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Roll of Poly Amines (Spermidine and Putrescine) on Protein, Chlorophyll and Phenolic Compounds in Wheat (Triticum aestivum L.) under Salinity Stress

Parvaneh Rahdari* and Seyed Meysam Hoseini

Department of Plant Physiology, Islamic Azad University, Tonekabon Branch, Iran

***Corresponding author*:** Parvaneh Rahdari

ABSTRACT: Salinity stress as a major adverse factor can lower leaf water potential, leading to reduced turgor and some other responses, and ultimately lower crop productivity in arid and semiarid zones. In order to, investigate effects of different concentrations of poly amines spermidine and putrescine on germination of wheat under salinity stress, this experiment was conducted as factorial based on completely randomized design with two factors include salinity and poly amines level, in biology laboratory of Islamic Azad University of Tonekabon Branch in spring of 2013. First factor was salinity level including 50 mmol of NaCl and the second factor was poly amines level including 0/5 mmol of spermidine and putrescine concentration. NaCl was reduced chlorophyll and protein concentration but poly amines improved chlorophyll and protein concentration under salinity stress in root and shoot. Phenolic compounds levels was decreased in root under salinity and poly amines treatment but in root not change. Results of this experiment indicated that poly amines reduction of destructive effects of salinity stress in wheat seedlings.

***Keywords*:** Salinity, Poly Amines, Phenolic Compounds, Wheat.

INTRODUCTION

Soil salinity is one of the major abiotic stresses that adversely affect plant productivity and quality (Zhu, 2001). Salinity stress had been paid attention by many farmers, and different methods for its solving had been thought which was effective in its time and place. But now days, regarding the progresses in physiology and biochemistry, the negative effects of environmental stresses should be reduced (Ozhan, 2013) that Application of plant growth regulators and poly amines are from them. It was estimated that up to 20% of irrigated lands in the world are affected by different levels of salinity and sodium content (Mostafazadeh-Fard et al., 2007). Wheat (*Triticum aestivum* L.) is one of the most important crops and main food for people in arid and semiarid areas (Ozhan, 2013). Plant hormone activated in tissues which are far from their production part. A growth regulator is an organic matter (more than a nutrient), which made, suppressed or changed qualifications of growth and improvement in very low concentration (lower than 1 mmol). Polyamines (PAs) (putrescine, spermidine and spermine) are group of phytohormone-like aliphatic amine natural compounds with aliphatic nitrogen structure and present in almost all living organisms including plants (Gill and Tuteja, 2012). The polyamines (PAs) putrescine (Put), spermidine (Spd) and spermine (Spm) have been shown to be involved in a variety of plant growth and developmental processes, including cell division, vascular differentiation, root initiation, shoot formation, flower initiation and development, fruit ripening and senescence and embryoid formation in tissue cultures (Unsal, 1995). Many of these functions are similar to those mediated by known plant hormones such as auxins, cytokinins, gibberellins, abscisic acid, and ethylene. In addition, direct and putative interactions of plant hormones and PAs are also known. However, in spite of these facts many plant physiologists still have some doubts about recognizing PAs as another class of plant growth regulators (Galston, 2005). PAs are small ubiquitous polycations involved in many processes of plant

growth and development and are well known for their anti-senescence and anti-stress effects due to their acid neutralizing and antioxidant properties, as well as for their membrane and cell wall stabilizing abilities (Zhao, 2008). It has been suggested that PAs play important roles in modulating the defense response of plants to diverse environmental stresses, which includes metal toxicity, oxidative stress, drought, salinity and chilling stress (Duan et al, 2009). It has been reported that exogenous application of PAs is also effective approach for enhancing stress tolerance of crops for enhanced crop productivity. Exogenous application of Put has been successfully used to enhance salinity that in this study was investigated.

MATERIALS AND METHODS

Plant material and treatment

This experiment was conducted as factorial based on completely randomized design in Plant Physiology Laboratory, Biology Department, Islamic Azad University, Tonekabon Branch in spring of 2013. . First factor was salinity level including 50 mmol of NaCl and the second factor was poly amines level including 0/5 mmol of spermidine and putrescine concentration. Seeds was put in 5% sodium hypochlorite solution of for 5 minute and then by distilled water washed for antiseptic of it (Falahati, 2006). Seeds was cultivated in petri dish, seedling transported in washed sand pot for controlling of their growth after 3 leaves stage. Hogland solution was added that get solution process performed every one week. Then salinity and poly amines treatment was imposed in one week and Determination of physiological factors was performed.

Total phenols content

Total phenolic content was determined with Folin-Ciocalteu reagent according to the method of Soland and Laima using gallic acid as a standard phenolic compound. In brief, 1 g of shoot and root samples were placed in an eppendorf tube, with 1 ml of methanol (80%), grinded at 4°C and centrifuged at 14000 × g for 15 min. The extract was mixed with 0.5 ml of Folin-Ciocalteu reagent (diluted 1:1 with water) then 1 ml of a 5% sodium carbonate solution was also added. After 30 min, absorbance was measured at 725 nm.

Photosynthetic pigments

The amount of photosynthetic pigments (chlorophyll a,b) were determined according to the method of Lichtenthaler, (1987). The samples (0.25 g) were homogenized in acetone (80%) and the extract was centrifuged at 3,0009g and absorbance was recorded at wavelengths of 646.8 and 663.2 nm for chlorophyll assay by a UV–Vis spectrophotometer (Unique, USA). Chl a and Chl b were calculated using the following formulas (Lichtenthaler, 1987):

Chl a = $(12.25\ A_{663.2} - 2.79\ A_{646.8})$
Chl b = $(21.21\ A_{646.8} - 2.79\ A_{663.2})$
Chl a+b = Chl a + Chl b

Protein determination

The concentration of protein was determined by the method of Bradford (1976) using bovine serum albumin (BSA) as a standard.

Statistical analysis

Experiments in completely random framework was performed. Data Analysis, variance and comparison of mean by Dunkan experiment and SPSS software was done and drawing of diagrams was used from Excel software.

RESULTS AND DISCUSSION

Chlorophyll Content

Analysis of variance showed that chlorophyll content was affected by salinity and poly amines at 0/05 of probability level (Table 1). Chlorophyll a was decreased under salinity stress that was meaningful in statistical level of 0/05, whereas not meaningful effected un chlorophyll b content. Also, poly amines was affected un chlorophyll a,b under salinity stress (Figure 1,2).

Table 1. Chlorophylls content under salinity and poly amines concentrations

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Blank	1/03+0/03^{b}	0/11+0/01^{b}	1/70+0/03^{b}
Putrescine	1/26+0/05^{a}	0/22+0/06^{a}	2/02+0/11^{a}
Spermidine	1/04+0/10^{b}	0/10+0/02^{b}	1/64+0/11^{b}
Blank	0/75+0/06^{c}	0/08+0/02^{b}	1/54+0/06^{b}
Salinity +Putrescine	1/01+0/04^{b}	0/12+0/05^{b}	1/72+0/06^{b}
Salinity +Spermidine	1/06+0/08^{b}	0/13+0/03^{ab}	1/69+0/11^{b}

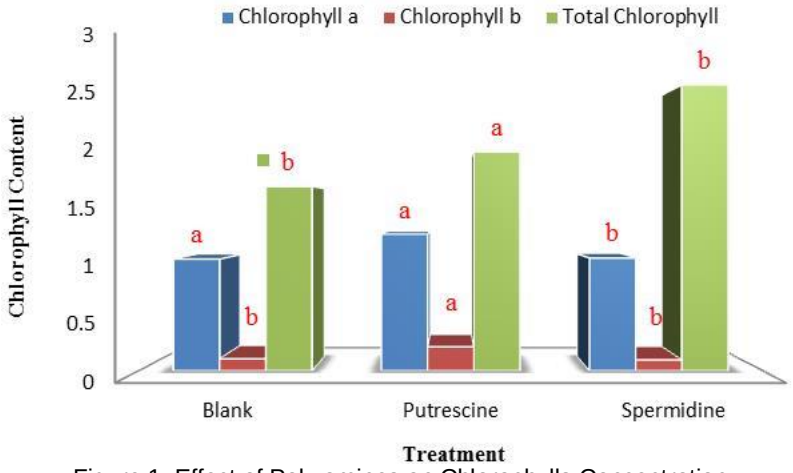

Figure 1. Effect of Poly amines on Chlorophylls Concentration

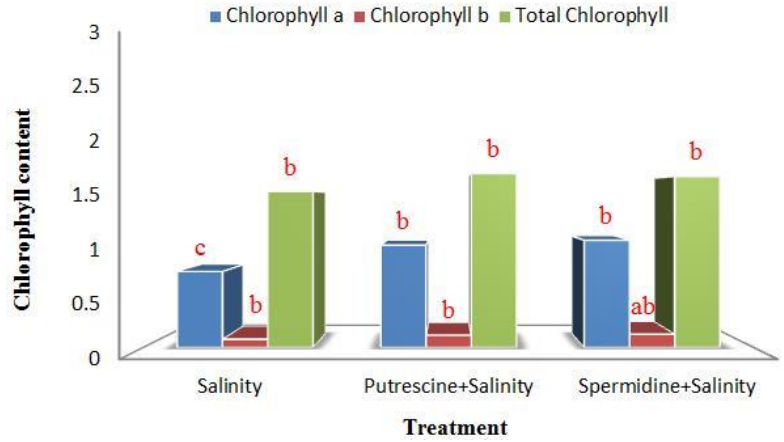

Figure 2. Effect of Salinity and Poly amines on Chlorophyll content

Total Protein and Phenol Content in Shoot

Salinity stress not effected on total phenol content in shoot but spermidine was increment total phenol content in shoot that meaningful in statistical level of 0/05. Also, total protein concentration in shoot was decreased under salinity stress that poly amines, also, decreased protein content in leaves (Table 2, Figure 3,4).

Table 2. Total of Protein and Phenol Content in Shoot

Treatment	Total Protein	Total Phenol
Blank	312+5^{a}	7/3+0/4^{c}
Putrescine	240+7^{b}	6/5+0/3^{c}
Spermidine	188+6^{cd}	9/76+0/2^{b}
Salinity	190+7^{d}	7/50+0/10^{c}
Salinity +Putrescine	221+6^{b}	4/87+0/3^{d}
Spermidine + Salinity	205+3^{c}	16/17+0/15^{a}

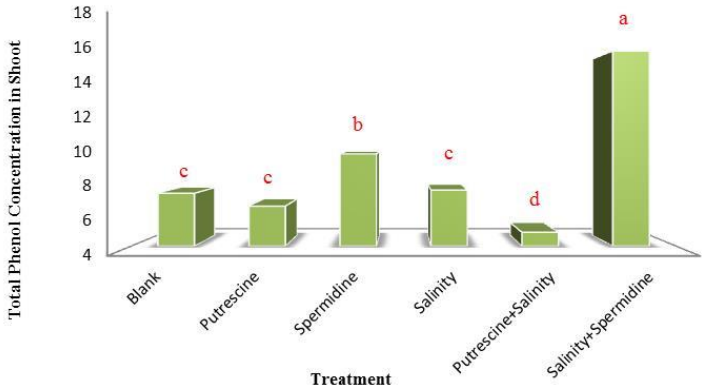

Figure 3. Total Phenol content in shoot

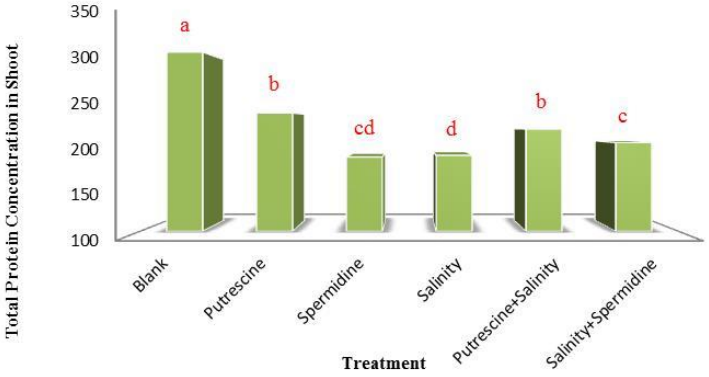

Figure 4. Total Protein content in shoot

Total Protein and Phenol Content in Root

Analysis of variance showed that salinity and poly amines was decreased phenol content in root that meaningful in statistical level of 0/05 (Table 3, Figure 5). Also, salinity and spermidine decrement protein content in root but application of putrescine was increased protein content in root (Table 3, Figure 6).

Table 3. Total of Protein and Phenol Content in Root

Treatment	Total Protein	Total Phenol
Blank	150+11[a]	7/70+0/11[a]
Putrescine	205+7[c]	4/30+0/13[e]
Spermidine	139+10[e]	5/61+0/10[d]
Salinity	122+4[d]	6/02+0/17[c]
Putrescine + Salinity	221+11[b]	5/60+0/16[d]
Spermidine + Salinity	117+8[d]	5/50+0/06[d]

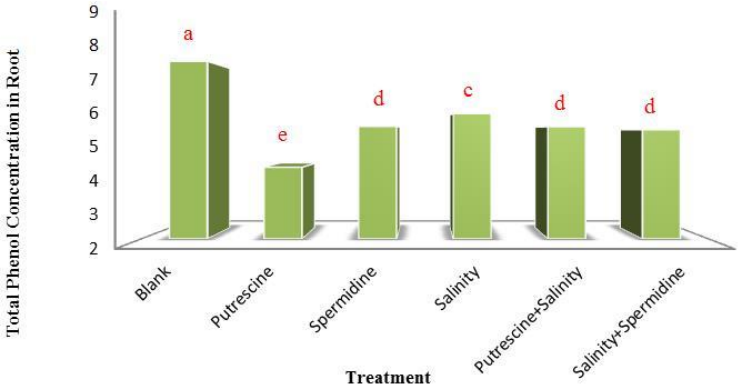

Figure 5. Total Phenol content in Root

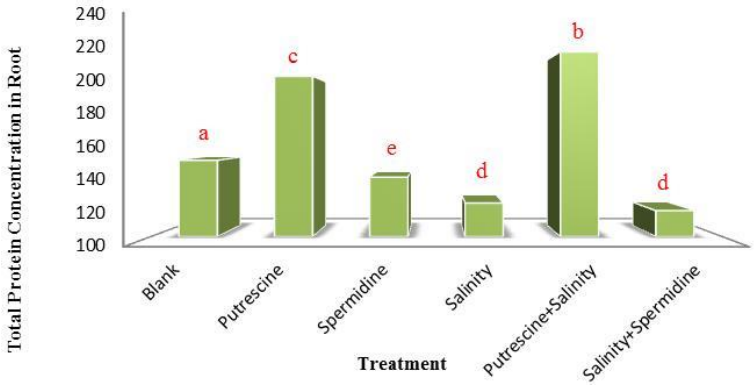

Figure 6. Total Protein content in Root

Discussion

Growth inhibition is a common response to salinity and plant growth is one of the most important agricultural indices of salt stress tolerance as indicated by different studies (Parida and Das 2005). Salinity treatment decreased the protein and chlorophyll content. Several studies have reported this reduction by salinity (Keutgen and Pawelzik, 2008; Kasukabe et al., 2006; Ibrahim et al., 2007). There are some complexities during stress, for example maybe made loose of energy resource and stress severity which maybe changed along the time. Opposite effect of these factors caused changes in metabolic balance. Therefore it caused changes in general appearance of plant. Application of exogenous treatment of hormone and poly amine such as spermidine and putrescine could reduced the effects of salinity. Using poly-amine due to the increased length of internode, the number of internode and leaf increased the growth of seedlings. Accordingly, bad effects of salinity on the vegetative growth significantly reduced. Polyamines are involved in plant defense to environmental stresses. In general, plant species and cultivars with high stress tolerance are endowed with a great capacity to enhance polyamine biosynthesis in response to environmental stresses including salinity (Amir et al, 2011). On the other hand, exogenous spermidine and putrescine application alleviated growth inhibition of Wheat and improved grain yield of plants under salinity. These results indicate that the accumulation of free spermidine and putrescine may be detrimental for growth and development of pomegranate plants.

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