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Impacts of salinity, *Pseudomonas* and salicylic acid on seed bean physiological properties and yield (*Phaseolus vulgaris*)

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Grains are the second most important dietary source for humans after cereals. The bean is one of the most important Grains. Because this plant is susceptible to saline conditions in our nation, especially in dry and semiarid locations, it is important to devise a plan to avoid a decrease in plant production. Bacterial stimulants, such as *Pseudomonas*, fortify plants against the detrimental effects of salt. By modulating physiological processes, salicylic acid increases plant tolerance to the detrimental effects of salt. In 2019, a randomized complete design research with three replications was carried out in the greenhouse of the agricultural department of Semnan University for this aim. Treatments included bacteria *Pseudomonas putida* with two levels (inoculated and non-inoculated), soil salinity with three levels (0, 2, and 4 ds/m), and two levels of salicylic acid (0 and 1 mM). According to the results, salinity, salicylic acid, and the combination of the two had a substantial influence on leaf relative water content. Salinity, *Pseudomonas*, and salicylic acid also had a substantial influence on chlorophyll index, 100 seeds weight, and protein seed percentage. Also important on seed production were *Pseudomonas*, saltiness, salicylic acid and the interaction impacts of salinity and salicylic acid. Salicylic acid and *Pseudomonas* were studied in general to minimize the influence of salinity on the indicators.

Keywords: Salicylic acid, Common Bean, *Pseudomonas*, Salinity

INTRODUCTION

Between peripheral pressures, salinity is recognized as a major limiting factor in the global production of legumes. Increased salination of agricultural land, on the other hand, is commonly anticipated (Gholamin and Khayatnezhad 2020, Khayatnezhad and Gholamin 2020, Bi, Dan et al. 2021, Guo, She et al. 2021, Hou, Li et al. 2021). Salinity affects plants in a variety of ways, particularly in terms of metabolic, anatomical, and morphological effects (Aletor 2021, Barth 2021). NaCl concentration in soil decreases fertility and, as a result of soil structure degradation, crop yield decreases (Alayi, Sobhani et al. 2020, Esmaeilzadeh, Fataei et al. 2020, Arjaghi, Alasl et al. 2021). Legume cultivation has the second

biggest country's cultivated area after grain and is cultivated in 1200000 acres, and produced 700000 tons. Legumes play a vital part in providing protein needs (Gholamin and Khayatnezhad 2020, Jia, Khayatnezhad et al. 2020, Huang, Wang et al. 2021, Khayatnezhad and Nasehi 2021, Ma, Khayatnezhad et al. 2021, Ren and Khayatnezhad 2021). Scientists suggest that during the plant's reproductive development stage, soil salinity can disturb several processes, reducing the yield available (Gholamin and Khayatnezhad 2020, Karasakal, Khayatnezhad et al. 2020, Peng, Khayatnezhad et al. 2021, Sun, Lin et al. 2021, Sun and Khayatnezhad 2021). Experiments on other plants likewise revealed a reduction in function when exposed to salt

(Karasakal, Khayatnezhad et al. 2020, Tao, Cui et al. 2021, Zhang, Khayatnezhad et al. 2021, Zhu, Saadati et al. 2021). The salt promoted the terminal bud, but the overall number of spikelets per spike and the number of wheat grains decreased, according to the researchers (Huang, Wang et al. 2021, Radmanesh 2021, Rodríguez 2021). Also, limit the number of pollen grains at this time, and lastly, salinity, decreased fertility, pollen count, and grain filling (Khayatnezhad and Gholamin 2020, Si, Gao et al. 2020) reduces salt stress percentage of the area to grains and seeds per seeds spike number in barley grain (Tao, Cui et al. 2021, Yin, Khayatnezhad et al. 2021, Zhu, Saadati et al. 2021).

Because bean plants are extremely susceptible to soil salt, it is critical to employ suitable methods to mitigate the negative impacts of salinity on yield and yield components of the plant. One of the fundamental techniques that should be explored is the use of a biological solution, such as the usage of bio fertilizers containing bacteria from the genus *Pseudomonas* (Hewitt 2021, Huma, Lin et al. 2021). The term "biologic fertilizer" refers not only to organic matter produced by dung manure, other plants, green manures, and so on, but also to tiny bacteria and fungous animals, particularly bacteria that stimulate plant growth or idiomatically (PGPR) and materials produced by their activity (Karasakal, Khayatnezhad et al. 2020, Li, Mu et al. 2021, Tao, Cui et al. 2021, Xu, Ouyang et al. 2021). This bacterial community increases plant development and yield through several methods in the rhizosphere. Some bacteria of the species *Azotobacter* and *Pseudomonas* have been identified (Gholamin and Khayatnezhad 2020, Yin, Khayatnezhad et al. 2021, Zhang, Khayatnezhad et al. 2021). Phosphate-solubilizing bacteria improves soil phosphorus solubility and plant development in phosphorus-deficient soil. The fundamental mechanism of phosphate solubilizing bacteria producing organic acids is claimed to be the most important function in phosphorus solubility in soil acid phosphatase. Organic acid production by phosphate-solubilizing bacteria is extensively established. Gloconic acid appears to be the most common mineral phosphate solubilizer. 2-Keto-gloconic acid is a well-known organic acid with phosphate solubility (Gholamin and Khayatnezhad 2021, Ma, Ji et al. 2021, Zhang, Khayatnezhad et al. 2021). *Pseudomonas* bacteria, particularly *Pseudomonas putida*, are key members of numerous rhizosphere microorganisms that research on the beneficial

effects of inoculation on plant development have been undertaken (Gholamin and Khayatnezhad 2020, Zhu, Saadati et al. 2021). According to Jalili et al. (2009), inoculating canola seeds with *Pseudomonas putida* enhances tolerance to salt p169, p108. Kabir et al. (2021) demonstrated that inoculating pea seeds with *Pseudomonas* leads to a rise in stem height, root length, and plant dry weight compared to the control treatment (Kabir, Arefin et al. 2021).

Salicylic acid, according to Kabir et al. (2007), can also play a significant role in helping the environmental disturbances. Salicylic acid is indeed an endogenous growth modulator of the phenolic chemical group, which plays a function in plant physiological processes. The role of salicylic acid is thought to be in the stimulation of blooming, growth, and development, the production of ethylene, and the influence on the opening and shutting of stomata and breathing. Salicylic acid improves salt tolerance in wheat seedlings, but it also promotes tolerance to salinity in dicotyledons like beans. Salicylic acid induces cell elongation and cell division, which may be benefited when working with other regulators like as auxin. There are reports on the influence of salicylic acid on the yield of various crops such as soybeans, cowpeas, and peas (Khayatnezhad and Gholamin 2021).

MATERIALS AND METHODS

This study was conducted in the Semnan University greenhouse in 2019 using randomized, factorial full block design including three replications. The levels of soil salinity (0, 2 and 4 dS/m), *Pseudomonas putida* (bio-fertilizer) and salicylic acid levels (0 and 1 mM) were investigated and inoculating the non-inoculated seeds was carried out with two levels. The seed amount required for each treatment was then put inside a plastic bag for seeds infected with *Pseudomonas putida*. For better attaching the inoculum with the seed, an inert substance surface was scratched to be moist and sticky, thus the quantity of sugar dissolved in water (per 100 grams of water 10 grams of sugar) was employed. Seed inoculum was introduced and thoroughly mixed after adding a solution of water and sugar to guarantee the soaking of the seeds. The seeds were then placed in different envelopes for two hours of drying in the same area (shade). Disposable gloves were utilized for each treatment to avoid mingling during planting. For salinity and soil salinity in the pot after it was formed, purified water and pure sodium chloride

were utilized. During the blooming stage, a solution of 1 mM Salicylic acid and likes scatter was applied. Pots with 21.5 cm diameter were utilized to carry out this test. Astronomers' figure owned the bean seeds utilized in this experiment. Firstly, 10 seeds were sown in each pot, but after emerging, bushes were thinned down and the number of plants per pot was increased to three.

A leaf from each plant was dissected at the conclusion of the growth season to measure the relative water content, and then precisely balanced by 0.001 were weighted. Following that, the samples were put in a plate containing distilled water for 12 hours before being weighed again after swelling. The samples were then oven-dried for 24 hours at 75° C before being weighed again.

The following equation was used for calculating the leaf relative water content:

$$RWC = (Fw - Dw / Tw - Dw) * 100 \text{ relation (1):}$$

The highest blooming stage was determined by taking chlorophyll meter readings and calculating the leaf chlorophyll index at the conclusion of the process plant, and also the weight (g) of 100 seeds and grain production per pot using a square meter. The content of seed protein was tested at the conclusion of the maturity phase by statistical software SAS, Kjidal Data analysis, and LSD mean comparison technique, with a 5% significance level.

RESULTS

The effects of acetylsalicylic acid, salt, and salicylic acid on leaf relative water content were shown to be significant using variance analysis. Salt, salicylic acid, and bacteria have a substantial influence on chlorophyll index, the weight of 100 seeds, and seed protein respectively. For grain production per square meter, the major influences and interactions of salicylic acid and salt were significant.

According to the findings of the study, at a salinity of 1 dS/m, the leaf water content was 58.5 %, compared to the greatest level of salinity, which was raised by 21%. As 1 mM salicylic acid was used, the content of leaf water increased by 20% when compared to no intake (Table 1). The interaction of salinity and salicylic acid, in which salicylic acid is utilized in various salinity therapies, was not substantially different. There was no significant difference between salicylic acid levels. In the case of salicylic acid, EC 2 and 3 dS m were also measured, and no statistically

significant difference was found (Fig. 1).

The findings of the study revealed that the 1 dS/m chlorophyll index has 37.5% salinity, and the greatest level of salinity was raised by 17%. As 1 mM salicylic acid was used, the chlorophyll index was 42.6%, and when there was no consumption, 42% was obtained. A chlorophyll index value of 6/37 was found in seeds infected with *Pseudomonas putida*, representing a 10% increase over non-inoculated treatments (Table 1).

According to the results, the EC 2 dS/m, the weight of 100 seeds to the 43.06 g increased 17% when compared to the maximum level of salinity. Seed weight was 49.87 when 1 mM salicylic acid concentration was used, rising 39 % from non-consumption. Also, *Pseudomonas putida* infected seeds, 100 seeds weighted at 25.42 mg, resulted in an 11% rise in this score (Table 1).

The results also revealed that at a salinity of 1 dS/m, the seed yield achieved 73.4 grams per square meter, a % increase over the greatest levels of salinity. When 1 mM salicylic acid was used, a yield of 73.3 grams per square meter was discovered, representing a 21% increase over no consumption. In seed infected with *Pseudomonas putida*, yield rises to 74.1 gram per square meter, a 25% increase over non-inoculated treatments (Table 1). The influence of salt on seed production was considerable in terms of seed yield per square meter (Fig. 2). In 2 dS/m salinity and 1 mM salicylic acid, the seed yield was 86.03 mg, a 47% increase over the same amount of salicylic acid intake. There were significant differences in grain production across different degrees of salt in the absence of salicylic acid. No significant difference was observed in salicylic acid between consumption and non-consumption modes at 3 ds/m salinity, although salicylic acid increased grain yield.

The content of grain protein of 26.62% increased 15% in EC of 0 dS/m and with the salinity of 4 dS. With 1 mM salicylic acid, grain protein content was 26.19%, which was 9% higher than the control state. Grain protein was also enhanced by 7% in seed infected with *Pseudomonas putida* as compared to seed not treated with bacteria (Table 1).

Table 1: Comparison of mean of salinity, salicylic acid and bacterium *Pseudomonas putida* on properties of the bean plan

		Leaf water content (%)	Chlorophyll Index	100 seeds weight (g)	Grain yield (g/m ²)	Protein seeds (g)
Salinity (ds/m)	0	58.5 a	37.5 a	41.53 a	73.37 a	26.62 a
	2	56.7 ab	37.9 a	43.06 a	70.91 ab	25.96 ab
	4	48.6 b	31.9 b	35.95 b	56.09 a	22.78 b
salicylic acid (mM)	0	49.4 b	30.01 b	30.49 b	60.26 b	24.04 b
	1	59.9 a	42.6 a	49.87 a	73.32 a	26.19 a
<i>Pseudomonas putida</i>	Non inoculated	52.8 b	33.9 b	37.84 b	59.48 b	24.41 b
	inoculated	56.5 a	37.6 a	42.25 a	74.1 a	25.83 a

In each column and in each treatment group, the average share of letters, are not significant difference.

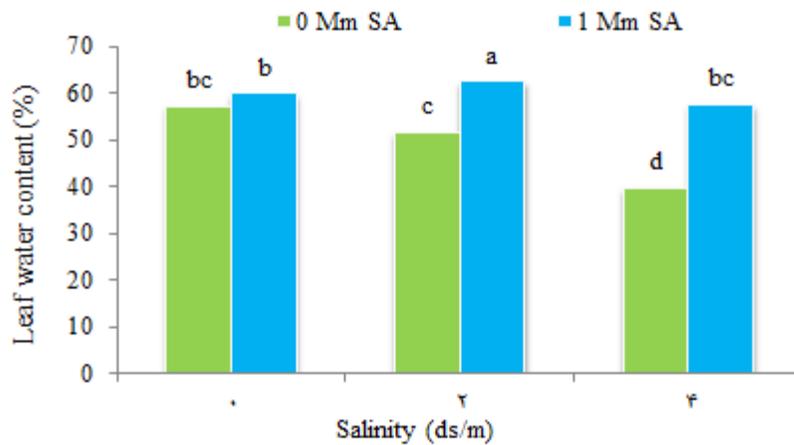


Figure 1: Effect of salicylic acid and salinity on Moisture content

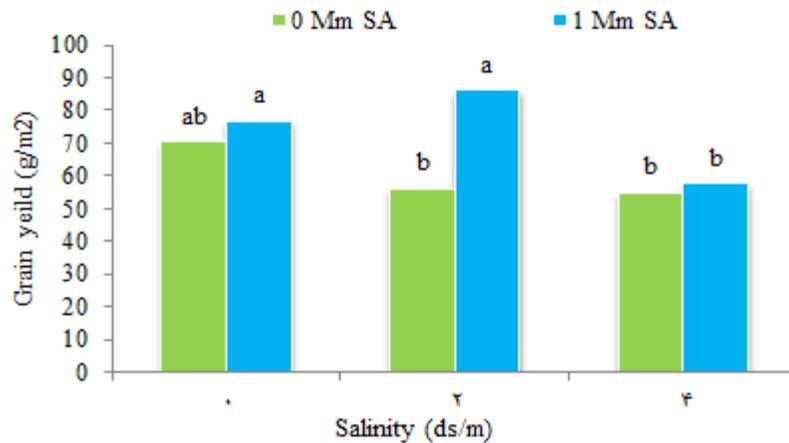


Figure 2: Effect of salicylic acid and salinity on seed yield of beans

DISCUSSION:

The highest amount of plant relative water content was achieved at a salinity level of 1 ds/m, and the lowest amount was obtained at a salinity level of 9 ds/m; when these two levels of salinity were compared, the plant relative water content decreased by 25.21 percent (Khayatnezhad and Gholamin 2021). Frick (2004) stated that when salinity increases, the content of the leaf relative water drops. The outcomes of transpiration, leaf development, and photosynthesis at lower levels will be defined. Due to the low absorption of water, roots growth and contraction decrease, strain on the plant increases, as does the density of abscisic acid, and as a result, plant growth decreases (Karami Chame, Khalil-Tahmasbi et al. 2016). According to Singh and Usha (2004), wheat treated with salicylic acid (3.1 mmol) produced seedlings with greater moisture content than untreated seedlings under normal and stressed circumstances (Singh and Usha 2003).

Khavary nezhad et al. (2002) showed that in sunflower, high levels of salinity reduced chlorophyll levels more than controls (Karami Chame, Khalil-Tahmasbi et al. 2016). Schutz and Fangmier (2001) reported that decreasing the quantity of chlorophyll in cells during extreme stress causes an increase in the generation of oxygen radicals (Schütz and Fangmeier 2001). These free radicals induce oxidation, which leads to the destruction of these pigments. Bashan et al. (2006) found that inoculating wheat seedlings with bacteria dramatically enhanced the quantity of photosynthetic pigments such as chlorophyll a and chlorophyll b (Cheng, Hong et al. 2021, Wang, Shang et al. 2021, Zheng, Zhao et al. 2021, Zhu, Liu et al. 2021). Treated Wheat seeds with a 5-10 mM salicylic acid solution generate plants that are considerably more pigmented, and higher salicylic acid concentrations reduce the quantity of pigment (Bashan, Bustillos et al. 2006).

According to Ashraf et al. (1997), the susceptibility of bean plants to salt stress reduces growth and yield (Ashraf 1997). There have also been reports of the impact of salicylic acid on the increase in several plants, including cowpea (Kumar, Dube et al. 1997). Many external stressors disturb the plant's hormonal balance. Many of the morphological and physiological consequences of stress are caused by hormonal changes. Ethylene is one of the plant hormones that rises in concentration in response to environmental stress (Karami Chame, Khalil-

Tahmasbi et al. 2016). According to research, salicylic acid, through its influence on ethylene production, causes plant resilience to environmental stressors (Shakirova, Sakhabutdinova et al. 2003). Salicylic acid improves stress resistance through raising the enzymes activity that function in response to stress. These results in increasing yield components, and grain production can be raised appropriately.

Proteins are one of the negative impacts of salt stress on plants, altering the foods balance and quality (Cachorro, Ortiz et al. 1993). Cachorro et al. (1993) discovered that with the protein Bivanij pea types, the plants were higher than the control plants and ranged from 116 mg to 127 mg in weight control while the plant seed was treated with 0.7. g salicylic acid According to Akbari et al. (2011), enhancing the protein content of seeds bacterial inoculation led to the growth increase, owing to phyto hormones (AKBARI, Ghalavand et al. 2009).

CONCLUSION

According to the researchers, salinity lowered the sunflower's leaf relative water content. Due to the rapid reduction in cell elongation and turgescence caused by saline conditions, the cell wall has hardened and thickened. Turgescence pressure will be low due to a drop in the leaves relative water content, resulting in reduced cell development. It is recommended to repeat the experiment in the following years to achieve more reliable results.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Masoud Radmanesh conducted, planned, Analyzed the data, wrote manuscript and interpreted the results and involved in manuscript preparation. All authors read and approved the final version.

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