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Research paper

Changes in Morphology and Catalase Activity in Response to Various Abiotic Factors from *Brassica Campestris*

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Abstract

Plants are always under the exposure of light and various abiotic factors. High and low level exposure of plants to these factors leads to generation of one or other types stress conditions in plants. These types of stress conditions are often visible in terms morphological changes like alternation in root and shoot length, leaf size and leaf colour and enzyme activities. As plants always remained fixed to their place, they could not move to avoid these stresses. Therefore, plants undergo various types metabolic shifts and changes in activities of various enzymes associated with their metabolism. Light and various types of solutes namely gibberellic acid, sucrose, polyethylene glycol, gibberellic acid magnesium chloride and glucose are the common abiotic factors inhabited by all the plants in any habitat. Catalase is one of the most important stress combating enzyme that are present in almost all the plants. The present study detailed the morphological changes in response to these abiotic factor and possible role of catalase during these processes. The present study was carried in *Brassica campestris* widely cultivated as a leaf vegetable and for edible oil used in cooking.

Keywords: Photomophogensis, catalase, abiotic stress

Introduction

Light is vital environmental factor acting on plants as the sole source of energy, external information, affecting their growth and development. Light has a variety of effects on plant like root and shoot length, leaf size and leaf colour and enzyme activity. Light is pivotal to a number of crucial processes that a plant undergoes on a regular basis in order to sustain life (Chory *et al* 1996). One of the main processes is photo-morphogenesis. There are also other changes light regulated such as change in morphology and/or cell structure and function, which occur as transient acclimatization to a changing environment. However, there is no uniform pattern of these changes these might be species specific. Some plants need a lot of light to sprout from their seeds, while others initially need little or can even find direct light detrimental to growth. Additionally, light during photomorphogenesis can even affect a plant's circadian rhythms. Lack of sufficient light in this process can often also result in plants that are lacking in normal color due to a lack of chlorophyll being produced (Kendrick *et al* 1995). Spectral changes of illumination evoke different morphogenetic and photosynthetic responses, which can vary among different plant species. Phytochrome-mediated

photomorphogenic responses are characterized by the complex variety of relationships between light input and physiological outputs, including germination, de-etiolation, shade avoidance, circadian rhythm, and flowering. Recent studies have resulted in several important advances, and have revealed the major consequences of phytochrome activity in terms of controlling protein subcellular localization, transcription, protein stability, and protein phosphorylation (Yun Jeong-Han *et al* 2007).

Similar type(s) of morphological and biochemical changes could result from exposures of plants to various environmental stresses. Moreover there is no uniform and distinct pattern of morphological and biochemical changes, these are highly species specific and concentration specific. For example, sprouting from seeds in some species require longer exposure and high intensity of light, while in others initially need little or even detrimental to their growth. Additionally, light during photomorphogenesis can even affect a plant's circadian rhythms. Lack of sufficient light in this process often result in plants lacking in normal color due to a lack of chlorophyll (Kendrick *et al* 1995). Therefore, to find relationship between light and environmental stimuli and to understand the cumulative effect of these factors, the present study was undertaken in *Brassica campestris* commonly known as field mustard, widely cultivated as a leaf vegetable and edible oil used in cooking that is extracted from its seeds. The present study investigates morphological changes *vis-à-vis* catalase activity of *B. campestris* under various external factors, like different wavelength of light i.e. under 700nm wavelength of light, 253.7nm of light (U.V) treatment and under complete darkness. The changes in catalase activities were also studied in response to gibberellic acid, sucrose, polyethylene glycol, and in combination of gibberellic acid magnesium chloride and glucose. The other objective was to find the role played by catalase during these stresses

Material And Method

Plant materials and reagent

The seeds of *B. campestris* were procured from grain market Jalandhar Punjab (India), were surface sterilized with 0.1% mercuric chloride solution for 20 minutes followed by rinsing with distilled water. Seeds (100 in number) were presoaked for 2h in 0.01 solution of GA_3 and then spread on moist filter paper placed on plastic trays (1.4cm ×2.9cm) and kept on germination chamber. Chemicals and reagents, were purchased from Sisco Research Laboratories (Mumbai, India). Sucrose and magnesium were purchased from Himedia Laboratories Pvt. Ltd (Mumbai, India).

Imposition of external cues

For light treatment, plants were exposed to different wavelength namely 253.7 nm (U.V), 700nm (normal) and under complete darkness (no light) at 25°C. For dark treatment trays were covered with black paper. Sampling was done after the interval of every 2 days for total duration of 6 days. For assessment of activity in response to different cues sprouted seeds were grown in the presence of Polyethylene Glycol (PEG; 2%, 5% w/v), Gibberrilic Acid (GA_3; 50Mm, 100µM), Sucrose (Suc; 2%, 5% w/v) and Magnesium Chloride ($Mgcl_2$; 50 µM). To avoid the depletion of carbon pool 5% glucose was added whenever the experiment was performed in dark.

For enzyme extraction:-

Crude protein was extracted by homogenization of fresh leaf tissues (0.3 g) in 50mM sodium phosphate buffer using a prechilled mortar and pestle and incubated overnight at 4°C. The homogenates were centrifuged at 12,000 rpm for 20 min at 4°C (Remi Instruments, India). The supernatant was collected and kept under -20°C for protein estimation and enzyme assay. The protein estimation was done by the method of Lowry et al., (1951). Protein in the unknown sample was estimated at 750 nm

using bovine serum albumin as standard and expressed per gm fresh weight basis. Total 9.1±2.2 mg of protein was found per gm fresh leaf of *B. campestris* .

Enzyme assay

Catalase activity was measured by recording the decrease in absorbance at 240 nm (Aebi, 1983). The reaction mixture contained 0.3 ml of enzyme extract and 2.8 ml of 0.1 M sodium phosphate buffer (pH 6.8) and the reaction was stored by the addition of 0.4 ml of 10mM hydrogen peroxide. The decrease of absorbance was recorded. Decrease of absorbance was recorded in every 15 sec up to 3 min. Catalase activity was expressed as nKat/ min/ mg of protein.

Result and Discussion

Changes in morphology and catalase activity in response to differential wavelength of light

Phytochrome-mediated photomorphogenic responses are characterized by the complex variety of relationships between light input and physiological outputs, including germination, de-etiolation, shade avoidance, circadian rhythm, and flowering (Yun Jeong-Han *et al.* 2007).

The shoot length was found to be of highest (Fig. 1) under complete darkness (7.2±0.57cm) and shortest under U.V light (3.5±0.5cm). In 700 nm (normal light) shoot length was found of intermediate (6.1±.28 cm) between dark and UV light. Similarly root length was found longest under complete darkness (7.8±0.76 cm) and shortest under UV light (4.8±0.28cm) and of intermediate length 6.8 ±.56 cm 700 nm of light the root was observed. The inhibition of shoot and root length is in agreement with previously results documented by Hopkins (1995). The responses might be result from direct damage to essential components or from UV absorption by specific photoreceptors or growh regulators (Ballaré et al.1995). Most of the anabolic processes are generally performed during the dark phase of photosyhtesis that justified the longest shoot and root length during dark period.

Leaf size was found to increase under UV light and leaf area was found to be 1.20 cm^2 as compared to normal light 0.75 cm^2 and decrease under complete darkness i.e 0.35cm^2 after 6 days of treatment (Fig. 2). Yellowing of leaves was also observed under dark. The epicotyl colour was also gets affected by the change in light wavelength the epicotyls colour under complete darkness was observed to be in whitish colour as compared to a little green colour under normal and UV of light. This was expected as the result of chlrosis of the plant.

The catalse activity was observed to be highest under UV light and lowest in normal light after 16 hrs duration of 700 nm of light, however intermediate catalase activity was observed under complete darkness (Fig. 3). This is similar to studies performed by Appleman and Pyfrom (1955), those reports that etiolated barley seedlings when exposed to visible radiation suffer a large decrease in catalase activity and a simultaneous increase in chlorophyll. Indrajith and Ravindran (2009) studied the impact of UV radiation on growth, biochemical and antioxidant enzymes activity on *Phyllanthus amarus* seedling. UV radiation significantly decreased the growth, development and changes in UV absorbing compounds such as anthocyanin and flavonoids. An enhance in catalase avtivity was found.. *Phyllanthusamarus* seedling tries to counteract high level of reactive oxygen species produce under UV stress through the increased activities of antioxidant enzyme.

Effect of chemical treatment on leaf area, root-shoot length and catalase activity

Highest leaf was recorded in response $MgCl_2$ treatment and this enhancing effect was naturalized by addition of glucose (Fig. 4). GA_3 was also found to enhance the leaf area as compared to control in a concentration dependent manner. However no appreciable change was observed in

response to sucrose as compared to control (Fig. 4), though higher concentration (5%) was found more effective than lower concentration concentration (2.5%). PEG treatment was found to decrease the leaf area in a concentration dependent manner; higher concentration (5%) had more pronounced effect on leaf area than lower concentration (2.5%).

Stunted growth was observed in response to Sucrose treatment at both concentrations (2%, 5%) as compared to control (Fig. 5 A). Lower concentration (2%) found to decrease the shoot length to 3.8±.35 cm as compared to respective control (6.1±.28 cm) after 6 days of treatment, similarly root length decreased to 4.2±.2 cm as compared to respective control (5.5±.5 cm) after 6 days of treatment. This effect was more stringent with higher concentration (5%) as shoot length 2.8±0.15 cm and root length of 3.8±0.2 cm was recorded after 6 days (Fig. 5 A table1). This is in agreement with study performed by Feng et al. (2010) the study highlighted the fact that high concentration of sucrose restrained germination and seedling development seriously. Addition of glucose and magnesium chloride was found to neutralize the effect of sucrose (Fig. 5 B table1). In addition to these changes sucrose was found to initiate the development of adventitious roots (Fig. 5 C). Leaf size was not much affected in this case. Also in this case adventitious root system was observed in contrast to tap root system observed normally in *B. campestris.* (Fig. 4)

An increase in leaf area was observed in response to both concentration of GA_3 (100µM and 50 µM) and values were 0.95cm^2 and 0.89 cm^2 respectively as compared to respective control and this value was 0.75 cm^2, (Fig. 4 , table1) . Under the application of 100µM GA_3 shoot length was found to decrease (3.5±0.5 cm) as compared to respective control (6.1±0.28 cm), however normal shoot length was observed under 50 µM concentration (Fig. 5D, table1). Root length was found to increase from normal 5.5±.5 cm to 7.1±.28 cm and 6.1±.37 cm under 100µM and 50 µM concentrations respectively after 6 days (Fig. 5 D, table1). Leaves were comparatively paler in response to gibberellic acid treatment, more paler at 100µM than at 50 µM concentration (Fig. 5 D). Pale leaves were observed under GA_3 treament similar studies were carried out by wheeler et al, (1962) he reported that GA increased chlorophyll content per leaf but increased leaf area more so that the chlorophyll per unit area decreased, and the leaves were paler than untreated leaves

Smaller leaf size was observed in response to PEG treatment as compared to control. At 5% PEG the leaf area decreased to 0.38 cm^2 as compared to respective control (0.75 cm^2) and this value was 0.50 cm^2 in response to 2.5% PEG (Fig.4). Shoot length was found to decrease and the values as 4.5±.25 cm and 5.3±.20 cm in response 5% and 2.5% of PEG treatment, respectively as compared to respective control (6.1±.28 cm). No observable change was observed in response to 2.5 % of PEG whereas higher concentration (5%) was found to inhibit the root length 4.5±.5 cm as compared to respective control and this value was 5.5± 0.5 cm (Fig. 5 E, table1)

Application of $MgCl_2$ (50 µM) made leaves more green and larger in size having 1.15cm^2 of area as compared to respective control (0.75 cm^2) whereas leaves under combined application $MgCl_2$ + 5% glucose treatment kept under complete darkness were more paler in colour, smaller leaf area 0.40 cm^2 (Fig. 5 F, table1). In the later case root and shoot length was found to be shorter than respective i.e 4.3±.32 cm and 3.5±.5 cm respectively. Short root and shoot length with $Mgcl_2$ combined with glucose were in accordance with Bas et al, (2003) who carries out similar studies in *Arabidopsis thaliana* and reported that glucose delays germination

Sucrose, PEG, GA_3 and $MgCl_2$ enhanced catalase activity treatment:-

The catalase activity of plant tissues fluctuates with changes in their metabolic activity or physiological condition. The measurement of these fluctuations can be

used as means of ascertaining the response of plants to various treatments. The measurement of catalase activity is a reliable and sensitive index to changes occurring in internal conditions of plants. (R.H Landon 1934). Catalase is a common enzyme found in nearly all plants that are exposed to oxygen, where it catalyse the conversion of hydrogen peroxide (H_2O_2) to water and molecular oxygen, thereby protecting cells from the toxic effects of hydrogen peroxide [$2H_2O_2 \rightarrow 2H_2O + O_2$].

An enhancing effect on the catalase activity was seen in response to all chemical used during this study (Fig. 6 A-E). Maximum activity was observed on the fourth day of all treatments. This increase was concentration dependent, higher concentration (5%) more effective in enhancing catalase activity than lower concentration (2%).

The increase in activity might be attributed to production H_2O_2. Similar increase in catalase activity was also documented by various workers in various plant species. Rajesh et al. (2006) performed the similar studies on mulberry plant and found that high concentration of $MgCl_2$ led to production of H_2O_2 and hence intiation of catalase activity. PEG increased in catalase activity as discussed by Ravi et al (2011). PEG induces water deificit which results in oxidative stress because of formation of ROS hence increase antioxidant enzymes such as catalase. Application of Giberellic acid enchanced the catalase activity, the similar changes had been reported by Kathleen et al, (1978) the study attributed changes to production oxidative bust.

In conclusion, treatments used in this study enhanced the activity of catalase along with the induction of various morphological changes in leave, shoot and root. The result suggests that various treatment might led to heavy production of oxidative bust particularly H_2O_2 production and which might elicit catalase activity as evident by marked enhancement of catalase activity in response to these treatments. Therefore, catalase possibility exist that catalase might be having protective role in cell by scavenging excess of H_2O_2 as this had capability of transforming H_2O_2 production in to water and protecting cells from oxidative damage.

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Table.1: Changes in Shoot, root and leaf in response to different treatments in *Brassica campestris*. Duration of various treatments and types of tissue has been mentioned above each column. chemical treatments has been mentioned in each row. The values represents the mean three biological replicates± standard deviation

Chemical reagents	Time of treatment					
	Day 2		Day 4		Day 6	
	Shoot	Root	Shoot	Root	Shoot	Root
Control	4.3±0.57	5.2±0.5	5.5±0.5	6.1±0.37	6.1±0.28	5.5±0.5
PEG (5%)	2.6±0.57	3±0.2	3.6±0.57	4.2±0.5	4.5±0.25	5.5±0.5
PEG (2.5%)	3.1±0.28	3.1±0.35	4.5±0.5	3.8±0.2	5.3±0.20	4.5±0.5
GA_3 (50µM)	3.5±0.5	4.5±0.5	5.5±0.5	5.5±0.5	6.2±.015	6.1±.037
GA_3 (100µM)	2.2±0.25	5.1±0.36	3.2±0.25	6.3±0.2	3.5±0.5	7.1±0.28
Sucrose 2%	2.5±0.40	2.5±0.5	3.1±0.1	3.6±0.2	3.8±.035	4.2±0.2
sucrose 5% w/v	1.5±0.57	1.8±0.4	2.1±0.36	2.5±0.5	2.8±0.15	3.8±0.2
$Mgcl_2$ 50 µM	3.4±0.36	3.2±0.28	4.6±0.28	4.5±0.1	5.5±0.5	5.7±0.5
$Mgcl_2$ 50 µM +5% glucose	2.1±0.28	2.2±0.25	2.8±0.15	3.1±0.2	3.5±0.5	4.3±.032

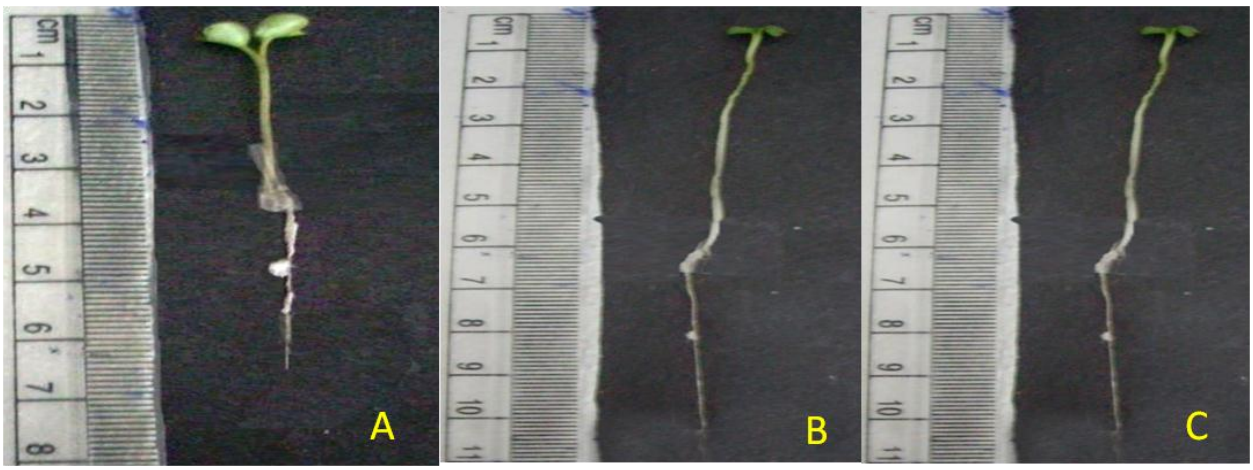

Fig. 1: Morphological changes in Shoot, root and leaf in response to different wavelength of light from *Brassica campestris*. Panel A: UV (253.7 nm), B: normal light (700 nm), C: in complete dark..

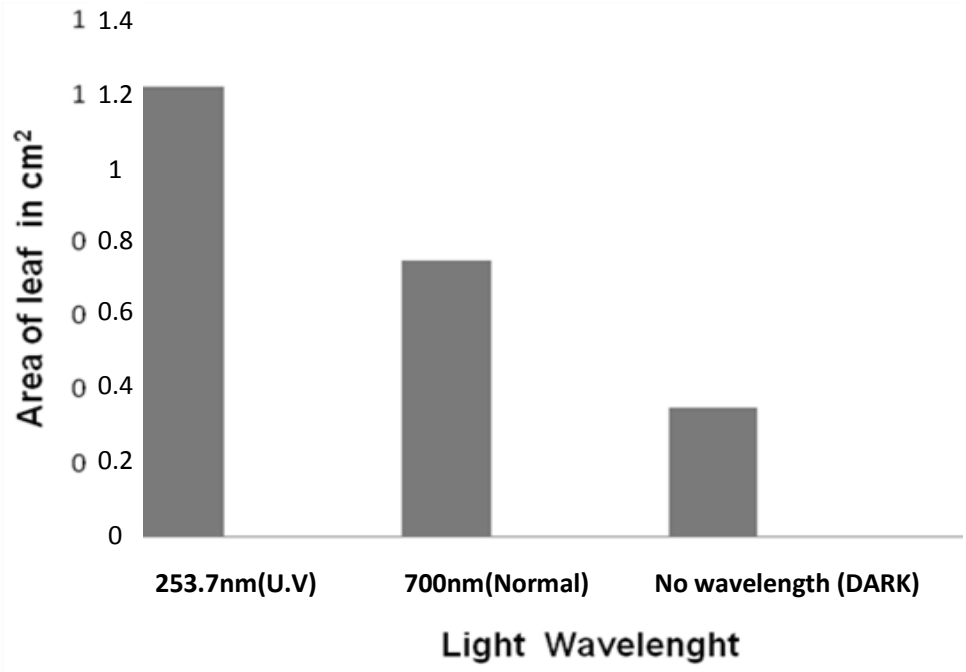

Fig. 2: Change in leaf area in response to different wavelength of light from *Brassica campestris*. Panel A: UV (253.7 nm), B: normal light (700 nm), C: in complete dark.

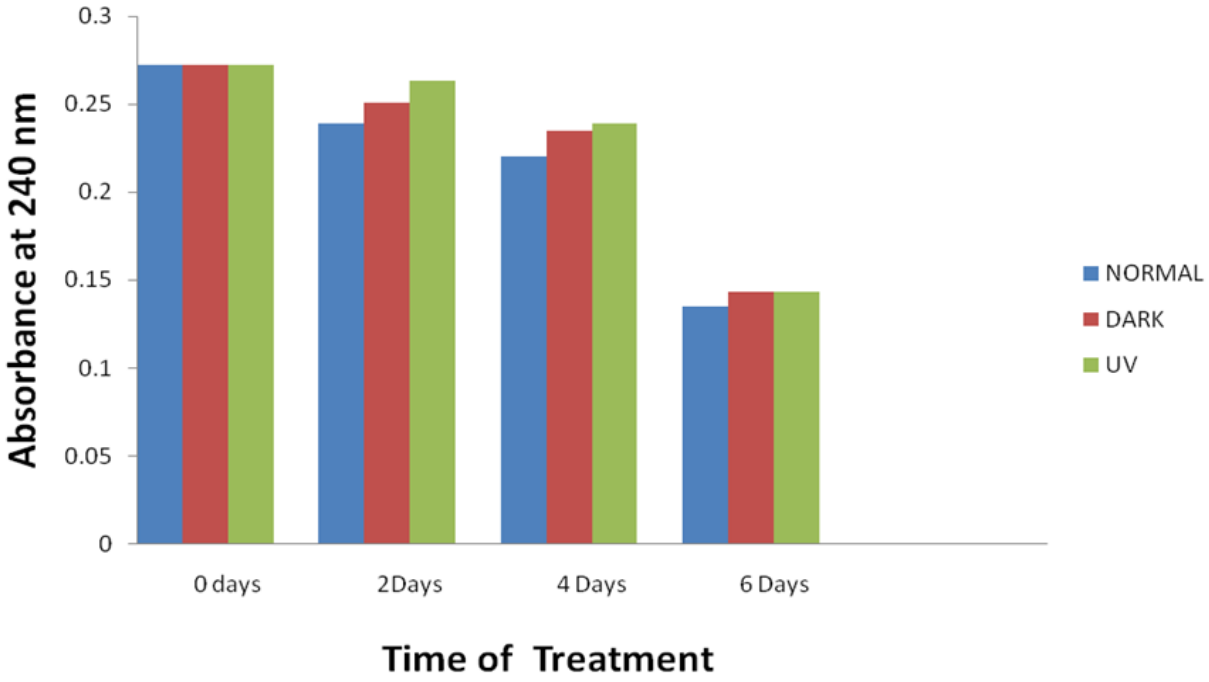

Fig. 3: Change in catalase activity (ΔA_{240}/min/mg protein) in response to different wavelength of light of *Brassica campestris*. Panel A: UV (253.7 nm), B: normal light (700 nm), C: in complete dark. Time of treatment has been mentioned on the horizontal axis whereas corresponding change in absorbance has been mention on the vertical axis.

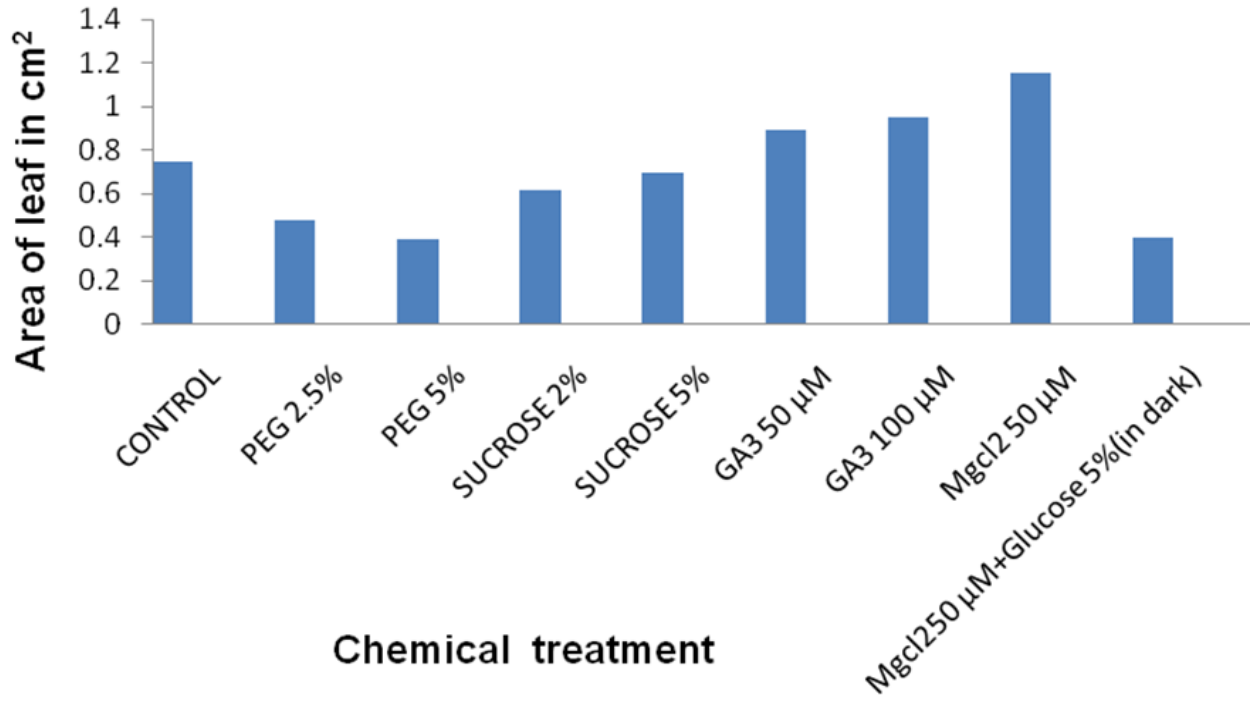

Fig. 4: Change in leaf area, in response to different chemical treatments from *Brassica campestris*. Treatment is mentioned below whereas leaf in cm^2 is on the vertical axis.

Fig. 5: Change in leaf, shoot and root length in response to different chemical treatments from *Brassica campestris*. Panel A: Sucrose (2%, 5%), B: sucrose+ glucose, C: sucrose, D: GA_3: (50µM, 100 µM), E: PEG (2.5%, 5%) and seedling kept in complete darkness. Length was measured in centimeters.

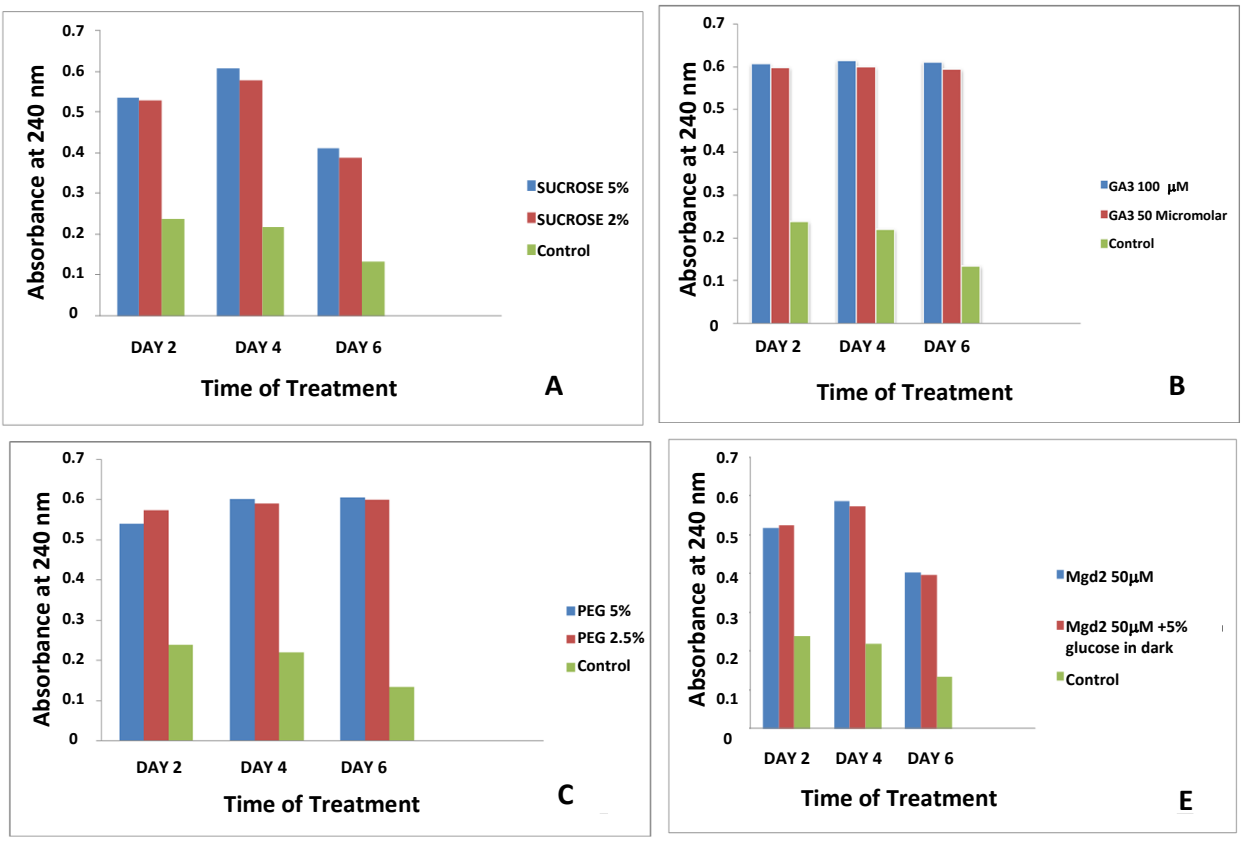

Fig. 6: Change in catalase activity (ΔA_{240}/min/mg protein) in response to different treatments from *Brassica campestris* . Panel A: Sucrose (2%, 5%), B: GA_3: (50µM, 100 µM), C: PEG (2.5%, 5%) and E: $MgCl_2$. Time of treatment and different concentrations has been mentioned on the horizontal axis whereas corresponding change in absorbance has been mention on the vertical axis.