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Ameliorative effects of calcium nitrate and humic acid on the growth, yield component and biochemical attribute of pepper (*Capsicum annuum*) plants grown under salt stress

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ABSTRACT

The present work was carried out in order to determine the effects of calcium nitrate and humic acid applications either separately or in combination on the growth and fruit yield quality of pepper plants under salt stress condition. Two different concentrations of calcium nitrate (Ca1; 60 mg/kg soil and Ca2; 120 mg/kg soil) were applied to the soil before sowing whereas humic acid applications (HA1; 750 mg/kg soil and HA2; 1500 mg/kg soil) were performed during plant growth (at the third true leaves stage). Plants were irrigated with saline water in a concentration of 100 mM NaCl. Data showed that applications of both concentrations of humic acid and lower concentration of calcium nitrate (Ca1) individually caused significant increases in growth parameters, RWC, photosynthetic pigments, minerals content, non-enzymatic antioxidants contents of the plants under normal and salt stress conditions. Moreover, fruit antioxidant compounds and quality of fruits (capsaicin, lycopene, β-carotene, total phenol, total flavonoids and antioxidant activity) were improved by using theses treatments. The combined treatment of Ca1 and HA2 was the most effective one on the previous criteria under salt stress conditions. Therefore, the usage of humic acid and calcium could be suggested to improve the soil properties, growth and antioxidant capacity of pepper plants and to mitigate the damage caused by salt stress.

1. Introduction

Abiotic stress such as salinity caused reduction in plant growth and productivity (Mohamed and Gomaa, 2012). Approximately 22% of the world agricultural land is saline (F.A.O., 2010). Egypt is one of the countries that suffer from severe salinity problems. For example, 33% of the cultivated land in Egypt suffers from salinity due to low precipitation and irrigation with saline water (El-Hendawy et al., 2004). Salinity stress affects on the physical and chemical properties of soil. The increase of Na^+ and high soil pH cause distortion of soil structure and reduction in hydraulic conductivity (Lauchli and Epstein, 1990). Salt stress affect on photosynthesis, lipid metabolism and protein synthesis (Parida and Das, 2005). Furthermore, the uptake and translocation of mineral nutrients within plant cells and their availability in the soil are affected by salt stress (Maathuis, 2006).

The major components of soil organic matter is composed of 65–70% humic substances such as humic acid and fulvic acid which causes increment in the growth of plant due to increasing the permeability of cell membrane, the uptake of phosphorus and oxygen, photosynthesis, respiration and the growth of root cell (Russo and Berlyn, 1990). The humic substances in the soil have directly and indirectly effects on the growth of plant (Chen and Aviad, 1990). The direct effect of the humic acid includes the uptake of these substances through the plant tissue causing an elevation in some biochemical aspects such as the amino acids, nutrient and vitamins. The indirect effect include the amelioration of soil properties such as gathering, airing, permeability, holding capacity of water, transport of ion and pH (Tan, 2003). Calcium is an essential nutrients and a divalent cation that helps in the stability of the surface of cell membrane, the effectiveness of the pH in cells and prevent the leakage of the solute from the cytoplasm of plant cell (El-Beltagi and Mohamed, 2013). Calcium plays an important role in the elongation and the division of plant cells, the cell membrane permeability, the metabolism of nitrogen and the translocation of carbohydrate (White, 2000). In addition, calcium has a positive role to help the plants to overcome salts stress (Ehret et al., 1990). The higher calcium levels in soil protect cell membrane from negative effects of salinity (Busch, 1995).

Pepper (*Capsicum annum*, Solananceae) is used as fresh vegetables or dried for use as a spice (Bosland and Votava, 2000). It contains a high amount of bioactive nutrients and antioxidant compounds such as

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ascorbic acid, carotenoids and phenolic compounds (Marin et al., 2004). The environmental and genetic factors affect the amounts of antioxidants in fruit (Slimestad and Verheul, 2005). Capsaicin is the major alkaloid compound which produced only in *Capsicum* fruits and used in the food, medicine and pharmaceutical industries. It has been used as an analgesic against arthritis pain and inflammation (Deal et al., 1991), an anticancer effect (Moore and Moore, 2003) and neurogenic inflammation (Szolcsanyi, 2004).

The objective of this study was to study the effects of calcium nitrate and humic acid applications separately or in combinations on vegetative growth, fruit yield and its chemical constituents of pepper plants grown under salt stress condition.

2. Materials and methods

2.1. Plant material and experimental conditions

Seeds of hot pepper (*Capsicum annuum* L.) were obtained from the Agricultural Research Centre, Egypt. The surface of hot pepper seeds was sterilized in distilled water and left to dry at room temperature (25 °C) and then sown directly in pots 30 cm in diameter; the pots were filled with 4 kg of soil (55% clay, 20% silt, 25% sand) in a greenhouse at Faculty of Education, Ain Shams University on seedlings were grown under favorable environmental conditions (day the 2nd April 2016. Pepper length 12-14 h, temperature 30-32 °C and humidity 65%). When emergence was complete (7 days), the percentage of seed germination was assessed and the seedling density was reduced to seven seedlings per pot. After 30 days of planting (third true leaves), all pots were randomized on rows in the greenhouse.

2.2. Humic acid, calcium nitrate and salt (NaCl) treatments

Two different growing media as saline and non saline were used in this study. Irrigation with salt water (100 mM) was carried out twice weekly over an eight-week period. Three different doses $CaNO_3$(0-60-120 mg/kg) and HA (0-750-1500 mg/kg) were used as individually and their combination were added to the pots before planting. After 10 weeks (70 days) of sowing, some growth parameters (shoot length, root length, number of leaves, fresh and dry weights of roots and stems) and some biochemical analyses such as relative water content, photosynthetic pigments, minerals content, anthocyanin, ascorbic acid, total phenol and total flavonoids contents of the shoots were determined. At the end of the study (15 weeks; 105 days), the harvested fruits were collected to determine the fruit length, fruit diameter, fresh and dry weight of fruit, in addition to some biochemical components in the harvested fruits (capsaicin, lycopene, β-carotene, total phenol, total flavonoids and antioxidant activity).

2.3. Biochemical analyses

2.3.1. Determination of relative water content of leaves

The fresh weight of leaves (FW) of pepper plants was recorded and then was kept in Petri dishes for 24 h immersed in distilled water. The turgid weight (TW) was measured after saturation of leaves with water. The leaves were dried at 70 °C to constant weight and then weighted (The DW). RWC values were then calculated using the formula (Mata and Lamattina, 2001).

RWC (%) = [FW-DW)/(TW-DW] * 100

2.3.2. Determination of photosynthetic pigments

Leaves of pepper plants were extracted in 80% acetone and then filtered. The filtration was used to determine chlorophyll a, chlorophyll *b* and carotenoids at different wave lengths using spectrophotometer as described by Vernon and Seely (1966).

2.3.3. Determination of minerals

The amount of nitrogen, phosphorus, potassium and sodium in shoots of pepper plants were estimated by flame photometry as described by Cottenie et al. (1982).

2.3.4. Determination of anthocyanin

Fresh weight of shoots was homogenized in methanol containing 1% (v/v) HCl and then filtrate. The filtration was read at 530 and 657 nm using spectrophotometer as described by Mancinelli et al. (1976).

2.3.5. Determination of ascorbic acid

A known weight of fresh shoots of pepper plants was ground in 6% trichloroacetic acid (TCA) and then was centrifuged at 12.000 rpm for 15 min. The filtrate was measured at 530 nm using spectrophotometer according to Mukherjee and Choudhuri (1983).

2.3.6. Determination of total phenols

A known weight of fresh shoots and fruits of pepper plants was extracted with 80% cold methanol (v/v) and then filtered through Whatman No. 1 filter paper. After filtration, the filtrate was used to determine total phenols according to Dihazi et al. (2003) and measured at 725 nm using spectrophotometer. Gallic acid was used as standard.

2.3.7. Determination of total flavonoids

The total flavonoids in shoots and fruits of fresh pepper were extracted with 80% cold methanol and then filtrate. The filtration used to determine total flavonoids according to the method of Bushra et al. (2009).

2.3.8. Determination of capsaicin content

Harvested fruits were dried in an oven at 50 °C until obtain constant weight. 0.04 g of powdered fruit was mixed with 1 ml acetone and placed on a shaker at room temperature for four h at 250 rpm. The mixture was filtered and the filtered solution was collected in 2 ml tubes. Each HPLC assay was conducted with 10 µL of filtered solution (Tsou et al., 1997). The HPLC was carried out on a Merck Lichrosorb RP- 18 column with HITACHI autosample L-7200. For each sample, 10 µl were injected into a HITACHI L-6200 Intelligent Pump. The mobile phase was a mixture of methanol (MERCK) and water at a ratio of 35/65 (v/v). The flow rate was 1 ml/min, and the flow time for each sample was 30 min. Capsaicin (Sigma, M2028) dissolved in 100% ethanol was used as the standard sample. Standard solutions of 50 and 100 ppm were used to calculate the capsaicin concentration in each sample (Avrdc, 1989).

2.3.9. Determination of carotenoids (lycopene and β–carotene)

A known weight of pepper fruits was homogenate with 60 ml of acetone–hexane (4:6) solvent. Two phases were separated and the upper solution was measured at 663, 645, 505 and 453 nm using spectrophotometer. Lycopene and β-carotene contents were calculated according to the Nagata and Yamashita (1992) equations:

Lycopene (mg 100 ml^{-1} of extract) = -0.0458 * A663 + 0.204 * A645 + 0.372 * A505 - 0.0806 * A453.

β -Carotene (mg 100 ml^{-1} of extract) = 0.216 * A663 -1.22 * A645 - 0.304 * A505 + 0.452 * A453.

Lycopene and β -Carotene were finally expressed as mg kg^{-1}

2.3.10. Determination of antioxidant activity

A known weight of pepper fruits was homogenate in methanol and then was filtered. The filtration was used to determine free radical scavenging activity using the 2,2,-diphenyl-2-picryl-hydrazyl (DPPH) method at optical density 517 nm (Turkmen et al., 2005). Antioxidant activity (%) was calculated using the following equation: