PEG imposed water deficit and physiological alterations in hydroponic cabbage

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Abstract

Drought is one of the major environmental problems in agricultural field worldwide. The present study investigated the effects of water deficit caused by exogenous application of polyethylene glycol (PEG) on cabbage (*Brassica oleracea* var. capitata) grown in hydroponic culture. Root length, shoot length, dry weight, and relative water content of the seedlings significantly decreased in dose dependent manner. Significant reduction in chlorophyll, sugar and protein content, and nitrate reductase activity were recorded. The increase in proline content was recorded as the concentration of PEG increased. Increasing concentration of PEG affected the antioxidant enzymes viz. superoxide dismutase, catalase, and peroxidase activity of the seedlings through the production of reactive oxygen species (ROS) in stress condition. PEG at higher concentrations significantly enhanced the activities of antioxidant enzymes. The cabbage seedlings induced antioxidative defense system to mitigate the adverse effect resulting from altered environmental condition.

Keywords: Antioxidants; cabbage; hydroponic culture; PEG-6000, proline; water deficit

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Introduction

Abiotic stresses viz., drought, salinity, and freezing cause water scarcity in plants (Ingram and Bartels, 1996). Drought is one of the most drastic abiotic stress problems faced in agricultural field worldwide. Abiotic stresses unfavorably affect growth and development of plant, resulting in crop failure and decreased crop yield (Wu et al., 2011; Mittler, 2006; Buchanan et al., 2000). Water is essential component for various physiological processes such as enzymatic reactions, solubilization, and metabolites transportation, hydrolytic breakdown of proteins, lipids, and carbohydrates in germinating seeds (Bewley and Black, 1994; Biaecka and Ke¸pczyn´ ski, 2010).

Polyethylene glycol (PEG)-6000 as a reliable marker in laboratory condition is used to test the effects of drought stress in plants. PEG acts as a non-penetrating osmotic agent resulting into increased solute potential and blockage of absorption of water by root system (Chutia and Borah, 2012; Ranjbarfordoei et al., 2000; Chezen et al., 1995). Jang et al. (1995) reported that stimulation of water stress by PEG induced drought stress on plants. PEG generated osmotic stress generally decreased the chlorophyll content and photosynthetic rate in plants (Ranjbarfordoei et al., 2000; Zhang and Kirkham, 1995).

Water deficit impairs the stomatal conductance and decreases photosynthesis causing oxidative stress mediated by

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overproduction of reactive oxygen species (Singh et al., 2010; Ünyayar et al., 2005). Water deficit reduces photosynthetic rate and impairs metabolic activities (Lawlor and Cornic, 2002), thus resulting in decreased plant growth. Plants subjected to water deficit experience reduced biomass (Specht et al., 2001), chlorophyll content (Massacci et al., 2008), relative water content (Lawlor and Cornic, 2002) and decreased grain yield and size (Edward and Wright, 2008).

Major organic osmolytes like protein and proline accumulate in plants species under various stresses. Accumulation of osmolytes is important for cellular homeostasis (Singh et al., 2010; Stewart et al., 1977).These organic compounds played adaptive role in stressed plants by mediating osmotic adjustment and protected subcellular structures (Chutia and Borah, 2012; Singh, 2003; Ashraf and Foolad, 2007). Nitrate reductase (NR) is the first enzyme of nitrate assimilation. NR is an important cytosolic enzyme and its activity is related to crop productivity (Lee and Stewart, 1978). NR activity in the leaves of higher plants is very sensitive to changes in the water status of the plant. NR activity decreased under water stress (Hsiao, 1973).

Plants develop several defense mechanisms to endure the stress. Production and expression of reactive oxygen species scavengers are important tools to increase the tolerance against oxidative stress (Singh et al., 2010; Sairam et al., 1995). The reactive oxygen species are detoxified through the activities of several antioxidant enzymes viz., superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) (Rubio et al., 2002; Ünyayar et al., 2005).

In present scenario, changes in the pattern of precipitation have been recorded due to the global warming. Sometimes prolonged precipitation is followed by dry period. In this condition plants experience water stress (Singh et al., 2010). The aim of the present study was to investigate the effects of water stress in hydroponic culture by the exogenous application of PEG. Seedling growth, pigment, protein, sugar, proline, and nitrate reductase activity were examined. Antioxidant enzyme activities were evaluated as plant defense against water stress.

Materials and Methods

Plant material and treatments

The certified seeds of *Brassica oleracea* var. *capitata* were purchased from certified seed agency Allahabad, India. The seeds were sown in nursery beds (1mx1m) in experimental plots in the Department of Botany, University of Allahabad, Allahabad (24^o 47' and 50^o 47'N latitude; 81^o 91' and 82^o 21'E longitude; 78 m above sea level). The seed beds were irrigated as and when required. Two-week-old seedlings from nursery were uprooted and washed with tap water followed by distilled water to clean root. The seedlings were transferred at the rate of 10 seedlings per box in transparent plastic boxes (height 9 cm, width 17 cm, length 23 cm) each containing 2L of Hoagland solution. Hoagland solution was prepared following the method of Hoagland and Arnon (1950). Polyethylene glycol (PEG-6000) at 10, 20, and 30% concentrations were prepared in distilled water and used for treatment. After one week of establishment of the seedlings in Hoagland solution, the nutrient medium was replaced with Hoagland solution (2 l/box) containing PEG according to the treatment. Untreated seedlings in nutrient solution were taken as control. The boxes were covered with black papers to avoid the algal growth. The experimental boxes were fitted with aerating tubes. Boxes were continuously aerated. The experiment was done in glass house. Biophysical and biochemical analyses were done after 72h of treatment.

Root length, shoot length, and dry weight

Root length (RL) and shoot length (SL) of the seedlings were measured with a metric scale and expressed in centimeters. Dry weight of the seedlings was recorded on an electronic balance. The samples were oven dried at 70^oC for 72h and then weighed independently for DW determination. DW was expressed in g per plant.

Relative water content

To measure the relative water content (RWC), leaf samples were cut into discs, weighed for fresh weight (FW) and were immediately floated on distilled water at 25°C in the dark. After 24 h the turgid weight (TW) of discs was measured

and they were dried in oven at 80°C for 48 h for dry weight. The RWC was calculated as per Bars and Weatherly (1962).

RWC (%) = (FW-DW)/ (TW-DW) x 100

Estimation of pigments and protein content

The photosynthetic pigments viz. chlorophyll a, chlorophyll b, and carotenoids from first fully expanded leaves were examined in 80% acetone and were quantified following Lichtenthaler (1987). Protein content was determined as per the method of Lowry et al. (1951). The amount of protein was calculated with reference to standard curve obtained from bovine serum albumin.

Sugar content

Sugar content was estimated following Hedge and Hofreiter (1962). About 0.1g of the sample was homogenized in 5.0 ml of 95% ethanol. After centrifugation, the sugar content was determined in the supernatant. The supernatant (1ml) was mixed with 4 ml of anthrone reagent and heated on boiling water bath for 8 min. Absorbance was taken at 620 nm after rapid cooling. Sugar was quantified with the standard curve prepared from glucose.

Extraction and assay of nitrate reductase enzymes

Nitrate reductase (EC 1.6.6.1) activity was assayed by modified procedure of Jaworski (1971) based on incubation of fresh tissue (0.25 g) in 4.5 ml medium containing 100 mM phosphate buffer (pH 7.5), 3% KNO_3 and 5% propanol. About 0.4 ml aliquot was treated with 0.3 ml 3% sulphanilamide in 3 N HCL and 0.3 ml 0.02% N-1-naphthyl ethylene diamine dihydrochloride (NEDD). The absorbance was measured at 540 nm. NR activity was calculated with a standard curve prepared from $NaNO_2$ and expressed as µ mol NO_2 g^{-1} FW h^{-1}.

Estimation of free proline

Extraction and determination of proline were performed according to Bates et al. (1973). Leaf samples were extracted with 3% sulphosalicylic acid. An aliquot was treated with acid-ninhydrin and acetic acid, boiled for 1 h at 100°C. The reaction mixture was extracted with 4 ml of toluene. The absorbance of chromophore containing toluene was determined at 520 nm. Proline content was expressed as µmol g-1 FW using a standard curve.

Extraction and assay of antioxidant enzymes

Enzyme extract was prepared by homogenizing 500 mg of plant leaves from each treatment in 10 ml of sodium phosphate buffer (0.1M, pH 7.0, 1% PVP). The homogenate was filtered through cheese cloth and centrifuged at 15,000 *g* for 30 min in cooling centrifuge (Remi instruments C 24). The supernatant was collected, stored at 4°C and used as enzyme extract for determining the activities of superoxide dismutase, catalase, and peroxidase.

Superoxide dismutase (EC 1.15.11) activities were determined by the nitroblue tetrazolium (NBT) photochemical assay method following Beyer and Fridovich (1987).The reaction mixture (4 ml) contained 63 µM NBT, 13 mM methionine, 0.1 mM ethylene diamintetra acetic acid (EDTA), 13 µM riboflavin, 0.5 M sodium carbonate, and 0.5 mL clear supernatant. Test tubes were placed under fluorescent lamps for 30 min and absorbance was recorded at 560 nm. One unit of enzyme was defined as the amount of enzyme which caused 50% inhibition of NBT reduction.

Catalase (EC 1.11.1.6) activities were assayed as per the method of Cakmak and Marschner (1992). The reaction mixture (2 ml) contained 25 mM sodium phosphate buffer (pH 7.0), 10 mM H_2O_2, and 0.2 ml enzyme extract. The activity was determined by measuring the rate of disappearance of H_2O_2 for 1 min at 240 nm and calculated using extinction coefficient of 39.4 mM^{-1} cm^{-1} and expressed as enzyme unit g^{-1}fresh weight. One unit of CAT was defined as the amount of enzyme required to oxidize 1 µM H_2O_2 $min^{-1.}$

Peroxidase (EC 1.11.1.7) activities were assayed following McCune and Galston (1959). Reaction mixture contained 2 ml enzyme extract, 2 ml sodium phosphate buffer, 1 mL 0.1 N pyrogallol and 0.2 ml 0.02% H_2O_2 and determined

Table1
Effects of PEG imposed water deficit on root length, shoot length, dry weight, and relative water content (RWC) of cabbage seedlings

Treatments	Root length (cm)	Shoot length (cm)	DW (g/plant)	RWC (%)
C	14.9±0.346 a	23.1±0.346a	1.332±0.069a	49.48±1.601a
P_1	9.35±0.086 b	18.2±0.173b	1.004±0.028b	41.46±1.172b
P_2	7.85±0.259 c	12.9±1.385c	0.879±0.018c	34.12±0.167c
P_3	6.7±0.115 d	8.65±0.490d	0.696±0.003d	25.08±1.747d

Mean±SE values followed by same letters are not significantly different at 0.05 (ANOVA and Duncan's multiple range test). n=3. C, control; P_1, P_2, P_3 were 10, 20, and 30% concentrations of PEG, respectively.

spectrophotometrically at 430 nm. One unit of enzyme activity was defined as the amount which produced an increase of 0.1 OD per minute.

Statistical analysis

Statistical significance was assessed at the $p<0.05$ level using one way ANOVA and means were separated by Duncan's multiple range test ($p<0.05$) with the help of SPSS 10 software. Means and standard deviation were calculated from 3 replicates.

Results

Growth and metabolism of plants were significantly affected by water deficit. The increase in water deficit in cabbage by PEG noticeably decreased the growth and development of the seedlings as compared to their relative control. Water stress caused significant ($P<0.05$) alterations in height of seedlings (Table 1). Water deficit caused a gradual decrease in RL, SL, DW and RWC with higher dosage of PEG. Maximum 55.03, 62.55, 47.74 and 49.31% inhibition in RL, SL, DW and RWC was recorded in 30% concentration of PEG, respectively.

Water deficit significantly decreased pigment content in the treated seedlings. However, maximum 73, 13.32, 44.83, and 41.72% inhibition in chl *a*, chl *b*, total chl (*a+b*) and carotenoids was noted in higher concentration of PEG. Control seedlings exhibited maximum pigment content (Table 2). Protein significantly ($P<0.05$) decreased in dose dependent manner under water stress. Maximum 58.84% reduction in protein was observed in P_3. Control seedlings exhibited a maximum amount of protein in the leaves (Table 3).

Sugar content was found to increase significantly as compared with control. Increase in sugar content was dose dependent with maximum 1.38 folds increase in P_3 (Table 3). Nitrate reductase activity (NR) in the leaves was slightly affected by water deficit. The maximum NR activity was recorded in control seedlings. A drastic decrease in NR activity was recorded in stressed seedlings with maximum 2.04 fold inhibition in P_3 treatment (Table 3).

Water stress significantly ($P<0.05$) enhanced the accumulation of proline in leaves of

Table 2
Effects of PEG imposed water deficit on the photosynthetic pigment contents of cabbage seedlings

Treatments	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total chlorophyll (mg/g FW)	Carotenoids (mg/g FW)
C	1.205±0.004a	0.594±0.156a	1.800±0.161a	1.026±0.288a
P_1	1.119±0.036b	0.553±0.020a	1.673±0.015ab	0.829±0.172a
P_2	0.925±0.005c	0.501±0.027a	1.426±0.027b	0.683±0.047a
P_3	0.561±0.016d	0.432±0.026a	0.993±0.042d	0.598±0.035a

Mean±SE values followed by same letters are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) n=3. C, control; P_1, P_2, P_3 were 10, 20 and 30% concentrations of PEG, respectively.

Table 3
Effects of PEG imposed water deficit on protein, sugar, proline content, and nitrate reductase activity of cabbage seedlings

Treatments	Protein (mg/g FW)	Sugar (mg/g FW)	Proline (µmol g^{-1} FW)	NR (µmol NO_2 g^{-1} FW h^{-1})
C	36.42±0.556a	42.89±0.243 a	0.103±0.001a	19.713±1.488 a
P_1	29.73±0.859b	45.06±0.323 b	0.124±0.003b	16.088±0.171 b
P_2	21.66±0.146c	52.89±0.293 c	0.149±0.002c	12.564±0.265 c
P_3	15.14±0.295d	59.36±1.219 d	0.269±0.008d	9.635±0.126 d

Mean±SE values followed by same letters are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) n=3. C, control; P_1, P_2, P_3 were 10, 20, and 30% concentrations of PEG, respectively.

the stressed seedlings. Maximum of 2.61 fold stimulation in proline content was recorded in P_3. Increase in proline was concentration dependent (Table 3).

SOD, CAT and POX, are important constituents of the antioxidative defense system. Enhanced activities of these enzymes were recorded under all treatments with PEG as compared with their respective control. Enhancement of 2.21, 1.67, and 2.18 times in SOD, CAT and POX activity was recorded in higher concentration of PEG (P_3) (Fig. I).

Discussion

Water status of the seedlings was differently influenced by PEG. The highest growth was recorded in control seedlings. Water stress resulted in decreased RWC (Jiang and Zhang, 2002). Loss of turgor pressure under water deficit condition adversely affected the rate of cell expansion and ultimately cell size and hence, reduced the shoot growth (Singh et al. 2009; Hale and Orcutt, 1987). Reduction of plant growth is one of the most prominent features of water stress. It is a part of drought avoidance mechanism (Ünyayar et al., 2005). Our results showed a significant reduction in plant growth in stressed seedlings which are in agreement with other reports (Singh et al. 2009; Patade et al. 2011; Idrees et al. 2010).

Water deficit decreased the contents of photosynthetic pigment to a larger extent. Water stress caused reduction of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids (Younis et al., 2000; Rahman et al., 2004). Decreased photosynthetic pigments reflect the level of water deficit (Smirnoff 1993). In our experiment also the decrease in pigment content

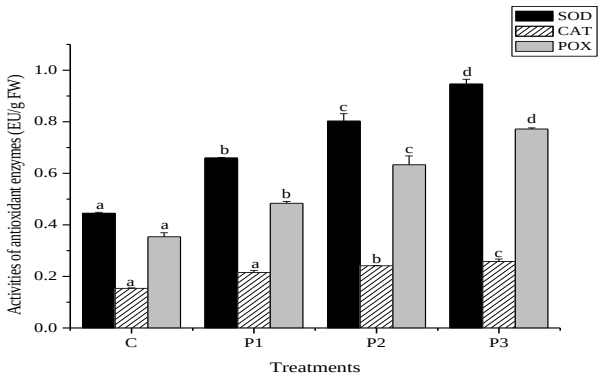

Fig. I. Effects of PEG imposed water deficit on antioxidant enzymes activity of cabbage seedlings. Mean±SE values followed by sa
letters are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) n=3. C, control; P1, P2, P3 were 10, 20
30% concentrations of PEG, respectively.

was expressed to a higher extent in the seedlings treated with higher concentrations of PEG.

ROS generated under water stress condition (Zgallai et al., 2006; Lara Nunez et al., 2006) which caused oxidative degradation of proteins (Pacifice and Davies, 1990). Inhibition of photosynthesis and other impaired metabolic activities resulted in decreased protein synthesis, which was evident from the correlation recorded in the decrease in photosynthetic pigments and protein contents (Singh et al. 2009). Another reason for the decrease in protein is its breakdown into amino acids which serve as osmolytes and defense enzymes (Stewart et al. 1977; Singh et al. 2009).

Our results of sugar content was in agreement with Patade et al. (2011) who reported that stressed plants accumulated significantly more total soluble and reducing sugars in stress condition. The elevated sugar level observed in stressed seedlings can be explained by less utilization of sugar for growth of seedlings subjected to stress (Asgharipour et al., 2011). Sugars act as osmoprotectant causing stabilization of cell membrane and maintenance of turgor pressure (Gupta and Kaur, 2005)

Kaiser et al. (1993) and Chen and Sung (1983) also recorded reduced NR activity under water deficit condition. Singh et al. (2009) reported decrease in NR activity in response to water deficit. Proline accumulation is positively related to drought tolerance (Reddy et al., 2004). Proline acts as an osmoprotectant as well as a compatible solute (Ueda et al., 2008). In our experiment, proline accumulation was recorded in dose dependent manner in the water stressed cabbage seedlings

Under oxidative stress condition several enzymes of the defense system increased tremendously to avoid the damage caused by ROS (Foyer and Noctor, 2003). Plants adapted to water stress maintained a high level of antioxidants (Singh et al. 2009). Antioxidant enzyme activities detoxify the reactive oxygen species which accumulated in the tissues of target species and caused deleterious effects (Mehdy, 1994; Singh et al. 2009).

Conclusions

In conclusion, the present study showed a deleterious effect of water stress on growth and metabolism cabbage seedlings giving an insight about the nature of drought induced.

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