similarity with the work of Dhokne[23] who noted a prominent decrease in leaf surface area after the application of salinity stress. The decline in leaf area reduced the net photosynthetic rate and biomass accumulation as well. Leaves of pea plants treated with HBR possessed more surface area which could be a sign of accelerated cell growth and division which is in agreement with Hayat's work[35].

The results of current research work showed a decrease in plant pigments with NaF administration which is accordant with the results of Ahmed[9] who reported a decline in photosynthetic pigments and photosynthetic efficiency of *Pisum sativum* L. Chlorophyll showed a reduction in response to NaF-induced stress. Consistency has also been found in the studies of Ahmed[44] who investigated the impact of NaF on Okra and observed a significant decline in photosynthetic pigments via NaF application. This decline in chlorophyll might be on account of chlorophyll breakdown under salt stress or suppression of chlorophyll biosynthesis, i.e., F-induced chlorosis. Stress caused by NaF reduced the carotenoid content which shows coherence with the work of Sachan and Lal[6]. A study administered by Ram[45] on the growth and development of watermelon seedlings revealed a reduction in Chl *a*, Chl *b*, total chlorophyll, and carotenoids in response to NaF-induced stress.

Treatment with 28-homobrassinolide has alleviated the salinity-induced stress. These findings are in accordance with Basit's results[46] who studied the impact of BRs on the growth and photosynthetic pigments in rice under abiotic stress conditions and found a prominent elevation in chlorophyll *a*, chlorophyll *b*, and total chlorophyll because of antioxidant application. Also, Eleiwa[47] found improvement in yield, yield attributes, crop productivity, and photosynthetic pigments of wheat plants because of BRs application. In addition, BRs perform a significant part in the development of plant and is found to be involved in a number of physiological responses[48]. Stress ameliorative attributes of BRs were revealed in earlier studies by regulation of cell wall loosening enzymes and prevention of loss of photosynthetic pigments caused by salinity stress[49, 50].

The considerable decline was recorded in gaseous exchange parameters of *Pisum sativum* L. due to salinity-induced stress. The rate of photosynthesis, stomatal conductivity, and rate of transpiration depicted substantial reduction in salt-treated plants. These outcomes are accordant with the studies by Dhokne[23] who found a significant decline in gaseous exchange parameters under salinity-induced stress. A decline in photosynthetic rate was on account of the reduction in water potential of leaf, relative water content, and stomatal conductivity and is accordant with findings of many workers like Ali in *Sorghum bicolor* [51] as well as Mohamed in *Brassica*[52]. The decrease in gas exchange parameters due to salinity stress was ameliorated by the treatment with HBR and improvement was recorded in the rate of photosynthesis, rate of transpiration, and stomatal conductivity after priming with antioxidant. These findings are in confirmation with the work outcomes of Sun[53] who depicted the improvement in maize seedlings under drought stress via BRs application. This improvement in gas exchange parameters could be on account of the positive impact of antioxidant on Rubisco activity which is regarded as a key enzyme in photosynthetic fixation. Sodium fluoride stress decreased the number of nodules but this deleterious effect was surmounted by the application of HBR. Nodules are a prominent feature of the leguminaceae family and are involved in nitrogen fixation. An increment in the number of nodules was reported in *Pisum sativum* L.[54] and *Cicer arietinum*[55] with the application of HBR.

Proline is a signaling molecule related to salt stress and is involved in the maintenance of sub-cellular structures and promotes osmotic adjustment. It also controls cell osmoregulation and provides protection to proteins during dehydration. Ahmed[9] reported higher proline content in pea plants under NaF stress which shows consistency with the results of the present study. Proline level is elevated owing to increased proline-rich protein formation under salt stress. Proline is regarded as an amino acid that accumulates in a larger amount in stress conditions and is categorized as a stress marker. Sodium fluoride stress elevates the proline content. Plants from seeds with treatment HBR+NaF showed enhanced proline levels in comparison with control. These findings are consistent with Shahid's work[54] and Geetika[56] who studied the effect of brassinosteroids to attenuate the deleterious impacts of stress conditions.

Sivakumar and Priya[57] studies the impact of salinity and phytoprotectants on black gram and found a notable decline in plant attributes due to salt stress which was alleviated by the implementation of plant growth regulators. In another study, HBR enhanced growth, biomass, chlorophyll concentration, rate of transpiration, stomatal conductivity, and photosynthetic rate in cucumber[44]. Similarly, seed priming with brassinosteroids also improved the performance of soybean under salinity stress[58]. The findings of the present study evaluated that phytohormone, 28-homobrassinolide ameliorated the salinity-induced stress and showed enhancement in the plant's morphological and biochemical attributes.

CONCLUSION

Sodium fluoride stress decreased the morpho-physiological attributes of pea plants and this is a matter of serious concern as Pakistan is an agricultural country and its land soils are polluted with hazardous concentrations of sodium fluoride (NaF) from a number of sources. However, these detrimental effects can be mitigated by the application of HBR. Application of 28-homobrassinolide via seed priming showed improved germination, growth, photosynthetic pigments, and biomass production of pea plants under NaF-induced stress. Additionally, priming with HBR resulted in high dry mass, nodule number, gas exchange parameters, and physiological properties which lead to excellent plant growth and production. The current study advocates the administration of HBR for stress mitigation and growth assurance of *P. sativum* under NaF stress. Further field investigations in this sector can have far-reaching economic repercussions as well as numerous advantages for farmers and relevant stakeholders.

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