Interaction of Applied Calcium, Potassium and Nitrogen with Adverse Changes in Brassica Napus L. Seedlings under Water Deficit Abiotic Environmental Stress

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Abstract—Water deficit is one of the most important abiotic environmental stresses responsible for substantial loss of crop yield, growth and agricultural extension. However, proper mineral nutrition can mitigate the adverse effects of water deficit and ensure the proper crop growth and yield and consequent food security. In this experiment, canola (Brassica napus) was provided with calcium, potassium and nitrogen through the growth medium by means of irrigation water prior to imposition of water deficit stress. It was observed that water deficit brought about negative changes in physiological and biochemical properties of canola. It was investigated that water deficit led to degradation in photosynthetic pigments, plasma membranes and water status of canola. However, fertilization of canola with calcium and potassium in increasing concentrations could protect chlorophyll and membranes and also improved the water relations of canola under water deficit condition. Application of nitrogen, however, could not prevent the detrimental effects of water deficit stress.

Keywords—Applied calcium, Nitrogen, Potassium, Water deficit

I. INTRODUCTION

W ATER stress is one of the most important limitations to the agricultural productivity worldwide, particularly in the arid and semiarid regions of the world [1]. Food and Agricultural Organization (FAO), has reported that about 45% of world’s agricultural land is exposed to water deficit stress [2]. Increased production of abscisic acid (ABA), an important plant growth regulator, is one of the responses of plants to water deficit stress, which causes the stomata closure to reduce the loss of water from the aerial parts of plants through transpiration [3]. Increased production of ABA decreases carbon fixation and in turn CO2 availability for photosynthesis leading to overproduction of AOS because of the imbalance between the production and utilization of electrons. AOS are highly toxic species and react with essential macromolecules leading to the subsequent damage of metabolic processes within cells[3]. Plants have acquired an internal protective antioxidant system which detoxifies the effects of AOS. Despite the internal antioxidant system, the negative effects of water deficit can also be reduced by external supplementation of minerals through the growth medium [4].

Calcium (Ca2+) is an important cation, usually involved in the structure of cell walls and membranes. It is one of the important Potassium (K+) is the most prominent plant solute and it has a role in improving water-use efficiency, osmotic potential and turgor pressure in plant tissues [6].

Brassica species are grown for edible oil throughout the world. Canola (Brassica napus L.) is one of the most important oilseed rapes grown especially due to its potential to produce good quality edible oil because of its low erucic acid content [7].

In this experiment, the effects of exogenous calcium, potassium and nitrogen, supplied through irrigation water via growth medium, were studied on chlorophyll content, RWC and membrane stability, as selected criteria for drought tolerance in Brassica napus L. seedlings.

II. METHODS AND MATERIALS

A. Plant Material and Experimental Plan

Brassica napus CV Bulbul-98 was grown in plastic pots. Before drought imposition, pots were arranged in groups in such a way that each group contained 6 pots. The first group, containing 6 pots, was taken as control and the remaining pots were supplemented with Ca2+ [as 30, 60 and 90 mM Ca(NO3)2.4H2O], K+ [as 50, 100 and 150 mM KNO3] and N [as 30, 50 and 100 mM NH4NO3] through irrigation water. After supplementation, the plants were irrigated for one week, followed by imposition of drought stress or water stress (WS) by stopping water supply for 10 days from half the pots in each group. Each treatment was replicated at least three times and five pots were maintained in each treatment.

B. Determination of Relative water content (RWC)

Leaf samples of uniform size were obtained and were immediately weighed to get fresh weight (Wf). Then, the
samples were completely immersed in double distilled water and were stored for 24 hours in dark at 4°C. After 24 hours, the samples were blotted dry on filter paper and weighed again to obtain the turgid weight (Wt). Then, samples were dried in oven at 70°C for 48 hours to find dry weights (Wd). Relative water content was measured as follows:

\[ RWC = \left( \frac{Wf - Wd}{Wt - Wd} \right) \times 100 \]  

**C. Determination of Electrolyte Leakage (EL)**

If the electrolyte leakage from the leaf disks was measured with a conductivity meter ( Consort C-931, USA). The initial conductivity (Ct) was found after incubating the samples at 25°C in 5 ml de-ionized water for about three hours with psi for 20 minutes. The samples were cooled down to 25°C and the final conductivity (Cf) was measured. The EL was calculated as:

\[ EL = \left( \frac{Ct}{Cf} \right) \times 100 \]

**D. Determination of Chlorophyll Content**

Total chlorophyll content was determined by Arnon method [8]. About 100 mg of leaf sample from each seedling was immediately frozen in liquid nitrogen and homogenized in 80% acetone followed by centrifugation at 15,000 rpm for 10 minutes at 4°C. The supernatant was collected and its absorbance was noted through spectrophotometer.

### III. RESULTS

**A. Relative Water Content (RWC)**

RWC shows the water status of plant tissues. Results of this experiment show that RWC of the seedlings was declined under drought stress to 54.61% ± 3.82 as compared to 88.27% ± 1.41 of the control and irrigated plants (Fig.1). At 30 mM Ca(NO3)2.4H2O, the RWC of the WS seedlings was 61.45% ± 6.52. Hence, increasing supply of external Ca(NO3)2.4H2O under drought stress condition increased RWC significantly and the largest RWC value observed was 72.14% ± 0.03 at the maximum addition of Ca(NO3)2.4H2O (90 mM Ca2+). Addition of KNO3 under water-deficit also enhanced RWC progressively and the RWC values observed were 59.48% ± 4.86, 73.67% ± 0.53 and 83.03% ± 2.00 at 50 mM, 100 mM and 150 mM KNO3... Nitrogen addition, however, decreased RWC under stress conditions and after addition of 30, 50 and 100 mM NH4NO3, the RWC values were 66.23% ± 2.29, 63.3% ± 1.60 and 59.71% ± 1.58 respectively. Under irrigated condition, the supplements did not show significant effect on RWC.

**B. Electrolyte Leakage (EL)**

EL shows damage to cell membranes as a result of abiotic stresses. The control and well-watered (WW) plants showed EL of about 5.18% ± 0.83 which was reduced to 41.85% ± 2.37 in WS plants (Fig.2). The plants grown in medium supplied with Ca(NO3)2.4H2O under WS condition, showed EL to a less extent than those grown in control and non-supplemented medium, though no significant differences were observed in EL of seedlings supplied with external Ca2+ under WW condition. Addition of 30 mM Ca(NO3)2.4H2O caused EL to come down to 38.20% ± 1.64, which was further decreased to 28.52% ± 0.73 and 19.59% ± 0.95 for addition of 60 mM Ca(NO3)2.4H2O and 90 mM Ca(NO3)2.4H2O respectively. Addition of KNO3 was also found to bring significant reduction in EL in WS plants, though, in WW plants, non-significant differences were observed in EL with increasing concentration of KNO3 added externally. It was found that at 50 mM KNO3 addition, EL was 45.22% ± 2.22 which was reduced to 30.30% ± 2.16 and 26.37% ± 0.99 at 100 mM and 150 mM KNO3 supplementation. Addition of NH4NO3, however, showed negative effect on EL of seedlings under WS condition and EL was found to increase with increasing concentration of exogenous NH4NO3. The EL value was 47.18% ± 0.77 at 30 mM NH4NO3, which was increased to 65.01% ± 2.22 at 100 mM NH4NO3 application.

**A. Chlorophyll Content**

The total chlorophyll content of leaf tissues of WW plants was 996 µg.g-1 FW ±13.63 which was reduced to 570.64 µg.g-1 FW ±13.71 in WS plants (Fig.3). At 30 mM Ca(NO3)2.4H2O, Chl content was about 1031 µg.g-1 FW ±33.03 in the WW plants and about 769.66 µg.g-1 FW ±29.40 in leaf tissues of WS plants. Under WS condition, Chl content was increased with increasing concentration of external Ca2+ and at 90 mM Ca(NO3)2.4H2O, the Chl content calculated was about 984.00 µg.g-1 FW ±4.32 which is about 42% larger than that of the non-supplements WS plants. At 50 mM KNO3 addition, the plants possessed about 836.33 µg.g-1 FW ±15.19 of Chl under WS condition which was increased to 845.00 µg.g-1 FW ±9.08 and 871.33 µg.g-1 FW ±14.61 in WS plants after external addition of 100 mM and 150 mM KNO3 respectively. Addition of NH4NO3 could not protect the chlorophyll content of the leaf tissues under WS condition. At 50 mM KNO3 addition, the Chl content of WW tissues was 966.0 µg.g-1 FW ±46.44 while it was 725 µg.g-1 FW ±6.68 in WS tissues. Addition of 50 mM and 100
mM NH4NO3 decreased the total Chl content of WS plants to about 669.33 µg.g-1 FW ±14.05 and 446.33 µg.g-1 FW ±11.84 respectively showing a negative correlation of added nitrogen with chlorophyll content under WS condition.

Our experiment showed that water stress reduced RWC of leaves significantly and application of calcium and potassium caused an increase in RWC. Leaf RWC shows the water status of plant tissues and hence it is used as one of parameters to estimate the degree and intensity of water stress [9]. Reduction in RWC under water stress, leads to reduction in leaf area and biomass which in turn leads to reduction in leaf mineral contents [10]. Increased RWC in response to applied Ca2+ and K+ in our experiment, is in agreement with the results of Ma et al.[11] and Umar [12]. Plants, under water stress condition, decrease their internal water potential to avoid desiccation and sustain water potential equilibrium and cell homeostasis. By doing this, osmolytes are accumulated in plant cells that have a role in maintaining the tissues water balance through osmotic adjustment processes [13].

It was found that water stress led to an increase in EL and decrease in Chl content of Brassica napus tissues and application of calcium and potassium decreased EL and increased Chl content under water stress condition. Ammonium nitrate application negatively affected RWC, EL and Chl contents of Brassica napus seedlings according to the results of our experiment. Drought damaged membrane and chlorophyll and supplemental calcium and potassium protected both membranes and Chl of Brassica napus. Basset (1998) found that calcium supplementation protected chlorophyll and cell membranes which were damaged by water stress in Vicia faba [14].

The water relations of Brassica napus were decreased by water stresses which were increased by supplemental calcium and potassium. Thus, it may be thought that calcium elevated tissue water content in Brassica napus which led to protection of membranes from severe dehydration which improved the growth of Brassica napus seedlings exposed to reduced soil moisture content. Calcium may be conceived to act as membrane stabilizer by promoting an array of physiological processes in WS seedlings. Recent research shows that calcium enhances the ability of plants to acquire adaptability to environmental stresses [15]. It not only plays an important role in preventing cell membrane damage and leakage but also contributes to the regularization of cell membrane structures under environmental stresses [16].

Recent investigation shows that potassium plays an important role in mitigating the harmful effects of water stress in crop plants. Fertilization of plants with exogenous potassium can promote root growth which in turn develops the uptake of soil moisture by plants through extended root system [17]. It increases the retention of water in plant tissues by decreasing the rate of transpiration [18]. It also regulates the osmotic potential and cell turgor and improves the functioning of stomata when plants are water stressed [19].

Umar (2006) pointed out that potassium application to sorghum, mustard and groundnut could ameliorate the harmful effects of water stress by enhancing RWC, above ground biomass and grain yield [20]. Potassium ameliorates the adverse effects of water stress mainly due to its property to maintain high pH in stroma and prevent the photo-oxidative
damage of chloroplast [21].

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REFERENCES