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Research Paper

Effect of foliar application of putrescine on free proline, soluble and insoluble carbohydrates in spring safflower (*Carthamus tinctorius* L.) under water deficit

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Abstract

Water deficit is the most common environmental stress factor that is effective on plant growth and development. Polyamines are as growth regulator in plants and increased their tolerance to abiotic stresses such as drought. In this research the effect of foliar application of putrescine (40 and 60 µM) on shoot and root soluble and insoluble sugars and leaf proline contents in safflower plants under different levels of water supply (100% and 40% field capacity) was studied as factorial arrangement based on complete randomized block design with three replications. The effects of water deficit and putrescine on proline content in leaf and soluble sugars content in shoot and root were significant. Shoot soluble sugars and leaf proline increased under drought stress, but insoluble sugars decreased. Proline content in leaf and soluble sugars in shoot and root significantly decreased by application of 40 and 60 µM putrescine under water deficit. Putrescine as osmolyte and reactive oxygen species scavenger reduced production and accumulation of compatible osmolytes. In non-stressed plants, effects of putrescine treatment on soluble sugars content in shoot and proline content in leaf were different related to concentrations. Insoluble sugars increased with both concentration of putrescine in shoot and decreased in root. Putrescine application could enhance resistance of safflower to drought without increasing osmolytes biosynthesis.

Keywords: Drought Stress; Osmolytes; Putrescine; Safflower

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1. Introduction

Abiotic stresses are defined as environmental conditions that reduce plan t growth (3). Drought stress is one of the most important environmental stresses that can negatively influence plants photosynthesis, chlorophyll content and as a result growth and production (1). Water deficit is characterized by reduction of water potential, CO_2 assimilation due to stomata closure, production of reactive oxygen species (ROS), oxidative damage in chloroplasts (19). The most detrimental to all biological systems under environmental stresses are reactive oxygen species (ROS) that include: $O_2^{\cdot-1}$, H_2O_2,

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$OH^{\cdot}$, and $^1O_2$ (9). The major targets of deleterious ROS action are cellular macromolecules as phospholipids, proteins, and nucleic acids. Drought can affect different physiological and biochemical processes in plants (15). Polyamines (PAs) are small, positively charged, organic molecules that are ubiquitous in all living organisms. The three common PAs in plants are putrescine (Put), spermidine (Spd) and Spm. PAs are involved in scavenging of the free radicals, and regulating osmotic potential and proline metabolism under abiotic stresses (8, 13). They have been deemed important in preparing the plant for stress tolerance (20). PA metabolism is increased in response to a variety of abiotic stresses - chemical or physical (8, 20).

Putrescine could have essential roles in defense mechanism of plants against environmental stresses (21). Large number of evidence suggested that exogenous application of putrescine could be used as tool to enhance plant tolerance under water deficit conditions. Roles of PAs in tolerance and/or amelioration of stress in plants include: serving as compatible solutes along with proline, glycine betaine and other osmolytes, interactions with macromolecules like DNA, RNA, transcriptional and translational complexes, and cellular and organellar membranes to stabilize them , role in directly scavenging oxygen and hydroxyl radicals and promoting the production of antioxidant enzymes (7). Safflower (*Carthamus tinctorius* L.) is important oilseed crop belonging to Asteraceae. It is well known that a particular environmental change may be stressful for one species but not for another living under the same conditions. Although, safflower is somewhat tolerant to drought stress, the physiological responses of this plant to polyamines such as putrescine under limited water supply is not clear. So, this research was conducted to investigate this subject in details.

2. Material and methods

This experiment was carried out in a greenhouse with a factorial arrangement based on randomized complete block design with three replications. The seeds of spring safflower (cv. Goldasht) were supplied by East Azerbaijan Agricultural and Natural Resources Research Center (Tabriz, Iran). The seeds were pretreated by 5% (v/v) sodium-hypochlorite solution for 5 min and sufficiently washed using distilled water. The seeds were cultivated in plastic pots (15×15 cm) containing perlite. Thereafter, all the pots were irrigated up to 100% field capacity (FC) in a greenhouse with 25-30ºC, 60% humidity and 16/8 h light/dark conditions. The water loss of the pots was compensated by distilled water for the first 7 days, 50% Hogland solution from day 7 to day 14, and after that time with 100% Hogland solution until three leaves stage of plants. At this stage, plants were treated by different levels of watering (100% and 40% FC) and after two weeks, plants were sprayed with putrescine (0, 40 and 60 µM) just once. The plants were gradually harvested after two weeks and biochemical traits were measured.

2.1 Soluble and insoluble sugars

Total soluble and insoluble sugars of leaf and root tissues were determined by the phenol sulfuric acid method as described by Kochert *et al*. (1978) (11). Briefly, 5 ml of ethanol (70%) was added to 0.05 g of dry shoot and root samples and maintained in refrigerator for one week. Then, the mixture was centrifuged at 10000 g for 15 min at room temperature and supernatant and obtained sediment were used for determination of soluble and insoluble sugars content, respectively. Glucose was used to prepare a standard curve and data was expressed as mg g^{-1} DW.

2.2 Proline content assay

For evaluation of free proline, 0.1 g of fresh leaf or root samples were homogenized by using 2 ml of sulfu-salicylic acid 3%. Then, homogenates were centrifuged at 2000 g for 10 min and supernatant used for quantification of free proline by spectrophotometer at 520 nm. The reaction mixture contained 2 ml of ninhydrin (25% w/v), 2 of ml acetic acid and 2 ml of the extract. Samples were placed for 1 h in boiling water. Reaction was then stopped by ice bath. Then, 2 ml of toluene was added to the mixture and the upper phase used to measure the absorbance (2). Free proline content was calculated as µg g^{-1} FW, using a standard curve which was prepared by L. proline (0 - 50 µL).

2.3 Statistical analysis

Analysis of variances (ANOVA) of the data were performed for all the traits by SAS (9.2) software. Means were compared according to Duncan multiple range test at $p \leq 0.05$ and Excel 2013 software was used to draw the Figures.

3. Results

The interaction of drought stress × putrescine treatment was significant ($p \leq 0.01$) for soluble sugars and free proline in shoots, leaves and roots (Table 1). Soluble sugars (in shoots, roots) and proline contents in leaves and roots significantly decreased with putrescine application under water deficit. In non-stressed plants, effect of putrescine on soluble sugars (shoots, roots) and proline contents

(leaves, roots) was different related to concentrations. 60 µM putrescine increased soluble sugars and proline in shoots of non-stressed plants. 40 µM putrescine improved proline in control plants (Table 2).

Table 1. Analysis of variance soluble, insoluble sugars and proline of safflower grown under different levels of watering in response to foliar application of putrescine

		Shoot		Leaf
Sources of variation	**df**	**Soluble sugars**	**Insoluble sugars**	**Proline**
Irrigation	1	319.41**	38.54**	6.81**
Putrescine	2	83.51**	38.30**	8.42**
Irrigation × Putrescine	2	40.21**	218.39**	8.49**
Error	12	14.877	8.07	1.52
(%) CV		3.68	3.06	8.57
		Root		
Sources of variation	**df**	**Soluble sugars**	**Insoluble sugars**	**Proline**
Irrigation	1	0.6164ns	0.57ns	4.09**
Putrescine	2	27.88**	26.02**	0.45**
Irrigation × Putrescine	2	10.91**	58.90**	0.66**
Error	12	2.46	5.01	0.085
(%) CV		6.52	6.10	3.87

ns, **: non-significant and significant at $p \leq 0.01$, respectively

Table 2. The effect of different rates of putrescine on soluble and insoluble sugars, proline contents in shoot, leaf and root of safflower plants under different levels of watering

		Shoot		Leaf
Water supply	**Put (µM)**	**Soluble sugars (mg g^{-1} DW)**	**Insoluble sugars (mg g^{-1} DW)**	**Proline (µg g^{-1} FW)**
	0	27/17±1.45^{d}	35.76±0.73^{a}	3.66±0.33^{bc}
100% FC	40	20.81±0.84^{e}	19.00±0.96^{e}	3.05±0.35^{cd}
	60	30.99±1.00^{c}	29.99±1.94^{b}	3.89±0.37^{b}
	0	39.42±1.44^{a}	21.20±1.78^{d}	7.36±0.14^{a}
40% FC	40	31.31±0.42^{bc}	28/78±0.51^{b}	4.09±0.41^{b}
	60	32.52±1.15^{b}	25.59±0.61^{c}	2.84±0.43^{d}
		Root		
	0	10.61±0.60^{a}	8.73±0.34^{c}	1.56±0.08^{d}
100% FC	40	5.75±0.61^{d}	9.13±0.59^{c}	2.27±0.16^{c}
	60	3.88±0.39^{e}	13.35±0.39^{b}	1.27±0.04^{e}
	0	8.17±0.44^{b}	16.11±1.04^{a}	3.05±0.05^{a}
40% FC	40	6.36±0.05^{cd}	7.51±0.76^{d}	2.48±0.04^{b}
	60	6.82±0.34^{c}	8.66±0.45^{c}	2.45±0.005^{b}

The data represent the mean of three replications ± SD.
Different letters indicate significant difference at $p \leq 0.05$.

4. Discussion

Drought stress had negative effect on growth of safflower plants. Plants produce high levels of osmolytes such as soluble sugars and proline for increasing resistance to drought (15). Increasing soluble sugars in stressed plants could be attributed to enhancing invertase and amylase activities and reducing consumption of soluble sugars due to decreasing plant growth. Proline is well-known compatible osmolyte (19) and also as osmoregulator it can protect membrane from damage by ROS (3, 18). Enhancing proline under water deficit could be the result of increasing pyrroline-5-carboxylate synthase (P5C5) activity. Proline biosynthesis is directly from Glu by P5CS (22). Converting Ornithine, Arginine and glutamine to proline also have role in enhancing proline (22) . Mohammadi et al., (2016) reported that

proline and soluble sugars increase under water deficit in safflower (15).

Decreasing proline in leaves and roots of plants treated with putrescine under drought stress could be due to enhancing chlorophyll synthesis, since both chlorophyll and proline are synthesized from same precursor (glutamate) (15). Also putrescine can mitigate detrimental effects of water deficit by improving water contents. Increasing chlorophyll contents and relative water contents was reported with polyamines application in mustard under drought stress (16). In addition, putrescine can act as osmoticum as well as scavenger of reactive oxygen species and mitigated harmful effects of the stress (10). Polyamines can act as compatible solutes along with proline, glycine betaine under stress condition (17). Because of their polycationic nature, polyamines possess free radical scavenging features and antioxidant activity and may confer plant tolerance to different biotic and abiotic stresses (5, 6). Reducing proline in plants by putrescine application could be due to decreasing activity of its biosynthetic enzymes. This finding is similar to those of Mostafaie *et al*., (2018) who suggested that putrescine and spermidine application reduced proline content under drought stress in mustard (16). Therefore polyamines can protect plants from stress injuries through their roles in osmotic adjustment and removing reactive oxygen species and enhancing tolerance. Reducing soluble sugars could be attributed to consumption of sugar sources for increasing phenolic compounds such as phenol and anthocyanins in stressed- plants. Putrescine was the best treatment for alleviating harmful effects of drought in safflower plants.

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