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Physiological changes induced by Salicylic Acid and Putrescine and their impact on drought tolerance in *Lentil (Lens culinaris L.)*

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ABSTRACT

Lentil (*Lens culinaris* L.) is very nutritive and consumed as pulse and in many other preparations. With its high nutritional value, lentil is primarily a major source of protein, essential nutrients such as calcium, zinc and iron for the vegetarian populations. Drought stress is the most prevalent environmental factor that limits growth, survival, and productivity of lentil. Drought stress causes a broad range of physiological changes and impairments of metabolic processes. PGRs, such as, salicylic acid (SA) evidenced to provide tolerance in plants against different abiotic stresses, such as heat, salinity, heavy metal toxicity, and drought. Putrescine (Put) also plays a positive role in reducing the adverse effects of abiotic stresses on plants through its acid neutralizing and cell wall stabilizing capabilities. Our result showed that drought stress decrease some characteristics such as leaf area contents, net photosynthesis, transpiration rate and RWC but increases proline and sugar content. The application of SA and PUT improved all the measured traits and induced drought tolerance in the treated plants.

Key words: Lentils, drought stress, PGRs, salicylic acid, putrescine, proline

INTRODUCTION

Pulse crops are energy rich plants and an important part of Indian dietary. Among pulses, lentil (*Lens culinaris*. L) is the most important pulse crop in the country. Lentil (*Lens culinaris* L.) is very nutritive and consumed as pulse and in many other preparations. With its high nutritional value, lentil is primarily a major source of protein, essential nutrients such as calcium, zinc and iron for the vegetarian populations. Drought stress is one of the most devastating environmental stresses, limiting the productivity of crop plants around the world. Drought stress causes a broad range of physiological changes and impairments of metabolic processes, which result in accumulation of reactive oxygen species (ROS) (Abid et al., 2018). Drought also causes a substantial reduction in crop productivity through negatively impacting plant growth, physiology, nutrient and water relations, photosynthesis, and assimilate partitioning. It has been shown that there is a significant correlation between the stomatal conductance and photosynthesis response under drought stress, which indicates that stomatal conductance play a major role in the reduction of leaf photosynthetic rates (Abid et al., 2018; Sarabi et al., 2019).

Plant growth regulators (PGRs) or hormones have been found to improve tolerance of plants against the damages caused by abiotic stresses. However, limited researches have been led to examine the possible benefits of exogenous

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application of PGRs under drouhgt stress conditions in lentil.

Salicylic acid (SA), a phytohormone, is a promising compound that can reduce the sensitivity of plants to environmental stresses through regulation of the antioxidant defense system, transpiration rates, stomatal movement, and photosynthetic rate (Nazar et al., 2015). It is evident that SA is a stress-signal molecule that activates abiotic stress -responsive gene expression (Li and Zhang, 1999), and induces the expression of biosynthetic enzymes and proteins in plants under environmental stresses (Nazar et al., 2015; Wang et al., 2019). Several studies have shown that the application of SA resulted in a positive effect by protecting plants against the oxidative damage caused by drought stress (Kang et al., 2012; Najafabadi and Ehsanzadeh, 2017; Wang et al., 2019; Sankari et al., 2019). In addition, SA is involved in the regulation of plant physiological processes including stomatal closure, chlorophyll and protein synthesis, nutrient uptake, transpiration, and photosynthesis (Shakirova and Sakhabutdinova, 2003).
Some studies have indicated that exogenous application of SA may lead to improvements in morpho -physiological traits that are involved in determination of plant yield of wheat (*Triticum aestivum* L.) (Shakirova and Sakhabutdinova, 2003) and maize (*Zea maize* L.). Furthermore, SA affects isoprenoid (a-Tocopherol, carotenoids, and monoterpenes) accumulation in leaves of plants especially under water stress. Putrescine (Put) plays a positive role in reducing the adverse effects of abiotic stresses and improves tolerance against drought. In fact, the putrescine was successfully applied to tolerate drought condition (Amri and Shahsavar, 2010).

Put application by spraying increased leaf area, height, leaf area, and grain yield of wheat plants owing to the increase in chlorophyll, water status, and the content of Pro, amino acids, and soluble sugars (Gupta et al.,2012). Zhu et al., 2019 showed that foliar Put application to lettuce subjected to drought conditions triggered a reduction in stomatal density, keeping chloroplast structure and cell turgor. Similarly, Shallan et al., 2012 described that Put application as pre treatment in cotton plants improved root to shoot ratio, leaf area, number and setting of bolls, seed cotton yield, total soluble sugars, pigments content, Pro content, total free amino acids, total phenols, total soluble proteins, total antioxidant capacity, and antioxidant enzyme activities. Put treatment also reduces the sensitivity of *Medicago sativa* plants to PEG-induced drought stress by reducing the activity of the hydrolytic enzymes and increasing the polysaccharide, protein and photosynthetic pigment contents, and photosynthetic activity (Zaid and Shedeed, 2006). Put has the ability to improve anatomical features, retaining chlorophyll concentrations and accumulating total soluble phenolic compounds in *Thymus vulgaris* plants, which leads to improved oil yield under drought conditions (Abd Elbar et al., 2019)

MATERIALS AND METHODS

Relative water content (RWC, %):

Leaf relative water content was measured from the first fully expanded leaf from the top in normal and flooded plants at pre-flowering stage. Leaf relative water content (RWC) was estimated by measuring the turgid weight of 0.5 g fresh leaf samples by keeping in the water for 4 hours followed by drying in the hot air oven till constant weight was achieved.

T*otal chlorophyll content:*

The total chlorophyll content was determined in the first fully expanded leaves from the top in normal and flooding stressed plants by the method of Yoshida. For this, 500 mg leaves were washed properly after that, leaves crushed with the help of mortar and pestle by using 5 mL of 80% acetone solution and a pinch of fine sand followed by centrifugation of the crushed material at 5000

rpm for 10 minutes and then supernatant was collected in the flask and final volume was made up to 50 mL by adding the 80% acetone. Absorbance was recorded at the wavelength of 645 and 663 nm by using the spectrophotometer (ELICO SL-196).

Soluble sugar content:

Soluble sugar content was determined in the first fully expanded leaves from the top in the normal and flooding stressed lentil plant by the method of Dubois and expressed as mg g^{-1} fresh weight.

Estimation of total protein content:

The total protein content was determined in the leaves of lentils in normal and stressed plants at pre-flowering stage by the method of Lowry. Two hundred mg leaf sample was homogenized with 10 mL 80% ethanol using mortar and pestle and centrifuged it at 4000 rpm for 20 minutes. The supernatant was kept aside and the residue was hydrolyzed with 5 mL of 1 N NaOH for overnight and next day centrifuged it again at 4000 rpm for 20 minutes. Supernatant was collected and residue was again extracted with 5 mL of 1 N NaOH after 1 hour of adding it and then centrifuged. Both the supernatant were mixed and volume was made up to 10 mL. Then 0.5 mL supernatant and 5 mL alkaline copper solution were added and mixture was left for 10 min and after that 0.5 mL folin reagent was added and incubated at room temperature for 1 hour. A blue colour developed and thereafter absorbance of the blue colour was recorded at 730 nm by using spectrophotometer. Bovine serum albumin (BSA) solution was prepared to get the standard curve.

Quantification of Total Phenolic content (TPC)

Total phenolic compounds present in the aqueous extracts of lentils were quantified spectrophotometrically through the Folin Ciocalteu test following the protocol of Singleton *et al.* with specific modifications. Gallic acid (GA) was used as standard and distilled water as the blank sample. In a 10 ml volumetric flask, 4 ml of distilled water were mixed with 0.4 ml of the standard solution, the blank sample, or the extract to be analyzed. 0.4 ml of Folin–Ciocalteu reagent was then immediately added and the solution was allowed to react for 5 min. At the end of this period, 4 ml of a 7% Na_2CO_3 solution was added, the mixture was stirred, and the volumetric flask was brought up to volume with distilled water. After 90 min of incubation in the dark and at room temperature (± 23 ° C), the solution absorbance was measured at 730 nm using a spectrophotometer. The TPC was expressed as mg equivalents of GA per g of dry matter (mg GAE/g).

Total Flavonoid content determination*:*

Total flavonoid content was determined by Aluminium chloride method 14 using quercetin as a standard. 1ml of test sample and 4 ml of water was added to a volumetric flask (10 ml volume). Add 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10% Aluminium chloride was added after 5 minutes. After 6 minutes incubation at room temperature, 1ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was make upto 10 ml with distilled water. Absorbance of sample was measured against the blank at 510 nm using a spectrophotometer. All the experiment was repeated three times for precision and values were expressed in mean ± standard deviation in terms flavonoid content (Quercetin equivalent, QE) per g of dry weight.

Proline estimation

Proline and total amino acids may also be extracted using a cold extraction procedure by mixing 20-50 mg fresh weight aliquots with 0.4-1 ml of ethanol:water (40:60 v/v). The resulting mixture is left overnight a 4°C, and then centrifuged at 14000 g (5 min). The cold extraction procedure can be repeated on the pellet and supernatants pooled and used for the analyses by using spectrophotometer.

RESULTS AND DISCUSSION

In present study we found that drought stress led to a remarkable decrease in chlorophyll a and b compared to the control. Foliar application of SA and PUT significantly enhanced chlorophyll a and b. There was a significant difference in chlorophyll contents in different hormone treated and drought stress plants (Table 1, Figure 1). Exogenously applied SA improved photosynthesis in both control and drought-stressed plants compared to untreated plants. In drought condition in lentils the amounts of RWC is decreased remarkably (Figure 2). Salicylic acid treated plants exhibit increased amount of RWC in comparision to PUT treated plants. Therefore the improvement in RWC by exogenous application of SA and PUT may be the result of osmotic adjustment because of accumulation of compatible solutes like proline. The results also show that protein contents increased when plants were subjected to drought stress (Figure 3). SA and PUT application to plants under drought stress remarkebly increased soluble protein content compared to the plants only treated with drought. The effect of drought stress on sugar content is shown in. The results showed that drought stress significantly enhanced the sucrose content compared to the control. Sucrose content was significantly higher in plants treated with SA compared to the control and PUT treated plants. This difference was even greater when the SA-treated plants were exposed to drought stress, as the highest sucrose content was observed in drought stressed-plants treated with SA. The effect of drought stress on flavonoid contents is shown in figure (Figure 5). The result showed that in drought stress, the flavonoids were remarkably increased. The plants treated with SA and PUT showed impressive results with increase amount of flavonoids. Under control condition there was no significance difference of leaf proline content during the experimental periods. But there was a large variation in proline content under drought stress and hormone treated plants e.g. SA and PUT (Figure 6). Proline contents in leaves of lentils remarkably increased under drought stress condition and slightly increased in hormone treated plants compared to control condition. Treatment with SA elevated the total phenolic content. PUT treated plant showed highest amount of total phenol content in comparison to SA and drought stress lentil (Figure 4).

Table 1. Effects of SA, PUT and drought on secondary metabolites of lentils under vegetative phase. Each value represents the Mean± SD of three replications

	Chlorophyll a+b	RWC	Protein content	Sugar content	Phenol	Flavonoids	Proline
Control	3.110±0.4899	0.028±0.12832	1.0±0.8164	0.026±0.13165	0.28± 0.64666	0.327±0.7132	0.136±0.67330
Salicylic acid	3.159±0.6638	0.137±0.05477	1.14±0.8717	0.39±0.50990	0.323±0.46404	0.622±0.41333	0.140±0.2160
Putrescine	3.782± 1.58797	0.030±0.11832	1.4±0.9486	0.37±2.856	0.33±0.46904	0.378±0.50497	0.137±0.17433
Drought	2.820±1.2465	0.024± 0.1	1.2±0.88881	0.35±0.48304	0.313±0.45752	0.608±0.63665	0.139±0.68068

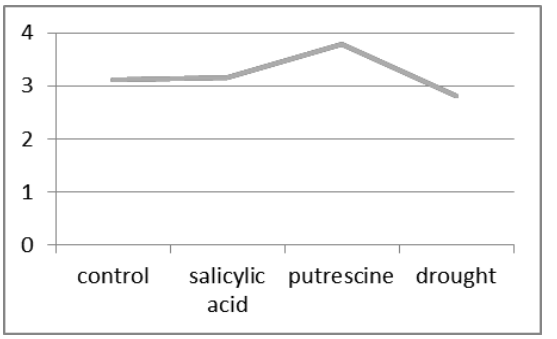

Figure 1. Effects of SA, PUT and drought on chlorophyll a+b of lentils under vegetative phase. Each line represents the Mean± SD of three replications

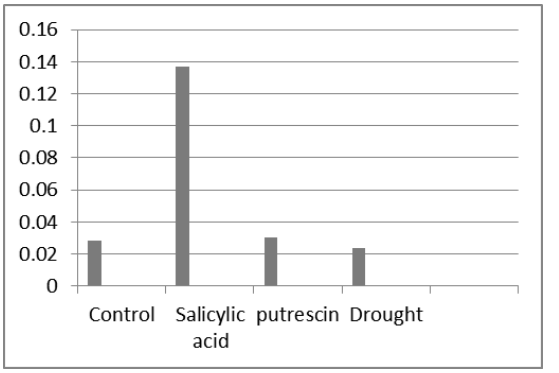

Figure 2. Effects of SA, PUT and drought on RWC contents of lentils under vegetative phase. Each column represents the Mean± SD of three replications

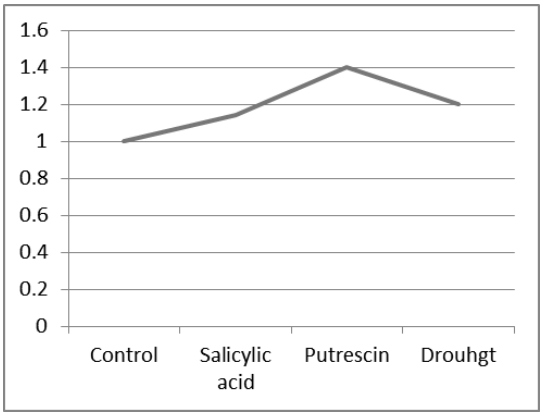

Figure 3. Effects of SA, PUT and drought on Protein contentsof lentils under vegetative phase. Each line represents the Mean± SD of three replications

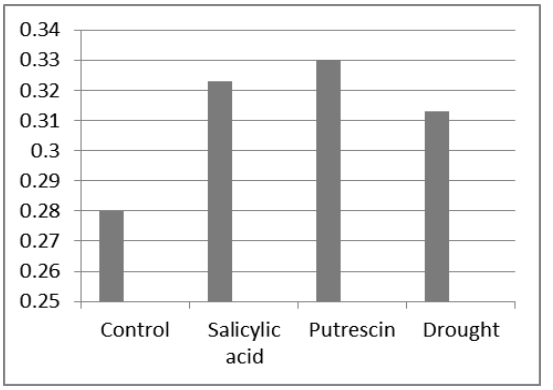

Figure 4. Effects of SA, PUT and drought on Phenol contents of lentils under vegetative phase. Each column represents the Mean± SD of three replications

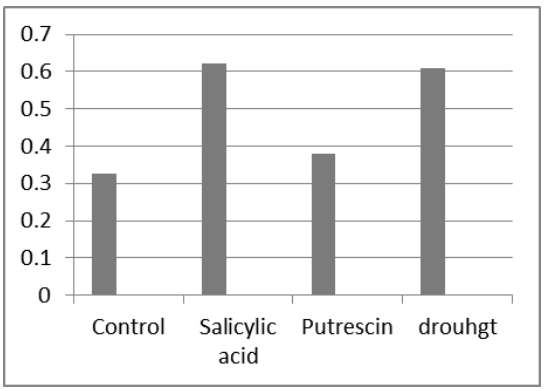

Figure 5. Effects of SA, PUT and drought on Flavonoids content of lentils under vegetative phase. Each columnrepresents the Mean± SD of three replications

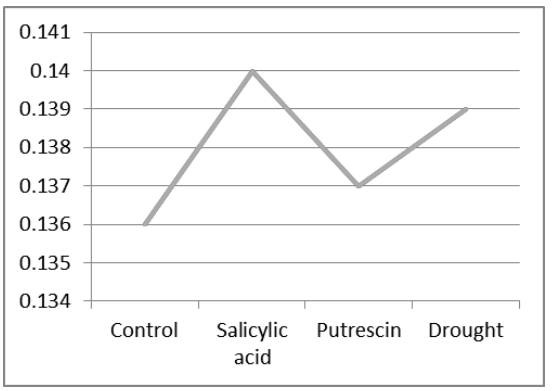

Figure 6. Effects of SA, PUT and drought on Proline contents of lentils under vegetative phase. Each line represents the Mean± SD of three replications

SA treatment enhanced the levels of soluble protein, and the abundance of many enzymes related to the accumulation of polypeptides in wheat under stressful conditions. Treating plants with SA induced an increase in abundance of protein spots (including ribulose-1,5- bisphosphate carboxylase activase, two Rubisco large subunit-binding proteins, carbonic anhydrase) (Kang et al., 2012), and appearance of two de novo polypeptides (630 and 141 KDa) (Azooz et al., 2011) in order to cope with drought stress. Previous studies demonstrated that the drought-induced reduction in chlorophyll content and chlorophyll fluorescence led to a decrease in photosynthesis and overall plant growth (Miller et al., 2010; Redzik, 2019). The SA and PUT treatment significantly enhanced the chlorophyll fluorescence and chlorophyll content values in lentil. Hassanzadeh et al., 2009 reported that decrease in RWC is related to the decrease in chlorophyll content and leaf fresh weight in sesame genotypes, however, the tolerant genotypes maintained higher RWC under stress condition and thus showed higher affinity for chlorophyll content and leaf fresh weight .Our results demonstrated that SA and PUT maintained efficient photo-system with improved water budget resulting in improved growth and productivity under drought stress condition. Higher accumulation of the total sugar content was also evidenced in our study due to SA and PUT treatment.

Our result showed that drought stress decrease some characteristics such as leaf area contents, net photosynthesis and RWC but increases proline and sugar content. The application of SA and PUT improved all the measured traits and induced drought tolerance in the treated plants. The results of this study support the hypothesis that Salicylic acid and Putrescin treatment might play an important role in modulating the physiological processes which eventually lead to protect plants under drought stress conditions. SA and PUT are of great potential to improve photosynthesis rate and chlorophyll content in lentils.

CONCLUSION

Drought is a major constraint on crop productivity worldwide and is expected to worsen in the near future. Hence, scientists are trying to understand different drought tolerance mechanisms of plants and to develop drought-tolerant crops. Phytohormones like SA and PUT trigger tolerance to drought stress via regulation of various morphological, physiological, biochemical and molecular processes. These phytohormones have great potential to develop drought tolerant crops.

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