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# Glutathione mitigates sodium fluoride toxicity in pea seedlings: morpho-physiological responses and biochemical analysis

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## ABSTRACT

**Purpose:** Soil salinity is an emerging threat to plant growth and crop production, resulting in substantial economic losses. Pea is one of the most important leguminous crops being grown all over the world being affected. Keeping in view the stress alleviation of glutathione, current investigation was designed to evaluate the beneficial role of seed priming with glutathione on morphological and biochemical attributes of pea under sodium fluoride stress.

**Methods:** For this purpose, pea seeds primed with 25, 50, and 70 µM $L^{-1}$ glutathione. Sodium fluoride caused a significant decline in seed germination percentage, root and shoot length. Glutathione primed seed improved the fresh and dry biomass, root nodules and proline content of Pea plant.

**Results**: The 50 µM $L^{-1}$ enhanced the growth rate by 50%, photosynthetic rate by 93% under NaF stress. Glutathione primed seed also enhanced the gas exchange parameters, i.e., transpiration rate and stomatal conductance, by 80% and 89%, respectively.

**Conclusions:** Consequently, the results of the current study demonstrated that seed priming with glutathione can reduce the sodium fluoride stress and improved the growth of pea.

***Key-words:*** *Sodium fluoride, Growth. Pea, Glutathione, Proline*.

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## INTRODUCTION

Plants during developmental stages are vulnerable to environmental stresses.[1]Any changes in environment that leads to metabolic, development and yield disruption are referred as stress. There are two types of stress: biotic and abiotic. Biotic stresses include insects, bacteria and viruses. Abiotic stresses include extreme temperature, flood, drought, light, salt and heavy metals. These stresses negatively influence plant growth and crop productivity.[2] The FAO (Food and Agricultural Organization) guessed the loss in the range of 20-40%, computing 31M dollars due to salinity only.[3] Abiotic pressures typically coexist with environmental factors, impacting all stages of development.[1] Even the Calvin cycle, electron chain reaction, photosynthetic enzyme activity, and stomatal function are affected by these condition.[4] Other abiotic stresses include water, which may be impacted by both drought and flooding, metals, excess or less nutrient, radiations, ozone, sulphur dioxide, and various mechanical causes Plants adapt with changing conditions by accumulation of phenolics and others as a response to adverse environmental conditions. [5]

Fluorine is a widespread pollutant and the 13th most prevalent element in the crust of earth. It is founded to decrease plant growth, chlorosis, burned leaf-tip, and necrosis.[7]There is ample fluorine in form of fluoride ion in environment, water, and soil. Its average concentration is 0.32g/kg soil.[6] Fluoride in high quantity is toxic for humans and plants in early seedling growth. Successful growth and survival mainly depend on how well seeds grow in its early stage. These stages are significantly affected by sodium fluoride due to its inhibitory activities. Fluoride causes serious disorders in living organisms. Its effects on plants are drastic especially on salinity sensitive plants and loss of economy. The best defense and survival mechanism in this situation for plants confined in stressful environments is the antioxidant system.[7] Efficient antioxidant systems are required for the betterment and survival of plants and are suitable enough to reduce any stress. But there are limitations to this system that they cannot cope with extremities. To cope with it there are various phytohormones. These phytohormones correlate with the antioxidant defense system to reduce the excessive production of ROS in Unfavorable conditions like salt stress.[8] Salinity also reduces the effectiveness of photosynthesis by decreasing amount of chlorophyll.[5]

A tripeptide called Glutathione is a basic thiol that is non-protein in nature, has low molecular weight and present both in plants and animals.[9]It helps in transportation and removal of toxic substances from plants.[10] Its main function is to stabilize sulfhydryl groups of protein within the cell in its actual oxidation state.[11] Glutathione oxidation-reduction state can be proved as a useful marker for unfavorable conditions like stress.[12] The other important function of glutathione-S-Transferase is to detoxify the herbicide.[13] In glutathione –ascorbate shuttle, glutathione act as a donor of diminishing identical.[10] As the seed is leading component of cultivation, and environmental stresses affects the strength of plantlet and their speed of germination drastically that results in loss of production. For this reason, there are various processes or techniques by which rate of seed germination can be enhanced and also help the seed to cope with different environmental stresses.[14]

The best technique to increase resistance against stress is seed priming. Different priming techniques are used to accelerate the germination rate of the crop and for better yield. It is a cheap and fruitful method to stimulate sprouting. In this method seed undergo in the well-controlled watered and dehydrating conditions. As a result, before germination the metabolic activity of seed boosts up.[4]Seed priming decrease the use of manure an increase the agricultural output of crop by organized germination. It also makes the seed resistant and this resistance is both non expensive and nonhazardous to environment. It also helps in increasing the uptake of essential nutrients, in the breakdown of light and temperature dormancy and in the maturation of the seedlings.[15] Seed priming is very useful for some members of family Poaceae, Cucurbitaceae and Leguminosae.[16] In spite of all these advantages some factors are there to affect the effectiveness of priming such as time duration, concentration level of the priming agents and place of storage.[17]

The leguminous seed-pod crop known as the pea (*Pisum sativum* L.) is a self-pollinated member of the Leguminosae family. On a dry matter basis, its seeds are high in protein and carbs (between 18 and 20 percent).[18] Pea is the primary legume among all the leguminous crops in all over the world. It is a frequently cultivated nutritive crop globally.[19] It provides various essential compounds like proteins and fibers as well as important antioxidant.[20] Seed coat of pea are a source of fibers that help in the better functioning of stomach and abdominal cavity.[2]

Given that peas are an excellent source of iron, minerals, and carbohydrates, their high nutritional worth cannot be overlooked.[6] But the crop production of pea is highly

reduced by salinity stress. To promote pea production all around the world, the present experiment was conducted to investigate the efficacy of antioxidants against salt stress.

## MATERIAL AND METHODS

*Priming of Seeds and Growth Environment*

Pea seeds were purchased from the Sky Seeds in Lahore. After five minutes of soaking in a 0.5% sodium hypochlorite solution, they were cleaned by rinsing three times with distilled water. Then, they were primed for 24 hours[21], in three different concentrations of glutathione (Glu) solution (25, 50, and 70 µM), followed by air drying at room temperature. The unprimed seeds were used as the control treatment. The pot experiment was carried out in the Botanical Garden, University of the Punjab. Using 24 pots, arranged according to randomized complete block design (RCBD), the experiment was commenced. Each treatment had three replicates. Five Glu primed or controlled seeds were uniformly sown in each pot. One seedling was permitted to develop after thinning in each pot after 10 days of sowing.

*Preparation and Application of NaF solution*

100ml of sodium fluoride was prepared by adding 0.1g NaF in 100mlof distilled water. NaF stress was applied in the form of soil drench method. 100ml of NaF was given to each pot twice a week. First NaF soil drench was given after 34 DAS (days of sowing). After 58 days of sowing, the harvest was taken. Measurements of biomass, morphological features, and other biochemical characteristics were examined.

*Measurement of Morphological Traits*

The morphological growth parameters, shoot length, root length, number of leaflets, leaflet area, number of nodules, and number of root hairs was noted. The Charleton and Foote 1965 [22], formula was used to determine the leaflet area.

Leaflet area=length of the plant × maximum width of plant × 0.75 (correction factor)

*Estimation of Biomass*

At 58 DAS, a biomass evaluation that included the fresh and dried weight of the root, shoot, and whole plant was recorded. Pea plants were harvested and dried for 72 hours at 70°C in an oven (Wiseven, Model WOF-105, Korea). The dry weight was determined using an electronic balance (Sartorius GMBH, model 1216MP 6E, Gottingen, Germany).

*Determination of Gas Exchange Parameters*

The stomatal conductance, rate of transpiration and photosynthesis of leaves of pea from each pot were measured at the experimental site. For this purpose, Infrared Gas Analyzer (LCA-4System ADC, Ltd) was used.

*Determination of Chlorophyll*

0.1g of plant sample was crushed using a pestle and mortar and immersed in an acetone for 48 hours in the dark to determine the amount of chlorophyll. Three different wavelengths of 645, and 663 nm were used to measure the chlorophyll a, b respectively using a spectrophotometer (UV-1800 Schimadzu). The number of carotenoids was determined at a wavelength of 480 nm [15].

*Evaluation of Proline*

A 2g sample of frozen leaves was kept for 30 minutes in 3% sulfosalicylic acid (5 mL) to extract the proline. A volume of 1 mL of glacial acetic acid, 1 mL of supernatant, and a 1:1 (v: v) ratio of Ninhydrin reagent was mixed after spinning at 4000 rpm for 20 min at 4°C. The mixture was centrifuged at 2000 rpm for 1 hour at 4°C after being immersed in a water bath at 100°C. At 520 nm, the absorbance was measured.[1]

*Statistical Analysis*

The one-way analysis of variance was performed using SPSS Version 20. The data provided shows the average values for three replicates. The means and standard errors of the repetitions were used to express the replicates average values. The statistical evaluation of changes in treatment means was performed using Duncan's multiple range tests (at $p \leq 0.05$). The Pearson correlation coefficients, heat-map analysis, and

## RESULTS

*Determination of Morphological Growth Attributes:* As compared to control, the plants that were treated with NaF had severe decrease in morphological growth parameter such as shoot length by 40%, root length by 15%, and total length by 19.6%. Root nodules and leaflet area were also decreased by 1.66 and 1.3 folds respectively. While priming with glutathione reduced the effect of NaF stress. As compared to NaF, Glu 1 treated plants showed increased in shoot length by 71%, root length and total length by 1-fold, number of root hairs by31%, tendrils by 36.5%, root nodules by 81%, and leaflet area by 88%.

The Glu 1, Glu 2 treated plants were far better as it increased shoot length, root length, total length, number of root hairs, tendrils by 11%, 24, 62%, 16%, and5%, respectively. Root nodules and leaflet area were also increased by 94 and 77 percent, respectively. But the above-mentioned parameters were decreased in Glu 3 treated plant due to toxicity of high concentration of glutathione. Morphological growth reduced when Glu 2 + NaF was applied as compared to Glu 2 only. It was reduced as shoot length, root length, total length, number of root hairs, tendrils, root nodules and leaflet area by 16%, 4%, 16%, 67%, 28%, 6%, 22%, respectively.

Priming with Glutathione reduced the effect of NaF on the pea seedlings. The maximum reduction of morphological growth attributes was found in the plant that was only treated with NaF while minimum reduction was found in the plant that was primed by using Glutathione 50µM (Table 1).

*Evaluation of Biomass Assessment:* As the NaF badly affected the morphology of pea seedlings, the biomass assessment was also affected. Due to NaF stress, fresh and dry weight of root and shoot was reduced. So, the total weight of seedlings was also reduced (Table 2). But there was a reduced effect of NaF on the seedlings that were treated with Glutathione. Glutathione treated seedling especially with Glu 2 (50 µM) showed better biomass assessment as compared to NaF treated seedlings and control. The total fresh and dry weight

of Glu 2 treated plants was increased by 6% and 8%, respectively as compared to NaF. While, in relation to Glu 2, Glu 2 + NaF treated plants showed decrease in total fresh weight by 1.4 folds and 3.4 for total dry weight it was decreased by 3.4 folds.

*Growth Indices:* Growth indices such as Germination percentage, seed vigor index and seed vigor mass were obtained by using mean of 3 replicates of each treatment. For Glu 1 their values were increased by 16%, 139% and, 9% folds, respectively in relation to NaF, while for Glu 3 Germination percentage was increased by 24%. But the results for Glu 2 were far better than Glu 1 and Glu 3. For that following increase were observed 37%, 180%, and43%, respectively. Glutathione mitigates the NaF stress as in Glu 2 +NaF the germination percentage and seed vigor index were increased by 24 and 127%, respectively as compared to NaF alone. Seed vigor mass was also increased by 3.7 folds. While the germination percentage was also increased by 13% and18% for Glu 1 +NaF and Glu 3 +NaF, respectively (Table 3).

*Determination of Gas Exchange Parameter:* As compared to control and NaF, it was observed that the rate of stomatal conductance, Transpiration and photosynthesis was high in plants that were treated with glutathione especially with Glu 2 (50 µM). There was a very low rate of photosynthesis and transpiration in the plants treated with NaF. Stomatal conductance was also very low in stress treated plants. The Photosynthetic rate for Control was 11% greater than that of NaF. While Glu 2 was 2.7 greater than Glu 1 and 1,6 folds greater than Glu3. Under stress, the photosynthetic rate of Glu 2+NaF was 38% greater than NaF only (Figure 1).

*Determination of Photosynthetic Pigment:* NaF significantly inhibited the synthesis of photosynthetic pigments in pea seedlings over control treatment. Stress of NaF affect the chlorophyll (*a, b*, total chlorophyll) and carotenoids content of pea seedlings. It was observed that the Glutathione treatment ameliorated the effect of NaF and help in the restoration of chlorophyll and carotenoids content in pea seedlings. The Chlorophyll *a* content was increased by 98% in Glu 2 and in Glu 1 and Glu 3 it was 95% and 97%, respectively (Figure 2).

*Determination of Proline content:* NaF caused the seedlings to accumulate more proline as compared to control and Glutathione treated seedlings. The amount of proline was very low in the seedlings that were treated with Glutathione as it ameliorates the stress. The maximum amount of proline was observed in stress treated (NaF) plants and minimum in Glutathione treated plant. It was 8 folds more than that of Glu 2 treated plant

and 10% than Glu 2+ NaF (Table 2).

*Pearson Correlation Analysis:* A Pearson correlation was also used to quantify a link between growth and physiological characteristics of *P. sativum* growing under NaF stress and Glutathione seed priming (Figure 3). Leaf Area has a positive correlation with Shoot length, Seed vigor index while it is negatively correlated with proline content. Root fresh weight is positively correlated with shoot fresh weight and total fresh weight of Pea plant. Chlorophyll *a* and *b* are negatively correlated with proline content of plant (Figure 3). Germination percentage has a strong positive correlation with carotenoids, total chlorophyll, transpiration rate, stomatal conductance while it is negatively correlated with proline. A heat map histogram was also illustrated to mark relationship between various morphological and biochemical attributes of Pea plant under different treatments. The figure illustrates that under Glu2, the plant has maximum positive response to photosynthetic rate. Under Glu2+NaF, the plant has weak to strong positive correlation to all the growth parameters while being strongly positive towards total dry weight of the Pea plant. These variables produce nearly identical findings to the correlation analysis (Figure 3).

*Principal Component Analysis:* Principal component analysis (PCA) loading plots were also created to demonstrate a link between the growth and biochemistry of *P. sativum* growing under NaF stress with the administration of glutathione seed priming (Figure 5). The first two major elements, Dim1 and Dim2, account for more than 71.6% of the total database and the biggest part of all components. Dim1 provides 45.1% and Dim2 contributes 26.5% of the whole dataset. The first set of variables with which PC1 is positively associated is as follows: Photosynthetic rate, Number of tendrils, Stomatal conductance, Total chlorophylls, Seed vigour index, Germination percentage, Chlorophyll *a*, Total plant length, Leaf area, Chlorophyll *b*, and Number of nodules. While in the dataset, those variables that are negatively correlated are as follows: Proline, Root fresh and dry weight, Shoot fresh and dry weight, Total dry weight and Seed vigor mass (Figure 3). Additionally, a heat-map histogram was displayed between the variables of the different treatments examined in this research (Fig. 6). Under Glu 2 treatment, a significant positive correlation between germination % and total fresh weight was found. Additionally, NaF and plant length overall had a negative correlation. These variables produce results that are comparable to those from the correlation analysis (Figure 3).

**Table 1:** Effect of Glutathione on the growth attributes of pea under NaF stress.

| Treatments | Shoot Lengths (cm) | Root Lengths (cm) | Total Length (cm) | Number of Leaflets | Number of Root hairs | Number of Tendrils | Number of Nodules | Leaf area ($cm^2$) |
|---|---|---|---|---|---|---|---|---|
| **Control** | 22.3±1.01c | 13.3±0.17b | 33.6±1.37b | 30.3±2.02a | 27±1.15a | 17.6 ±1.45a | 72.3±1.45d | 7.46±0.04bc |
| **NaF** | 13.3±0.88a | 11.3±0.14d | 26.5±1.02a | 35±1.73ab | 28.3±1.20a | 20±1.15ab | 43.3±0.88a | 5.78±0.08a |
| **Glu 1** | 22.8±1.15ab | 11.6±0.21bc | 26.6±1.35a | 37±1.73bc | 37.3±1.45b | 17.3±1.45a | 81.6±0.88f | 9.42±0.21d |
| **Glu 2** | 25.5±0.14d | 14.4±0.12f | 43.1±10.32c | 42±1.15cd | 43.3±0.88c | 25.6±1.20cd | 77.3±0.88e | 7.45±0.07bc |
| **Glu 3** | 23.5±0.88c | 10.0±0.88a | 43.6±4.12 | 45.6±2.33d | 35.3±1.45b | 27.6±1.45d | 57.3±1.76b | 11.3±0.22e |
| **Glu 1+NaF** | 16.7±0.88b | 11.9±0.12c | 31.8±4.11ab | 59±1.73e | 34.6±1.45b | 16±1.15a | 46±1.73a | 7.09±0.002b |
| **Glu 2+NaF** | 21.3±1.201c | 13.7±0.14e | 36.5±1.03b | 68.6±0.88f | 72.6±1.45e | 18.6±0.88a | 72.6±1.45d | 9.1±0.208d |
| **Glu 3+NaF** | 19.3 ±1.201c | 12.8±0.14d | 36.6±0.2b | 38.6±1.76bc | 65.3 ±1.45d | 23±1.15bc | 65.3 ±1.45c | 7.87±0.001c |

Data exhibit means ±SD of 3 replicates. Non-identical letters specify significant dissimilarity between the treatments ($P \leq 0.05$).C= Un contaminated Control, NaF = contaminated Control (100 mL NaF), Glu 1 = 25 µM Glutathione, Glu 2 = 50 µM Glutathione, Glu 3 = 75 µM Glutathione, Glu 1 + NaF= 25 µMGlu + 100 mL NaF, Glu 2 + NaF= 50 µMGlu + 100 mL NaF, Glu 3 + NaF= 75 µMGlu + 100 mL NaF.

**Table 2:** Effect of glutathione on the biomass assessment of pea under NaF stress.

| Treatments | Shoot fresh weight (g) | Root fresh weight (g) | Total fresh weight (g) | Shoot dry weight (g) | Root dry weight (g) | Total dry weight (g) | Proline Content ($\mu molg^{-1}FW$) |
|---|---|---|---|---|---|---|---|
| **Control** | 7.10±0.003a | 0.627±0.01b | 7.73±0.012a | 0.92±0.012a | 0.14±0.002a | 1.06±0.013a | 1.024 ±0.059d |
| **NaF** | 10.57±0.23e | 0.77±0.014c | 11.5±0.395ab | 1.12±0.015a | 0.08±0.001a | 1.23±0.016a | 2.282±0.004a |
| **Glu 1** | 8.7±0.0057b | 0.65±0.017b | 9.35±0.023ab | 0.68±0.290a | 0.14±0.009a | 0.82±0.289a | 0.195±0.002d |
| **Glu 2** | 9.86±0.01d | 0.803±0.008c | 10.6±0.017ab | 1.067±0.009a | 0.12±0.012a | 1.187±0.019a | 0.251±0.023g |
| **Glu 3** | 8.52±0.01b | 0.012±0.011a | 18.683.224ab | 1.99±0.141b | 0.13±0.012a | 2.11±0.151b | 1.01±0.001b |
| **Glu 1+ NaF** | 9.01±0.005c | 0.55±0.018a | 15.2±5.69ab | 1.95±0.150b | 0.47±0.347a | 38.04±0.02b | 1.13±0.0058c |
| **Glu 2+ NaF** | 15.6±0.020f | 1.3±0.009d | 20.41±3.48b | 3.91±0.447c | 0.32±0.035a | 150±0.012c | 2.03±0.001f |
| **Glu 3+ NaF** | 9.68±0.0153d | 0.64±0.009b | 10.33 ±0.032a | 1.09±0.444a | 0.16±0.019a | 55.63±0.009a | 1.33±0.015e |

Data exhibit means ±SD of 3 replicates. Non-identical letters specify significant dissimilarity between the treatments ($P \leq 0.05$). C= Un contaminated Control, NaF = contaminated Control (100 mL NaF), Glu 1 = 25 µM Glutathione, Glu 2 = 50 µM Glutathione, Glu 3 = 75 µM Glutathione, Glu 1 + NaF= 25 µM Glu + 100mL NaF, Glu 2 + NaF= 50 µM Glu+ 100 mL NaF, Glu 3 + NaF= 75 µM Glu+ 100 mL NaF.

**Table 3:** Effect of glutathione and NaF on the growth indices of pea.

| Treatments | Growth indices | | |
|---|---|---|---|
| | Germination Percentage (%) | Seed Vigor index (SVI) | Seed vigor mass (SVM) |
| **Control** | 50.67 | 1576.00 | 49.37 |
| **NaF** | 53.333 | 869.333 | 41.144 |
| **Glu 1** | 62.00 | 2079.33 | 45.00 |
| **Glu 2** | 73.333 | 2434.000 | 59.556 |
| **Glu 3** | 66.67 | 2175.42 | 48.65 |
| **Glu 1 + NaF** | 60.00 | 1749.33 | 145.11 |
| **Glu 2 + NaF** | 66.67 | 1979.33 | 152.96 |
| **Glu 3 + NaF** | 63.33 | 1953.33 | 149.59 |

Each treatment mean is sum of three replicates. C= Control, NaF = NaF 100 mL, Glu 1 = Glu 25µM, Glu 2 = Glu 50µM, Glu 3 = Glu 75µM, Glu 1 + NaF= 25 µM Glu + 100 mL NaF, Glu 2 + NaF= 50 µM Glu+ 100 mL NaF, Glu 3 + NaF= 75 µM Glu+ 100 mL NaF.

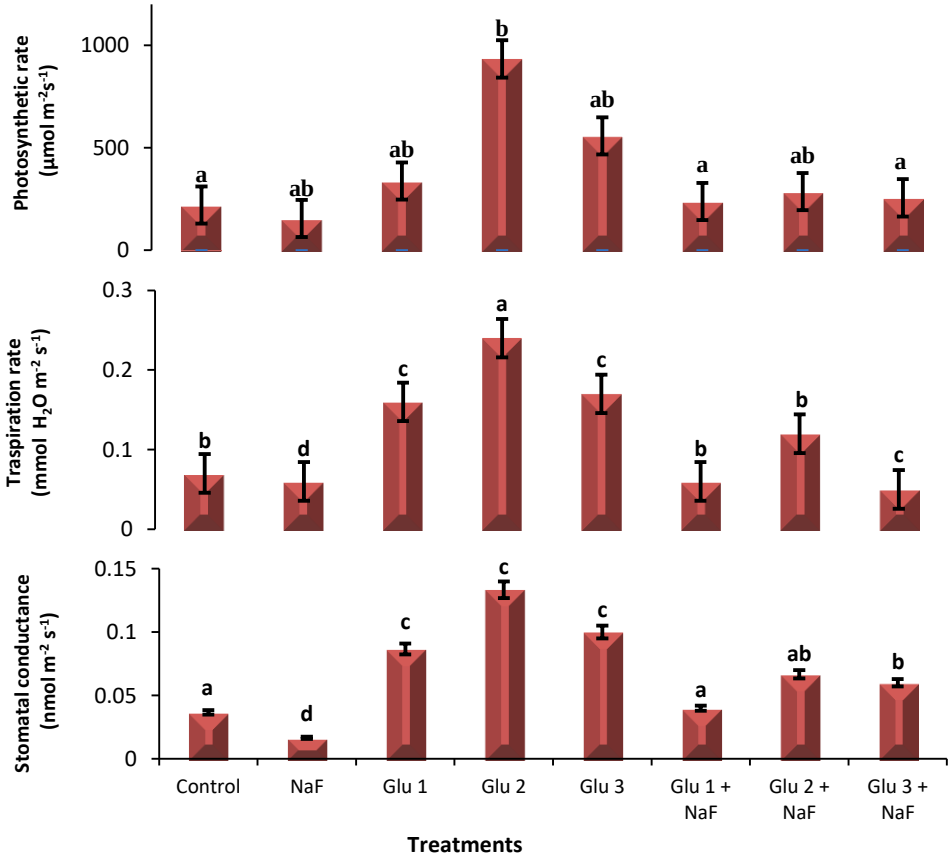


**Figure 1: Effect of glutathione and NaF on gas exchange parameters of pea.** Data exhibit means ±SE of 3 replicates. Non-identical letters specify significant dissimilarity between the treatments (P ≤ 0.05).C= uncontaminated control, NaF = contaminated control, Glu1=25 µM Glutathione, Glu2=50 µM Glutathione, Glu3=75µM Glutathione, Glu 1+NaF= 25µM Glu 100 mL NaF, Glu 2+NaF=50 µM Glu+ 100 mL NaF, Glu 3+NaF=75µM Glu+100 mL NaF.

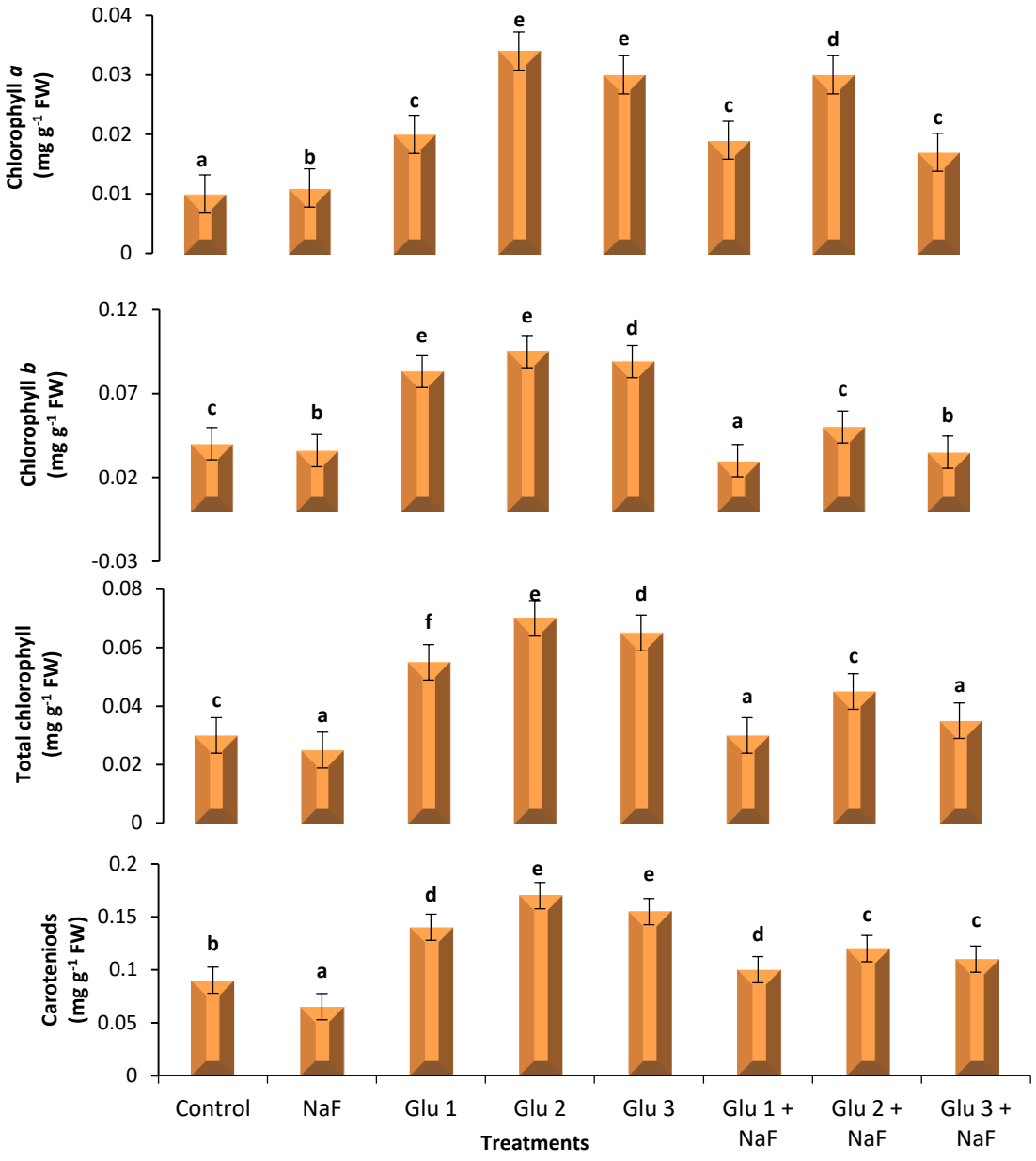


**Figure 2: Effect of glutathione and NaF on photosynthetic pigments of Pea.** Data exhibit means ±SE of 3 replicates. Non-identical letters specify significant dissimilarity between the treatments (P ≤ 0.05). C= uncontaminated control, NaF = contaminated control, Glu1=25 µM Glutathione, Glu2=50 µM Glutathione, Glu3=75µM Glutathione, Glu 1+NaF= 25µM Glu 100 mL NaF, Glu 2+NaF=50 µM Glu+ 100 mL NaF, Glu 3+NaF=75µM Glu+100 mL NaF.

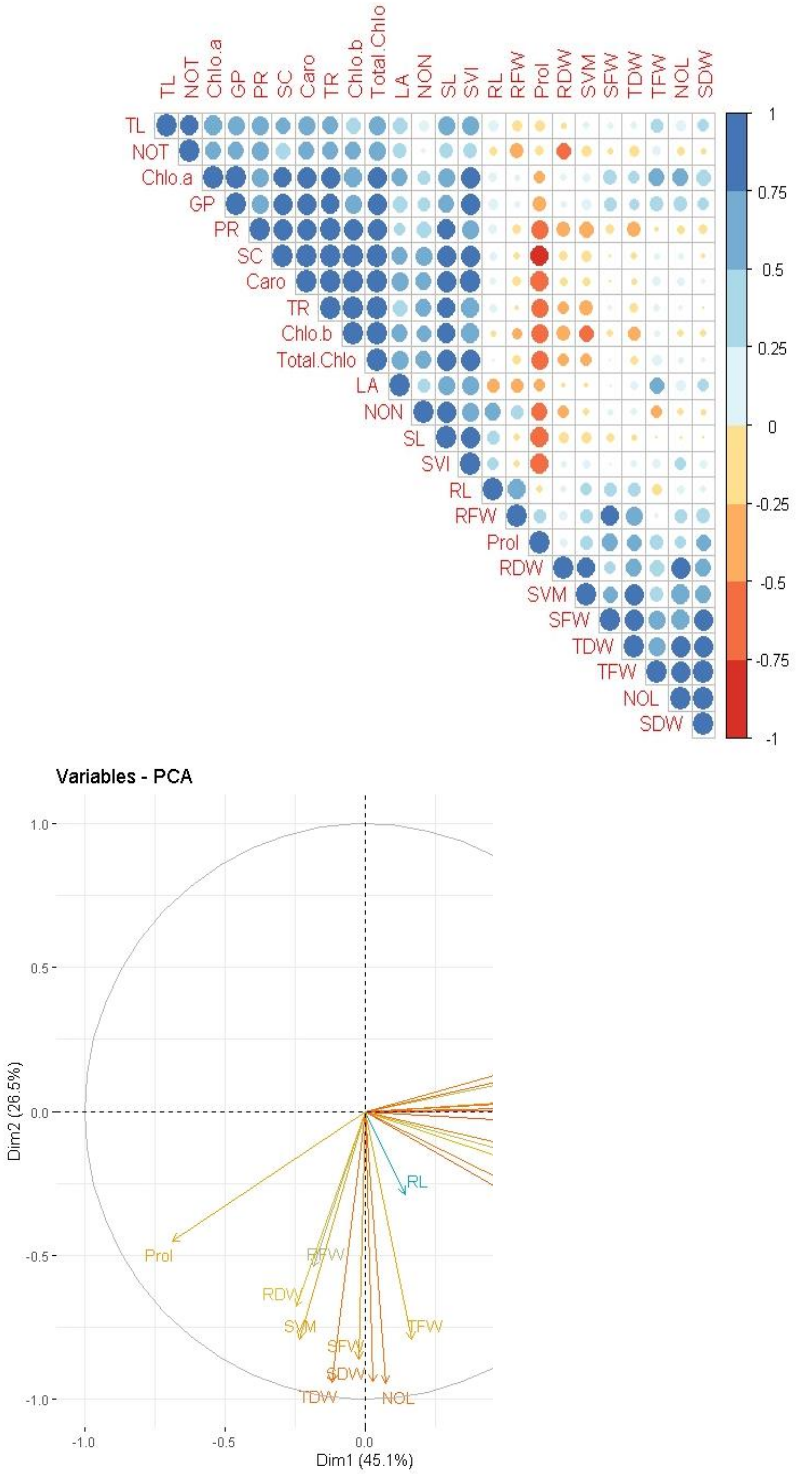


**Figure 3: Correlation and Loading plots of principal component analysis showed a relationship between growth and physiological parameters of pea grown under salt stress of NaF**. Different abbreviations used in the figure are as follows: TL (total length)NOT (number of tendrils), GP (germination percentage), PR (photosynthetic rate), SC (stomatal conductance),Caro (carotenoid contents),TR (transpiration rate),Chl *b* (chlorophyll *b*), Total *Chlo* (total chlorophyll), LA (leaf area), NON (number of nodules), SL (shoot length), SVI (seed vigor index), RL (root length), RFW (root fresh weight),Prol (proline content),RDW (root dry weight), SVM (seed vigor mass), TDW (total dry weight), TFW (total dry weight), NOL (number of leaflets), SDW (shoot dry weight).

## DISCUSSION

Sodium fluoride is an environmental pollutant as it contaminates soil, water and vegetation even in its slightest amount. Pea is one of the legumes used in various forms as fresh vegetable, as dry beans and in the form of canned food.[23] It is found in investigations that high concentration of sodium fluoride badly effects the production of plant.[6]By using varied NaF concentrations, the current study examined the effects of stress caused by fluoride in the production of the pea plant. Fluorine is the 13th most abundant element in the earth crust and hence is a major pollutant of environment. Fluorine and fluorine compounds are posing a global concern in recent times.[7] Studies are establishing the bad effects on growth and development [24], leaves mutilation,[16]and decreasing chlorophyll in the leaves.[11]

According to the results of the current study, plant pigments tend to gradually diminish when exposed to sodium fluoride and priming with glutathione lessens the stress-inducing effects of NaF (Figure 2). These findings are very similar with the previous research where NaF stress also reduced surface area of leaves which leads to reduced net photo-synthetic rate and biomass [25], and this is also analogous to the results of current study (Table 1). This research founds that fluoride stress caused a loss in photosynthesis by decreasing the photosynthetic pigments and decreasing the yield of pea (Figure 2), a result similar to Ahmad et al. 2020. [5]

Present study used Glutathione as basic seed priming agent, worked as antioxidant thus helps improving stress tolerance. Priming before sowing the seeds improves overall performance because the seed is already on the stage where the metabolic processes are started giving the them an edge on un-primed seeds. Another advantage of priming is the synchronization of seed metabolism, which guarantees consistent emergence and growth across the entire seed lot.[3] The current study used priming techniques to maximize the in-built capacity of the seeds to cope with the stresses. Glutathione was used as prime which increased radical length and dry weight initially and reduced the effects of stress possibly due to increase in production of some of plant hormones like auxin and cytokinin[1] which is in comparison to current investigation where glutathione primed seeds showed a better morphological and physiological growth. (Table 1 and 2).

There is another plant health indicator called seed vigor and can be computed for all the germination and growth parameters.[18] This research study resulted that with the inclusion of NaF, there is reduced germination and decrease in root and shoot lengths. Addition of sodium fluoride in the concentrations of 200 ppm also decreased seed vigor measured as vigor index. On the contrary, plants that have been treated with Glutathione (50 µM) have increased vigor index. In a nutshell, plants exposed to NaF exhibit a significant decline in their plant health index, but seeds saved by glutathione do well. *Cicer arietinum* under NaF stress shows similar results in seed vigor.[26]

This conclusion of this experiment is comparable to that made by Jayashree et al. 2021[22], who found that fluoride impairs the setting of pods and decreases the dry weight of the pod. When compared to the control plants, there is a considerable decrease in the production of the NaF-treated plants. This study also demonstrates that the existence of NaF in the mesophyll cells alters physiological and morphological traits such the quantity of leaves, plant height, fruiting, biomass, and yield. It occurs because NaF disturbs the metabolism of various minerals and lowers the chlorophyll content, according to Ahmed et al 2020[5], which is a reasonable analogy to the current study where the preceding parameters demonstrate a declining trend (Table 1 and Table 2). Additionally, it is known that leaves with a high NaF concentration develop chlorosis and necrosis, which slows growth and reduces yield. According to reports, a high salt content diminishes the number of flawless flowers and fruit setting as well as causes the development of fruit with flaws.[27] The idea that NaF causes changes in metabolism that reduce plant development is also confirmed by the study's findings.

Sodani *et al.,* 2021[28] has studied that excess of sodium fluoride reduces the root and shoot length reported in *Triticum aestivum* and *Oryza sativa,* which is consistent to the results of our study where NaF caused a detrimental effect of shoot and root length. (Table 1). Matthews *et al.*

(2017)[29] described that gas exchange between leaves and atmosphere is regulate by stomata and salt stress reduced their efficiency. So, the rate of transpiration is also affected as in the case of present study. The rate of transpiration was decreased in the plants that were treated with NaF while the plants that were not treated with stress had a better rate of transpiration. Figure 1 demonstrated that enhanced rate of transpiration was observed in plant treated with Glutathione 2 (50 µM).

In case of salinity, there is additional $Na^+$ buildup in leaves which altered the chlorophyll and /or limiting its biosynthesis.[6] First indicator of fluoride toxicity in plants is destruction of chloroplast which results in loss of chlorophyll. Increased in sodium fluoride is inversely proportional to the total content of chlorophyll.[13]In this study, the plants that were treated with NaF became yellow due to the loss of chlorophyll. But the plants that were treated with Glutathione remained green. So, the results of our study are in favor of previously reported study (Figure 2).

Plants exposed to stressful environment accumulates large amount of osmolytes such as proline as an adaptation response. It is organic in nature and a source of energy. It mainly stabilizes the sub-cellular structures and osmotic regulations. Additionally, it facilitates cell osmo-regulation by acting as an enzyme regulator. Proline also protects the proteins during drought and salinity.[30] [31] increased amounts of proline in shoot reflects better protection under oxidative stress. It plays a vital role as an osmolyte by triggering water uptake to maintain cellular turgidity.[32]

Our findings also indicated that, in contrast to other plants, the plant that had undergone stress had a high concentration of proline. Proline acts as an antioxidant to protect the cell from the damaging effects of free radicals. Plants are better able to withstand severe salt stress thanks to the quick synthesis of proline-rich proteins.[15] In the current investigation, the administration of glutathione decreased the proline content whereas higher NaF concentrations raised it (Table 2).

## CONCLUSIONS

Exogenous application of glutathione in the form of seed priming agent successfully mitigated the salt stress of sodium fluoride (NaF) and improved Pea plants' growth and biomass production. The present study applied NaF to pea plants biweekly to produce stress conditions. It had detrimental effects on the growth parameter, such as morphological parameters and biomass assessment as well as on the biochemical responses of pea plants. To alleviate this stress, Glutathione was used as a priming agent. Out of three different concentrations of Glutathione, Glu 3 (75 µM) showed some toxic effects due to higher concentration. Glu 1 (25 µM) proved helpful to cope with stress to some extent, but the Glu 3 (50 µM) showed the best results. It not only mitigates stress but also enhanced the morpho-physiological responses of pea plant. So, the present study revealed that the problem of salinity could be resolved by using Glutathione as a priming agent.

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