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PROLINE, PROTEIN, RWC AND MSI CONTENTS AFFECTED BY PACLOBUTRAZOL AND WATER DEFICIT TREATMENTS IN STRAWBERRY CV. PAROS

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ABSTRACT. Drought is one of the critical environmental stresses that affect growth and development of plants. Plants are damaged directly and indirectly under drought stress. Increasing water stress tolerance in plants is crucial. The aim of this study was to investigate the effects of different water stress levels (-1, -5, and -10 bars) and paclobutrazol application (0 and 50 mg^{-l}) on strawberry cv. Paros. According to analyses of variance there were significant effects of drought stress and paclobutrazol application on leaf area, leaf dry weight, leaf relative water content (RWC), cell membrane stability index (MSI), proline and protein content of leaves. Leaf area, leaf dry weight, leaf relative water content and cell membrane stability index decreased in drought stress, especially at -10 bars. Proline and protein contents were enhanced by increasing water stress levels. Paclobutrazol application increased leaf relative water content and cell membrane stability index, proline and protein contents of leaves. Leaf relative water content was 68.77% in -10 bars drought stress that increased to 79% in paclobutrazol treatment. Also, cell membrane stability index was 69.65% in severe drought stress and reached to 77% in paclobutrazol treatment. According to the results pacloburazol is a benefit substance to ameliorate drought stress effects in strawberry cv. Paros.

Key words: Strawberry (*Fragaria×ananassa*); Drought stress; Morphological traits; Biochemical characteristics; Physiological traits; Abiotic stress.

INTRODUCTION

Plants are damaged directly and indirectly by water stress. Direct damages consist on desiccation of foliage, buds, bark and roots. Indirect injury includes inhibition of photosynthesis, the resulting slowing of plant growth, and the inability to produce defensive chemicals. Water stress has been defined as the induction of turgor pressure below the maximal potential pressure (Fitter and Hay, 1987). Changes in protein expression, accumulation, and synthesis have been observed in many

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plant species as a result of plant exposure to drought stress during growth (Cheng *et al.,* 1993).

The use of practical methods to mitigate drought stress practically by chemical treatment is likely to increase productivity under drought stress conditions. Hence application of antitranspirants (such as paclobutrazol, cycocel and daminozide), which are helpful tools in reducing transpiration losses, is becoming popular (Parkash and Ramachandran, 2000). Paclobutrazol belongs to the triazole family. They can protect plants against various stresses, including water deficit, high and low temperatures, UV-B radiation, air pollutants, fungal pathogens, and flooding. Paclobutrazol induces mild stress tolerance in seedlings and adult plants; more specifically paclobutrazol has been reported to protect plants against drought stress (Fletcher *et al.,* 2000).

The present study was carried out to investigate the effects of water deficit on strawberry (cv. Paros) and impact of paclobutrazol against drought stress.

MATERIALS AND METHODS

An experiment was done in the research greenhouse of Department of Horticultural Science, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran. Paros cultivar of strawberry was used in this study. The photoperiodic pattern of this cultivar is as a short-day plant and also it is resistant to powdery mildew and leaf spot diseases. During research, the average temperature of the greenhouse was 25 ºC.

A factorial experiment arranged in a complete randomized design with three replications that each replication had four trial units. Factors were drought stress and paclobutrazol treatments. Treatments were three levels of drought stress (-1 bar as control, -5 bars as moderate stress, and -10 bars as severe stress) and two concentrations of paclobutrazol (0 and 50 mgl^{-1} for each pot). Plants were cultivated in the 10 lit plastic pots. Mixed soil (one part sand + two parts field soil + one part organic manure) was used as culture medium. Gypsum blocks have been used to determine soil moisture content (Dela, 2001). The treatments were done when the plants had good growth and establishment (four months after cultivation). During the experiment leaf samples were taken and frozen (in -80 ºC) for measurement of future different traits (Caus *et al.,* 2012).

Leaf area was measured using a leaf area meter system (Leaf Area Meter AM 200). For evaluating leaf dry weight, 10 leaves from each treatment were took in an oven in 70 ºC, for 48 h, and then leaf dry weight were determined using a digital balance.

Leaf relative water content (RWC) was determined from the fully expanded young leaves according to (Galmes *et al.,* 2007). Leaf relative water content was calculated according to the equation:

$$RWC(\%)=\frac{\text{Fresh weight}-\text{Dry weight}}{\text{Saturated weight}-\text{Dry weight}}\times 100$$

Fresh weight is the samples fresh weight, dry weight is the dry weight after oven-drying the leaves at 70°C for 48 h, and saturated weight is the turgid weight after rehydrating the leaves at 4°C.

Cell membrane stability index (MSI) was measured according to Sairam *et al.* (1997). Cell membrane stability index was calculated according to the equation:

$$MSI = \frac{1 - C_1}{C_2} \times 100,$$

where C_1 is electrolyte leakage content after exposed in 40 ºC and C_2 is electrolyte leakage content after exposed in 100 ºC. Proline content of the leaves was determined by Bates *et al.* (1973). For doing that, 1 g of plant material was homogenized in 10 ml of 3% aqueous sulfosalcylic acid and the mixture was then centrifuged at 6000 rm for 5 min. Then 2 ml of upper phase of centrifuged was reacted with 2 ml acid ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 hour at 100°C and the reaction terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene, mixed for 15-20 seconds. The chromophore containing toluene was aspirated from the aqueous phase and the absorbance was read at 520 nm, using a spectrophotometer. The proline concentration was determined from standard curve and calculated using formula that presented by Bates *et al.* (1973). Protein contents of the samples were determined according to Bradford (1976) at 595 nm colorimetric wavelength, using a spectrophotometer.

MSTATC software was used for data analysis and means comparison was performed with Duncan's test at the 5% level of significance ($P < 0.05$).

RESULTS AND DISCUSSION

The results of analysis of variance (*Table 1*) show the effect of paclobutrazol and drought stress on different parameters of strawberry cv. Paros. As seen in *Table 1*, paclobutrazol application had significant effects at 0.05 level of probability on leaf area and protein content and 0.01 level of probability on leaf dry weight, leaf relative water content, cell membrane stability index and proline content. Also, drought stress significantly affected all the mentioned parameters at 0.01 level of probability except protein content; it had significant effect at 0.05 level of probability on protein content. Interaction effect of paclobutrazol and drought stress had no significant effect on the parameters.

According to the means comparison there were significant differences between control (-1 bar) and other treatments (-5 bars and -10 bars) for leaf area (*Table 2*). Also, paclobutrazol application had significant effect on leaf area (*Table 3*). The highest leaf area obtained in control and the lowest observed in -10 bars drought stress. In this research, leaf area (LA) was reduced by both drought stress and paclobutrazol that is in agreement with Movahed *et al.* (2012) report. They studied the ameliorative effects of paclobutrazol on different characteristics of grapevine under drought stress condition. Also, Navarro *et al.* (2007) reported that paclobutrazol treatment significantly reduced the number of leaves per plant and the leaf blade area compared with the control.

Table 1 - Analysis of variance of the effect of drought stress and paclobutrazol application on leaf area (LA), leaf dry weight (LDW), leaf relative water content (RWC), cell membrane stability index (MSI), proline content and protein content of strawberry cv. Paros

Source of variance	df	LA (cm^2)	LDW (g)	RWC (%)	MSI (%)	Proline (mg/g)	Protein (mg/g)
Paclobutrazol (P)	1	554.82*	0.07**	242.15**	87.34**	0.033**	0.020*
Drought stress (D)	2	1796.83**	0.21**	519.93**	402.76**	0.023**	0.014*
P × D	2	014.32^{ns}	0.006^{ns}	8.12^{ns}	6.78^{ns}	0.002^{ns}	0.002^{ns}
Error	12	65.499	0.003	8.39	4.57	0.001	0.002
Cv(%)	-	16.87	12.31	3.84	2.84	12.90	12.90

ns, ** and *: respectively, non significant and significant at the 0.01 and 0.05 levels of probability.

Table 2 - Effects of different drought stress levels on traits of strawberry cv. Paros

Treatment	LA (cm^2)	LDW (g)	RWC (%)	MSI (%)	Proline (mg/g)	Protein (mg/g)
Drought stress						
-1 bar	403.1a	4.01a	86.11a	84.59a	0.199c	0.48b
-5 bars	258.5b	2.36b	71.57b	71.28b	0.263b	..0.53ab
-10 bars	201.7b	1.84c	68.77b	69.65b	0.324a	0.58a

Similar letters in each column are not statistically different at 5% level of probability using Duncan test.

Table 3 - Effects of paclobutrazol application on different traits of strawberry cv. Paros

Treatment	LA (cm^2)	LDW (g)	RWC (%)	MSI (%)	Proline (mg/g)	Protein (mg/g)
Paclobutrazol						
0 mg	321a	3.12a	72b	72b	0.22b	0.51b
50 mg	254b	2.26b	79a	77a	0.31a	0.57a

Similar letters in each column are not statistically different at 5% level of probability using Duncan test.

Leaf dry weight was sharply decreased as drought stress increased (*Table 2*). The lowest leaf dry weight obtained at -10 bars drought stress (1.84 g) and the highest obtained at -1 bar (4.01 g). There were significant differences between all treatments of drought stress for this parameter. Paclobutrazol treatment also decreased leaf dry weight. Saffan (2008) reported that there was a decrease in the leaf dry weight of wheat, barley, kidney bean and mung bean under both salinity and water stress. Our result is in agreement with Saffan (2008).

Leaf relative water content is considered as an important criterion of plant water status. Leaf relative water content (RWC) showed a decrease as water stress increased. It decreased from 86.11% (-1 bar) to 68.77% (-10 bars). Paclobutrazol treatment increased leaf relative water content and there was a significant difference between paclobutrazol treated and untreated treatments. Water limitation has an impact on plant growth and development. Li et *al.* (2011) showed that drought treatment significantly reduced the leaf relative water content in *Cotinus coggygria* seedlings. The leaf relative water content values decreased with increasing water stress on patumma plant (Jungklang and Saengnil, 2012). Increasing of leaf relative water content in paclobutrazol treatment may attribute to decreasing leaf area. The suspension of photosynthesis, increase in respiration, and proline and abscisic acid (ABA) accumulation occur when leaf relative water content is lower than 80% (Gonzalez and Gonzalez-Vilar, 2001). In addition, leaf relative water content reflecting the metabolic activity in tissues (Flower and Ludlow, 1986). Bolat *et al.* (2014) reported that in apple rootstocks leaf relative water content and chlorophyll index decreased while electrolyte leakage increased with the increase of water stress in apple rootstocks. Paclobutrazol reduces evapotranspiration and decreases plant moisture stress by enhancing the relative water content of leaf area and develops resistance in the plants against biotic and abiotic stresses (Yadav *et al.,* 2005).

Drought stress decreased cell membrane stability index and there were significantly differences in cell membrane stability indices obtained from control and other levels of drought stress (*Table 2*). It is also worthwhile to mention that paclobutrazol application dramatically increased cell membrane stability index in strawberry (*Table 3*). Orabi *et al.* (2010) reported that in cucumber (*Cucumis sativus* L.) treatment with salicylic acid or paclobutrazol showed remarkable decreases in the content of malondialdehyde and electrolyte leakage as compared with the control. So, it shows that paclobutrazol application increases cell membrane stability. Also, in the other study paclobutrazolalso provides maximum protection from heat by decreasing electrolyte leakage and during drought stress from loss of membrane integrity and ion leakage in wheat seedlings (Kraus and Fletcher, 1994; Gilley and Fletcher, 1997). These outcomes may be attributed to the evident increases in antioxidant enzymes activities (Hussein and Orabi, 2008).

There were significant differences between proline content of leaves in different drought stress levels (*Table 2*). The proline content of leaves increased with increasing water stress. The paclobutrazol treatment also increased the proline content (*Table 3*). These results were in agreement with the results of the trial conducted by Movahed *et al.* (2012) on grapevine. The results of the current study showed that under drought stress, paclobutrazol treatment and proline content of leaves in strawberry was increased.

When plants are subjected to water stress, proline is synthesized from glutamic acid, which acts as osmoprotectant for keeping the water balance in cells and the outer environment (Delauney and Verma, 1993). Proline plays a role as compatible solute under environmental stress conditions. For example, high levels of proline can be found in pollen and seeds, protecting cellular structures during dehydration (Lehmann *et al.,* 2010). Also, under drought stress conditions, paclobutrazol treatment increased proline content in black locust (*Robinia pseudoacacia*) seedlings (Sheng and Zeng, 1993). Accumulation of proline permits osmotic adjustment, which results in water retention and avoidance of cell dehydration (Blum, 2005). The higher proline content could be due to increase activity of ornithine aminotransferase and pyrroline 5-carboxylate reductase, the enzymes involved in proline biosynthesis as well as due to the prevention of proline oxidase, proline catabolising enzymes (Debnath, 2008).

In stresses strawberry, both the higher levels of drought stress and paclobutrazol treatments could increase the protein contents (*Tabs. 2 and 3*). This effect was more obvious in -10 bars of water stress (*Table 2*). According to Jamalian *et al.* (2008), by increasing the concentration of paclobutrazol in strawberry, the protein content of the leaves was increased.

CONCLUSIONS

Nowadays, drought stress study has been one of the main directions in world plant biology and biological breeding. Water stress affected all studied parameters. Enhancing water stress levels decreased leaf area, leaf dry weight, leaf relative water content and cell membrane stability index and increased proline and protein content of the leaves. Paclobutrazol treatment increased leaf relative water content, cell membrane stability index, proline and protein contents in strawberry. So, paclobutrazol application has an important role in inducing tolerance of strawberry cv. Paros against water stress.

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