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# Effect of Drought Stress on Photosynthetic Parameters and Gene Expression in *Brassica juncea* L. (Czern. and Coss.)

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**A B S T R A C T**

Drought stress limits the plant growth resulting in drastic decline in the photosynthetic yield globally. The present investigation was carried out to study the expression of drought induced pp2c and lhcb genes in Brassica juncea cvs. RH0116 (drought tolerant) and RH8812 (drought sensitive) via semi-quantitative RT-PCR using actin as constitutive gene. Brassica plants were grown in net house and subjected to drought stress by withholding water at flowering stage. We observed that pp2c gene was upregulated and was expressed in both shoots and roots during drought. The drought tolerant genotype exhibited higher accumulation of pp2c transcript than RH8812. However, lhcb gene was found to be down regulated during drought and its expression was present only in shoots. Seeds of both the genotypes were grown on MS medium with various levels of stress treatments using Mannitol and ABA. Seed germination was arrested at initial stage only and reduction in seed germination was significant among treatments and no seedling growth was observed in case of ABA treatment. Physiological parameters such as relative water content, osmotic potential, electrolyte leakage and chlorophyll fluorescence were also evaluated in stressed and control plants of both the genotypes and we found that physiological response was consistent with the changes in gene expression.

## Introduction

*Brassica juncea* L. Czern & Coss. (Indian mustard) is a crucifer, used as a source of food spice and folk medicine all over the world (Tian and Deng, 2020). It is the most widely cultivated oilseed crop in the Indian subcontinent (Akhtar *et al.*, 2020), with very high acerage (6.9 million hectares) and production (7.2 million metric tonnes) (USDA, 2018-19). *B. juncea* also known as Oriental mustard, brown mustard, chinese mustard or leaf mustard is an annual herb (Lin *et al.*, 2011) and is an allotetraploid (AABB, 2n = 36). It has evolved through multiple hybridization events between *B. rapa* (AA, 2n

= 20) and *B. nigra* (BB, 2n = 16) (Nagaharu, 1935).

Plants, being sedentary organisms are continuously challenged by a wide variety of abiotic stress such as drought, cold, high temperature, UV radiation, heavy metals and salinity hampering a lot quality and production of crops (Soda *et al.,* 2015). Abiotic stresses are responsible for more than 50% decline in crop yield worldwide (Rodziewicz *et al.,* 2014). Drought is a major abiotic stress which affects majority of the world's crop plants.

Drought tolerance in crops is a complex trait as it involves biochemical, physiological and morphological mechanisms (Kalina *et al.,* 2016). Being a multigenic and quantitative trait, it is quite difficult to understand the molecular mechanism of abiotic stress tolerance. Also, there are hundreds of genes underlying the plant response to stress. In addition, drought cannot be forecast and hence plays a vital role in destabilizing the crop productivity (Amudha and Balasubramani, 2011). Plants have evolved acclimation and adaptation mechanisms to cope up with drought that includes avoidance, escape from stress and dehydration tolerance of the protoplast.

RH0116 is a drought tolerant variety of *Brassica juncea*, whereas RH8812 is drought sensitive, developed by CCS Haryana Agricultural University, Hisar (Aneja, 2014). RH0116 is also known to be tolerant to high temperatures (Sharma and Sardana, 2013). RH8812 was released in the year 1996, its average yield is 22q/ha, it gets matured at 142 days and possesses thick siliquae (www.hau.ernet.in). In this paper, we have discussed comparative study of these genotypes as they offer magnificent source to understand the mechanism of drought stress tolerance in *Brassica juncea*.

Photosynthesis is of central importance to plant growth and development and involves collection of light and transfer of solar energy to the reaction centers of PSII using LHC (light harvesting chlorophyll a/b- binding) proteins (Fanna *et al.,* 2016). They are often associated with chlorophyll and xanthophyll and serve as antenna complex to drive photosynthetic electron transport (Gao and Li, 2015). LHC proteins are mostly found in thylakoids and encoded by nuclear genes (Kong *et al.,* 2016).

PP2Cs or Protein phosphatase 2C play crucial role in plant processes such as cell differentiation, growth, metabolism, environmental stress signaling pathways and represent the major group of protein phosphatases in plants (Yang *et al.,* 2018). These enzymes are monomeric with catalytic and regulatory domains present upon the same polypeptide. They need divalent metal ions such as $Mn^{2+}$ or $Mg^{2+}$ for their activity (Sugimoto *et al.,* 2014). PP2Cs including PP2CA and ABI1/HAB1 branches are key negative regulators of ABA signaling (Zhang *et al.,* 2013). Combined inactivation of specific PP2Cs involved in ABA signaling could provide an approach for improving crop performance under drought stress conditions (Rodriguez, 2006).

To have additional cognizance to the function of lhcb and pp2c in drought tolerance, we examined their transcript levels in drought tolerant and sensitive cultivars of *Brassica juncea*. Development of PCR for detecting the presence of rare transcripts has revolutionized gene identification. Gene expression strategies such as semi-quantitative RT-PCR offers a sensitive, versatile and rapid method to analyse drought stress related gene expression. It requires gene specific primers for studying the differential gene expression (Chen *et al.,* 2005). In this way, it can be useful for identification of certain key genes

that play role in drought stress tolerance and overexpression of such genes can lead to increased productivity in case of drought (Aneja *et al.,* 2015). In the present study, we also analyzed certain physiological parameters viz. seed germination studies, chlorophyll fluorescence, relative water content, osmotic potential and electrolyte leakage.

The dominant phase in the plant life cycle begins with seed germination (Yan *et al.,* 2014) which proceeds by water uptake under favourable conditions leading to the activation of metabolic processes (Atia *et al.,* 2011). ABA (Abscisic acid) hormone plays vital role in dormancy and germination control (Nambara *et al.,* 2010). Drought stress induced by mannitol is also widely documented in many crop species (Ullah *et al.,* 2014). Relative water content depicts absolute amount of water that a plant needs to attain saturation. Hence it is an important indicator of state of water balance of the plant (Arjenaki *et al.,* 2012). Electrolytes such as $K^+$, release outside the cell after a cell dies and loses membrane structural integrity. Hence electrolyte leakage can be used as a proxy for the extent of cell death (Hatsugai and Katagiri, 2018). Estimating the fluorescence emission from chlorophyll molecules of photosynthesizing plants is a commonly used non destructive technique involved in photosynthetic research. It is the most reliable test enabling the varietal discrimination as per their drought tolerance (Sayar *et al.,* 2008).

## Materials and Methods

### Plant material

Indian mustard cultivars (RH8812 and RH0116) seeds were procured from Department of Genetics and Plant Breeding, CCSHAU, Hisar.

### In vivo drought stress treatment

Plants (RH0116 and RH8812) were seeded and grown in pots in nethouse. Plants were regularly watered with equal amounts of water (to keep the water level equal) and Hoagland Solution (50 ml each) to supply them adequate amounts of nutrients for growth. At the stage of flowering, one half of the plants of both the genotypes was segregated as control (in which watering was continued) and the second set of plants was subjected to drought stress condition by withholding watering.

Samples of shoot and root tissues were taken from the control and stressed plants and were stored at -80°C for RNA isolation and for analyzing the physiological parameters such as relative water content, osmotic potential and electrolyte leakage.

Post sampling, the drought stressed plants were rehydrated by equal amounts of water. The day wilting disappeared samples were taken again for analysis.

### Germination studies in Indian mustard under drought stress condition

Seeds of both the cultivars (RH0116 and RH8812) were grown on MS medium having varying levels of stress treatments using mannitol (100µM, 200µM, 300µM and 400µM) and ABA (10, 50 and 100µM). Physiological attributes like seed germination pattern, shoot and root lengths were estimated after 2 weeks.

### Physiological studies

The control, stressed and rehydrated Brassica plants were grown in nethouse and the following physiological parameters were studied:

## Relative water content

For the estimation of RWC, two fully expanded leaves (of *in vivo* grown plants of each variety RH8812 and RH0116) were cut into tiny pieces and weighed to record the fresh weight (Yamasaki and Dillenburg, 1999).

The leaf samples were then hydrated till they became fully turgid by immersing in de-ionized water in a closed petriplate for 4 hrs. Thereafter the samples were removed from water and any surface moisture was soaked using filter paper and immediately weighed to record full turgid weight.

Then the samples were oven- dried at 70°C for 24 hrs and weighed to record the dry weight of the sample. The experiment was performed in triplicates and RWC was estimated using following formula

$$\text{RWC}(\%) = 100 \times \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}}$$

## Osmotic potential

Osmotic potential of leaf was estimated using Vapour pressure osmometer (Wescor INC., USA). The leaves were excised from control, stressed and rehydrated plants and were sealed in syringes separately and immediately frozen at -20°C. The samples were thawed for 60 min at 25°C prior to the measurement of osmotic potential.

Then the sap was released from the syringe. A filter paper disc was immersed in the sap and quickly placed inside the vapour pressure osmometer chamber.

The chamber was sealed and the osmotic potential values were recorded. For calibrating the osmometer, standard solutions of NaCl (0.1 mM- 1.0 M) were used.

## Electrolyte leakage

The relative integrity of plasma memberane was determined as the Percentage Electrolyte leakage (Gong *et al.,* 1998). Fresh leaves were excised into small pieces and put in test tubes.

The test tubes were incubated at 52°C for 1 hr in a water bath and 10 ml of deionised water was added to it and kept overnight.

The initial electrical conductivity (EC1) was estimated on the next day. Thereafter the samples were autoclaved to release all the electrolytes, cooled and then, the final electrical conductivity (EC2) was estimated. The leakage percentage of electrolytes was calculated as (1-EC1/EC2) X 100.

## RNA Isolation and cDNA synthesis

Total RNA was isolated from the control, stressed and rehydrated plant samples (shoot and root tissues) by means of Trizol method (Chomczynski, 1993) with some modifications as described in Aneja *et al.,* (2015).

The RNA samples were reverse transcribed by using oligo dT primer (0.5 µg), total RNA (1 µg), dNTPs mix (4 mM), reverse transcriptase enzyme (200 units) and RNase inhibitor (20 U).

## Semi- quantitative RT-PCR

For gene expression analysis, the primers for pp2c and lhcb genes were synthesized by using sequences from Arabidopsis (Zhang *et al.,* 2008). The primers used for PCR analysis are given in table 1.

| Gene | Forward Primer | Reverse Primer | Annealing temperature (˚C) |
|---|---|---|---|
| ***pp2c*** | 5'CAAATGGCTGGGATTTGTTGC 3' | 5'AAGACGACGCTTGATTATTCCTC 3' | 51.0 |
| ***lhcb*** | 5'CAACGATCTCCTCCGCAAA3' | 5'CTTGACGGTACGACGCATGAT3' | 50.5 |
| ***BjActin*** | 5'TGGCATCACACTTTCTACAA3'` | 5'CAACGGAATCTCTCAGCTCC3' | 54.3 |

PCR reaction was performed in a 20 µl reaction volume containing 10X PCR buffer (containing 2.5 mM MgCl2), 200 µM of dNTPs, 0.6 µM of primers (forward and reverse), 1U Taq DNA Polymerase and template (cDNA- 20 ng). PCR cycling conditions consisted of an initial denaturation at 94°C for 2 min; 36 cycles of 92°C for 1 min; annealing for 30 sec and 72°C for 1 min; and a final extension at 72°C for 10 min.

### Germination studies in *Brassica juncea* under drought stress

Seeds of *Brassica juncea* cvs. RH0116 and RH8812 were grown on MS medium with varying levels of stress treatments using ABA (10µM, 50µM, 100µM) and Mannitol (100µM, 200µM, 300µM, 400µM). Physiological attributes such as seed germination, shoot and root length were recorded after 2 weeks.

## Results and Discussion

### Analysis of lhcb and pp2c gene expression in *Brassica juncea* cvs. RH8812 and RH0116 under drought stress condition

The transcripts of lhcb were expressed only in the shoots and not in roots (Fig. 1) while pp2c gene transcript was present in both roots and shoots (Fig. 2). The expression of lhcb gene was down regulated while that of pp2c gene was upregulated in the plants exposed to drought stress conditions. The transcripts of lhcb gene were absent in the drought stressed plants and present in the control and rehydrated plants. The transcripts of pp2c gene were expressed only in the drought stressed plants and the expression level was higher in the drought tolerant genotype vs drought sensitive genotype. Housekeeping gene actin was expressed in the roots and shoots of all the plant groups viz. control, drought stressed and rehydrated plants (Fig. 3).

### Physiological Studies

### Seed germination under drought stress condition induced by mannitol and abscisic acid (ABA)

The percent seed germination and the average length of root and shoot decreased as the concentration of mannitol was increased 100 µM, 200 µM, 300 µM and 400 µM (Table 1 and Fig. 4-6). The average root and shoot length was minimum at 400 µM and highest in control. The root/shoot ratio in the mannitol- stressed seedling was lesser as compared to the control, however, the root/shoot ratio increased with the concentration of mannitol (Fig. 7). Root/shoot ratio of the tolerant genotype, RH0116, was always higher than the sensitive genotype, RH8812 at the respective concentration of mannitol. The shoot length in RH0116 and RH8812 decreased by 4.21%, and 4.48% at 100 and 200 µM mannitol, respectively and the respective decrease at 300 and 400 µM was 16.45% and 16.41%. Similarly, the root length in RH0116 and RH8812 decreased by

14.54% and 10.50% at 100 and 200 µM mannitol, respectively and the respective decrease at 300 and 400 µM was 26.06% and 22.50%.

Seed germination was observed in both RH0116 and RH8812 in drought stress condition induced by ABA at concentration of 10 µM, 50 µM and 100 µM in MS media. In RH0116 and RH8812, the seed germination was highest in the control (MS media without ABA) (93.33% vs 60.00%) and least at 100 µM ABA (50.00% vs 33.30%). However, no seedling growth was observed in the seeds germinated in the drought stress induced by ABA at any concentration (Fig. 8-11, Table 1)

## Physiological parameters in *Brassica juncea* (cvs. RH8812 and RH0116) under drought stress

The physiological parameters such as Relative water content, Osmotic potential, Electrolyte leakage and Chlorophyll fluorescence were observed in the *in vivo* grown stressed, control and rehydrated groups of *Brassica* plants, and two weeks old *Brassica juncea* (cvs. RH8812 and RH0116) seedlings (grown *in vitro*) subjected to air drying stress (for 15 min, 30 min, 1 hr and 2 hr).

## Chlorophyll fluorescence in *Brassica juncea* (cvs. RH8812 and RH0116) under drought stress

With the help of PEA analyzer, chlorophyll fluorescence was observed was observed in the *Brassica juncea* (cvs. RH8812 and RH0116) plants under drought stress conditions in the plants grown in *in vivo* conditions and in *in vitro* grown seedlings. The ratio of minimum fluorescence to maximum fluorescence (Fo/Fm) was higher in the stressed plants as compared to control plants in both the conditions (*in vivo* and *in vitro*). In the rehydrated and control plants of both the genotypes (sensitive as well as tolerant), similar kind of observation was seen. While the ratio of variable fluorescence to maximum fluorescence (Fv/Fm) was higher in the control as compared to stressed plants of both the genotypes. However, the increase was more in the RH8812 (drought sensitive) genotype; and almost same in the rehydrated and control plants in both the genotypes (Table 2). In the *in vitro* grown seedlings, the increase in the Fo/Fm ratio and decrease in the Fv/Fm ratio was observed with the increased duration of the stress (from 15 min to 2 hr) in both the cultivars of *Brassica juncea* (Table 3).

## Relative Water Content in *Brassica juncea* (cvs. RH8812 and RH0116) under drought stress

Relative water content (%) in the *in vitro* grown seedlings of *Brassica juncea*, decreased as the duration of drought stress (15 min, 30 min, 1 hr and 2 hr), induced by air drying, increased. RWC (%) was highest in the control plants and minimum in the plants exposed to stress for two hr, in both the cultivars (Fig. 12). The decrease in the RWC (%) was more for the drought sensitive variety, RH8812 (from 84.55% to 33.85%), as compared to the drought tolerant variety, RH0116 (from 79.10% to 39.55%).

## Osmotic potential and Electrolyte leakage in *Brassica juncea* (RH8812 and RH0116) under drought stress

As the duration of stress was increased (15 min, 30 min, 1 hr and 2 hr), decrease in osmotic potential was observed in the *in vitro* grown seedlings (Fig. 13). Maximum decrease in osmotic potential was observed at 2 hours of drought treatment i.e. -1.37 MPa in RH8812 and -1.35 MPa in RH0116. Similarly, the electrolyte leakage percentage in the *in vitro* grown plants also increased as

the duration of drought stress, induced by air drying, was increased (15 min, 30 min, 1 hr and 2 hr), in both the genotypes. Maximum electrolyte leakage was observed after the stress of 2 hr of stress treatment, as the electrolyte leakage (%) increased from 15% to 30% in RH0116 and from 17% to 38% in RH8812 as compared to control (Fig. 14).

**Table.1** Seed germination (%age) in *B. juncea* (cvs. RH0116 and RH8812) under drought stress induced by Mannitol and abscisic acid (ABA)

| | | Per cent germination | |
|---|---|---|---|
| **Treatment** | | RH0116 | **RH8812** |
| **Mannitol** | Control | 83.33% | **80.00%** |
| | 100 µM Mannitol | 80.00% | **56.66%** |
| | 200 µM Mannitol | 63.33% | **53.33%** |
| | 300 µM Mannitol | 70.00% | **46.66%** |
| | 400 µM Mannitol | 60.00% | **40.00%** |
| **ABA** | Control | 93.33% | **60.00%** |
| | 10 µM ABA | 73.00% | **40.00%** |
| | 50 µM ABA | 66.00% | **40.00%** |
| | **100 µM ABA** | **50.00%** | **33.30%** |

**Table.2** Chlorophyll fluorescence in the *Brassica juncea* plants (RH8812 and RH0116) in *in vivo* conditions

| | RH8812 Stressed | RH8812 Control | RH0116 Stressed | RH0116 Control | RH8812 Rehydrated | RH8812 Control | RH0116 Rehydrated | RH0116 Control |
|---|---|---|---|---|---|---|---|---|
| $F_o/F_m$ | 0.32 | 0.29 | 0.32 | 0.28 | 0.28 | 0.29 | 0.30 | 0.30 |
| $F_v/F_o$ | 1.70 | 2.33 | 1.82 | 2.55 | 2.32 | 2.34 | 2.31 | 2.30 |
| Fv/Fm | 0.54 | 0.67 | 0.58 | 0.71 | 0.65 | 0.68 | 0.69 | 0.69 |

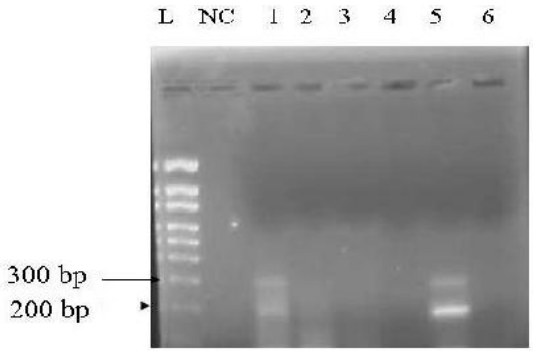
(where, $F_o$ = minimum fluorescence, $F_m$= maximum fluorescence, $F_v$ = variable fluorescence)

**Table.3** Chlorophyll fluorescence in the *Brassica juncea* plants (RH8812 and RH0116) in *in vitro* conditions

| | RH8812 | | | RH0116 | | |
|---|---|---|---|---|---|---|
| | Fo/Fm | Fv/Fo | Fv/Fm | Fo/Fm | Fv/Fo | Fv/Fm |
| Control | 0.35 | 1.88 | 0.66 | 0.41 | 1.45 | 0.59 |
| Stressed (15 min) | 0.65 | 0.53 | 0.34 | 0.67 | 0.50 | 0.34 |
| Stressed (30 min) | 0.78 | 0.28 | 0.22 | 0.76 | 0.32 | 0.24 |
| Stressed (1 hr) | 0.85 | 0.18 | 0.15 | 0.89 | 0.12 | 0.11 |
| Stressed (2 hr) | 0.91 | 0.10 | 0.09 | 0.93 | 0.20 | 0.19 |
| Rehydrated (15 min) | 0.75 | 0.32 | 0.24 | 0.77 | 0.29 | 0.22 |
| Rehydrated (30 min) | 0.75 | 0.33 | 0.25 | 0.73 | 0.36 | 0.26 |
| Rehydrated (1 hr) | 0.74 | 0.34 | 0.25 | 0.67 | 0.49 | 0.33 |
| Rehydrated (2 hr) | 0.75 | 0.32 | 0.24 | 0.76 | 0.30 | 0.23 |

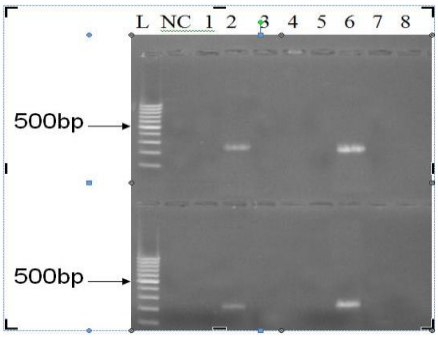
(where, $F_o$ = minimum fluorescence, $F_m$= maximum fluorescence, $F_v$ = variable fluorescence)

**Fig.1** Agarose gel electrophoresis of *lhcb* transcripts in *Brassica juncea* genotypes under drought stress



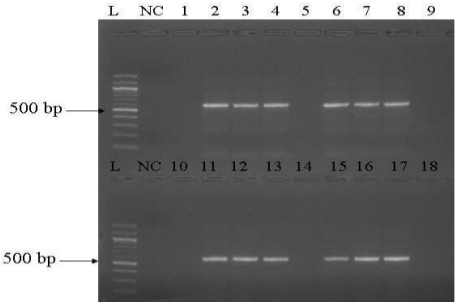

Lane L-Ladder; NC-Negative control; 1-RH0116 control; 2-RH0116 root; 3-RH8812 root; 4-RH0116 stressed; 5-RH8812 control; and 6-RH8812 stressed

**Fig.2** Agarose gel electrophoresis of *pp2c* transcripts in *Brassica juncea* genotypes under drought Stress




Lane L-Ladder; NC-Negative control (NC); 2-RH8812 control (shoot); 3-RH8812 stressed (shoot); 4-RH8812 rehydrated (shoot); 6-RH0116 control (shoot); 7-RH0116 stressed (shoot); 8-RH0116 rehydrated (shoot); 11-RH8812 control (root); 12-RH8812 stressed (root); 13-RH8812 rehydrated (root); 15-RH0116 control (root); 16-RH0116 stressed (root); 17-RH0116 rehydrated (root).

**Fig.3** Agarose gel electrophoresis of *actin* transcripts in *Brassica juncea* genotypes under drought stress




Lane L-Ladder; NC-Negative control (NC); 2-RH8812 control (shoot); 3-RH8812 stressed (shoot); 4-RH8812 rehydrated (shoot); 6-RH0116 control (shoot); 7-RH0116 stressed (shoot); 8-RH0116 rehydrated (shoot); 11-RH8812 control (root); 12-RH8812 stressed (root); 13-RH8812 rehydrated (root); 15-RH0116 control (root); 16-RH0116 stressed (root); 17-RH0116 rehydrated (root)

**Fig.4** *In vitro* seed germination response in *Brassica juncea* cvs. RH0116 and RH8812 subjected to various levels of mannitol treatment (a= mannitol 0 % i.e. control, b= mannitol 100 µM, c= mannitol 200 µM, d= mannitol 300 µM, mannitol 400 µM)

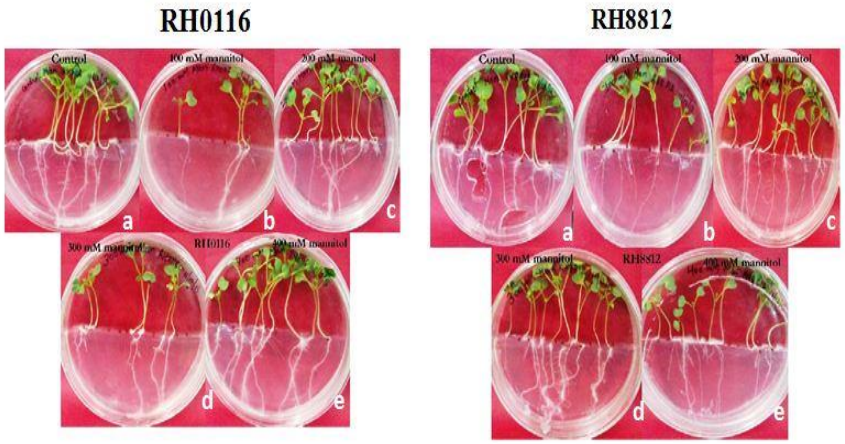



**Fig.5** Shoot length in the germinated seedlings of *B. juncea* (cvs. RH0116 and RH8812) under drought stress induced by different concentrations of mannitol (100, 200, 300 & 400 µM)

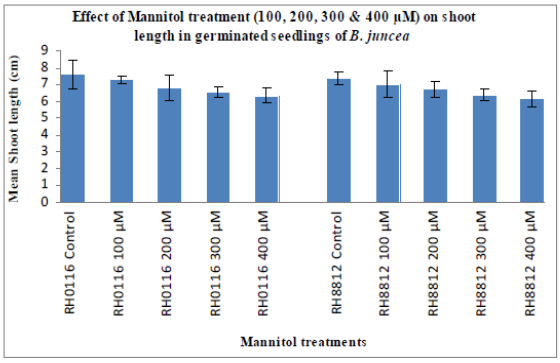



**Fig.6** Root length in the germinated seedlings of *B. juncea* (cvs. RH0116 and RH8812) under drought stress induced by different concentrations of mannitol (100, 200, 300 & 400 µM)

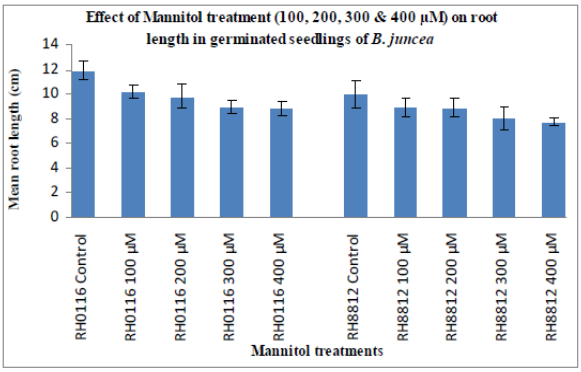


**Fig.7** Root:Shoot ratio in the germinated seedlings of *B. juncea* (cvs. RH0116 and RH8812) under stress induced by mannitol treatment (100, 200, 300 & 400 µM)

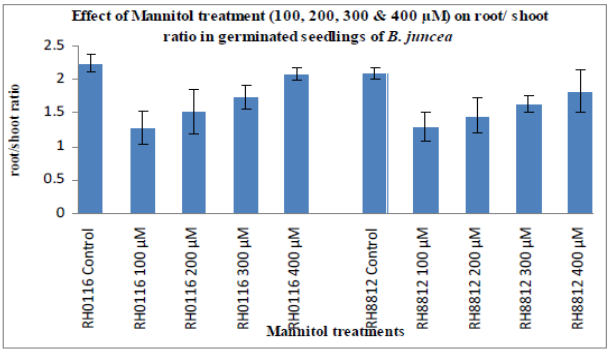



**Fig.8** *In vitro* seed germination response in *Brassica juncea* cvs. RH0116 and RH8812 subjected to various levels of ABA treatment (a=ABA 0 % i.e. control, b=ABA 10 µM, c= ABA 50 µM, d= ABA 100 µM)

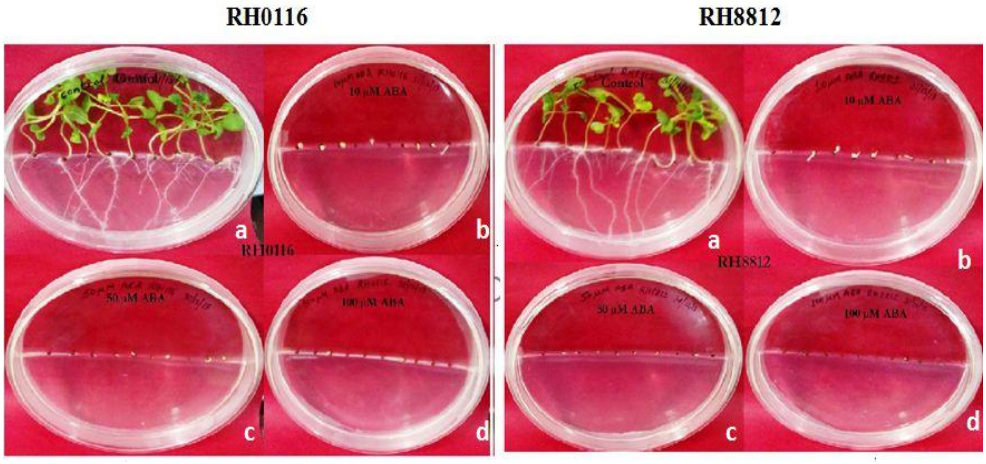



**Fig.9** Shoot length in the germinated seedlings of *B. juncea* (cvs. RH0116 and RH8812) under drought stress induced by different concentrations of absicic acid (ABA)

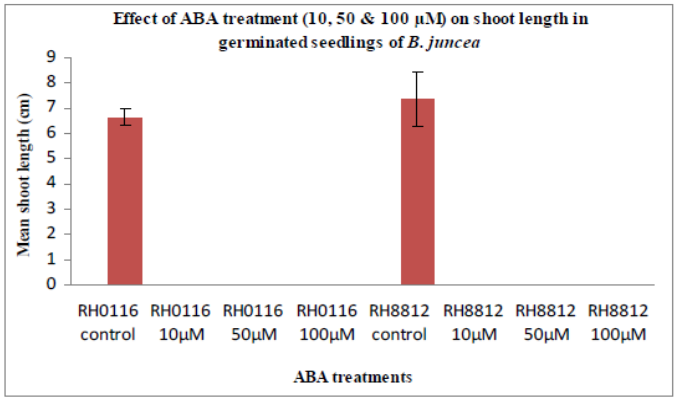


**Fig.10** Root length in the germinated seedlings of *B. juncea* (cvs. RH0116 and RH8812) under drought stress induced by different concentrations of absicic acid (ABA)

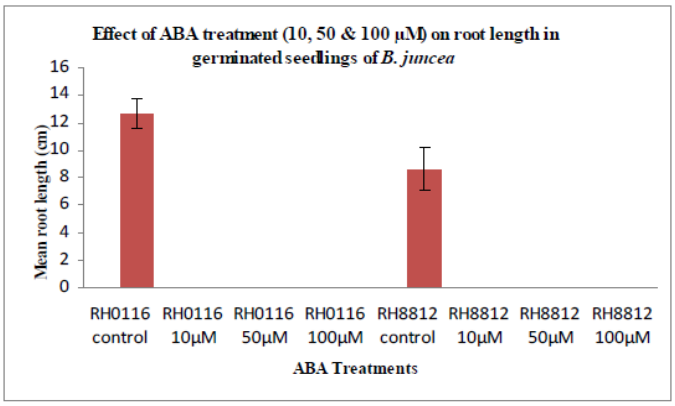



**Fig.11** Root:Shoot ratio in the germinated seedlings of *B. juncea* under stress induced by abscisic acid (ABA) treatment (10, 50 & 100 µM)

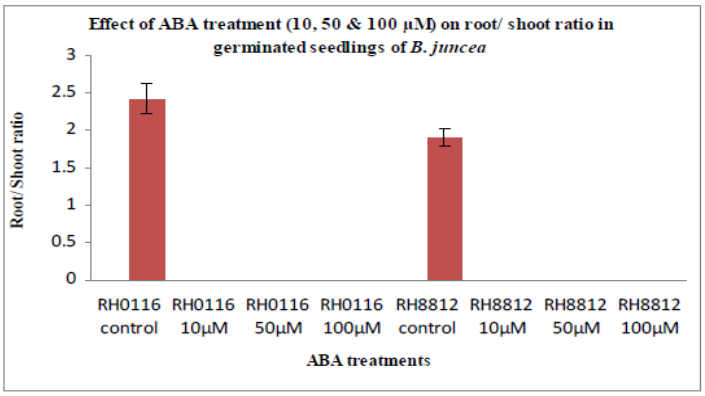



**Fig.12** Relative water content (%) under *in vitro* drought stress condition in *Brassica juncea* plants

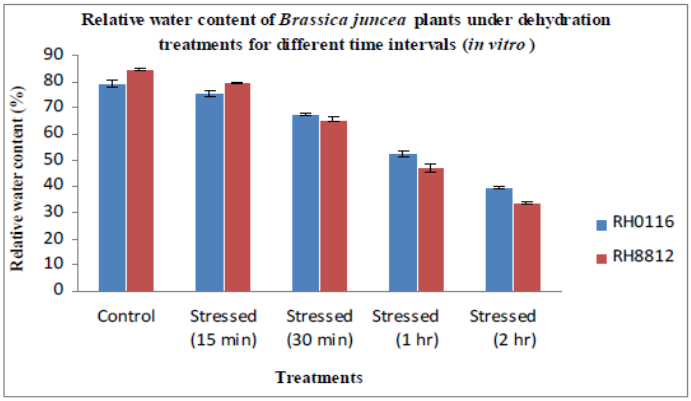


**Fig.13** Osmotic potential under *in vitro* drought stress condition in *Brassica juncea* plants

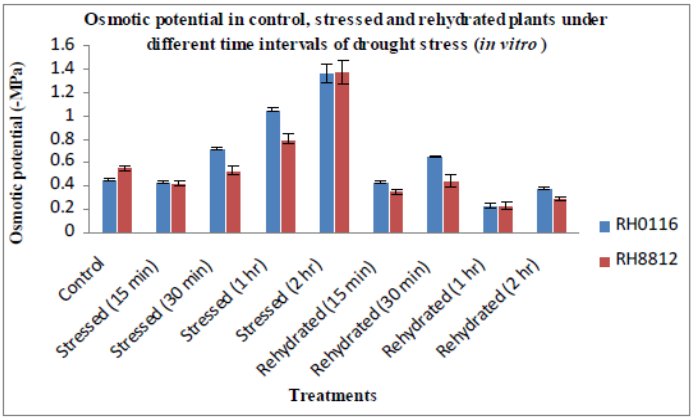
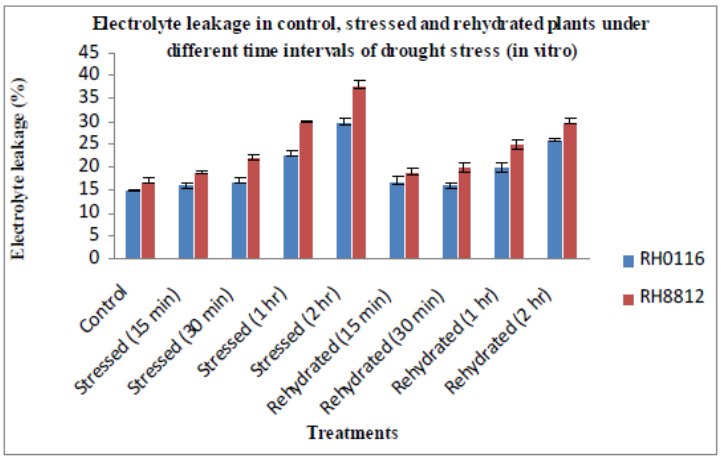



**Fig.14** Electrolyte leakage under *in vitro* drought stress condition in *Brassica juncea* plants




Water is a critical resource for plant growth and development and its associated physiological phenomenon and due to its limited supply in arid and semi-arid ecosystems, plants have to suffer water deficit which further hampers photosynthesis and net productivity of plants in arid ecosystems (Luo *et al.*, 2014). Also it is foreseen that as a result of global warming, plants would be exposed to such stresses in near future (IPCC, 2007). Having crops with better WUE (water use efficiency), achieved via genetic engineering or breeding technologies is the most reliable solution to the problem of water scarcity (Chaerle *et al.*, 2005).

## Analysis of physiological parameters in *Brassica juncea* under drought stress

Physiological and biochemical modifications due to abiotic stress in plants are related to altered expression of genes (Saibo *et al.*, 2009) and affected by the intensity, duration and rate of progression of the stress imposed (Chaves *et al.*, 2009). However, a particular stage e.g. germination, seedling or flowering could be the most critical one for drought stress depending upon the species. Also studies have proven that in specific genotypes, drought induced gene expression was in consistence with the physiological response (Hazen *et al.*, 2005; Street *et al.*,

2006). Uptake of water after the seed dormancy is broken paves the way for germination to occur (Atia *et al.,* 2011), which further initiates the dominant phase of life cycle of higher plants (Yan *et al.,* 2014).

Mannitol (or manna sugar) with the chemical formula $C_6H_8(OH)_6$, is a sugar alcohol and is white, crystalline solid. It controls the osmotic potential in culture media so as to induce drought (Nilanthi *et al.,* 2015). Abscisic acid (ABA) is a hormone which plays an important role in dormancy as well as controls germination (Nambara *et al.,* 2010).

In the present study, we have recorded seed germination, shoot and root length 2 weeks post drought stress treatment by using Mannitol (100, 200, 300, 400 µM) and ABA (10, 50, 100 µM) under *in vitro* condition. It was observed that seed germination was arrested at the initial stage and reduction in seed germination was significant among mannitol treatment. There was differential seed germination in case of ABA treatment, however, no seedling growth was observed in case of ABA treatment. Bibi *et al.,* (2010) reported in sorghum that physiological and morphological parameters are affected by drought stress. Okcu *et al.,* (2005) observed that drought stress resulted in shorter shoots and in some cases, longer roots. Also, a longer root is characteristic feature of drought tolerant variety. In our study also we found that the average shoot and root lengths of the *in vitro* grown seedlings after mannitol treatment decreased with increased dose of mannitol in both the genotypes, however more reduction was recorded in RH8812 when 400 µM mannitol was used.

Since plant growth is directly linked with its RWC, changes in the water status of plant due to drought also needs to be focused upon (Hayatu *et al.,* 2014). RWC (relative water content) and leaf water potential are important parameters for studying physiological response of plant in case of drought (Marchese *et al.,* 2010; Silva *et al.,* 2010). Medeiros *et al.,* (2012) stated that these parameters decrease in most plants under drought stress. Arjenaki *et al.,* (2012) studied the effect of drought on chlorophyll content, RWC (relative water content) and mineral elements in wheat. It was observed that chlorophyll content, RWC and ion concentration of K and Na were varying among resistant and sensitive genotypes. Similarly we also found rapid decline in RWC when Brassica plants were subjected to water stress. The decline was greater in sensitive check as compared to RH0116. Also RH0116 (tolerant) exhibited higher recovery of RWC upon rehydration of stressed plants. *In vitro* studies also reflect similar kind of response (Personal data). Kumar *et al.,* (2013) characterized temperature tolerant Brassica genotypes on the basis of RWC and they observed that late sown Brassica showed higher decline in RWC than the early sown. In Plantago, RWC showed sharp decrease upon intensifying stress (Rahimi *et al.,* 2010). Saura-Mas and Lloret (2007) also found similar observations in case of woody species. It clearly supports our results as increasing the duration of air drying from 15 min to 2 h in Brassica seedlings decreased the RWC progressively (Personal data).

Our results also reveal that the values of osmotic potential were more negative in case of drought subjected plants than control plants. Also as the duration of drought stress treatment increased, the seedlings showed more negative osmotic potential. The decline was higher in sensitive variety than tolerant. Alikhan *et al.,* (2010) also reported reduced relative water contents, osmotic potential under water stress in rapeseed.

*Brassica juncea* seedlings were evaluated for heat stress tolerance by Wilson *et al.,* (2014).

They observed that electrolyte leakage was more in susceptible genotypes as compared to the tolerant ones. Similarly we also found that electrolyte leakage was increased when the plants were subjected to drought stress as compared to control plants and the increase was relatively more in sensitive variety (RH8812) as compared to the tolerant variety (RH0116). Sayar *et al.,* (2008) also reported that leakage was found to be significantly more in sensitive varieties and the values increased as the dehydration time increased. We also observed progressive increase in the values of electrolyte leakage with increased drought stress treatment. This suggests that electrolyte leakage parameter is an indicator for assessment of drought, heat or cold stress in crop plants.

Chlorophyll fluorescence technique has helped in understanding photochemical and non photochemical processes occurring in thylakoid membrane of chloroplast. It provides useful information about leaf photosynthetic performance of many plants under drought stress (Baker and Rosenqvist, 2004). In the present study, the value of ratio of minimum fluorescence to maximum fluorescence (Fo/Fm) was found to be more in stressed plants as compared to the control plants and same in the rehydrated and control plants in both the genotypes (sensitive as well as tolerant). Many researchers (Oyetunji *et al.,* 2007; Arji *et al.,* 2008; Xu *et al.,* 2008; Guerfel *et al.,* 2009) observed that drought significantly decreased maximum efficiency of PSII (*F*v/*F*m) and maximum effective quantum yield of PSII. We also observed that the ratio of variable fluorescence to maximum fluorescence (Fv/ Fm) was lower in the stressed plants as compared to the control plants of both the genotypes, however, the decrease was more pronounced in the drought sensitive genotype. Faraloni *et al.,* (2011) also reported that the Fv/Fm ratio decreased by 90% in the "susceptible" cultivars of olive, whereas the "tolerant" ones did not show any decline in Fv/Fm. Sharma *et al.,* (2012) observed that a temperature of 38˚C with a pretreatment at 33– 35˚C was sufficient to obtain significant heat induced differences in *F*v/*F*m among the cultivars, which were absent in the control plants. Khaleghi *et al.,* (2012) also reported that in young olive plants cv. Dezphul, the irrigation treatments had significant effect on chlorophyll a, total chlorophyll (chl a+b) and Fv/Fm ratio.

Differential gene expression gives rise to physiological changes. Such genes turn on/ off in response to stress. In previous reports, we have reported that hsp gene which is drought/ heat inducible is activated under drought stress in Indian mustard (Aneja *et al.,* 2015). In the present study, we studied the induction of lhcb and pp2c genes in response to drought stress in two important cultivars of *Brassica juncea* RH8812 (drought sensitive) and RH0116 (drought tolerant) using semiquantitative RT-PCR approach. We used actin (a constitutive or housekeeping gene) as positive control in our experiments. We found that the actin transcript was induced in roots as well as shoot tissues irrespective of the treatment, i.e. it was present in control, stressed and rehydrated plants with similar expression.

## Expression of *pp2c* in *Brassica juncea* under drought stress conditions

Protein kinases and phosphatases regulate many biological processes by catalyzing phosphorylation and de-phosphorylation of proteins. Major group of protein phosphatases are monomer enzymes and represented by Type 2 C protein phosphatases (PP2Cs) in plants. They need divalent ion ($Mg^{2+}$ or $Mn^{2+}$) for activity and act on serine/ threonine residues, hence catagorized under protein serine/threonine phosphatases (Bhalothia *et al.,* 2018). PP2Cs are found to be

evolutionarily conserved from prokaryotes to eukaryotes (Sugimoto *et al.,* 2014), found in archaea, bacteria, fungi, plants and animals (Yang *et al.,* 2018). They negatively regulate ABA responses (Zhang *et al.,* 2013) and MAPK cascade pathways and play crucial role in stress signal transduction in plants (Cao *et al.,* 2016). Rodriguez (2006) suggested that if specific PP2Cs involved in ABA signaling are inactivated in combination, it could offer an approach to improve crop performance in drought.

In the present study, the expression of *pp2c* gene was induced in the plants subjected to drought stress condition and not in the control and rehydrated plants. The gene was expressed in both shoots and roots of the stressed plants, however, the expression was higher in drought tolerant genotype as compared to drought sensitive genotype. This is also supported by Cohen *et al.,* (2010) in poplar where they found that Protein phosphatase type- 2C (PP2C) was up-regulated in both shoot and root tissues. Guo *et al.,* (2009) also observed that 18 genes, including those encoding ⊿l-pyrroline-5-carboxylate synthetase (P5CS), protein phosphatase 2C-like protein (PP2C) were differentially expressed in all genotypes under drought in barley. Zhang *et al.,* (2013) also supported our observation that the plant PP2CA genes appear to be expressed ubiquitously in various organs, albeit at varying levels. Wei and Si Pan (2014) reported that Group A PP2Cs were negative regulators of ABA signalling pathway and key regulators of drought tolerance in plants.

## Expression of *lhcb* in *Brassica juncea* under drought stress conditions

The *Lhcb* gene family in green plants encodes highly homologous, conserved, numerous light-harvesting Chl *a/b*-binding (LHC) proteins that harness and transfer light energy to the reaction centers of PSII (Teramoto *et al.,* 2001). The Lhc family is bifurcated into Lhca or Lhcb depending upon whether the encoded gene product belongs to photosystem I (PSI) or photosystem II (PSII) respectively. Members of the Lhcb subfamily express differentially which plays a role in acclimating plants to excess light (Caffarri *et al.,* 2005). The LHCB proteins are mostly complexed with chlorophyll and xanthophyll and serve as antenna complex. Xu *et al.,* (2012) reported that the outer antenna proteins LHCBs are probably the most abundant membrane proteins. *Lhcb* gene expression is regulated by mainly light (Humbeck and Krupinska, 2003), chloroplast retrograde signal (Nott *et al.,* 2006), oxidative stress and ABA (Staneloni *et al.,* 2008). In our experiments, the expression of this gene was found to be downregulated under drought stress conditions, therefore, the transcripts were present in the control and rehydrated plants and absent in the stressed plants. However, the transcripts were present only in the shoots and not in the roots. Several studies support our findings where it has been postulated that the Lhcb genes were down-regulated in stress conditions such as cold (Seki *et al.,* 2002), high-salinity (Seki *et al.,* 2002), drought (Hazen *et al.,* 2005; Guo *et al.,* 2009), exogenously applied ABA (Staneloni *et al.,* 2008), high light (Heddad and Adamska, 2000) and infection by Puccinia triticina (Manickavelu *et al.,* 2010). Breeze *et al.,* (2011) also reported that LHCBs were found in the same cluster of downregulated genes together with many others encoding subunits of the PSI and PSII complexes. The pattern of the rarely expressed *Lhc* genes was always found to be more similar to that of *PsbS* and the various light-harvesting-like genes, which might indicate distinct physiological functions for the rarely and abundantly expressed Lhc proteins. In general, *Lhc* genes are most strongly expressed when light harvesting is limiting

for plant growth, i.e. in low light but otherwise optimal conditions. Since the need for efficient light harvesting is lower under high light conditions, *Lhc* gene expression is down regulated in strong light (Klimmek *et al.,* 2006). Kleine *et al.,* (2007) also found that *Lhcb* expression in the wild type was reduced to 25% of the control level after high light treatment and similarly reduced in all investigated mutants. Xu *et al.,* (2012) reported that *Lhc* gene expression levels are very high in leaves but low in non green tissues, or even absent in many such tissues and in our study also, its expression was detected only in the shoots and not in the roots. The regulation of the *LHCB* expression is considered to be one of the important mechanisms for plants to modulate chloroplast functions (Nott *et al.,* 2006; De Montaigu *et al.,* 2010; Pruneda-Paz and Kay, 2010; Thines and Harmon, 2010). The exploration of genetic variation in genes encoding LHCPs may facilitate a better understanding of functions of LHCPs and provide useful information and selection tools for plant breeders to improve plant with high photosynthesis efficiency (Xia *et al.,* 2012).

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