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Research Article

EVALUATION OF BIOCHEMICAL RESPONSES OF ONION (*ALLIUM CEPA* L.) SEEDLINGS UNDER DROUGHT STRESS

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ABSTRACT

Drought is one of the most important global problems for agriculture. Plants adapt and respond to water stress by inducing different defense mechanisms by changing their cellular metabolism. The present study is aimed to gauge the effect of drought (with holding watering for six days) by screening the nine cultivars of onion seedlings, by monitoring the following parameters like chlorophyll content, relative water content (RWC), quantitative analysis of proteins, sugars, proline, phenols, flavonoids, and tannins. Drought stress decreased the chlorophyll content in all the cultivars. Relative water content was maintained in Arka lalima under stress when compared with other cultivars. Protein, sugars, and proline content increased in all cultivars under drought conditions. Maximum proline levels were examined in prema 178, arka kirthaman and arka lalima under stress conditions. Secondary metabolites such as flavonoids, phenols and tannins levels enhanced in stressed seedlings. Among all nine cultivars of onion arka kirthaman, arka lalima and prema 178 showed highest response to drought stress when compared to the control. Further physiological and molecular investigation is needed to know the drought tolerance mechanism in onion cultivars.

INTRODUCTION

The scarcity of fresh water for agriculture around the world threatens the productivity of crop lands. Irregular distribution or scarcity of most of the rainfall in rain fed areas at any specific stage of the crop leads to drought (Bouman and Aureus, 2009). Four-tenths of the world's agricultural land lies in arid and/or semi-arid regions. Water stress is one of the most serious environmental stresses which limit production by regulating plant growth and development. Crop plants habituate to such drought stress conditions by invoking various physiological, biochemical and molecular responses (Maksup *et al.* 2014). The change in response include the over production of enzymes responsible for the biosynthesis of detoxification enzymes and osmolytes (Huseynova *et al.* 2016). Habash *et al.* 2014 reported physiological and a transcriptome response in durum wheat under drought stress. Glycine betaine, proline, sugars and some inorganic ions are small compatible solutes synthesized and accumulated under water stress (Chaves *et al.*, 2003). These solutes maintain cellular dehydration and membrane integrity and this in turn furnish tolerance against cellular dehydration and drought (Ramanjulu and Bartels, 2002). Sucrose, stachyose, sorbitol, raffinose, galactinol and trehalose are sugars included in raffinose family oligosaccharides. These sugars play important role in the drought tolerance mechanism of plants and seeds (Seki *et al.*, 2007). In addition to all these, reactive oxygen species is also synthesized in drought stress.

Proline, an amino acid play a versatile role in the water stress defense mechanisms. Non-enzymatic scavenging mechanisms include accumulation of proline, glycine betaine (Ashraf *et al.*, 2007). Amino acid proline is deposited in big amount in response to environmental stresses and it occurs widely in higher plants (Ozturk *et al.*, 2002), proline acts as an compatible solute for osmotic adjustment. In plants secondary metabolites are known to increase during water stress. Accumulation of secondary metabolites observed by Bartwal *et al.* 2013 is a defense mechanism of plants to adapt themselves under drought stress by altering their cellular metabolism. Increased synthesis of flavonoid content in wheat leaves under water stress was observed by Ma *et al.* 2014.

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MATERIALS AND METHODS

Onion seeds Collection, Germination and Drought Treatment

Nine cultivars of onion seeds were selected and collected (Agrifound rose, Bellary, Nasik red, Prema-178, Arka Kirthiman, Arka Lalima, Gavran Light Red, and Light Red & Red Diamond) and allowed to germinate for 10 days at 27 ^{o}C in plant growth chamber. Seasons and places where they are cultivated are reported by Sai Sudha *et al*. 2019. The 10 day old seedlings were subjected to non lethal drought stress by withholding watering for six days under controlled growth chamber conditions.

Chlorophyll and Relative water content (RWC)

Onion seedlings (0.05g) were homogenized in 80% acetone (v/v) for estimating the total chlorophyll content (Arnon 1949). For relative water content, 20 seedlings from each cultivar were weighed immediately (FW) after harvesting (Sai Sudha *et al*. 2015). Relative water content was calculated by the following formula.

Relative water content (FW-DW) ÷ (TW-DW) X 100
Where, FW- Fresh weight, DW- Dry weight, TW- Turgid weight.

Proteins, Sugars and Proline levels

Determination of protein concentrations was done by Lowry method (Lowry *et al*. 1951). The total soluble carbohydrates extraction was made with 50 mg of seedlings and sugars were determined by anthrone method. Proline was assayed according to the method described by Bates *et al* (1973). Fresh seedlings of 500 mg were homogenized in 5 ml of 3 % (w/v) sulphosalicylic acid. Acid ninhydrin and glacial acetic acid were added and incubated for 1 h in a boiling water bath at 90^{0}C followed by an ice bath then toluene was added and measured at 560 nm against blank.

Total Phenols, Total Flavonoid and Tannin Content

The total phenolic content was determined by the spectrophotometer method (Harborne 1973). 1 ml of sample was mixed with Folin- Ciocalteu phenol reagent and Na_2CO_3 and mixed thoroughly. The mixture was kept in the dark for 90 min at 23˚C, and then the absorbance was read at 750 nm. The TPC was expressed as milligrams of Gallic acid equivalents (GAE) per g of dried sample. The total flavonoid content of the sample was determined by using ammonium chloride colorimetric method as described by Chang *et al*. (2002) with slight modifications. Absorbance of the mixture was measured at 415 nm and the calibration curve was prepared by using quercetin as standard. Tannins were quantified according to Van-Burden and Robinson (1981). 0.5 g of the sample was mixed with distilled water in a mechanical shaker. To the Filtrate 0.1 M $FeCl_3$ and 0.1 N HCl and 0.008 M potassium ferrocyanide was added and absorbance was read at 120 nm.
Statistical analysis

RESULTS AND DISCUSSION

Chlorophyll Content and Relative water Content

Total chlorophyll content reduced in all cultivars under drought stress (Fig. 1). Negative correlation between chlorophyll content and drought has been reported many researchers which is in accordance with our results (Lakshmi sahitya *et al*. 2018). When compared to controls relative water content varied and decreased in all the cultivars under drought. In controls relative water content level was maximum in Light red i.e. 91 % and minimum in Nasik red i.e. 72 % (Fig. 1). Under stress conditions more relative water content was observed in Arka lalima and lesser quantity in Gavran light red.

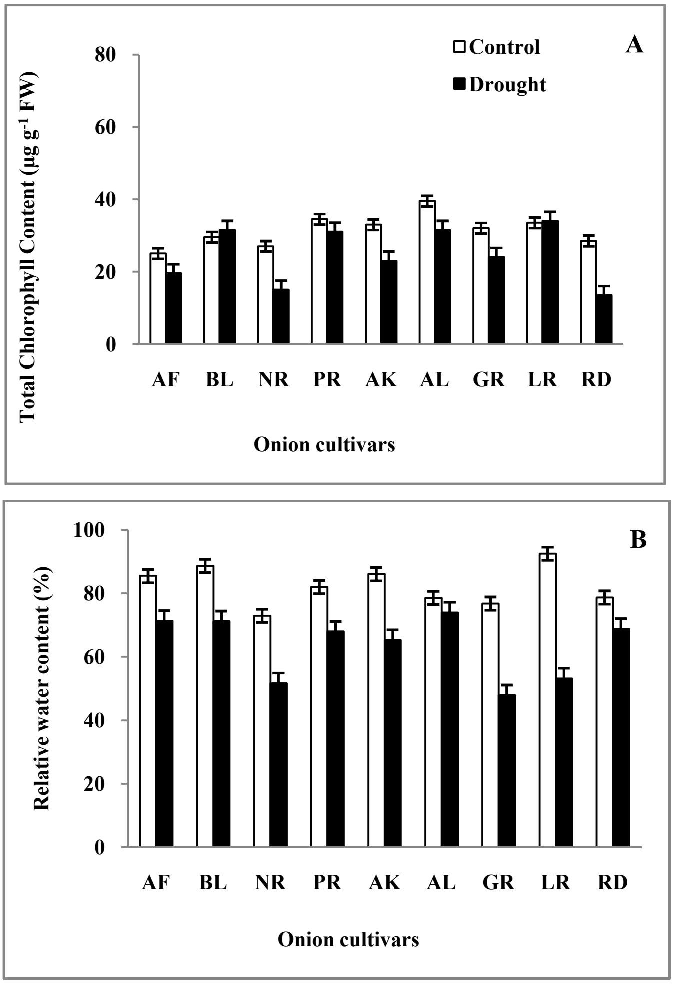

Figure 1 Effect of drought stress on (A) total chlorophyll content and (B) relative water content in different onion seedlings. Each bar represents the mean (±SE) of three replications.

Protein, Sugar and Proline levels

Under withholding water condition protein, sugars and proline accumulation varied and increased in all the cultivars when compared with controls (Fig. 2). Overexpression of drought-inducible galactinol synthase gene (*ArGolS2*) in transgenic *Arabidopsis* enhanced drought tolerance because of the accumulation of galactinol and raffinose (Taji *et al*., 2002). Maximum protein quantity was observed in Arka kirthman and Prema-178 and minimum in Nasik red (Fig. 2). Highest proline content was monitored in Arka kirthman and Arka lalima in stress. The correlation between the accumulation of proline and tolerance of drought stress has been demonstrated by overexpression of proline-5-carboxylate synthase gene *P5CS* or by antisense suspression of the proline dehydrogenase (*ProDH*) gene in various plants (Bartels and Ramanjulu 2005).

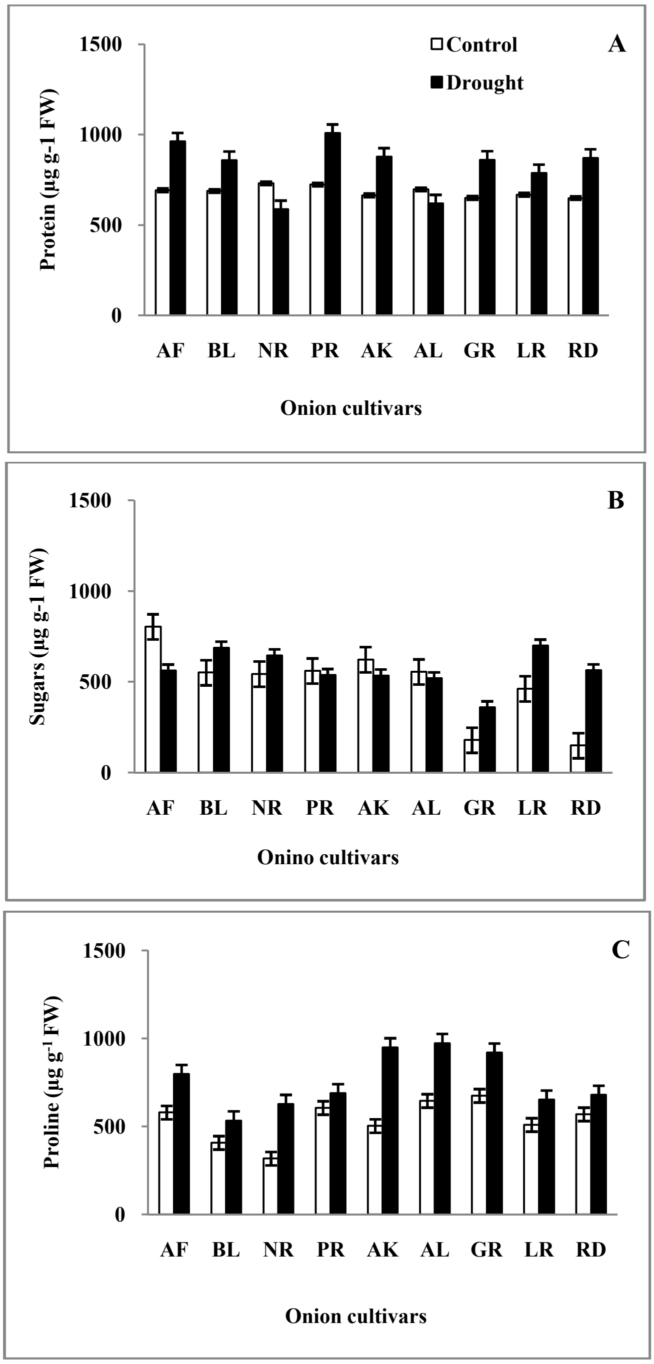

Figure 2 Effect of drought stress on (A) Protein (B) Sugars and (C) Proline in different onion seedlings. Each bar represents the mean (±SE) of three replications.

Total phenols, Total flavonoid and Tannin content

Drought stress enhanced total phenols, flavonoids content in amaranthus leafy vegeatable (Sarker and Oba 2018). Total phenol, flavonoid and tannin content of the nine cultivars were different both in control and drought (Fig. 3). Greatest total phenol, flavonoid and tannin levels were observed in all the cultivars under drought conditions. More phenolic content were examined in Arka kirthiman and Prema-178. Under drought stress highest flavonoid levels were observed in Arka kirthiman, Prema-178 and Gavran light red (Fig. 3). The increase in tannin content was very high in stress when compared with controls in all the cultivars.

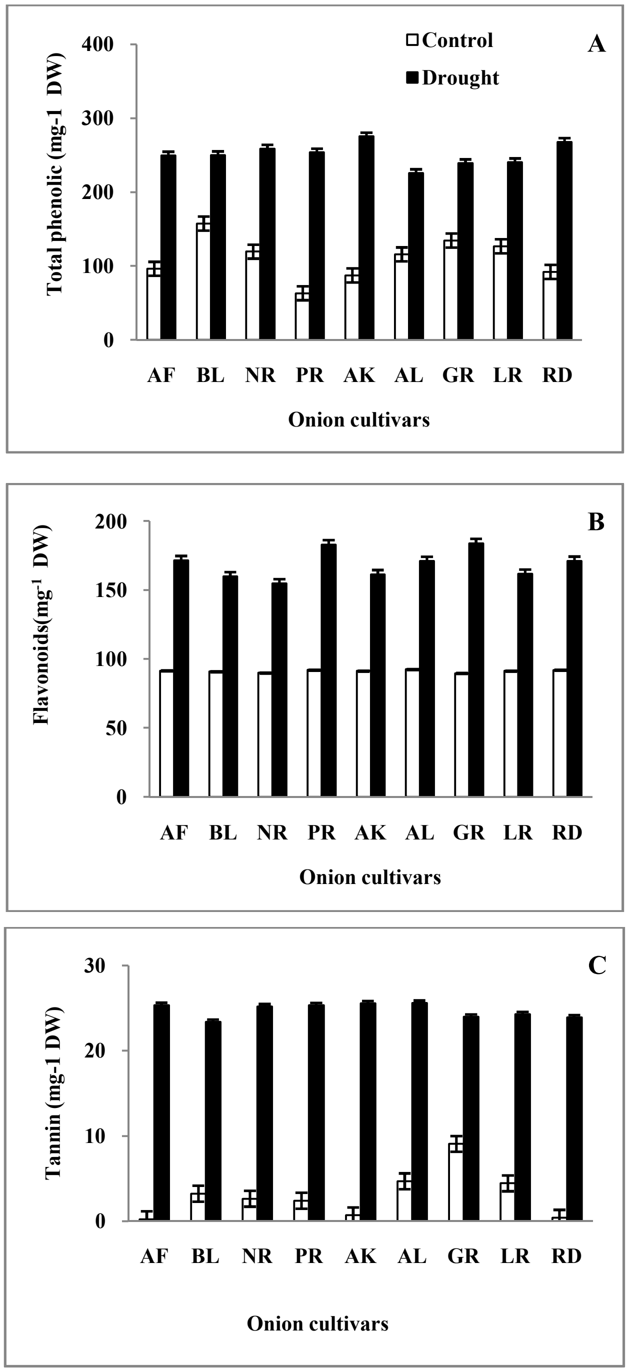

Figure 2 Effect of drought stress on (A) Phenol (B) flavonoids and (C) tannins in different onion seedlings. Each bar represents the mean (±SE) of three replications.

CONCLUSION

The present investigation was performed to evaluate the physiological and biochemical responses of onion germplasm under withheld water stress. Assessment of Nine onion cultivars revealed the negative correlation between chlorophyll, relative water content, and positive correlation between proteins, sugars, proline, phenols, flavonoids, tannins and drought. Three onion germplasm like arka kirthman, arka lalima and prema 178 exhibited better response among all the cultivars.

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