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Seed priming with 28-Homobrassinolide enhances growth and physiochemical characteristics of radish under sodium fluoride stress

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ABSTRACT

Purpose: Abiotic stresses harm a plant's growth and biomass production. The use of 28-Homobrassinolide (HBL) helped plants to cope with a variety of abiotic stresses. The current study aimed to assess the seed priming with HBL by reducing the toxicity of Sodium Fluoride (NaF) in radish plants.

Methods: *Raphanus sativus* L. (Radish) under NaF stress decreased plant growth, photosynthetic activity, and chlorophyll pigment production. Radish seedlings were primed with HBL concentrations of 1µM, 5µM, and 10µM1 as HBL1, HBL2, and HBL3, respectively. The 200 ppm NaF was applied using soil drench method. Seeds that had been pre-treated with HBL and non-treated were cultivated in petri plates and pots.

Results: It was observed that HBL2 increased growth attributes including root length (17.18%), shoot length (24.53%), Number of leaves (10.33%), leaf area (8.7%) and biomass production like shoot fresh weight (68.30%), root fresh weight (79.61%), root dry weight (76.1%), and shoot dry weight (14.28%) when compared to control. When compared to HBL2+NaF treatments, HBL 2 increased Chlorophyll *a* chlorophyll *b* total chlorophyll and carotenoids content by 25.9, 23.81 21.74, 42.85 % respectively. Furthermore, HBL2 also enhanced the net photosynthetic rate (26.91%), rate of transpiration (0.11%), and stomatal conductance (47.36%) as compared to control. Proline level was also increased under HBL2+NaF treatment (16.91%) as compared to HBL2 which are not under NaF-stress. The radish seed germination, root growth, and shoot growth were all enhanced by the treatment of HBL. Additionally, priming with HBL treatment improved stomatal conductance (*gs*), net photosynthetic rate (*A*) and rate of transpiration (*E*). Overall, seed primed with 5µMHBL was found to be the most effective method for increasing radish NaF tolerance.

Conclusions: The improved seedlings growth treated with 28-Homobrassinolide, suggested that seed priming with HBL enhance plant tolerance to NaF stress.

Key-words: *Fluorine; Sodium Fluoride; Raphanus sativus.; growth; priming; 28-Homobrassinolide*

INTRODUCTION

Abiotic stresses that inhibit plant growth and reduce crop yield include drought, flooding, high temperatures, low temperatures, salinity, and heavy metals (HM) [1]. Abiotic stresses are estimated to be responsible for more than half of all yield reductions [2]. These stresses disrupt plant growth and development, severely restrict plant distribution, and lower crop yield [3].

Salinity poses a serious threat to the agricultural system by limiting crop development, growth, and productivity [4]. Soil salinity has a direct impact on agricultural productivity [5]. Salinity reduces crop plant production, especially in dry and semi-arid areas. In the 831 Mha total salt-affected area of the globe, saline soils cover 397 Mha, and sodic soils cover 434 Mha. Pakistan's salt-affected area is 4.5 Mha [6]. Salt deposition in soil solutions decreases water and nutrient absorption. Osmotic stress, ion toxicity, nutritional imbalances, and a water shortage result from this [7].

Fluoride (F) is a chemical that exists naturally in the soil, water, and atmosphere of the earth. In several regions of the world, excessive environmental fluoride levels have been related to serious health issues. Fluoride significantly affects how plants behave physically and biochemically, as well as how they grow and produce [8]. The most significant sources of fluoride in agriculture are coal-fired power plants, and particularly fertilizer (superphosphates) made in factories. Even at low ambient fluoride concentrations, plants can undergo a variety of physiological and biochemical changes without any visible negative effects. Fluoride is not a necessary nutrient for plants [9]. In addition to preventing germination, causing ultrastructure malformations, lowering photosynthetic capacity, altering membrane permeability, lowering productivity, and lowering biomass, high levels of F in plants also impair other physiological and biochemical processes. Fluoride is known to cause problems with a variety of physiological processes, including decreased plant development, chlorosis, leaf tip burn, and leaf necrosis. Fluoride is taken up by plant roots and then transported to the surface by xylematic flow [10].

Seed priming produce a physiological state known as a primed state before germination, promoting various cellular responses [11]. Plants are hence best prepared to respond quickly to additional stresses [12]. Early and uniform germination and a general improvement in several growth aspects are characteristics of seedlings developed from primed seeds, which are noticeable in their life span [13]. Under normal conditions as well as diverse biotic and abiotic challenges, seed priming boosted the percentage and rate of germination and improved seedling growth and crop output. Plants can increase their ability to quickly and successfully overcome various stresses through seed priming. As a result, phytohormone seed priming has become a crucial strategy for reducing the impacts of abiotic stress [14].

Phytohormones are those insignificant substances that are structurally unrelated but significantly necessary to control show plastically plants grow in response to environmental triggers by taking adaptive measures in adverse conditions. Endogenous plant hormones are extremely important, according to research analysis, identification, and effective detection and monitoring [15].

A plant growth regulator called brassinolide can be found in nature and helps plants survive in cold climates [16]. Brassinolide is now being synthesized in several high-activity forms, including 24-Epibrassinolide and 28-Homobrassinolide. Along with controlling plant development and growth, brassinolide also helps to shield plants from damaging environmental factors [17,18]. It has been found that treatment with vegetables. Additionally, it is primarily grown in Asian nations brassinolide lowers the undesirable threats caused by membrane lipid peroxidation, boost the activity of protective enzymes, and increases plant drought tolerance [19,20].

The *Raphanus sativus* L. (radish) belongs to the *Raphanus* genus of the *Brassicaceae* family. Worldwide, radish is a very significant biennial root, particularly in China, Japan, and Korea. Radish seeds can also be used to make edible oil, and the seedling and taproot are frequently picked as vegetables [21]. It is an important source of antioxidants and minerals for human diets [22]. Due to its great nutritional content, the tap root of radishes has been eaten all across the world in pickles, salads, and curries. The surface of radish can be white, red, purple, yellow, green, or even black.[23,24]. It has certain significant nutrients in both the roots and leaves that are crucial to the human body [25].

The purpose of our research was how 28-Homobrassinolide, affects morphological growth, biomass assessment, photosynthetic pigments, and gas exchange properties in radish plants under Sodium Fluoride stress.

MATERIAL AND METHODS

Screening of Antioxidants

Seeds of the F1 cultivar of radish (*Raphanus sativus* L.) were purchased from the Roshan Seed Center, Lahore. The radish seeds were sterilized in 0.5% sodium hypochlorite for five minutes, followed by three washes in distilled water. Radish seeds were primed for 24 hours using 28-Homobrassinolide (1µM, 5µM, and 10µM), also known as HBL1, HBL2, and HBL3, and Triacontanol (TRIA) solutions (10µM, 20µM, and 30µM), also known as TRIA1, TRIA2, and TRIA3.After properly washing the triacontanol and 28-Homobrassinolide primed seeds in distilled water, they were allowed to air dry at room temperature. Radish seeds that had not been pretreated served as the control treatment.

Five seeds were placed on three layers of Whatman filter paper (No. 1) in the petri dishes for each treatment. The NaF solution or distilled water on the allocated petri plate was sucked out and replaced as needed with 10mL of fresh solution or distilled water. Filter papers were changed every three days. Three replicates of each treatment were available. Seedlings were carefully taken from petri plates after 15 days to assess various signs of growth and stress. Each treatment fresh root and shoot weight was measured and analyzed. Root and shoot samples were dried in an oven to determine the dry weight. The antioxidants that had the highest growth, biomass, and germination % were evaluated, compared, and chosen for a further pot experiment. As a result of our conclusion that 28-Homobrassinolide performed better in the germination test than Triacontanol so screened it out for the pot experiment.

Experimental Site for Pot Experiment

The pot experiment was carried out at the University of Punjab's Botanical Garden in Lahore (31 30' 17" North, 74 18' 24" East). 28-HBL primed and controlled seeds were uniformly sown in each pot that was allotted to them. The 200ppm NaF treatment was applied as a soil drench twice a week during the experimental work. There were 3 replicates of every treatment. No stress was applied to control and HBL1µM, HBL5µM, and HBL10µM pots but provided with 100ml distilled water till the harvest. In the experiment a randomized complete block design (RCBD) was used with three replicates. To examine their physio-biochemical traits one one-month-old seedlings were carefully removed. At the time of harvest, morphological parameters were obtained.

Measurement of Growth Traits

The seedlings were harvested after 40 days of germination. The seedlings were separated and washed. The roots and shoots fresh weight as well as their length, leaf area, and the number of leaves were all measured. Afterward plant samples were dried in an oven (Wiseven, Model WOF-105, Korea) for 48 hours to determine their dry weight. The leaf area was calculated through Charleton and Foote's (1965) formula:

Evaluation of Gas Exchange Parameters

Using a portable gas-exchange system (LCA-4System ADC, Ltd), the net photosynthetic rate (*A*), rate of transpiration (*Ei*), and stomatal conductance (*gs*) were measured from plant leaves at 9 am [26].

Plant Photosynthetic Pigments

Following Arnon, (1949), the concentrations of the photosynthetic pigments in plants, including chlorophyll *a*, chlorophyll *b*, and total chlorophyll content, were determined. The concentration of carotenoids was determined using Davis, [27]method. Fresh leaves were crushed with a mortar and pestle, extracted with 10 ml of an acetone solution at an acetone concentration of 80%, and then refrigerated. The extract was centrifuged at 4°C for 5 minutes at 10,000 rpm. Using a (Shimadzu UV-1800) Spectrophotometer, the optical density of the supernatant was measured at 480 nm, 645 nm, and 663 nm.

Estimation of Proline

A 0.25g ice-cold sample of plant leaves was taken for proline analysis, and 10 ml of 3% Sulpho-salicylic acid was added and thoroughly vortexed and filtered. Ninhydrin (2.8g), 48.16 ml 85% phosphoric acid, and 72.8 ml acetic acid were combined with 2ml of filtrate. The homogenized mixture was kept in a water bath (N.S Engineering Concern, Model XMTG-9000) at 100°C.To stop the reaction, the mixture was cooled for one hour. The liquid was then violently vortexed for 30 seconds while 4 mL toluene was added to the mixture. The aspiration was done using chromophore-containing toluene. 30 minutes or so were spent keeping the solution at 25 °C. Shimadzu UV-1800 spectrophotometer was used to measure the absorbance at 520 nm, and the results were compared to the L-proline standard curve [28].

Statistical Analysis

Using one-way ANOVA and SPSS software, the collected data was analyzed. Duncan's multiple range test was performed to separate means for significant treatments at $p \leq 0.05$. The shown values are the means of four replications ± SE. With the help of the IBM-SPSS Statistics 20 software, the data were precisely calculated. Furthermore, the R Studio software was used to calculate Pearson Correlation coefficients, heat-map analysis, and principal component analysis between the *Raphanus sativus* measured variables.

RESULTS

Seed priming with 28-Homobrassinolide regulates growth parameters of radish under NaF stress. Additionally, 28-Homobrassinolide-primed seeds exhibited improved gas exchange rates and biochemical attributes.

Screening of Antioxidants: All seedlings had their estimated and computed shoot length, root length, fresh weight of the shoot and root, germination percentage, and growth indexes. The length of the radish roots, the length of the shoots, the quantity of leaves, and the assessment of biomass were all affected by Sodium Fluoride. As indicated in Table 1, as compared to the Tria 2 treatment, radish seeds treated with HBL2 had longer length of root, length of shoot and germination percentage about1.92%, 55.48%, and 55.55%, respectively. Table 1 showed that Sodium Fluoride has significant negative impacts on the fresh and dry weights of root and shoot. When compared to the Tria 2 treatments, radish seedlings treated with HBL2 exhibited increase in fresh weight of root, fresh weight of shoot, dry weight of root, and dry weight of shoot of 22.22%, 4.54%, 33.33%, and 12.5% respectively (Table 2). As 28-Homobrassinolide showed significantly better results described above so it gets screened out for further experiment.

Growth Indexes: For seedlings treated with HBL, the growth indices, such as Seed Vigor Index (SVI), and Seed Vigor Mass, were calculated (SVM). When compared to the control, radish seedlings treated with HBL2 exhibited an increase in the Seed Vigor Index, and seed mass of 27.27%, 64.69%, and 84.13%, respectively (Table 3).

Evaluation of Growth Attributes Traits: Table 4 showed how NaF stress and HBL priming affect the plant root length, shoot length, number of leaves, and leaf area in addition to their fresh and dry weight. The seedlings treated with sodium fluoride had shorter root and shoot lengths, fewer leaves, less leaf area, and lower root and shoot fresh and dry weight than the control plants. The detrimental effects of NaF on development features were lessened by the application of HBL. When compared to the NaF treatment, radish seed primed with HBL2 resulted in seedlings that had lengthened shoots by 1.43 fold, roots by 1.27 fold, the number of leaves by 1.63 fold, the area of the leaves by 1.46 fold, shoot fresh weight by 2.12 fold, and roots by 2.37 fold. Sodium Fluoride stress hindered seed germination. In comparison to controls seedlings facing NaF stress showed reduced fresh and dry weight of roots as well as decreased fresh and dry weight of shoot. Furthermore, HBL2 treatments increased root fresh weight, shoot fresh weight, root dry weight, and shoot dry weight by 79.61%, 68.30%, 76.19%, and 14.28%, respectively in comparison to control conditions. (Table 5). On the other hand, *Raphanus sativus* L. seeds primed with HBL improved growth characteristics and reduced NaF stress.

Estimation of Photosynthetic pigments: In comparison to the control treatment, sodium fluoride dramatically reduced the production of photosynthetic pigments in radish seedlings. Between the HBL3 treatment and the control, there was no discernible difference in the overall amount of photosynthetic content. Figure 1 shows that sodium fluoride stress decreased the amounts of carotenoids (68.75%), chlorophyll *a* (18.18%), chlorophyll *b* (52.15%), and total chlorophyll (40%) compared to control conditions (Figure 2). However, it was shown that treatment of HBL2+ NaF affected the NaF-induced reduction of chlorophyll concentration. The HBL2+NaF treatment increased the content of chlorophyll *a* (7.69%), chlorophyll *b* (23.52%), total chlorophyll (70.37%), and carotenoids (16.67%) in comparison to the control conditions. Compared to HBL2+NaF treatments, HBL 2 increased the levels of chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids by 25.9, 23.81, 21.74, and 42.85%, respectively.

Evaluation of Gas Exchange Parameters: The results showed a prominent reduction in gas exchange characteristics of seedlings under NaF stress in comparison to the control treatment (Figure 3). When compared to the control, Sodium Fluoride treatment alone decreased net photosynthesis (*A*), stomatal conductance (*gs*), and transpiration rate (*E*) by 27.33%, 26.67% and 27.27% respectively. Furthermore, the detrimental impacts of NaF were lessened and the measured gas properties improved when HBL was given to plants that had been exposed to the salt. Nevertheless, HBL 2 overturned this harmful effect and increased photosynthesis (*A*), stomatal conductance (*gs*), and transpiration rate (*E*) by 26.91%, 47.36%, and 0.11% respectively when compared with the control.

Evaluation of Proline Content: According to the findings, 40.75% more proline was being synthesized in NaF-affected radish plants than in control plants. The proline content of the plants raised from primed seeds with HBL under NaF stress increased by 9.42%, 16.91%, and 20.77%, in comparison to the plants raised from HBL1, HBL2, and HBL3, that are not under NaF stress (Figure 4). Less proline content was found in the plants supplemented with HBL and not subjected to NaF stress.

Correlation Between Growth, Physiological Attributes and

Biochemical Parameters in Raphanus sativus: The Pearson correlation analysis was carried out to quantify the relationship between different studied parameters of *Raphanus sativus* grown in control and saline soil (NaF)with or without the application of 28-Homobrassinolide (Figure 5). It showed that all the studied parameters were in significantly positive correlation with each other i.e., Germination percentage, seed vigor mass, seed vigor index, Chlorophyll a, chlorophyll b, total chlorophyll content, carotenoids, proline, leaf area, number of leaves, stomatal conductance, transpiration rate, photosynthesis rate, shoot length, root length, shoot fresh weight, root fresh weight and shoot dry weight.

Heat map Histogram: A heat-map histogram was also illustrated between growth and physio-biochemical attributes of various treatments studied under control and NaF-contaminated soil with and without the seed priming of 28-Homobrassinolide. (Figure 6). Heat map depicts that various parameters have similar trends as compared with correlation analysis.

Principal Component Analysis: *Raphanus sativus* cultivated in control and NaF-contaminated soil with and without seed priming with 28-Homobrassinolide were compared for growth, physiological, and biochemical characteristics using principal component analysis (PCA). In Figure 7, The biggest percentage of all components is found in the entire dataset, which contains 87.2%. Dim1 contributes 80.6% of this whole dataset, and Dim2 contributes 6.6%. It depicts that all the studied parameters were in significantly positive correlation i.e., Germination percentage, seed vigor mass, seed vigor index, Chlorophyll a, chlorophyll b, total chlorophyll content, carotenoids, proline, leaf area, number of leaves, stomatal conductance, transpiration rate, photosynthesis rate, shoot length, root length, shoot fresh weight, root fresh weight and shoot dry weight.

Table 1: Effect of 28-Homobrassinolide and Triacontanol on Seedling Length and Germination Percentage of 15 days Radish seedlings under NaF-stress.

Treatments	**Growth Traits**		
	Shoot length (cm)	**Root length (cm)**	**Germination%**
C	8.2±0.08e	7±0.05b	86.67±6.67ab
NaF	6±0.05a	4.1±0.05a	73.33±6.67a
HBL1	7.26±0.08c	7.8±0.05d	80±11.54a
HBL2	9.36±0.08g	10.6±0.06f	93.33±6.67ab
HBL3	8.90±0.05f	9±0.57g	66.67±6.67ab
HBL 1+NaF	6.96±0.06b	7.60±0.05c	66.67±6.67b
HBL 2+NaF	8.3±0.08e	8.4±0.03e	73.33±6.67ab
HBL 3+NaF	7.9±0.05d	7.7±0.05cd	60±11.54b
Tria1	4.±0.05cd	7.4±0.02c	73.33±6.67ab
Tria2	6.02±0.04g	10.4±0.057f	60±11.54a
Tria3	5.16±0.08f	9.12±0.006f	80±11.54c
Tria 1+NaF	4.2±0.057e	6.4±0.03b	60±11.54a
Tria 2+NaF	4.63±0.12f	8.2±0.0de	73.33±6.67ab
Tria 3+NaF	3.63±0.08c	7.6±0.05c	66.67±6.67a

Each treatment mean is sum of three replicates and ± represents standard error (SE). Non-identical letters specify significant differences among the treatments at p≤0.05. C = control, NaF= 200ppm NaF, HBL 1= 1µM HBL, HBL2= 5µM HBL, HBL 3= 10µM HBL, and Tria1=10 µM, Tria2=20µM and Tria3=30.

Table 2: Effect of 28-Homobrassinolide and Triacontanol on Seedling fresh and dry weight of 15 days Radish seedlings under NaF-stress

Treatments	**Biomass Assessment**			
	Shoot FW (g plant^{-1})	**Root FW (g plant^{-1})**	**Shoot DW (g plant^{-1})**	**Root DW (g plant^{-1})**
C	0.15±0.005b	0.05±0.0009b	0.06±0.0034c	0.005±0.0007bc
NaF	0.11±0.05a	0.03±0.005a	0.04±0.0006a	0.002±0.0006a
HBL1	0.19±0.005a	0.07±0.005c	0.05±0.001ab	0.0005±0.0001a
HBL2	0.23±0.005e	0.11±0.0001d	0.12±0.001c	0.0009±0.0001a
HBL3	0.21.001c	0.08±0.001c	0.06±0.001a	0.0006±0.0005a
HBL1+NaF	0.14±0.005c	0.04±0.005b	0.04±0.001ab	0.0003±0.0001a
HBL2+NaF	0.18±0.003f	0.08±0.004f	0.06±0.004b	0.0006±0.0001a
HBL3+NaF	0.16±0.007df	0.06±0.0036e	0.05±0.005a	0.0004±0.0001a
Tria1	0.17±0.005cd	0.08±0.0006d	0.07±0.006d	0.0006±0.0006bc
Tria2	0.22±0.0006f	0.09±0.0001e	0.09±0.0006f	0.0008±0.0006d
Tria3	0.18±0.003e	0.07±0.0006d	0.08±0.0009g	0.0007±0.006d
Tria 1+NaF	0.15±0.003d	0.05±0.006b	0.06±0.0005c	0.0004±0.0003b
Tria 2+NaF	0.18±0.005de	0.06±0.003c	0.07±0.006e	0.0006±0.0004bc
Tria 3+NaF	0.16±0.00058bc	0.05±0.006b	0.05±0.002b	0.0002±0.0006b

Each treatment mean is sum of three replicates and ± represents standard error (SE). Non-identical letters specify significant differences among the treatments at p≤0.05. C = control, NaF= 200ppm NaF, HBL 1= 1µM HBL, HBL2= 5µM HBL and HBL 3= 10µM HBL and Tria1=10 µM, Tria2=20µM and Tria3=30 µM.

Table 3: Effect of HBL on Growth indexes (Seed vigor Index and Seed Vigor Mass) of 15 days Radish seedlings under NaF stress.

Treatments	Growth Indexes	
	SVI	SVM
C	1116.67±99.7b	4.498±0.40ab
NaF	676±73a	2.91±0.29a
HBL 1	1207±180bc	4.77±0.68ab
HBL 2	1838±131.46d	9.17±0.65c
HBL 3	876±174.46cd	3.57±0.688ab
HBL 1+NaF	876±174.46bc	3.57±0.68ab
HBL 2+NaF	1230±114.3bc	5.525±0.50b
HBL 3+NaF	623.60±336.25ab	2.91±1.25a

Each treatment mean is sum of three replicates and ± represents standard error (SE). Non-identical letters specify significant differences among the treatments at p≤0.05. C = control, NaF= 200ppm NaF, HBL 1= 1µMHBL, HBL2= 5µMHBL, HBL 3= 10µMHBL

Table 4: Effect of HBL on Morphological parameters of radish harvested at 40 DAS under NaF-stress.

Treatments	Growth Traits			
	Shoot FW (g plant^{-1})	Root FW (g plant^{-1})	Shoot DW (g plant^{-1})	Root DW (g plant^{-1})
C	14.2±0.05b	1.03±0.08b	1.89±0.02b	0.21±0.005c
NaF	11.23±0.08a	0.78±0.007a	1.75±0.008a	0.12±0.0058a
HBL1	17.29±0.15d	1.63±0.01c	1.91±0.003b	0.22±0.003cd
HBL2	23.90±0.46f	1.85±0.05d	2.16±0.04c	0.37±0.008f
HBL3	19.25±0.05e	1.71±0.058e	1.93±0.006b	0.25±0.012d
HBL1+NaF	14.84±0.05b	1.16±0.12b	1.86±0.02b	0.16±0.012b
HBL2+NaF	18.90±0.05e	1.48±0.02c	2.14±0.005c	0.31±0.005e
HBL3+NaF	15.4±0.33c	1.15±0.03d	1.75±0.006a	0.17±0.006b

Each treatment mean is sum of three replicates and ± represents standard error (SE). Non-identical letters specify significant differences among the treatments at p≤0.05. C = control, NaF= 200ppm NaF, HBL 1= 1µM HBL, HBL2= 5µM HBL, HBL 3= 10µM HBL

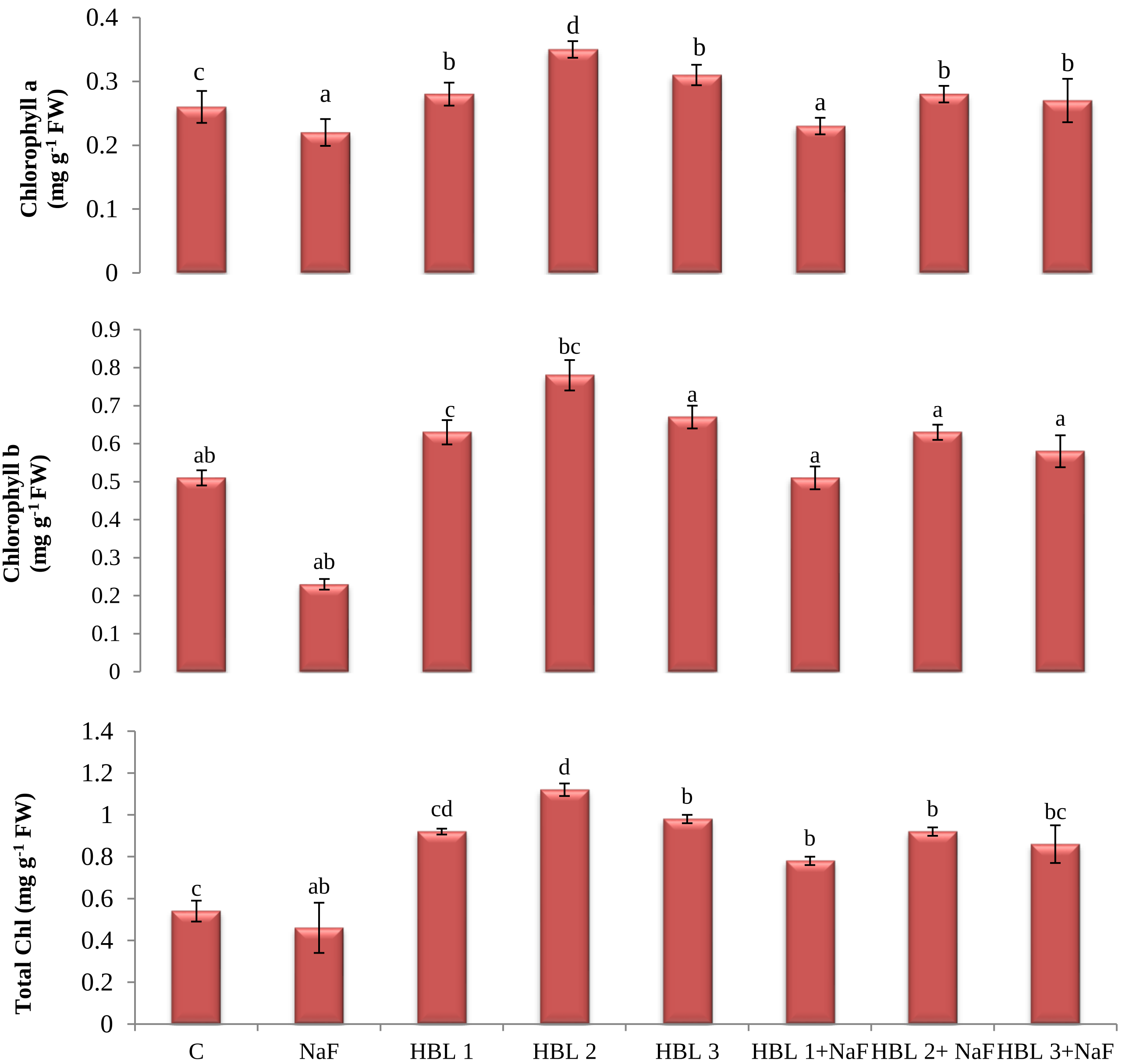

Figure 1: Effect of HBL on Chl *a*, Chl *b* and total chlorophyll content of radish plants under NaF stress. Each treatment mean is sum of three replicates and ± represents standard error (SE). Non-identical letters specify significant differences among the treatments at p≤0.05. C = control, NaF= 200ppm NaF, HBL 1= 1µM HBL, HBL2= 5µM HBL, HBL 3= 10µM HBL.

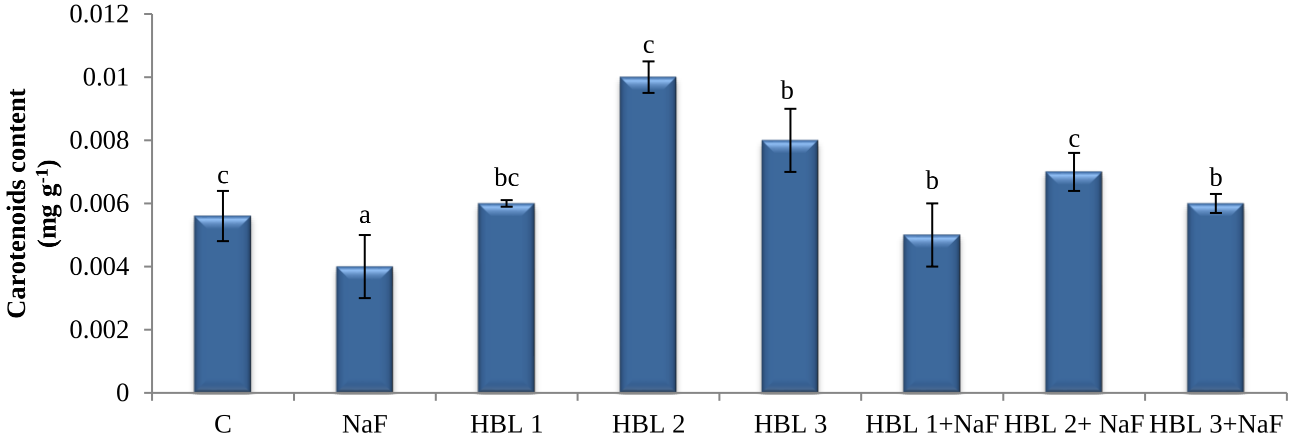

Figure 2: Effect of HBL on carotenoid content of Radish plants under NaF stress. Each treatment mean is sum of three replicates and ± represents standard error (SE). Non-identical letters specify significant differences among the treatments at p≤0.05. C = control, NaF= 200ppm NaF, HBL 1= 1µM HBL, HBL2= 5µM HBL, HBL 3= 10µM HBL

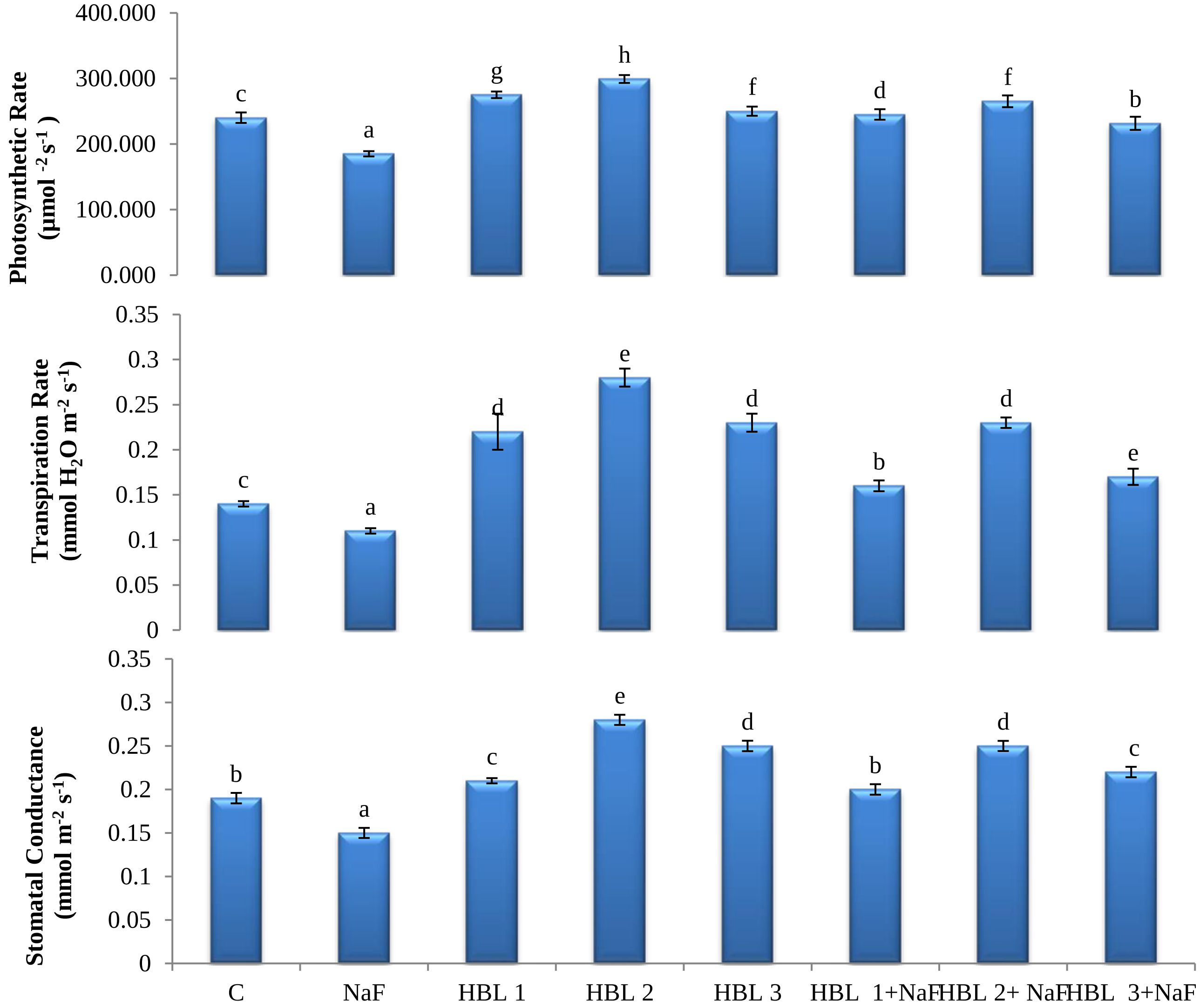

Figure 3: Effect of HBL on photosynthetic rate *(A)*, transpiration rate *(E)* and stomatal conductance *(gs)* of radish plants under NaF- stress. Each treatment mean is sum of three replicates and ± represents standard error (SE). Non-identical letters specify significant differences among the treatments at p≤0.05. C = control, NaF= 200ppm NaF, HBL 1= 1µM HBL, HBL2= 5µM HBL, HBL 3= 10µM HBL

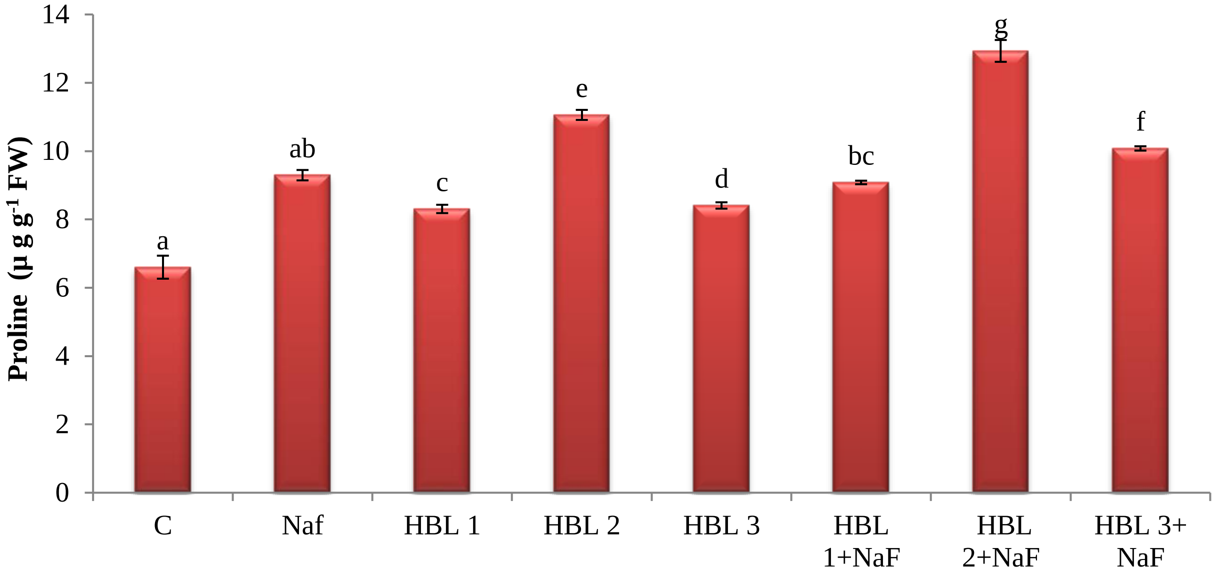

Figure 4: Effect of HBL on Proline content of Radish plants under NaF-stress. Each treatment mean is sum of three replicates and ± represents standard error (SE). Non-identical letters specify significant differences among the treatments at p≤0.05. C = control, NaF= 200ppm NaF, HBL 1= 1µM HBL, HBL2= 5µM HBL, HBL 3= 10µM HBL

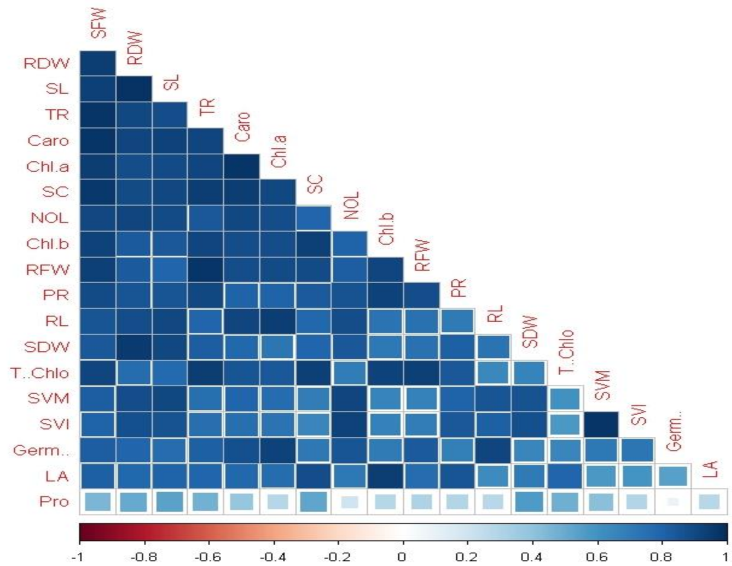

Figure 5: Correlation between different growth and physio-biological traits of *Raphanus sativus* grown in control and saline soil (NaF) with or without the application of 28-Homobrassinolide. The abbreviations are as follows: (Germ.) Germination percentage, (SVI) seed vigor mass, (SVM) seed vigor index, (SL) shoot length, (RL) root length, (SFW) shoot fresh weight, (RFW) root fresh weight, (SDW) shoot dry weight, (RDW) root dry weight, (LA) leaf area, (NOL) number of leaves, (SC) stomatal conductance, (TR) transpiration rate, (PR) photosynthesis rate, (Chl. *a*) chlorophyll *a*, (Chl. *b*) chlorophyll *b*, (Total. Chlo) total chlorophyll content, (Caro) carotenoids and (Pro) proline.

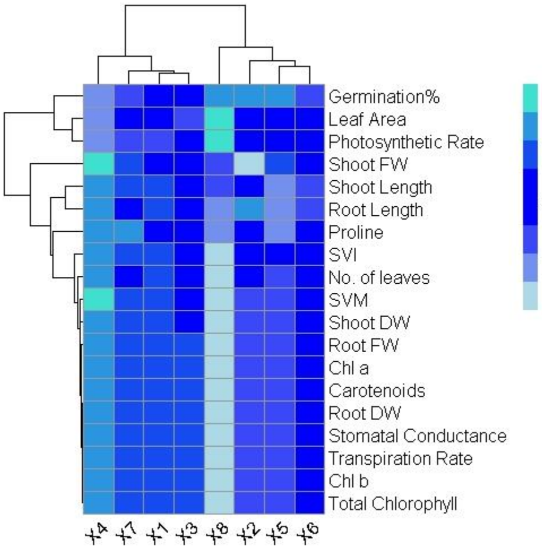

Figure 6: Heatmap histogram correlation between different growth and physio-biological traits of *Raphanus sativus* grown under control and NaF contaminated soil with and without the seed priming of 28-Homobrassinolide. Parameters under study are as follows: Germination percentage, leaf area, (SVI) seed vigor mass, (SVM) seed vigor index, shoot length, root length, (Shoot FW) shoot fresh weight, (Root FW) root fresh weight, (Shoot DW) shoot dry weight, (Root DW) root dry weight, number of leaves, stomatal conductance, transpiration rate, photosynthesis rate, (Chl. *a*) chlorophyll *a*, (Chl. *b*) chlorophyll *b*, total chlorophyll content, carotenoids and proline. The different treatments are represented as (X1) (Control), (X2) (NaF 200ppm), (X3) (HBL 1µM), (X4) (HBL 5µM), (X5) (HBL 10µM), (X6) (NaF 200ppm + HBL 1µM), (X7) (NaF 200ppm + HBL 5µM) and (X8) (NaF 200ppm + HBL 10µM).

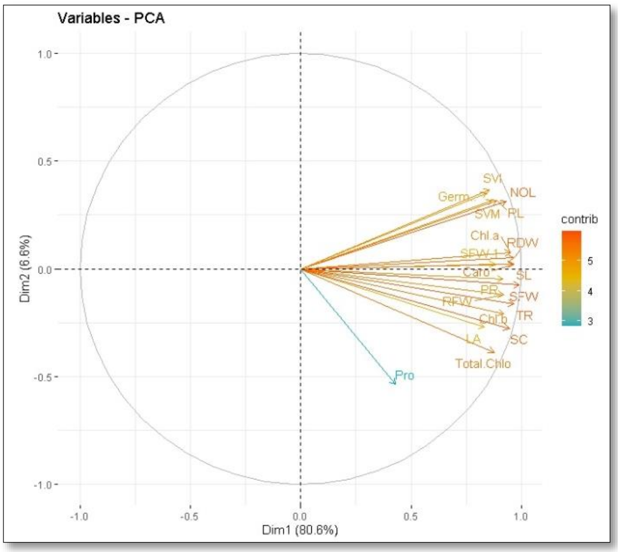

Figure 7: Loading plots of principal component analysis showed a relationship between growth and physio-biological traits of 28-HBR primed *Raphanus sativus* grown under NaF. Different abbreviations used in the figure are as follows: (Germ.) Germination percentage, (SVI) seed vigor mass, (SVM) seed vigor index, (SL) shoot length, (RL) root length, (SFW) shoot fresh weight, (RFW)root fresh weight, (SDW) shoot dry weight, (RDW) root dry weight, (LA) leaf area, (NOL) number of leaves, (SC) stomatal conductance, (TR) transpiration rate, (PR) photosynthesis rate, (Chl. *a*) chlorophyll *a*, (Chl. *b*) chlorophyll *b*, (Total. Chlo) total chlorophyll content, (Caro) carotenoids and (Pro) proline.

DISCUSSION

It is well known that sodium fluoride significantly affects a variety of physiological processes in plants, such as the reduction of chlorophyll content and plant development [29]. The significant reduction in radish seedling growth reported during the current experiment may be due to sodium fluoride (NaF) toxicity's adverse effects on the physiological and metabolic activities of seed and emerging seedlings. Fluoride (F) contamination of the world's soil, water, and vegetation has been an ongoing issue. Fluoride toxicity impacts various morphological, physiological, and biochemical processes, including seed germination parameters, growth, development, mineral nutritional status, photosynthesis, respiration, metabolic activity, yield, and yield characteristics [30]. Fluoride-treated plants are reported to have significantly lower growth and related characteristics than control plants, such as seedling germination percentage, roots and shoot length, plant height, and fresh and dry biomass [29]. The experiment's findings demonstrated that, when compared to the control, Fluoride harmed the morphological and physiological characteristics of the plants similar to the finding of [31].

Seed priming, a low-cost technique, helps farmers boost the rate of germination. Priming boosts seed germination and encourages earlier more coordinated, and healthier crop stands. [32]. Seed priming increases the activity of enzymes that can enhance yield characteristics [33]. The present study was based on the assessment of Sodium Fluoride-induced stress on growth, and biomass attributes of radish. The outcome demonstrated the impact of Fluoride stress on the F1 hybrid variety of radish plants and revealed that the HBL2 plants that weren't exposed to NaF had the least detrimental effects on growth and biomass parameters (Table 4 and 5). Fluoride, especially at 200 and greater levels of F, decreased the growth of seedlings (shoot length and root length) and seed germination. The outcomes were comparable with [34].

Early seedling growth was increased when 28-Homobrassinolide was applied; reducing the growth inhibition caused by salinity. A plant growth regulator (HBL) has a significant impact on how well plants perform as well as leaf photosynthesis. When HBL was applied to cotton seedlings under NaF stress, photosynthetic activity increased [35]. The use of HBL improved the growth of lateral roots, the appearance of new roots, and the ability of cucumber seedlings to absorb nutrients. Exogenous HBL (5μM) at low concentration improved root growth. Higher HBL (10μM) concentrations resulted in the inhibition of root development (Table 4). The 28-Homobrassinolide encourages cell elongation in the root's meristematic zone. To maintain normal root growth rates through the regulation of root meristem size, balanced HBL signaling is necessary. The 28-Homobrassinolide promoted a strong root system and enhanced root absorption. The 28-Homobrassinolide are necessary for the development of lateral or adventitious roots and they work in concert with auxin to encourage the growth of these types of roots. Therefore, boosting root activity with HBL may lead to improved root physiology [36].

In the current study application of HBL mitigates salinity by promoting the root length, shoot length, shoot weight, and root weight, which was comparable to the results of [37]. On morphological characteristics such as the number of leaves, shoot length, and root length, salinity showed damaging effects. The parameters used by [38] showed that the trend was declining with increasing concentration. According to the findings of the current study, the harmful effects of Sodium Fluoride caused a decrease in physiological parameters such as shoot length, root length, and dry weight. This coincides with previous research by [39]. To demonstrate the impact of NaF toxicity on wheat crop seed germination and seedling growth, [40] conducted their study. Fluoride in high concentrations inhibits the germination of seeds and the early growth of plants.[38]

In the current investigation, HBL2 plants had the largest leaf area and the highest average value. In comparison to the control, the NaF-treated plants showed the highest percentage reduction value (Table 4). The findings were consistent with Mustafa et al [41]. When Fluoride (F) came into contact with leaves and epidermis, it damaged stomata and disrupted the mechanism of stomatal opening [42]. Additionally, [43] reported that there was severe necrosis, chlorosis, and yellowing of the leaves, which resulted in a reduction in leaf area. Along with the loss of apical dominance, chlorosis caused by F may similarly resemble symptoms of an iron, magnesium, and boron deficiency [44]. Low water temperatures stressed plants, and this stress reduced crop roots' ability to absorb N, P, and K nutrients. Plant metabolic activity has diminished, which is the cause of this drop. In addition to lowering protein and chlorophyll concentrations, the decreased intake of N, P, and K also affects the structure of chloroplasts, electron transfer, and the distribution of exciting energy. As a result, photosynthesis may be inhibited, which could lead to a plant suffering chilling injuries [45].

Other research has demonstrated that a small concentration of HBL given to plants can enhance Nitrogen, Phosphorous, and potassium uptake in *Camellia oleifera* [46] and increase Nitrogen, Phosphorous, and potassium contents in banana plants under normal temperatures HBL also improves plant root water uptake, controlled cell physiology, supported normal physiological and biochemical metabolism, and increased plant resistance to low temperatures [47]. In contrast to the tissues of the control plants under cold stress, rice plants treated with HBL had higher Nitrogen, phosphorus, and potassium concentration in their leaf, stem, and sheath tissues. HBL also encouraged photosynthesis and increased the metabolism and translocation of carbohydrates in the treated plants because it stimulated chlorophyll content. The NaF had a detrimental effect on the photosynthetic apparatus and carotenoid concentration of stressed radish plants [48].

Chlorophyll is the primary pigment used in the photosynthesis of green plants. Chlorophyll concentration has an immediate impact on plant photosynthetic rates. In rice, cooling damage can make the chloroplast's structure weaker, make the membrane more permeable, and stop chlorophyll from accumulating [49]. The entire metabolic and physiological system of rice is harmed, and the chloroplasts themselves suffer damage as well as accelerated chloroplast aging and decreased leaf photosynthetic capacity. Researchers discovered that under cold stress, HBL applied topically improved the chlorophyll content in pepper and maize [50,51]. In the current study, chlorophyll content was the maximum for HBL2 plants and showed the highest average value (Figure 1). The regular functioning of photosynthesis is greatly influenced by the production of chlorophyll pigments [52]. Our results were supported by other researchers who noted several plant species exhibiting comparable reduction in photosynthetic properties [53].

Under both normal and stress conditions, 28-Homobrassinolide treatments were reported to improve photosynthesis in maize and Tung tree seedlings [54]. When exposed to NaF stress, radish seedlings demonstrated a decrease in net photosynthetic rate due to a disruption in water relations. However, according to the results of the current study, the plants whose seeds had been treated with HBL2 displayed the most photosynthetic pigment (Figure 1). Under stress conditions, photosynthetic rate and stomatal conductance decreased [55]. Due to a decrease in CO_2 availability in the mesophyll cells, salt stress caused stomatal closure and lowered the rate of photosynthetic activity [56]. Low leaf CO_2 availability, reduced photosynthesis, reduced transpiration rates, and restricted stomatal conductance are all effects of high salt concentration circumstances that lead to morphologically reduced plant biomass [57, 58]. The application of HBL 2(5µM) enhanced the transpiration rate, intercellular CO_2 concentration, and stomatal conductance in radish seedlings and showed the highest protective role against NaF stress (Figure 3).

Proline is an essential osmolyte that regulates cellular molecular structure and function, maintains turgor, and provides a considerable source of energy and nitrogen, claims [59, 60]. Under some stress circumstances, proline also controls the redox environment and scavenges elevated ROS levels [61]. P5CS, also known as pyrroline-5-carboxylase synthase, is a helpful enzyme for converting glutamate into proline [62]. Proline safeguards protein structure and cell membrane integrity by controlling turgor, osmotic pressure, and water transport within the cell through processes of tolerance against the oxidative condition of stress [63]. By establishing a connection between HBL metabolism and proline synthesis, higher proline synthesis appears to be a method used by radish plants to tolerate NaF stress. Thus, it may be inferred that the higher proline content in HBL-primed plants helps to reduce NaF-induced phytotoxicity. Glutamate is a typical precursor of proline [64]. Our findings showed that plants under NaF stress and radish plants treated with HBL had the highest proline concentration (Figure 4).

CONCLUSION

The results of the present study suggest that 28-homobrassinolide (HBL) priming promotes the growth of radish plants by reducing the phytotoxic effects Sodium Fluoride (NaF) stress. Poor germination, root growth, shoot growth, and biomass are all characteristics of radish plants that are decreased due to NaF stress. Overall, seed primed with 5µM HBL was found to be the most effective method for increasing radish NaF tolerance. The 28-Homobrassinolide improved gas exchange parameters, such as transpiration rate (*E*), net photosynthesis (*A*), and stomatal conductance (*gs*). This experiment showed that HBL reduced the stress caused by NaF. HBL served as a priming agent that enhanced radish germination in challenging environmental circumstances and increased the therapeutic value of mature leaves. To completely comprehend the NaF stress tolerance mechanism in real-world settings, more research is necessary.

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