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Interactive effect of drought and nitrogen on growth, some key physiological attributes and oxidative defense system in carrot (*Daucus carota* L.) plants

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ABSTRACT

Exogenous application of nitrogen is an efficient means of enhancing plant stress tolerance through modulation of a number of physio-biochemical processes. Thus, the present investigation was carried out to assess the effectiveness of foliar applied nitrogen (100, 200 and 300 mg L^{-1}) on growth and some key physiological attributes in carrot plants under drought stress [50% field capacity (FC)]. Drought-induced increase was observed in leaf free proline and glycinebetaine (GB), as well as total phenolics. However, there was no significant effect on shoot and root dry weights, ascorbic acid, and MDA, but chlorophyll *a* and ratio of chlorophyll *a/b* significantly decreased under water deficit conditions. Foliar-applied urea as a source of nitrogen significantly increased the shoot and root dry weights, root length, chlorophyll *b*, total chlorophyll, leaf free proline, GB, total phenolics, activities of catalase (CAT) and peroxidase (POD) enzymes and contents of total soluble proteins, but it decreased chlorophyll *a/b* ratio. So overall, exogenously applied nitrogen particularly 300 mg L^{-1} was very effective in improving the drought tolerance of carrot plants by enhancing the levels of chlorophyll pigments and up-regulating the oxidative defense system.

1. Introduction

Plants, by virtue of their natural habitats, are prone to experience stressful environmental conditions. Undoubtedly, such stresses impose a myriad of adverse effects on growth and metabolism of most plants (Tuna et al., 2007; Chegah et al., 2013; Tatrai et al., 2016; Li et al., 2017). Of several abiotic stressful factors known, water scarcity is the most devastating one in terms of hampering growth, yield and quality of most crop plants (Yuyan et al., 2007; Mahmood et al., 2009; Ashraf, 2010; Hamayun et al., 2010; Riaz et al., 2010; Tatrai et al., 2016). Plants growing under water deficit regimes exhibit considerable perturbance in different physiological phenomena such as nutrient accumulation, water relations, photosynthesis, protein and carbohydrate metabolisms, oxidative defense system, etc. (Ashraf, 2009; Hasanuzaman et al., 2010, 2014). Dahal et al. (2016) reported that drought can also disturb the energy balance in plants and increase the production of reactive oxygen species (Ashraf, 2009; Hasanuzzaman et al., 2010, 2014).

Carrot (*Daucus carota* L.) is a favorite vegetable in almost all countries of the world because, it's some of the biochemicals are of great value for human nutrition and for preventing several human disorders and diseases such as cancer, obesity, diabetes, heart diseases, renal and liver problems (Simon, 2000; Gallichio et al., 2008; Zhang et al., 2009; Arscott and Tanumihardjo, 2010; Dias, 2014). Carrot is referred to as a nitrophilous vegetable because it has the ability to accumulate large quantity of nitrates (Boskovic-Rakocevic et al., 2012). Thus, nitrogen fertilization must be done with great care. However, one of its prominent biochemicals, β-carotene, for which carrot has gained a ground throughout the world, is influenced by increasing external N supply (Chenard et al., 2005; Boskovic-Rakocevic et al., 2012). For example, Musa et al. (2010) described that exogenously applied nitrogen significantly increased the β-carotene at maturity level.

One of the most important mineral nutrients for normal growth of plants is nitrogen, which is absorbed by plants in inorganic forms as nitrate (NO_3^-) or ammonium (NH_4^+) from the root growing medium (Glibert et al., 2016). Although nitrogen is an important part of DNA, RNA, proteins and chlorophyll, it performs a vital role in many metabolic processes (Marschner, 1995; Bungard et al., 1997; Glibert et al., 2016). To sustain normal and consistent growth of plants, a proper level of nitrogen is necessary to cope with environmental cues (Wang et al., 2008; Zhu et al., 2014; Boschma et al., 2015; Chang et al., 2016). In creeping bentgrass (*Agrostis stolonifera* L.), heat tolerance improved by

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foliar application of NH_4NO_3, due to limited lipid peroxidation and maintenance of scavenging ability of antioxidants (Fu and Huang, 2003; Wang et al., 2012; Chang et al., 2016).

In view of the afore-mentioned studies, it was hypothesized that addition of appropriate amount of N to the growth medium of carrot plants could mitigate the drought stress induced adverse effects on growth and metabolic processes in carrot plants. Thus, the principal objective of the present study was to examine the interactive effect of drought and nitrogen on growth and physio-biochemical attributes including the oxidative defense system in carrot, a potential vegetable crop grown widely in a large number of countries of the world.

2. Materials and methods

The study was conducted to evaluate the interactive effects of drought and nitrogen on carrot (*Daucus carota* L.) plants. The seeds of cultivar T-29 were obtained from the Vegetable Section of Ayub Agricultural Research Institute (AARI), Faisalabad (Punjab), Pakistan. A three-factor factorial completely randomized experiment with four replicates was conducted in the Botanical Garden of the Government College University, Faisalabad during November 2015 to January 2016. Plastic pots were used for this experiment filled with 7 kg sandy loam soil. Ten seeds were sown in each pot. Thinning of the plants was done after seed germination. After four weeks of germination, two levels of water [(100% field capacity (control) and 50% field capacity (drought)] were started. The moisture contents of the droughted pots were maintained and regularly monitored by keeping the weight of each pot equal to that calculated for 50% and 100% field capacities daily through addition of normal irrigation water if required. After three weeks of drought stress treatments, the plants were subjected to different levels (0, 100, 200 and 300 mg L^{-1}) of nitrogen. All these concentrations were prepared by dissolving urea into distilled water along with 0.1% Tween-20 as a surfactant. Each level of N was applied once at the rate of 15 mL per plant. After two weeks of foliar application, two plants from each pot were uprooted carefully. Then shoots and roots were washed with distilled water. After this, shoots and roots were detached and their lengths recorded. After recording fresh weights of shoots and roots, they were kept in an oven at 65 °C for 3 days. After three days, dry weights of the shoots and roots were recorded.

2.1. Leaf free proline contents

According to the method of Bates et al. (1973), fresh leaf material (0.5 g) was ground in 10 mL of 3% sulfosalicylic acid (w/v) and the extract was filtered; two mL of the filtrate was taken in a test tube and 2 mL of acid ninhydrin solution added to the filtrate along with 2 mL of glacial acetic acid. Then the test tubes were heated in a water bath at 100 °C for 1 h. Thereafter, the test tubes were ice-cooled and 4 mL of toluene was added. The optical density of the mixture was recorded at 520 nm using a UV–vis spectrophotometer.

2.2. Estimation of glycinebetaine (GB)

According to the method of Grieve and Grattan (1983), dry leaf sample (0.5 g) was ground in 10 mL of 0.5% toluene solution and the extract was kept overnight at 4 °C. Then, it was centrifuged at 12000 × *g* for 10 min. To 1 mL of the supernatant, 1 mL of 2*N* sulfuric acid were added. Thereafter, 0.5 mL of the mixture was taken in a test tube and added 0.2 mL of potassium tri-iodide solution (KI_3) to it. The mixture was shaken and cooled. After it, 2.8 mL of ice cooled distilled water along with 6 mL of 1,2 dichloroethane were added. Finally, two layers of the mixture were formed, the upper layer discarded and the absorbance of the lower layer noted at 365 nm using a spectrophotometer.

2.3. Determination of hydrogen peroxide (H_2O_2)

In a pre-chilled pestle and mortar, fresh leaf material (0.5 g) was homogenized in 5 mL of 0.1% (w/v) trichloro-acetic acid (TCA) following Velikova et al. (2000). Then, the extract was centrifuged for 15 min at 12000 x *g*. To an aliquot (0.5 mL) of the supernatant, 0.5 mL of potassium phosphate buffer (pH 7) was added followed by the addition of 1 mL of potassium iodide (KI). Then the mixture was vortexed and its absorbance observed at 390 nm using a spectrophotometer.

2.4. Determination of malondialdehyde (MDA)

According to the method of Carmak and Horst (1991), the thiobarbituric acid (TBA) assay following Carmak and Horst (1991) was used for determining lipid peroxidation in carrot leaf samples. Fresh leaf material (0.5 g) was homogenized in 5 mL of 5% (w/v) TCA, and it was centrifuged at 12000 × *g* for 15 min. To 4 mL of the supernatant, 4 mL of 0.5% (w/v) TBA in 20% TCA was added. The mixture was kept at 95 °C for 30 min and ice cooled, then mixture was vortexed for 5 min at 7500 × *g*, its absorbance was measured at 532 and 600 nm.

2.5. Determination of total phenolics

The method of Julkunen-Tiitto (1985) was employed to determine total phenolics in leaf tissues using the Folin-Ciocalteu's phenol reagent. Fresh leaf material (0.1 g) was ground in a mortar and pestle using 5 mL of 80% acetone. Then, the extract was centrifuged at 10,000 × *g* for 10 min. An aliquot of 0.1 mL of the supernatant was taken in each test tube and 2 mL of distilled water was added, followed by addition of 1 mL of Folin-Ciocalteu's phenol reagent. The mixture was shaken well and 5 mL of 20% sodium carbonate was added and made its volume up to 10 mL with distilled water. The absorbance was recorded at 750 nm using a spectrophotometer.

2.6. Chlorophyll contents

According to the method of Arnon (1949), fresh leaves (0.5 g) were cut and dipped in 80% acetone overnight at −4 °C. Then, the extract was centrifuged for 5 min at 10,000 × *g*. Absorbance of the supernatant was recorded at 645 and 663 nm using a spectrophotometer. The concentration of chlorophyll *a* and chlorophyll *b* were calculated using the following formulae:

$$\text{Chlorophyll } a = \{12.7(OD_{663} - 2.69(OD645) \times V/1000 \times W\}$$

$$\text{Chlorophyll } b = \{22.9(OD_{645} - 4.68(OD_{663}) \times V/1000 \times W\}$$

2.7. Antioxidants

Fresh leaves (0.5 g each sample) were ground in a pestle and mortar containing 5 mL phosphate buffer (50 mM) of pH 7.8. Then the extract was centrifuged at 15,000 × *g* at 4 °C for 20 min. The supernatant was used for determining the activities of the antioxidant enzymes, peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT). The enzyme activities were recorded on the basis of total soluble proteins.

2.7.1. Superoxide dismutase

The method described by Giannopolitis and Ries (1977) was followed for measuring the activity of superoxide dismutase (SOD). One mL reaction mixture containing nitroblue tetrazolium (50 µL), riboflavin (50 µL), L-methionine (100 µL), leaf extract (50 µL), triton-X (100 µL), buffer (250 µL) and H_2O (400 µL) were added in the test tube. All test tubes were kept under light for 15 min. Then absorbance of the mixture in each test tube was recorded at 560 nm using a spectrophotometer.