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ARTICLE

Physiological Responses of Pea Plants to Salinity and Gibberellic Acid

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ABSTRACT

Pea is a seed legume. It is rich in cellulose fibre and protein. It is also a significant source of minerals and vitamins. In this paper, we set out to better characterize the physiological responses of *Pisum sativum* L. to the combined effects of NaCl, 100 mM and gibberellins (GA3). Our analysis revealed that NaCl caused a decrease in growth resulting in a reduction in root elongation, distribution and density, leaf number and leaf area, and a decrease in dry matter of roots and shoots. However, the contribution of GA3 in the salty environment induced an increase in these different parameters suggesting an improving effect of this hormone on growth of pea in presence of salt. NaCl also led to a disturbance of the photosynthetic machinery. Indeed, level of chlorophyll pigments (a and total) and photosynthetic activity were decreased compared to the control plants. However, the exogenous supply of GA3 restored this decrease in net CO_2 assimilation, but not in chlorophyll content. Additional analyses were performed on the effect of salinity/GA3 interaction on osmolytes (soluble sugars and starch). Our results showed an increase in sugars and a decrease in starch in the presence of 100 mM NaCl. The salt-GA3 combination resulted in compensation of soluble sugar contents but not of starch contents, suggesting a beneficial effect of GA3 under saline stress conditions. Level of three main polyamines putrescine, spermidine, and spermine increased significantly in all organs of salt-treated plants.

KEYWORDS

Pisum sativum L.; salinity; gibberellic acid; growth; sugars; polyamines

Abbreviations

Car	Carotenoids
Chl	Chlorophylls
DW	Dry weight
FW	Fresh weight
GA3	Gibberellic acid
PAs	Polyamines
PA	Photosynthetic activity
Put	Putrescine
Spd	Spermidine
Spm	Spermine

1 Introduction

Soil salinity is a major constraint that negatively affects physiological and biochemical aspects of plant, leading to a reduction in their yield. In addition, it induces osmotic stress, physiological drought and ionic imbalance, thus deactivating plant's vital cellular functions [1].

Although plant responses to abiotic stresses depend on various factors, phytohormones are considered the most important endogenous substances for modulating physiological and molecular responses, a critical requirement for plant survival [2]. Indeed, phytohormones play a key role in the control and regulation of plant physiological mechanisms, both under favorable and constraining conditions. According to Khan et al. [3], these phytohormones are considered important endogenous substances and are often involved in plant tolerance or sensitivity mechanisms. Thus, one of the strategies for plant defense against environmental stress, such as salinity, is to change concentrations and ratios of endogenous phytohormones [4]. Indeed, a change in the external environment corresponds to a signal for the plant that will influence its development. This may involve hormones as intermediaries between receptors capable of perceiving these signals and transforming them into information that can be used by plants, and effectors translating this information into specific responses [5].

In order to improve the performance of plants under saline stress, researchers have now turned their attention to the use of growth regulators, such as GA3, which play an important role in regulating the response of cotton plants to the external environment and in controlling gene expression under saline stress [6]. Reduced plant growth under drought or salinity may be due, at least in part, to reduced gibberellin production or the inability of plants to respond to this hormone [7]. In response to salinity, Geng et al. [8] reported that salt-treated Arabidopsis plants showed a decrease in bioactive GAs levels. Thus, the chemical composition and concentration of salt present in the soil could modulate the GAs metabolism. Several studies have confirmed the importance of GA3 in improving synergy and crop performance under saline conditions. This improving effect of gibberellic acid can affect several parameters including growth and photosynthesis. Renu [9] reported that multiple positive effects of GA3 in response to salt in beans include improved growth parameters, increased photosynthetic pigments, reducing sugars and sucrose, and stimulation of the synthesis of existing and new proteins.

Pea is one of the most important grain vegetables, traditionally cultivated in many regions of the world. Pea grains are a rich source of dietary proteins, carbohydrates, dietary fiber, vitamins, and minerals [10]. Pea is one of the most tolerant vegetables to salt stress as 50% yield reduction in this crop was noted at 100 mM NaCl. Nonetheless, its productivity was markedly reduced at elevated levels of salt stress [11]. In Tunisia, this species is cultivated mainly in the northern regions. It occupies the 4th place after broad bean, chickpea and field bean. The two favourable seasons for this crop are autumn and spring. Saline soils in the world, which extend over 1.5 million hectares, present a major constraint for growth and development of cultivated pea plants. Therefore, some strategies such as exogenous application of plant growth regulators (such as gibberellic acid (GA3)) could be used to reduce the negative effects of salinity and improve growth performance [12].

Polyamines (PAs) are aliphatic amines present in all plant cells [13]. These PAs can be in free forms, linked to macromolecules (proteins and nucleic acids) or to molecules of low molecular weight (such as hydroxy-cinnamic acids). Currently, these substances are considered to be a novel group of plant regulators, and appear to be involved in several aspects of plant development such as growth, differentiation and senescence [14]. PAs are notably involved in the initiation and floral development as well as in fruit growth. PAs and aromatic amines also seem to be involved in plant's response to biotic and abiotic stresses [15]. Recently, a large body of research shows that plant PAs are involved in the acquisition of tolerance to such stresses as high and low temperatures, salinity, hyperosmosis, hypoxia and atmospheric pollutants [16].

We have opted for an experimental approach in which gibberellic acid is added to culture medium of salt-enriched plants, in order to study the possibility of improving their production potential. The objective of this project was to study the effects of a saline treatment and application of gibberellic acid (GA3) on growth, mineral nutrition, chlorophyll, carbohydrate (sugar and starch contents) and polyamine contents of pea grown only on a saline medium.

2 Material and Methods

2.1 Plant Material and Culture Conditions

This study focused on the Lincoln variety of pea (*Pisum sativum* L.). It is widespread in Tunisia, with a long cycle (60 days). Seeds of this variety were provided by the Laboratory of Seed Legumes of INRAT, Tunisia.

Pea seeds were disinfected with 5 g L^{-1} calcium hypochlorite solution for 10 min, rinsed with distilled water, then placed for 4 h in darkness in beakers filled with water. Subsequently, they were placed in Petri dishes lined with filter paper soaked in distilled water, at a rate of 15 seeds per dish, and then placed in an oven at 23°C in the dark for 3 days. Germination was indicated by the emergence of a 3 cm long radicle.

Petri dishes containing 3-day-old *Pisum sativum* L. seedlings were placed in an air-conditioned growing room under a bright ceiling, where growing conditions were controlled: brightness (16 h day/8 h night), temperature (22°C day/18°C night), effective radiation of 200 µmol.m^{-2} s^{-1} and hygrometry (60% humidity during the day/80% humidity at night).

Seedlings of *Pisum sativum* L. from sowing and aged 6 days were transplanted at a rate of 6 per bucket containing 5 litres of nutrient solution diluted 4 times. At the age of 15 days, plants were divided into four batches. The first batch, serving as a control, was grown on basic Long Ashton [17] nutrient medium, containing 1.5 mM $MgSO_4$, 3 mM KNO_3, 1.6 mM KH_2PO_4, 0.3 K_2HPO_4, 3.5 mM $Ca(NO_3)_2$, 2 mM NH_4NO_3, 10^{-3} mM $MnSO_4$, 5×10^{-4} mM $ZnSO_4$, 5×10^{-4} mM $CuSO_4$, 10^{-2} mM H_3BO_3, 5×10^{-5} mM $(NH_4)_6Mo_7O_{24}$, and 3 µM FeEDTA. For other three batches, either 100 mM NaCl (second batch) or 10^{-6} mM gibberellic acid (GA3) (third batch) or both (fourth batch) was added to the same medium. The four treatments were as follows:

0 mM GA3 + 0 mM NaCl = control

10^{-6} mM GA3 + 0 mM NaCl = GA3

0 mM GA3 + 100 mM NaCl = NaCl

10^{-6} mM GA3 + 100 mM NaCl = GA3 + NaCl

After 15 days of treatment, six plants were harvested and separated into leaves, stems and roots. Several parameters were determined before (root elongation and leaf number) and after final harvesting of plants from the four treatments (fresh and dry weights, leaf area, water content and Na^+, K^+ contents of different organs).

Root elongation was measured on the total length of the whole root system (all primary and lateral roots together).

Root density was estimated as the ratio between the dry weight and the roots length [18].

The specific leaf area was calculated as the ratio of the total leaf area to the leaf dry weight [19] and the leaf thickness was calculated as the ratio of leaf water content and the total leaf area [20].

The degree of leaf succulence and the degree of leaf sclerophylly were determined as the ratio of the amount of water and dry weight to the total leaf area, respectively [21,22].

Water content (mL g^{-1} DW) were estimated by the difference between the fresh weight (FW) and dry weight (DW), referred to the unit mass of DW.

Another five plants per treatment were harvested and used for determination of chlorophylls, carotenoids, proteins, carbohydrates (soluble sugars and starch) and polyamine contents.

2.2 Chlorophyll and Carotenoid Contents

Leaf fragments were incubated in 80% acetone solution. After 72 h in the darkness and at 4°C, the density of acetone extracts was measured with a "Beckman" spectrophotometer at the absorbances (A) of 470 nm, 646 and 663 nm to calculate contents of chlorophyll a, chlorophyll b, total chlorophyll, and total carotenoids according to the equation proposed by Lichtenthaler [23].

Chlorophyll a ($\mu g\ ml^{-1}$) = (12.25 × A663) − (2.79 × A646)

Chlorophyll b ($\mu g\ ml^{-1}$) = (21.5 × A646) − (5.10 × A663)

Total chlorophylls ($\mu g\ ml^{-1}$) = (7.15 × A663) + (18.71 × A646)

Carotenoids ($\mu g\ ml^{-1}$) = (1000 × A470 − 1.82 × Chla − 85.02 × Chlb)/198

2.3 Measurement of Photosynthetic Activity

The measurement of this parameter was carried out inside the growth cabinet, with a portable open mode gas analyser system with a cylindrical cuvette (model LCA4; Analytical Development Company, Hoddesdon, UK). Six separate plants were tested. Leaves from the 3rd node. The leaf area inside the chamber was 2.5 cm^2. The temperature and light intensity were those of the growth cabinet (22°C and 150 $\mu mol\ s^{-1}\ m^{-2}$ PAR, respectively) with a mass flow rate of 201.5 $\mu mol\ s^{-1}$ and 1182 vpm CO_2. Measurements were performed 4 h after the start of the photoperiod.

2.4 Protein Content

Frozen roots, stems and leaves of *P. sativum* plants were ground in poly-vinyl-polypyrrolidone (PVP) and homogenized in 50 mM potassium phosphate buffer (pH 7), containing 0.1 mM Ethylenediaminetetraacetic (EDTA), 0.1 mM phenyl-methyl-sulfonyl-fluoride (PMSF) acid and 1 mM DTT. The mixture was centrifuged at 12,000 *g* at 4°C for 30 min. Supernatants were stored at −20°C for protein and enzyme analysis. The protein content of individual extracts was calculated by the Bradford reaction [24] utilizing bovine serum albumin (BSA) as a reference standard.

2.5 Determination of Carbohydrates (Soluble Sugars and Starch)

Soluble sugar and starch levels were determined in roots, stems and leaves of plants from all four treatments. Determination of soluble sugars and starch was carried out according to the technique described by Albouchi et al. [25], the standard range was established by pure glucose. Optical density was measured at a wavelength of 640 nm.

2.6 Analyze Free Polyamine Contents

Roots, stems and leaves samples for polyamine analysis were kept in ice until taken to the laboratory and stored at −80°C. Free polyamines (putrescine, spermidine and spermine) were extracted from samples with 5% $HClO_4$, dansylated and analyzed by HPTLC as described by Sarjala et al. [26].

Five individual plants of each treatment were used; each measurement was technically replicated 4 times.

2.7 Statistical Analysis

Statistical analysis was performed with Statistica™ software, using ANOVA and means were compared according to Duncan's multiple-range test at 5% level of significance.

3 Results

3.1 Effects of NaCl and GA3 on Plant Growth and Development

Morphological parameters. Morphogenesis was followed by root elongation and root density, leaf counts and measurements of leaf area. Specific leaf area, degree of succulence and degree of leaf sclerophylly were deduced from the different growth parameters measured.

Exposure to saline stress resulted in statistically significant decreases in elongation and root density of 18% and 17%, respectively, compared to the control. The addition of gibberellic acid (GA3) to nutrient solution with or without NaCl significantly improved elongation of these organs. In fact, root elongation was highest on GA3-treated plants, intermediate on control and GA3 + NaCl-exposed plants, and lowest on NaCl-treated plants. As for root length density, the highest value of this parameter was observed on control plants, while this parameter was similar on plants treated with GA3, NaCl or GA3 + NaCl (Table 1).

Table 1: Effects of NaCl and GA3 on root elongation, root distribution and root density, leaf number, total and individual leaf area, specific surface, leaf thickness, leaf succulence and leaf sclerophylly in 36-day old *P. sativum* plants. Measurements made after 15 days of treatment in the presence of different concentrations of NaCl and GA3. Mean of six plants and confidence interval for $P = 0.05$. Mean values with the same letter were not significantly different at $P < 0.05$ (ANOVA and mean comparison with Duncan test)

	Treatments			
Parameters	Control	GA3	NaCl	GA3 + NaCl
Root elongation (cm)	34 ± 0^{b}	38 ± 1^{a}	28 ± 1^{c}	34 ± 0^{b}
Root density (mg cm^{-1})	11 ± 1^{a}	10 ± 1^{ab}	9 ± 1^{b}	9 ± 1^{b}
Leaf number	48 ± 4^{b}	72 ± 11^{a}	39 ± 4^{c}	49 ± 4^{b}
Total leaf area (cm^2 $plant^{-1}$)	327 ± 21^{a}	366 ± 41^{a}	207 ± 5^{b}	226 ± 18^{b}
Individual leaf area (cm^2 $leave^{-1}$)	6.86 ± 0.26^{a}	5.13 ± 0.22^{b}	5.38 ± 0.49^{b}	4.63 ± 0.12^{c}
Specific leaf area (cm^2 mg^{-1})	0.50 ± 0.04^{b}	0.44 ± 0.03^{c}	0.63 ± 0.07^{a}	0.37 ± 0.04^{c}
Leaf thickness (mm)	24 ± 2^{b}	13 ± 1^{c}	55 ± 6^{a}	39 ± 6^{b}
Leaf succulence (mg cm^2)	16.2 ± 1.3^{b}	11.1 ± 0.7^{c}	18.3 ± 0.6^{b}	23.4 ± 1.9^{a}
Leaf sclerophylly (mg cm^2)	2.0 ± 0.2^{b}	2.3 ± 0.1^{b}	1.6 ± 0.2^{c}	2.7 ± 0.3^{a}

For the photosynthetic organs, the highest value was recorded for the number of leaves of GA3-treated plants. Presence of 100 mM NaCl induced a slower leaf settling in order of 19% compared to control. However, it was similar to those of the control plants for the GA3 + NaCl treated plants. Regarding leaf expansion parameters, the highest values were recorded for total leaf area of control and GA3-treated plants and single leaf area of control plants and for specific area of NaCl-treated plants. Salinity reduced total and single leaf area, but increased specific area by 27% compared to the control. The addition of GA3 to NaCl culture medium did not lead to an improvement in total leaf area, where values were similar to those of NaCl-treated plants, nor in individual leaf area, where values were lower than those of GA3 or NaCl-treated plants, nor in specific leaf area, where values were lower than those of the controls and similar to those of GA3-treated plants (Table 1).

In calculating thickness of these organs, the highest values were recorded for leaf thickness in NaCl-treated plants and for leaf succulence and leaf sclerophylly in GA3 + NaCl-treated plants. The leaf thickness of GA3 + NaCl treated plants showed similar values to those of the control plants, while recording low values for GA3 treated plants. In terms of succulence and sclerophylly leaves, the presence

of NaCl in the medium did not affect the first parameter, which showed values similar to those of the control plants, but it decreased the second parameter by 21% compared to the control (Table 1).

3.1.1 Growth

Examination of Fig. 1 shows that the highest values were recorded for the aerial organs of GA3-treated plants. In the presence of 100 mM NaCl, the dry weights of roots, stems and leaves were reduced by 32%, 28% and 50%, respectively, compared to control plants. On the other hand, cultivation on medium with GA3 + NaCl, significantly improves the growth of all organs, especially the growth of aerial organs, where the values are comparable to those of the control plants. The values recorded by the roots of GA3 + NaCl plants were intermediate between those treated with NaCl and those of both controls and GA3-treated plants.

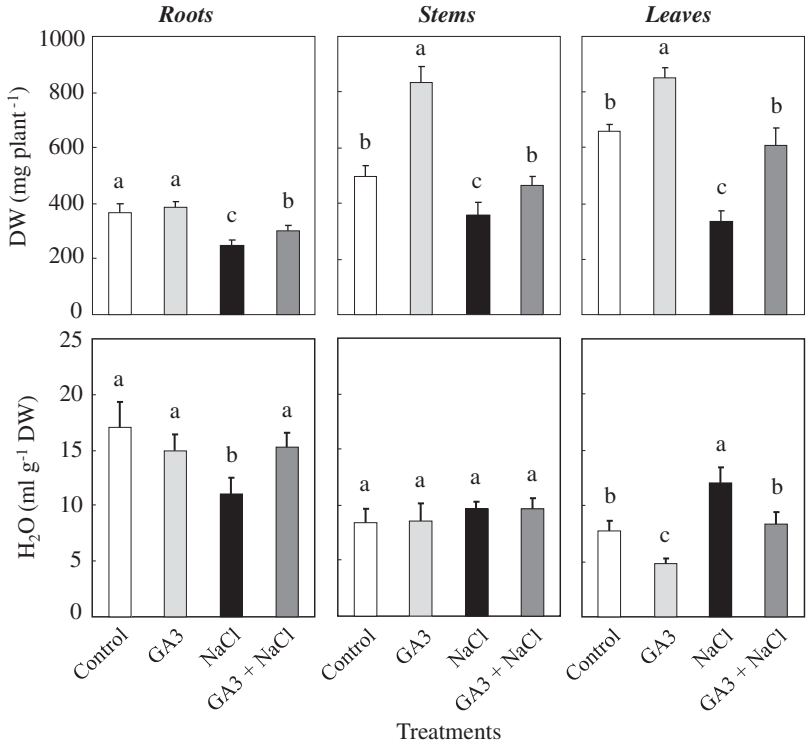

Figure 1: Effects of NaCl and GA3 on dry matter masses and water content of roots, stems and leaves in 36-day old pea plants. Measurements made after 15 days of treatment in the presence of different concentrations of NaCl and GA3. Mean of six plants and confidence interval for $P = 0.05$. Mean values with the same letter were not significantly different at $P < 0.05$ (ANOVA and mean comparison with Duncan test)

3.1.2 Organ Hydration

There was a depressive effect of salt on root hydration, no change in stems and a stimulating effect in leaves. Indeed, the water content decreased by about 35% and increased by 44% compared to control plants in roots and leaves, respectively (Fig. 1). However, the addition of GA3 to the culture medium with or without NaCl improved the hydration of the roots, where the values reached those of the control plants. The same was observed for the leaves of GA3 + NaCl-treated plants. However, the hydration of these organs seemed to be even lower in the presence of GA3 only. As for the stems, the water contents did not seem to be affected by either salt or GA3 with or without NaCl (Fig. 1).

3.1.3 Ionic Characteristics

Examination of Fig. 2 shows that Na^+ accumulation in the different organs of plants grown on saline media was almost identical, in the order of 5 mmol g^{-1} DW. The addition of GA3 to the culture medium decreases the Na^+ levels in these organs, especially in the aerial parts. The values recorded on this treatment were even lower than those recorded on the control medium. Moreover, the values recorded by the plants treated jointly with NaCl and GA3 are at a lower level (stems and leaves) or equal (roots) to those of the plants treated with salt.

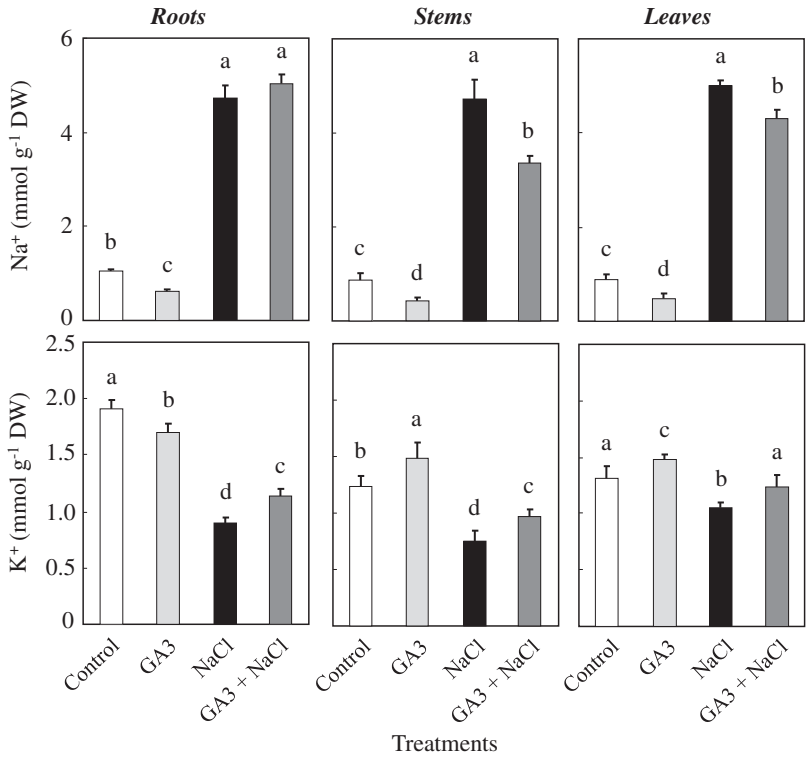

Figure 2: Effects of NaCl and GA3 on sodium and potassium content of roots, stems and leaves in 36-day old pea plants. Measurements made after 15 days of treatment in the presence of different concentrations of NaCl and GA3. Mean of six plants and confidence interval for $P = 0.05$. Mean values with the same letter were not significantly different at $P < 0.05$ (ANOVA and mean comparison with Duncan test)

In the control medium (without NaCl or GA3), roots were the most potassium-rich organs compared with aerial organs (Fig. 2). The presence of salt (NaCl, 100 mM) significantly reduced K^+ levels, more markedly in roots than in stems and, to a lesser extent, in leaves. This reduction was 53%, 40% and 20% of the control, respectively. However, this effect seems to be attenuated by the addition of GA3 in a saline medium, especially in leaves where values were similar to those of the control plants. For all organs, the addition of GA3 to the NaCl-free medium results in lower (roots) or higher (aerial parts) K^+ uptake and accumulation than measured in control plants (Fig. 2).

3.2 Effects of NaCl and GA3 on Photosynthetic Parameters

3.2.1 Chlorophyll and Carotenoid Content

Based on hypothesis that the effect of salt on photosynthesis may be due to a disturbance in metabolism of chlorophylls (Chl) and carotenoids (Car), we determined the contents of these pigments in leaves taken at

the end of cultivation from plants grown in presence of different concentrations of NaCl and GA3 (Table 2). It can be seen that the highest values were reported in the leaves of control and GA3 treated plants and also in NaCl treated plants only for chlorophyll b and carotenoids. NaCl caused a decrease in total chlorophyll contents due to that of fraction a (respectively 32% and 22% of control for Chl a and Chl tot). On the other hand, chlorophyll b and carotenoids appear stable and insensitive to salt. When calculating the Chl a/b and Chl/Car ratios, we notice a slight statistically significant decrease due to the effect of salt treatment. On saline medium with added GA3, the level of accumulation of photosynthetic pigments decreases further and reaches a level lower than that of the control leaves. Despite this decrease, the Chla/Chlb and Chl/Car ratios remained comparable to those of salt-treated plants. The addition of GA3 alone to the medium leads to a pigment status comparable to that of control plants.

Table 2: Effects of NaCl and GA3 on the level of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids in 36-day old pea plants. Measurements made after 15 days of treatment in the presence of different concentrations of NaCl and GA3. Mean of six plants and confidence interval for $P = 0.05$. Mean values with the same letter were not significantly different at $P < 0.05$ (ANOVA and mean comparison with Duncan test)

	Treatments			
Photosynthetic parameters	Control	GA3	NaCl	GA3 + NaCl
Chlorophyll a, mg g^{-1} FW	33.9 ± 3.4^{a}	34.8 ± 3.9^{a}	23.1 ± 1.1^{b}	18.8 ± 1.7^{c}
Chlorophyll b	10.7 ± 1.0^{a}	13.7 ± 2.8^{a}	10.8 ± 1.3^{a}	8.1 ± 1.2^{b}
Chlorophyll totale	44.1 ± 2.9^{a}	47.2 ± 4.1^{a}	34.4 ± 2.7^{b}	27.2 ± 1.9^{c}
Chl a/b	3.2 ± 0.2^{a}	2.6 ± 0.6^{ab}	2.2 ± 0.2^{b}	2.4 ± 0.3^{b}
Carotenoids (Car)	11.8 ± 0.8^{a}	11.4 ± 0.8^{a}	11.2 ± 0.8^{a}	9.0 ± 0.6^{b}
Chl/Car	3.7 ± 0.2^{b}	4.1 ± 0.2^{a}	3.1 ± 0.4^{c}	3.0 ± 0.2^{c}

3.2.2 Photosynthetic Activity

On control medium, as on medium with GA3, photosynthetic assimilation showed the highest values, corresponding to 7 µmol m^{-2} s^{-1} (Fig. 3). On the other hand, a net reduction in this activity, of about 66% compared with control, was noted in leaves of plants treated with NaCl alone. Simultaneous addition of NaCl and GA3 raised photosynthetic activity to a higher level than that of organs of salt-treated plants, although it was still lower than that of control plants or plants enriched with GA3. This shows a regulating effect of GA3 in presence of NaCl on net assimilation of CO_2 by pea (Fig. 3).

3.3 Effects of NaCl and GA3 on Total Proteins

The analysis of Fig. 4 showed that the aerial parts were richer in protein than the roots. The highest values were observed in the aerial parts of control and GA3-treated plants, whereas in the roots, plants grown on GA3-enriched medium showed high protein levels that exceeded those of control plants (Fig. 4). In the presence of 100 mM NaCl, soluble protein contents were reduced more significantly in aerial organs than in underground organs. The addition of GA3 to the salt-supplemented culture medium restored this decrease in the roots where similar values to those of the controls were observed. While for the aerial parts, the values were intermediate between the control or GA3-treated plants and the plants treated with 100 mM NaCl (Fig. 4).

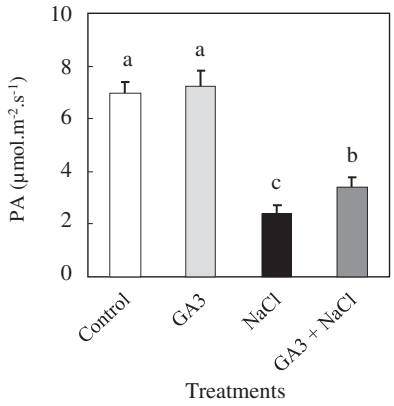

Figure 3: Effects of NaCl and GA3 on photosynthetic activity in 36-day old pea plants. Measurements made after 15 days of treatment in the presence of different concentrations of NaCl and GA3. Mean of six plants and confidence interval for $P = 0.05$. Mean values with the same letter were not significantly different at $P < 0.05$ (ANOVA and mean comparison with Duncan test)

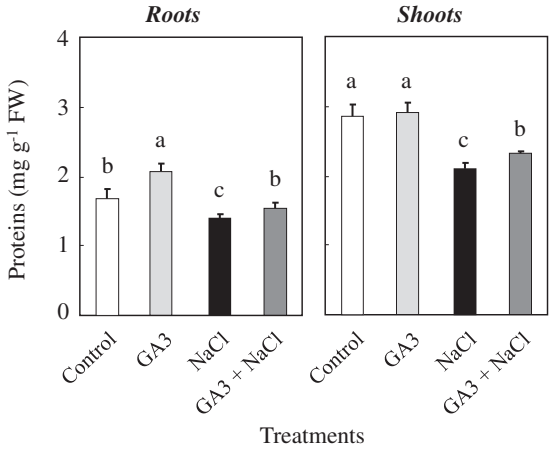

Figure 4: Effects of NaCl and GA3 on soluble protein content of roots and shoots in 36-day old pea plants. Measurements made after 15 days of treatment in the presence of different concentrations of NaCl and GA3. Mean of five plants and confidence interval for $P = 0.05$. Mean values with the same letter were not significantly different at $P < 0.05$ (ANOVA and mean comparison with Duncan test)

3.4 Soluble Sugar and Starch Contents

Fig. 5 showes that salt stress induced an increase in soluble sugar levels in all organs. Leaves (62 mg g^{-1} FW) and, to a lesser extent, stems (36 mg g^{-1} FW) showed the highest levels. However, this increase was more notable in stems than in other organs. The presence of GA3 alone significantly lowered soluble sugars to levels below those of control plants in roots and leaves, while in stems the values were similar to those of control plants. GA3 + NaCl-exposed plants maintained sugar levels lower than those of salt-treated plants, but still higher than those of control plants in roots and stems. For the leaves, the values were similar to those of the control plants.

Regarding starch contents, Fig. 5 shows that the highest values were recorded in the control plants. An inhibitory effect of NaCl on starch synthesis was observed in all organs. The addition of GA3 to the NaCl-enriched medium did not improve the levels of these metabolites in either the roots or aerial organs.

The values recorded were lower than those of salt-treated plants for aerial parts, those of roots, the values were similar to those of NaCl stressed plants. The addition of GA3 to the control medium also seems to have a depressive effect on starch levels in the different plant organs, explaining the stronger effect of the GA3-NaCl combination on these levels.

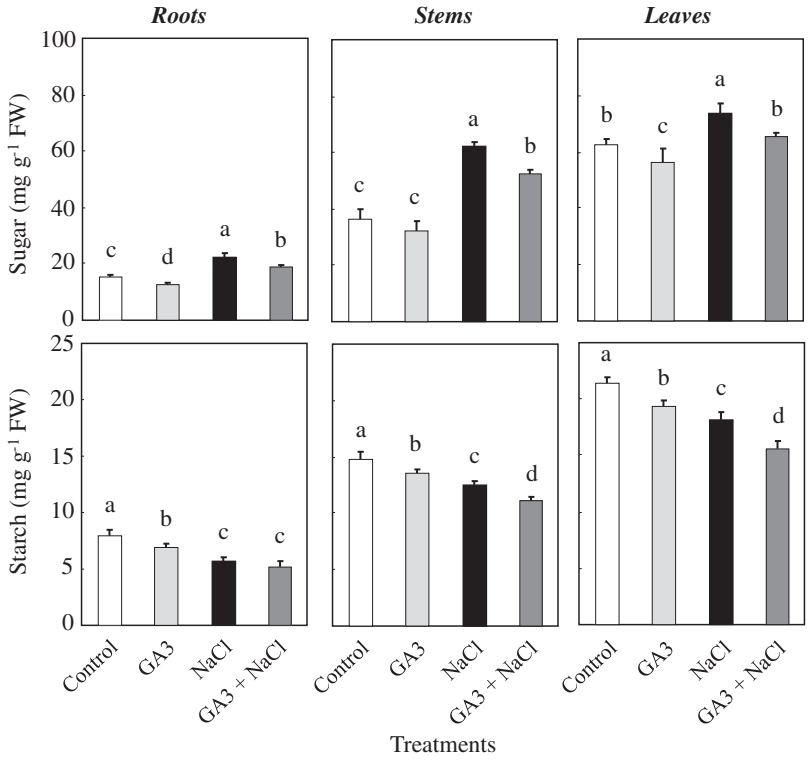

Figure 5: Effects of NaCl and GA3 on sugar and starch content of roots, stems and leaves in 36-day old pea plants. Measurements made after 15 days of treatment in the presence of different concentrations of NaCl and GA3. Mean of five plants and confidence interval for $P = 0.05$. Mean values with the same letter were not significantly different at $P < 0.05$ (ANOVA and mean comparison with Duncan test)

3.5 Polyamine Contents

Fluctuations in polyamine contents of roots, stems and leaves in treatments of NaCl, 0 and 100 mM were shown in Table 3. For control plants (without salt), contents of putrescine, spermidine and spermine were higher in roots, both roots and leaves, and in stems, respectively. The changes in polyamines under salt stress increase considerably with the exception of spermidine at the root level, where no significant modification was recorded compared to those of the control plants (Table 3). The increase for Put, Spd and Spm compared to controls (without NaCl) was 2, 1, 2-fold for the roots and 7, 7 and 3-fold for the stems, 4, 2, 4-fold for the leaves, respectively, under saline stress conditions (Table 3).

4 Discussion

Presence of salt in growing medium of pea plants, after two weeks of treatment, affects roots reduce elongation, distribution and root density compared to controls. However, GA3-salt combination increases root elongation and distribution compared to plants treated with NaCl. In contrast, root density remained unchanged. Indeed, gibberellic acid stimulates plant growth by increasing wall extensibility. Ijaz et al. [27] showed that in rice, KS-282 plants were capable of reducing root density by diminishing root

architectural parameters to maintain the resources under osmotic and ionic stress at the early phase of salt treatment compared to the sensitive Super Basmati plants. Robin et al. [28] showed that under salt stress, the length and density of absorbing hairs decreased. It was proposed that the Salt Overly Sensitive (SOS) pathway is implicated in the modulation of salt-sensitive absorbing hairs. Overexpression of triptych (TRYs) TFs from the halophyte Limonium bicolor in Arabidopsis revealed their implication in the salt tolerance absorptive hair development pathway [29]. In the literature, phytohormones, such as GA3, activate the proton pump of plasma membrane. Protons are pumped from cytosol to the cell wall. According to some authors, the resulting decrease in pH has led to a loosening of the wall structure, either through the rupture and reconstitution of non-cellulosic polysaccharides normally binding cellulose microfibrils, or through the action of a new class of proteins called expansins, which break hydrogen bridges between wall polysaccharides [30]. Indeed, gibberellins promote a transverse arrangement of microtubules, located immediately below the plasma membrane, and control the orientation of those of cellulose and, consequently, the great longitudinal growth or root elongation [31].

Table 3: Effects of NaCl on Polyamines contents of roots, stems and leaves in 36-day old pea plants. Measurements made after 15 days of treatment in the absence or presence of NaCl. Mean of five plants and confidence interval for $P = 0.05$. Mean values with the same letter were not significantly different at $P < 0.05$ (ANOVA and mean comparison with Duncan test)

	Treatments	
Polyamines, µmol g^{-1}FW	0 mM NaCl	100 mM NaCl
Roots		
Putrescine	9 ± 1^{b}	20 ± 1^{a}
Spermidine	11 ± 0^{a}	12 ± 1^{a}
Spermine	9 ± 1^{b}	21 ± 1^{a}
Stems		
Putrescine	5 ± 1^{b}	37 ± 1^{a}
Spermidine	6 ± 1^{b}	43 ± 2^{a}
Spermine	12 ± 1^{b}	40 ± 3^{a}
Leaves		
Putrescine	6 ± 1^{b}	24 ± 1^{a}
Spermidine	11 ± 2^{b}	26 ± 2^{a}
Spermine	7 ± 1^{b}	27 ± 1^{a}

At photosynthetic organ level, our results showed that presence of 100 mM NaCl increased leaf thickness and specific leaf area. The latter parameter was considered critical in several physiological studies as it describes leaf's efficiency in capturing light as a function of its biomass [32]. In addition, a specific leaf area was positively related to relative growth rate, rate of leaf change, leaf nutrient concentration and photosynthetic capacity. By inferring the degree of succulence and the degree of sclerophylly, we showed that salt did not affect the former, while it caused a decrease in latter parameter. On the other hand, addition of GA3 in salty environment decreased leaf area, specific leaf area and thickness and increased leaf count, succulence and sclerophylly. Our results therefore suggest that plants subjected to treatment (NaCl + GA3) maintained a better cellular water state, reducing salt-induced water stress [33]. According to these authors, the increase in leaf sclerophylly can be considered a physiological

trait in response to stressful conditions, such as salinity. Therefore, this increase in LS indicates higher leaf thickness, with more layers of photosynthetic tissue, which may be an important factor in reducing the deleterious effects of salinity on plant growth [33].

The response of glycophytes to excess salt in environment is often manifested by a decrease in plant growth and yield [34]. Indeed, culture of *P. sativum* on hydroponic medium under different treatments with NaCl and GA3 led, at the end of 15 days, to a reduction in biomass in presence of salt, a strong increase in presence of GA3 and a restoration in response to combined effects of salt and GA3 compared to control. Concerning the effect of NaCl, our results were reminiscent of those of Reddy et al. [35] who showed that reduction in growth can be attributed to changes in plant water relationships under salt stress, resulting in inhibition of meristematic activity and cell elongation. As for the effect of GA3 (with or without NaCl) on dry biomass production, results found were confirmed by those of Altaey [36] in peper. These results can be explained by the fact that GA3 controlled developmental processes in the plants, contributing to the growth of cells by inducing cell elongation and an increase in intermodal length.

Salinity of the environment induces a disturbance in nutritional balance, thus limiting absorption, transport and accumulation of ions essential for growth [11,37]. Our results showed a strong accumulation of Na^+ in all organs of plants treated with NaCl 100 mM. However, a decrease in these levels was observed in plants grown on medium with added salt and GA3, but only in aerial organs and not in roots, where a slight increase was noted compared to plants treated only with NaCl. Tuna et al. [38] suggested that this additional accumulation could be related to root elongation, and thus the increase in their surface area of uptake in response to application of gibberellic acid. GA3 application may also enhance plant growth due to improved carbohydrate metabolism [39]. The growth stimulation induced by the application of GA3 to wheat stressed by zinc oxide nanoparticles was attributed to the enhanced nutritional status [40]. The ability of plants to protect themselves from salt stress is highly dependent on status of their potassium nutrition. Balkaya [41] reported that the enhancement of Na^+ content in the leaves of plants leads to K^+ deficiency due to the antagonistic effects of sodium and potassium ions. Any change in this status, particularly in case of potassium deficiency, affects growth by limiting cell expansion and inhibiting photosynthetic processes [42], and to avoid cell damage, plant cells need to maintain adequate potassium levels in cytosol. In this study, K^+ samples were reduced in all organs whose roots were in contact only with salt. However, simultaneous application of NaCl and GA3 attenuated inhibitory effect of salt on potassium nutrition. This was consistent with results found by Wang et al. [43] who showed that application of GA3 increased K^+ level in root and shoot of okra seedlings grown in presence of 100 mM NaCl.

One of the causes of reduced growth under saline stress is a slowdown in photosynthesis, which can be explained by stoma closure and/or alteration of photosynthetic system [44,45], resulting from the formation of proteolytic enzymes, such as chlorophyllase, an enzyme responsible for the degradation of chlorophylls under stress conditions, oxidation of chlorophyll and disruption of pigment-protein complex stability [46]. The carotenoid and chlorophyll assay, which was conducted in this experimental study, showed a salt-related decrease in carotenoids, chlorophyll a and total chlorophylls. Ozturk et al. [47] showed that salt also significantly decreases chlorophyll a, total chlorophyll a and β-carotene content in pea, which is the same in tomato [48]. At the same time, addition of GA3 to culture medium with NaCl did not improve chlorophyll status of leaves, but decreased it further compared to that of leaves treated with salt. This suggests the absence of a GA3-regulating effect on metabolism of these pigments. According to literature, the involvement of this hormone in the reduction of chlorophyll content was reported by Leite et al. [49] who found that GA3 application increased leaf area in soybeans and therefore decreased chlorophyll content per unit leaf area.

Lipids and proteins, main constituents of biological membranes, are an important element in regulation of selective permeability of cell membrane and play a role in salt resistance of plants [50]. Environmental stresses, such as salinity, affect their composition and structure, leading to increased cell permeability and loss of membrane integrity [50]. Our results, like those of Khalid et al. [51], show that salt decreases the protein content. However, the negative impact of salinity on protein content may be explained by the osmotic effect [52] or by a decline in amino acid availability and denaturation of enzymes implicated in the synthesis of amino acids and proteins under salt stresses [53]. At the same time, addition of GA3 and NaCl to culture medium restored protein content in these organs. Shahzad et al. [54] also showed that addition of GA3 to salt medium stimulated soluble protein synthesis in maize, compared to salt intake. This can be explained by activation of protein biosynthesis, including antioxidant enzymes that play a primary role in detoxification of plant tissues, by eliminating reactive oxygen species (ROS) produced under these conditions [52].

Apart from the role of compatible solutes as osmolytes used in osmotic adjustment and in protecting cells from dehydration, sugars (trehalose), sugar alcohols (sorbitol and mannitol), amino acid (proline) and betaines have also been shown to be particularly effective in protecting cytoplasmic proteins and cell membranes from desiccation. They are involved in several metabolic processes and act as molecular signals in regulation of different genes and especially in photosynthesis, sucrose metabolism and osmolyte synthesis. In the present study, soluble sugar contents of plants treated with salt increased in different organs (roots, stems, leaves) compared to other treatments. This stimulation was concomitant with a decrease in starch content that could be attributed to its hydrolysis [55].

Puterscine content increased considerably in roots, stems and leaves on salt exposure (Table 3). Similarly, Zhou et al. [56] found that at high salinity, Put content was significantly increased in tomato leaves. It is believed that higher aliphatic polyamines, such as Spd and Spm, are part of the endogenous plant protection; their accumulation is associated with the plant resistance to salinization [15]. The present data show that Spd and Spm (Table 3) enhanced during salt exposure, and this trend was better expressed in pea stems and leaves. As endogenous protectors Spd and Spm could contribute to stabilizing cell membranes and/or affecting cell ionic balance through activation of H^+-ATPases [57]. It was assumed that Spm was involved in the direct scavenging of ROS at salt stress because its molecule is most positively charged [58].

Similarly, Jouve et al. [59] observed an increase in polyamines in aspen treated with 150 mM NaCl, along with increasing amount of other osmoprotectants as sucrose, proline, mannitol and raffinose, and concluded that polyamines are more or less directly related to antioxidant or osmoregulatory mechanisms that function at salt stress. It is obvious that under salinity conditions the endogenous PAs accumulate, which correlates with the information that these plant growth regulators provide protection in terms of salinity and mitigate negative oxidative stress effects.

In conclusion, application of gibberellic acid on pea plants caused an improvement in growth under salt stress. This improvement results in an increase in parameters of growth, nutrition and photosynthetic activity and an improvement in sugars and starch level. These results suggest an improving effect of this hormone on growth of pea plants in presence of salt.

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