Effects of exogenous polyamines on leaf area of sunflower grown in salinity stress

Tuzluluk stresi altında büyütülen ayçiçeği bitkilerine dışsal uygulanan poliaminlerin yaprak alanına etkisi

Research Article

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ABSTRACT

This study investigated the effects of polyamines, which were applied exogenously in growth period of sunflower (*Helianthus annuus* L. cv. Santafe) plants grown, under controlled conditions, on leaf pairs and total leaf surface area. Salt was used in 3 different concentrations (50, 100 and 200 mM NaCl) and also, spermine, spermidine and putrescine was applied in 3 levels (0.01, 1 and 2 mM). The leaf and stems of sunflower plants grown in salt culture solutions in Hoagland and at various concentrations for 45 days were sprayed with polyamine solution every other 3 days and 8 times in total as from the 15th day.

The total leaf areas and areas of leaf pairs decreased depending on increasing salt concentration. Spermine, spermidine and putrescine sprayed to plants were able to show positive effect only under specific and limited conditions. Putrescine was determined to be the most effective polyamine in removing the inhibitory effect of salt on leaf area.

Key Words
Sunflower, Salt stress, Polyamines, Leaf area

ÖZET

Bu çalışmada kontrollu iklim koşullarında büyütülen ayçiçeği (*Helianthus annuus* L. cv. Santafe) bitkilerinin büyüme döneminde uygulanan dışssal poliaminlerin yaprak çiftleri ve total yaprak yüzey alanı üzerine etkileri çalışılmıştır. Çalışmada 3 farklı tuz derişimi (50, 100 ve 200 mM) ile üç farklı düzeyde (0.01, 1 ve 2 mM) putresin, spermin ve spermidin kullanılmıştır. Hoagland ve değişik konsantrasyonlardaki tuzlu kültür çözeltilerinde 45 gün büyütülen ayçiçeği bitkilerinin yaprak ve gövdelerine 15. günden itibaren 3`er gün arayla toplam 8 kez poliamin çözeltisi püskürtülmüştür.

Total yaprak alanı ve yaprak çifti alanları artan tuz derişimine bağlı olarak azalmıştır. Bitkilere püskürtülen Spermin, Spermidin ve Putresin kültür ortamına eklenen tuz ve poliamin düzeyine bağlı olmak üzere sadece belirli ve sınırlı koşullarda olumlu etki gösterebilmiştir. Tuzun yaprak alanı üzerinde yapmış olduğu engelleyici etkiyi kaldırabilmek açısından en etkili poliamin Putresin olarak tespit edilmiştir.

Anahtar Kelimeler
Ayçiçeği, Tuz stresi, Poliaminler, Yaprak alanı

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INTRODUCTION

Polyamines (PAs) are low molecular weight polycations found in all living organisms. Spermidine (Spd), spermine (Spm) and their diamine obligate precursor, putrescine (Put), are major PAs in plant cells. PAs have been implicated in a wide range of regulatory processes such as promotion of growth, cell division, DNA replication and cell differentiation [1]. Because of their polycationic nature at physiological pH, PAs can be strongly bound to the negative charges in cellular components, such as DNA, membrane phospholipids, pectic polysaccharides, and proteins, and thus mediate their biological activity [2].

PAs are involved in the stress tolerance of higher plants, which encounter various abiotic stresses, including salt stress [3, 4]. Since PA level changes significantly upon exposure to environmental stress, it has been hypothesized that they are part of a plant defense mechanism against stress [5]. Because the levels of PAs increase during the adaptation to stress in a variety of plants, it is thought that they are also involved in these processes.

Recently, some published studies showed that increased endogenous levels of PAs induced by spraying suitable concentration of exogenous PAs are able to improve photosynthetic efficiency in several environmental stresses. Exogenous application of PAs improved tolerance against several abiotic stresses [6]. A positive response to exogenously applied PAs has been reported in rice and sorghum [7, 8]. Nayyar et al. [9] found that exogenous application of Put and Spm substantially improved the drought tolerance in soybean. Alternatively, exogenous PA application is a convenient and effective approach for enhancing the salinity tolerance of plants and eventually improving plant productivity under high salinity. Indeed, exogenous PA application has been successfully used for enhancing the salinity tolerance of plants [10]. Exogenous treatment of PAs enhanced the growth of wheat plants under control and salt stress conditions. It has been demonstrated by several authors that a foliar spraying of Put may enhance the behavior of rice in the presence of NaCl [11]. The leaves are the commercially important plant parts, and by estimating leaf area, the production could be predicted. The leaf area of a plant is essential to understand the interaction between plant growth and environment. Plant yield and quality are affected by photosynthesis and transpiration rate, which are closely related to leaf area; making leaf area a key variable in most developed models to simulate carbon and water dynamics [12]. However, a number of studies in literature that investigate the effects of PAs, exogenously applied to plants grown in salt conditions, on leaf area is limited and inadequate, and such comprehensive study has not been encountered, yet. On the basis of these observations, we have made an attempt to investigate the effects of the exogenous application of Put, Spd and Spm, the three major PAs, on leaf areas in salt-stressed sunflower plants.

MATERIALS AND METHODS

Plant Material

This study was done with seeds of sunflower (*Helianthus annuus* L. cv. Santafe) that are cultivated as an important agricultural plant. The seeds were obtained from Seed Improvement and Certification Centre in Turkey. For this purpose, 10 different cultures of studied sunflower (*Helianthus annuus* L.) which are Frankasol, Fleuron, Isoleic, Santafe, Eurosol, Printasol, IS897, Odil, İcarsol, Briosol. "Santafe" was chosen because of its rapid germination and its moderately salt tolerant.

Seed Germination and Plant Growth Method

The seeds of uniform size were surface-sterilized according to Ellis et al. [13]. Then, they imbibed Hoagland (Hoag.) (control) and NaCl solutions at different concentrations (50, 100 or 200 mM NaCl) for 24 h. The imbibed seeds were germinated at 25 ± 1°C in a dark growth cabinet. Four-day old seedlings were transferred to plastic growth boxes containing vermiculite supplied with the same culture solution. 6 seedlings were placed in each growth boxes in three repeats. The growth experiments were also carried out in a growth cabinet at 25 ± 1°C, relative humidity of 65 ± 5% and 15-h-light/9-h-dark periods. During the growth period, the plants were cultivated on a half-strength Hoag. solution (control plants) and on the same Hoag. solution supplemented with 50, 100 or 200 mM NaCl.

Spm, Spd or Put were sprayed on the stems and leaves of plants growing on vermiculite. PAs sprayed was prepared with distilled water in 3 different concentrations (0.01, 1 and 2 mM). PA application was begun on the 15th day as from the date that seeds were soaked with culture solution, the spraying process was done every three days, 8 times in total, on the leaves and stems of plants. Distilled water was sprayed on plants in the control group on the same days. Growing plants in growth cabinet for 45 days as from planting day by using this method was found adequate to measure leaf areas.

Measurement of Leaf Area

Images of leaves obtained in this study was transferred to a computer via a scanner. These leaf areas were calculated using Adobe Photoshop (version 6) software. The standard was brought out by retrieving pixels of known areas during calculation. Pixels obtained at the end of measurement were determined in cm^2 [14]. The measurement of standard areas revealed a very high correlation ($R^2 = 0.999$) between the number of measured pixel (15480 pixel/cm^2) and the tested areas. Leaf measurements were performed on all developing plants.

Statistical Analysis

SPSS for Windows (version 17.00) software program was used to make statistical analysis. The assays were carried out in triplicate and the results are expressed as mean values ± standard error. The test performance levels were statistically analyzed by one-way multivariate analysis of variance (one-way MANOVA). All of the applications performed during the study were determined as an 'independent variable', and areas belonging to defined different leaf pairs were determined as 'dependent variable'. Variance-covariance matrixes in Box's M test were homogenous ($P = 0.075$; $P>0.05$). $P = 0.00$; $P<0.05$ was found for all leaf area pairs in Levene test and the variance of groups was not homogenous. There was a difference between applications because the value of Wilks's Lambda was $P = 0.00$; $P<0.05$. Tamhane's T2 test (conservative pairwise comparisons test based on a t test) was used to determine which groups were different from each other since difference between groups was determined as a result of variance analysis and variance did not distribute homogenously. Leaf areas were also calculated for each treatment and compared within and with the control.

RESULTS

Salinity and Leaf Area

Salinity was significantly inhibited both total the leaf area and leaf area pair depending on concentration ($P<0.05$). This inhibition was found to be 41.80% in culture medium containing 50 mM NaCl in Spm application, 65.01% in culture medium containing 100 mM, and 85.60% in culture medium containing 200 mM when compared to control group (Hoag.). As this inhibition for Spd application was found to be, respectively, 44.23%, 67.47% , and 81.62%, it was 36.75%, 58.48% and 92.58% for Put. There was a decrease in leaf pairs as well as similarly increasing salt concentrations in comparison with control (Table 1, 2, 3).

Exogenous polyamine applications and leaf area

The measurement results obtained concerning leaf areas belonging to each leaf pair of PAs and the total leaf areas at the end of study are summarized on Table 1, 2 and 3.

Exogenously applied Spm, Spd or Put remained generally ineffective on total leaf area ($P <0.05$). But all of the Put levels sprayed on plants cultivated in only Hoag. culture medium increased total leaf area ($P<0.05$).

Combined treatments of polyamines and salt

PAs exogenously applied to plants grown under salinity stress showed different effects on leaf pairs. Spm levels applied exogenously provided a certain increase in the leaf areas of leaf pairs 2, 3, 5, 6, and 7 when compared with their control; but this increase was not considered significant. However, while leaf areas of leaf pairs 8 and 9 were determined in 2 mM Spm application exogenously

Table 1. Effect of foliar application of Spm on leaf pairs and total leaf surface area of salt-stressed plants (mean±SE).

	Pairs of Leaves									Total Leaf area (cm^2)/ per plant
Treatment	1.	2.	3.	4.	5.	6.	7.	8.	9.	
Control (Hoag.)	4.298±0.109	5.693±0.133	5.768±0.197	5.254±0.261	4.274±0.216	2.870±0.248	2.395±0.283	_	_	30.55±0.84
50 mM NaCl	3.440±0.108	4.395±0.132	3.682±0.163	2.964±0.160	1.855±0.190	1.445±0.102	_	_	_	17.78±0.69
100 mM NaCl	2.773±0.130	3.375±0.180	2.520±0.217	2.019±0.149	_	_	_	_	_	10.69±0.48
200 mM NaCl	1.617±0.178	1.673±0.089	1.113±0.087	_	_	_	_	_	_	4.40±0.13
0.01 mM Spm + Hoag.	4.158±0.122	5.557±0.108	5.565±0.121	5.272±0.116	4.347±0.150	3.089±0.168	2.198±0.194	_	_	30.19±0.80
0.01 mM Spm + 50 mM NaCl	3.514±0.197	4.464±0.079	3.811±0.127	2.798±0.167	2.429±0.209	1.985±0.188	_	_	_	19.00±0.45
0.01 mM Spm + 100 mM NaCl	3.020±0.104	3.606±0.111	2.699±0.162	1.915±0.141	_	_	_	_	_	11.24±0.42
0.01 mM Spm + 200mM NaCl	1.695±0.093	1.757±0.041	1.967±0.057	_	_	_	_	_	_	5.42±0.13
1 mM Spm + Hoag.	3.755±0.176	5.163±0.157	5.146±0.187	4.959±0.211	4.232±0.197	3.142±0.229	2.271±0.268	_	_	28.67±1.20
1 mM Spm + 50 mM NaCl	3.458±0.102	4.386±0.133	3.830±0.148	3.113±0.171	2.148±0.300	2.322±0.254	_	_	_	19.26±0.82
1 mM Spm + 100 mM NaCl	2.582±0.120	3.548±0.088	2.667±0.138	1.954±0.084	_	_	_	_	_	10.75±0.30
1 mM Spm + 200 mM NaCl	1.768±0.148	2.077±0.080	1.527±0.152	_	_	_	_	_	_	5.37±0.23
2 mM Spm + Hoag.	3.470±0.316	5.224±0.128	5.402±0.157	5.258±0.217	4.672±0.189	3.555±0.228	2.890±0.252	2.540±0.088	1.933±0.153	34.94±1.10
2 mM Spm + 50 mM NaCl	3.117±0.171	3.879±0.223	3.222±0.221	2.472±0.223	2.262±0.157	1.697±0.141	_	_	_	16.65±0.83
2 mM Spm + 100 mM NaCl	2.632±0.120	3.441±0.117	2.812±0.151	2.009±0.114	_	_	_	_	_	10.89±0.27
2 mM Spm + 200 mM NaCl	1.746±0.102	2.071±0.139	1.491±0.121	_	_	_	_	_	_	5.31±0.19

Table 2. Effect of foliar application of Spd on leaf pairs and total leaf surface area of salt-stressed plants (mean±SE).

	Pairs of Leaves									Total Leaf area (cm^2)/ per plant
Treatment	1.	2.	3.	4.	5.	6.	7.	8.	9.	
Control (Hoag.)	4.425±0.109	6.029±0.133	6.307±0.165	5.802±0.241	4.713±0.298	3.124±0.319	1.862±0.190	1.524±0.105	_	33.78±1.08
50 mM NaCl	3.352±0.119	4.361±0.159	3.747±0.242	2.737±0.271	2.075±0.136	1.447±0.094	1.120±0.097	_	_	18.84±0.78
100 mM NaCl	2.811±0.072	3.261±0.140	1.963±0.207	1.619±0.134	1.340±0.082	_	_	_	_	10.99±0.43
200 mM NaCl	1.855±0.144	2.026±0.170	1.473±0.120	0.855±0.041	_	_	_	_	_	6.21±0.33
0.01 mM Spd + Hoag.	3.944±0.135	5.426±0.207	5.966±0.323	5.542±0.382	4.560±0.318	3.173±0.255	1.797±0.212	1.353±0.092	_	31.76±1.49
0.01 mM Spd + 50 mM NaCl	3.688±0.091	4.790±0.131	4.242±0.181	3.114±0.236	2.275±0.302	2.079±0.110	1.625±0.087	_	_	21.81±0.43
0.01 mM Spd + 100 mM NaCl	3.249±0.062	3.737±0.071	2.312±0.096	1.388±0.013	_	_	_	_	_	10.69±0.15
0.01 mM Spd + 200 mM NaCl	1.932±0.028	2.012±0.180	1.123±0.075	0.925±0.085	_	_	_	_	_	5.99±0.39
1 mM Spd + Hoag.	3.785±0.310	5.330±0.254	5.635±0.208	5.229±0.237	4.248±0.278	3.212±0.244	2.185±0.175	1.635±0.118	1.153±0.109	32.41±1.35
1 mM Spd + 50 mM NaCl	3.417±0.092	3.059±0.225	3.709±0.189	2.759±0.299	1.844±0.202	1.298±0.162	_	_	_	17.41±0.67
1 mM Spd + 100 mM NaCl	2.741±0.097	3.246±0.138	2.027±0.108	1.550±0.093	1.012±0.016	_	_	_	_	10.57±0.35
1 mM Spd + 200 mM NaCl	1.712±0.114	1.776±0.192	1.248±0.129	1.482±0.122	2.086±0.042	_	_	_	_	8.30±0.23
2 mM Spd + Hoag.	4.273±0.188	6.063±0.218	6.308±0.241	5.760±0.288	4.772±0.345	3.985±0.293	2.599±0.227	1.779±0.058	_	35.54±1.83
2 mM Spd + 50 mM NaCl	3.655±0.076	4.599±0.197	3.844±0.099	2.800±0.099	1.872±0.121	0.913±0.066	_	_	_	17.68±0.39
2 mM Spd + 100 mM NaCl	2.987±0.044	3.508±0.065	2.315±0.069	1.303±0.032	_	_	_	_	_	10.11±0.17
2 mM Spd + 200 mM NaCl	1.624±0.298	1.747±0.043	1.367±0.057	_	_	_	_	_	_	4.74±0.85

Table 3. Effect of foliar application of Put on leaf pairs and total leaf surface area of salt-stressed plants (mean±SE)

	Pairs of Leaves										Leaf area (cm^2)/ per plant
Treatment	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	
Control (Hoag.)	4.172±0.100	5.873±0.366	5.649±0.274	5.102±0.367	4.295±0.363	3.063±0.317	2.029±0.183	_	_	_	30.18±1.50
50 mM NaCl	3.477±0.108	4.703±0.261	4.160±0.179	2.951±0.222	2.360±0.249	1.447±0.084	_	_	_	_	19.09±0.64
100 mM NaCl	3.088±0.111	3.923±0.088	2.982±0.091	1.618±0.090	0.920±0.067	_	_	_	_	_	12.53±0.34
200 mM NaCl	1.108±0.238	1.131±0.261	_	_	_	_	_	_	_	_	2.24±0.49
0.01 mM Put + Hoag.	4.203±0.129	6.805±0.225	6.292±0.269	5.937±0.339	4.611±0.256	3.466±0.166	2.303±0.141	1.587±0.056	0.154±0.12		35.36±1.12
0.01 mM Put + 50 mM NaCl	3.406±0.194	4.794±0.117	4.209±0.187	2.813±0.118	1.609±0.114	1.101±0.059	_	_	_	_	17.93±0.43
0.01 mM Put + 100 mM NaCl	3.170±0.085	4.063±0.102	3.277±0.135	1.874±0.157	1.632±0.257	1.072±0.089	_	_	_	_	15.09±0.52
0.01 mM Put + 200 mM NaCl	2.739±0.106	1.498±0.129	1.018±0.089	_	_	_	_	_	_	_	5.255±0.40
1 mM Put + Hoag.	4.070±0.169	5.848±0.224	6.574±0.249	6.250±0.404	5.061±0.435	3.630±0.229	3.131±0.267	2.094±0.149	1.743±0.03	0.967±0.23	39.37±1.87
1 mM Put + 50 mM NaCl	3.598±0.114	4.737±0.207	4.096±0.125	2.956±0.154	1.901±0.181	1.320±0.99	_	_	_	_	18.61±0.58
1 mM Put + 100 mM NaCl	3.106±0.084	3.943±0.088	2.964±0.168	1.998±0.118	1.530±0.058	1.162±0.075	_	_	_	_	14.70±0.42
1 mM Put + 200 mM NaCl	2.893±0.021	1.561±0.146	_	_	_	_	_	_	_	_	4.45±0.15
2 mM Put + Hoag.	4.319±0.288	5.981±0.283	6.452±0.124	5.991±0.136	4.979±0.357	3.733±0.292	2.636±0.241	1.949±0.209	1.022±0.16		37.06±1.01
2 mM Put + 50 mM NaCl	3.698±0.177	4.485±0.267	3.454±0.292	2.705±0.134	1.630±0.136	2.668±0.308	_	_	_	_	18.64±0.77
2 mM Put + 100 mM NaCl	3.160±0.091	3.780±0.105	2.884±0.143	1.833±0.137	1.363±0.106	1.153±0.075	_	_	_	_	14.17±0.39
2 mM Put + 200 mM NaCl	1.596±0.119	1.606±0.138	1.388±0.139	_	_	_	_	_	_	_	4.59±0.27

applied plants grown in Hoag. culture solution, there was no plant growth in other applications.

Increases in certain ratios were determined for Spd applied cultures; however, similarly it was not considered significant (P >0.05). As leaf did not develop in other culture media in only leaf pair 9, the average leaf surface area of plants, which were grown in Hoag. culture medium and to which 1 mM Spd was sprayed, was 1.153 ± 0.109 cm^2.

Exogenously applied Put showed a more distinct effect on leaf areas (Table 3). 0.01 and 1 mM Put exogenously applied to plants grown in only 200 mM NaCl medium in leaf pair 1 caused an increase in comparison with its control (P < 0.05) (Figure 1). 0.01 mM Put applied to plants grown in Hoag. culture solution in leaf pair 2 increased in comparison with its control (P < 0.05). 0.01 mM and 2 mM Put applied to plants grown in 200 mM NaCl medium in leaf pair 3 increased in comparison with its control (P < 0.05). Each three Put levels applied to the plants grown in Hoag. culture solution increased on leaf area for leaf pairs 4, 5, 6, 7, 8, and 9, but this was considered significant at only certain Put concentrations. Only 2 mM Put applied to plants grown in 50 mM culture solution in leaf pair 6 increased leaf area in comparison with its control (P < 0.05). In leaf pair 10, only the group, which was grown in Hoag. culture solution, and to which 1 mM Put was applied, developed.

Polyamine concentrations and leaf area

On the other hand, when we evaluate Spm, Spd and Put applications in terms of the same concentration application; in terms of the effect of PAs applied 0.01 mM on leaf pairs, 0.01 mM Put application given to plants grown in Hoag. solution for leaf pair 2 increased in comparison with Spm (22%) and Spd (25%) having the same concentration (P < 0.05) (Figure 2). Measurements of only 0.01 mM Put application were made for plants grown in 100 mM NaCl medium for leaf pair 5 and 6, and plants on which Spm and Spd applied did not develop. Leaf areas obtained from plants, which were grown in Hoag. culture solution for leaf pair 8 and to which 0.01 mM Spd and Put applied, found high because there was no plant development for Spm (P < 0.05).

In the applications of 1 mM PA, 1 mM Put application to plants grown in 200 mM culture solution in leaf pair 1 increased leaf surface area in comparison with Spm and Spd (P < 0.05). Put application given to plants grown in Hoag. and other culture solutions on other leaf pairs were determined to be generally more effective in comparison with Spm and Spd.

In the applications of 2 mM PA, Spd and Prg applied to plants grown in Hoag. and 50 mM NaCl were found more effective on leaf area for leaf pairs 1, 2, 3 and 4 compared to Spm; however, results were considered significant only for some concentrations.

DISCUSSION

The reduction in leaf area of plants under salt stress has been reported wheat [15] and tomato [16]. This reduction may be the result of slower leaf expansion [17] or the reduction of photosynthetic activity [18]. Franco et al. [19] indicate that the reduction in seedling leaf area can be a good selection criterion to facilitate rapid screening for salt tolerance in muskmelon.

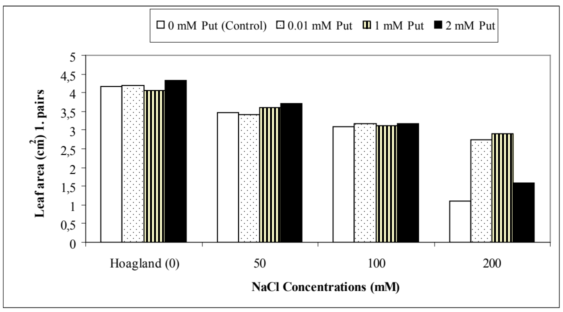

Figure. 1. Effect of foliar application of Put on 1. pair of leaves surface area of salt-stressed plants.

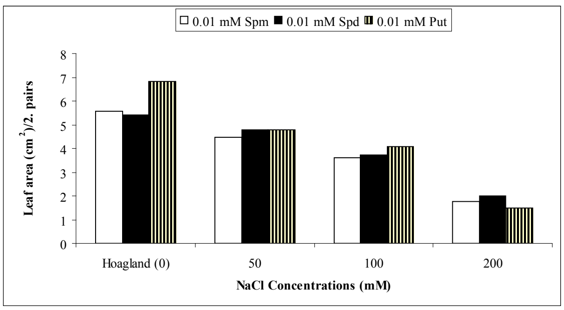

Figure 2. Effect of foliar application of 0.01 mM Spm, Spd or Put on 2. pair of leaves surface area of salt-stressed plants.

Several researchers obtained positive results with Put application especially under salinity stress. Krishnamurthy [20] expressed that Put applied to rice plants significantly increased stem growth, fresh and dry weigh, and grain yield. Amri et al. [10] also reported that Put. compared to Spd, could significantly increase the leaf area. On the other hand, exogenous Put application alleviated growth inhibition and improved the grain yield of rice plants under salinity [21]. Amin et al. [22] indicated that under stress-free conditions, there was an increase in the leaf area of onion (*Allium cepa* L. cv. 'Giza 20) 25, 50 and 100 mgL^{-1} Put applied. Several authors have demonstrated that a foliar spraying of Put may improve the behavior of rice in the presence of NaCl [11]. However, as is seen, the results related to leaf areas made by exogenous Put are not as certain or distinct as the results obtained by these researchers. We are of the opinion that the inhibition the salt caused may be associated with partial elimination of some concentration by applying Put in particular, stabilization of cell membranes, which were damaged due to possibly salinity, the use of exogenous PAs, and preservation of water content of tissues.

As is seen, PAs exogenously applied to plants grown in different salt concentrations were found to be generally ineffective on leaf surface area except for certain and limited conditions. We believe that the reason behind this is the fact that sunflower plants are insensible to exogenous polyamines applied during growth stage. Apparently, a plant may respond to one PA in a different manner compared to another.

Our findings are in accordance with only a few previous studies in this respect. Suleiman et al. [23] showed that treatment with PAs had no effect on leaf area regardless of salinity level in spinach. Leaf area decreased in response to Spd treatment at all salinity levels as compared to controls and plants treated with 5 mM Put. Ndayiragije and Lutts [24] reported that Put, Spd and Spm did not alleviate salinity-induced growth suppression in rice.

These results are contrast to other reports which showed that exogenously applied PAs consistently and significantly increased leaf area of salt stressed maize [25]. Shu et al. [26] also reported the inhibitory effect of salt stress on the values of leaf area was completely alleviated as a result of Spd treatments in cucumber plants. Zhang et al. [27] indicated that 10 mM Put applied on leaves of cucumber plants grown in salt culture medium increased leaf surface area.

In the present study, we examined whether or not PAs could influence the leaf area of sunflower grown under saline conditions. However, in contrast with reports on other species, analysis of our growth data showed that PAs treatment did not ameliorate salinity stress in sunflower. The growth of sunflowers was not influenced by Spm or Spd, either applied via a foliar spray. This incongruence may be explained mainly by the use of different cultivars and plant age, growing conditions and the level and duration of salt stress. It is unclear as to the reason we were not able to achieve similar results for sunflower to those reported for other plant. We considered the possibility that the PAs were not able to penetrate the cuticle of the leaves. Future work with PAs may be based on studies where PAs are applied by presoaking the seeds.

In conclusion, it does not appear from our data that the PAs; Spm, Spd or Put can ameliorate the effect of salinity stress on leaf area in sunflower. However, salinity stress significantly influenced leaf area. The adverse effects of salinity with regard to leaf area can be ameliorated, to some entent, by the exogenous application of Put. But, differently from sunflower variety resistant to medium level salinity to be studied in future, results should be put forward by studying salt resistant and sensible varieties, as well.

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