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Original article

Impact of Salt Stress on Pea (*Pisum sativum*) Physiological Features in Lab Settings

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Abstract
Soil salinity is a growing problem that threatens agricultural output worldwide. By investigating the "impact of salinity on pea (Pisum sativum) physiological features grown in laboratory conditions," this study aimed to address this issue. The study was carried out in the laboratory of the Faculty of Science at Omar Al-Mukhtar University in Libya in the fall of 2023. The effects of salt stress on growth, photosynthetic rate, and chlorophyll concentrations were investigated using four genotypes of peas (Pisum sativum): Local 1, Wando, Lincoln, and Green Arrow. Different genotypes of pea seeds were cultivated in pots using fine sand as the growth medium. The plants were exposed to salt stress at 0, 25, 50, and 75 mM NaCl following 30 days of germination. After the experiment, the growth of the plants was considerably reduced as the salt increased. Following two weeks of salt treatment, all genotypes showed a marked drop in photosynthetic rate and chlorophyll concentrations as salinity increased. Among the several cultivars, it was discovered that Green Arrow exhibited salinity-sensitive behavior, while Wando and Lincoln from Local 1 were salt-tolerant.
Keywords. *Pea, Growth, Photosynthesis, Chlorophyll Content, Salinity*.

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Introduction

One of the main abiotic stressors that negatively impacts plant development and productivity globally is salinity. It impairs a number of physiological functions, including photosynthesis, water uptake, and nutrient balance, which eventually results in decreased yield and biomass accumulation (1). A significant crop high in protein, the legume *Pisum sativum* (pea) is highly vulnerable to salt stress, especially in its early growth stages (2). Osmotic and ionic imbalances brought on by salt stress lower the turgor pressure and water potential of leaves, which hinders cellular growth and function (3). Furthermore, oxidative stress brought on by salt causes an excess of reactive oxygen species (ROS), which can harm proteins, lipids, and nucleic acids (4). One of the physiological characteristics most impacted by salinity is photosynthetic activity. Salt stress has been shown to decrease gas exchange efficiency, stomatal conductance, and chlorophyll content in Pisum sativum (5). Furthermore, salt stress can decrease nutrient uptake, especially potassium and calcium, which are necessary for membrane stability and enzyme activation, as well as change the activity of antioxidant enzymes (6).

To improve agricultural sustainability in saline-prone areas and create salt-tolerant cultivars, it is crucial to comprehend how pea plants physiologically react to salt stress. An essential edible leguminous seed crop for human sustenance is the pea (*Pisum sativum*). Its seeds have 18–20% dry matter, of which 5-8% is protein and 10-12% is carbohydrates (7,8). In addition to having immediate effects on yield and quality, salinity is an abiotic stressor that impacts pea leaf growth, photosynthesis, mineral nutrition, stomatal conductance, transpiration, water and ion transport, and increases sugars, amino acids, and various ions. Disorders such as nonspecific chlorosis, stunted leaf size, and decreased shoot growth were brought on by salinity (9). As a result, plants in saline environments develop several defenses against the osmotic and ionic shocks brought on by excessive salt stress. Pea water consumption reduced when soil salinity rose as a result of saline water applications.

When plants are cultivated in saltwater circumstances, photosynthetic activity declines, resulting in decreased plant growth, leaf area, chlorophyll concentration, and chlorophyll fluorescence. Crop performance at various growth stages is impacted by this decline (10). The current experiment's goal was to investigate the physiological reactions of peas in saline environments. One important environmental component that impacts plant growth and development, including *Pisum sativum* (pea), is salt stress. Researching how salt stress affects peas in experimental settings can help improve agricultural practices for dealing with saline conditions and offer important insights into plant physiology.

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Materials and methods

Plant Composition and Growth Environment Four distinct genotypes of seeds—Local 1, Wando, Lincoln, and Green Arrow—with differing capacities for salt tolerance were planted in plastic containers with fine sand as the growth medium. The seedlings were trimmed to five after two weeks of germination, with seven seeds per pot. The study was carried out in the laboratory of the Faculty of Science at Omar Al-Mukhtar University in Libya in the fall of 2023.

For 30 days following germination, plants were cultivated in Hoagland solution in non-saline circumstances. The salt treatment was then started. After dissolving sodium chloride (NaCl) in double-distilled water, the final concentrations were 0 (Control), 25, 50, and 75 mM. Three salinity levels—low salinity (25 mM), intermediate salinity (50 mM), and high salinity (75 mM)—were developed for the current study after these levels were filtered from a variety of salinity treatments in a different exploratory experiment. Thus, three saline regimes—low, moderate, and severe salt stress—were used to assess the investigated pea genotypes' performance. The intended salinity levels, 25, 50, and 75 mM, were produced by progressively raising the salinity level (25 mM) every day until the final concentrations (50 and 75 mM) were attained after three days to prevent the osmotic shock. Salt-stressed plants were cultivated for two weeks. 200 mL of half-strength Hoagland solution per pot was used to water the plants.

Typically, the plants were watered with Hoagland solution every day, but occasionally, this schedule was adjusted based on how wet the rooting medium (sand) was. Fresh and Dry Biomass, Internodal Distance, and Leaf Count. Each plant's internodal distance was measured in millimeters using a measuring tape. For every plant, the number of leaves was also counted. Each plant's fresh biomass was measured using an electric balance. For every treatment, the fresh biomass average was determined. Following a 72-hour incubation period at 70°C, the dry biomass of the entire plant was assessed. A computerized electric balance was used to measure the dry biomass, and the means for each treatment were determined. On undamaged, completely grown leaves, the photosynthetic activity (Pn) was measured (11).

The quantity of chlorophyll present in three leaves is used to measure the amount of chlorophyll for each genotype and NaCl concentration. To extract it, 35 milliliters of 96% ethanol are used to boil one gram of fresh weight leaves. The chlorophyll concentration is determined spectrophotometrically at 654 nm from the ethanolic supernatant after centrifugation (10 min at 4.000 g) (12).

The experiment was set up using a completely randomized design (CRD) with two components (genotypes and salinity) in a factorial configuration. Statistical software (Statistix 7.1) was used to do a two-way analysis of variance on the data, and comparisons with P-values 0.05 were deemed substantially different based on HSD values (13).

Results and discussion

In general, when the NaCl osmotic potential increased, all of the parameters under investigation steadily decreased (Tables 1-6). Growth characteristics were adversely affected by NaCl osmotic potentials. The influence of NaCl content on internodal distance in several plant species is illustrated by the data in Table 1. The findings demonstrate the effect of salt stress on plant growth by showing that a significant decrease in internodal distance occurs for all species under study as the concentration of NaCl rises. For instance, as the concentration of NaCl rises from 0 to 75 mM, the internodal distance in the "Local 1" genotype falls from 5.6 ± 1.4 cm to 2.5 ± 0.6 cm. These findings provide credence to the theory that salt stress impairs cell growth by altering osmotic balance and ion toxicity, which subsequently impacts the plant's capacity for cell elongation (1). The "Lincoln" and "Wando" genotypes, on the other hand, exhibit higher tolerance to salt stress than the other species because they continue to grow at higher NaCl concentrations. This may be explained by their enhanced osmotic control and defense systems against ion toxicity, which enhance their capacity to withstand elevated salt concentrations (14). Therefore, it can be said that different plant species react differently to salt stress, which allows for more research to determine the molecular mechanisms underlying these species' salt tolerance.

Table 1. Effect of NaCl on Internodal Distance (cm)

Genotypes	NaCl (mM)			
	0	25	50	75
Local 1	5.6±1.4	4.4±1.5	3.1±0.3	2.5±0.6
Wando	6.5±2.4	5.1±1.3	3.6±0.5	2.2±0.7
Lincoln	6.2±1.3	5.2±1.5	4.2±0.7	2.6±0.8
Green arrow	5.7±1.1	4.5±1.5	3.2±0.8	1.7±0.3

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The impact of rising NaCl concentrations on the fresh biomass of four pea (*Pisum sativum* L.) genotypes—Local 1, Wando, Lincoln, and Green Arrow—is shown in Table 2. The findings show that fresh biomass consistently decreases in a dose-dependent manner across all genotypes when salinity rises from 0 to 75 mM. This decrease emphasizes how salt stress inhibits plant growth; a phenomenon that has been extensively studied in the literature. The main ways that salt stress hinders plant growth are through osmotic stress and ion toxicity, which harm cellular metabolism, water absorption, and nutritional balance (1) At 75 mM NaCl, fresh biomass values drastically decrease, as seen in Table 2, with the Green Arrow and Wando genotypes showing a 47.7% decrease in comparison to control conditions. Similar declines were observed in Lincoln and Local 1 (48.3% and 40.8%, respectively), indicating that all genotypes are susceptible to salinity, albeit to differing degrees.

Lincoln had the largest fresh biomass (11.8±5.4 g) of the studied genotypes in non-saline environments, suggesting a promising start to growth. This benefit, however, was lost at higher salt levels, where it performed similarly to Local 1 (both at 6.1 g under 75 mM NaCl), highlighting the fact that initial vigor and salinity tolerance are not always correlated. Salinity-induced oxidative stress, which damages membranes and prevents cell growth, could also be the source of the observed decline in fresh biomass (15)(3). In addition to impairing photosynthetic efficiency and hormonal communication, salinity also hinders development. The marginally smaller decline in Local 1 points to a relatively superior tolerance mechanism from an agronomic and breeding standpoint, making it a contender for additional research. For sustainable crop production in saline-prone locations, it is essential to identify genotypes that are tolerant of salt. To validate and describe the tolerance mechanisms found in this work, more physiological, biochemical, and molecular investigations would be helpful.

Table 2. Effect of NaCl on Fresh Biomass

Genotypes	**NaCl (mM)**			
	0	**25**	**50**	**75**
Local 1	10.3±4.1	10.4±4.5	8.4±2.3	6.1±2.3
Wando	10.9±3.7	9.7±4.2	7.9±1.8	5.7±2.5
Lincoln	11.8±5.4	10.2±3.6	8.2±2.7	6.1±1.8
Green arrow	10.7±4.5	9.4±4.6	7.2±2.9	5.6±2.5

Table 3 shows how four pea genotypes (Local 1, Wando, Lincoln, and Green Arrow) responded to rising NaCl concentrations (0–75 mM) based on variations in dry biomass. The findings show that salt tolerance varies by genotype, with biomass typically decreasing under salinity stress, while the pattern and degree of decline vary by genotype. All genotypes show their maximum dry biomass in 0 mM NaCl (control), which ranges from 2.08 g (Local 1) to 2.95 g (Lincoln), suggesting similar growth in non-saline circumstances.

An overall decrease in biomass is shown when the concentration of NaCl rises, which is in keeping with the fact that salt stress inhibits plant growth because of osmotic stress, ion toxicity, and nutritional imbalance (1). At 75 mM (2.01 g), there is only a modest loss in biomass, suggesting a less significant salt-induced reduction (3.4%). Local 1 appears to be moderately tolerant, retaining rather steady biomass across all salt concentrations. This implies effective ionic homeostasis or osmoregulatory systems that lessen the consequences of salt stress. In contrast, Wando exhibits a notable decrease in biomass at 50 mM (1.26 g) and 75 mM (1.08 g), which is over 50% less than the control. This suggests a high sensitivity to salinity, which could be brought on by ineffective osmotic adjustment or poor exclusion of harmful Na+/Cl+ ions (3).

Lincoln's biomass gradually decreases as the concentration of salt rises. It begins with the maximum control biomass (2.95 g), but after 75 mM, it decreases to 1.57 g, signifying a 46.8% decrease. Under mild salinity, Lincoln retains a comparatively higher biomass than Wando and Green Arrow, indicating intermediate tolerance despite being sensitive. With a noticeable drop of 62.6% from 2.70 g (0 mM) to 1.01 g (75 mM), Green Arrow exhibits significant sensitivity. This genotype appears to be extremely sensitive even at low salinity levels, as evidenced by the sharp drop at 25 mM (1.73 g). These findings suggest that salinity significantly affects pea dry biomass in a genotype-dependent manner. Wando and Green Arrow are more vulnerable, while Local 1 is the most tolerant genotype, exhibiting the least amount of biomass loss. Genetic diversity in processes including osmotic adjustment, antioxidant capability, and ion transport regulation may be the cause of these variations (16). Because genotypes like Local 1 may be viable candidates for creating salt-tolerant cultivars, this information is helpful for breeding projects aimed at places affected by salt.

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Table 3. Effect of NaCl on Dry Biomass

Genotypes	NaCl (mM)			
	0	25	50	75
Local 1	2.08±0.57	2.38±0.40	2.18±0.31	2.01±0.51
Wando	2.15±0.63	2.64±0.54	1.26±0.32	1.08±0.21
Lincoln	2.95±0.64	2.54±0.58	2.06±0.17	1.57±0.41
Green arrow	2.70±0.55	1.73±0.66	1.17±0.18	1.01±0.24

Table 4 shows how four pea (Pisum sativum L.) genotypes—Local 1, Wando, Lincoln, and Green Arrow—respond to increasing NaCl concentrations (0, 25, 50, and 75 mM) in terms of the number of leaves per plant. The detrimental effect of salinity stress on vegetative growth is seen by the steady decrease in leaf number that occurs across all genotypes as NaCl concentration rises. Particularly in salt-sensitive species like peas, salinity causes osmotic and ionic stressors that disrupt cellular metabolism and water intake, frequently resulting in decreased growth and biomass accumulation (1). The Lincoln genotype in this study exhibits the greatest number of leaves (80 leaves/plant) under control circumstances, but at 75 mM NaCl, this number decreases by 25%. At the greatest salinity level, Wando and Local 1 exhibit a similar pattern, with leaf numbers declining by almost 25% and 18.5%, respectively. With a 22% decrease in leaf number already seen at 25 mM NaCl and a nearly 22% overall loss from control to 75 mM NaCl, Green Arrow seems to be the most sensitive genotype. This genotype's early sensitivity to salt stress is indicated by the rather sharp decline between 0 and 50 mM NaCl.

Salt-induced suppression of cell division and expansion, harm to photosynthetic tissues, and potential hormonal abnormalities are all responsible for this reduction (15). The discrepancies across genotypes point to innate variability in mechanisms of salt tolerance, which may include osmoprotectant accumulation, antioxidant activity, or ion homeostasis (16). These results highlight the necessity of screening and breeding salt-tolerant genotypes for sustainable production in saline circumstances and are in accordance with previous publications that show cultivar-dependent responses to salinity in legumes.

Table 4. Effect of NaCl on the number of leaves/plants

Genotypes	Nacl (mM)			
	0	25	50	75
Local 1	70±8	67±15	64±14	57±16
Wando	71±9	68±8	66±10	53±14
Lincoln	80±0.9	73±11	68±9	60±8
Green arrow	72±13	64±8	58±6	56±7

All four genotypes—Local 1, Wando, Lincoln, and Green Arrow—clearly show a progressive decrease in photosynthetic rate (Pn) as NaCl concentrations (0–75 mM) rise, as shown in Table 5. This decrease reflects a well-established physiological response in plants under salinity stress: salt-induced photosynthetic inhibition. A similar baseline capacity for carbon absorption under non-stressed conditions was suggested by the relatively similar Pn values (~7.4 to 8.4 μmol CO_2 m^{-2} s^{-1}) displayed by all genotypes at 0 mM NaCl. However, photosynthesis significantly decreased as NaCl levels rose. Green Arrow showed the sharpest loss, especially between 25 and 50 mM NaCl (from 4.5 to 3.4 μmol CO_2 m^{-2} s^{-1}), and it reached a minimum of 2.2 at 75 mM.

There are multiple interrelated physiological systems responsible for this inhibition: 1) Reduced CO_2 uptake due to stomatal closure (1) Ion toxicity, specifically from the buildup of Na^+ and Cl^-, which impairs photosynthetic enzymes and chloroplast function (3) Oxidative stress, which damages membranes and impairs photochemistry (16). Greater salt tolerance may be the result of improved osmotic adjustment, ion compartmentalization, or antioxidant defenses, as Lincoln and Local 1 genotypes maintained comparatively higher Pn levels at increasing salinity, particularly at 75 mM. The Green Arrow genotype, on the other hand, displayed the highest sensitivity, which could be due to either a diminished ability to maintain ion homeostasis or weakened stress signaling pathways that shield photosynthetic equipment from salinity. These results are in line with earlier research that found underlying variations in Na+

exclusion, chlorophyll retention, and antioxidant enzyme activity were associated with genotype-specific variation in photosynthetic resilience under salt stress (17,18).

Table 5. Effect of NaCl on Photosynthetic Rate (Pn)

Genotypes	NaCl (mM)			
	0	25	50	75
Local 1	7.8±3.3	6.5±1.3	4.6±0.7	2.8±0.1
Wando	7.4±2.4	6.6±2.6	4.7±0.8	2.2±0.2
Lincoln	8.1±2.7	6.7±2.2	4.8±0.5	2.6±0.1
Green arrow	8.4±2.5	4.5±1.5	3.4±0.8	2.2±0.3

All genotypes' total chlorophyll content (mg/g fresh matter) gradually decreases when the NaCl concentration rises from 0 to 75 mM, as shown by the results in Table 6. Because of ionic toxicity, osmotic stress, and oxidative damage, salinity stress, which is brought on by high NaCl levels, is known to negatively impact photosynthetic pigments, especially chlorophyll (2). Lincoln had the greatest initial chlorophyll content (3.76 mg/g FM) among the genotypes under study under control conditions (0 mM NaCl). At 75 mM, it showed a relatively modest decline (1.80 mg/g FM), indicating a considerably stronger tolerance to salt-induced chlorophyll degradation. On the other hand, at 50 mM NaCl, Local 1's chlorophyll content dropped sharply from 3.22 to 1.22 mg/g FM before slightly increasing at 75 mM (1.55 mg/g FM). This could be a sign of experimental variability or a stress adaptation response. Green Arrow showed one of the sharpest drops at the greatest salt concentration (1.16 mg/g FM at 75 mM), indicating its vulnerability to salinity, even if its chlorophyll level was rather modest under control (3.13 mg/g FM).

At moderate salt concentrations, Wando and Lincoln maintained comparatively higher amounts of chlorophyll, suggesting improved chlorophyll retention ability and maybe more potent ion compartmentalization or antioxidative defense mechanisms (3). According to earlier research, salt-induced disruption of chloroplast structure, suppression of chlorophyll production, and increased chlorophyllase activity are responsible for the observed decrease in chlorophyll content across all genotypes. To improve crop production in saline environments (19)**.** Breeding programs must take into account the genotypic heterogeneity in salt tolerance mechanisms suggested by these studies.

Table 6. Effect of NaCl on Total Chlorophyll Content (mg/g Fresh Matter)

Genotypes	NaCl (mM)			
	0	25	50	75
Local 1	3.22±0.099	2.92±0.088	1.22±0.077	1.55±0.056
Wando	3.48±0.094	2.98±0.051	1.92±0.062	1.44±0.085
Lincoln	3.76±0.092	3.08±0.072	2.41±0.096	1.80±0.062
Green arrow	3.13±0.088	2.71±0.088	2.37±0.050	1.16±0.073

Conclusion

Under carefully monitored laboratory circumstances, this study demonstrates the negative impact of salinity on the physiological characteristics of pea plants (*Pisum sativum*). For all investigated genotypes, increasing salt stress dramatically decreased photosynthetic rate, hindered plant development, and decreased chlorophyll contents. Nonetheless, it was clear that salinity tolerance varied by genotype. Wando and Lincoln—along with Local 1—showed stronger tolerance to salinity than Green Arrow, indicating their potential for cultivation in saline-prone settings. These results highlight how crucial it is to choose and cultivate salt-tolerant pea varieties to lessen the negative effects of soil salinity on agricultural output.

Conflict of interest. Nil

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