

# Physiological Responses of *Brassica napus* Genotypes to Combined Drought and Salt Stress

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## ABSTRACT

Physiological responses of *Brassica napus* genotypes were studied when drought and salinity stress were applied together. Irrigation of plants, which first started with 150 mM NaCl solution, was stopped when soil EC (electrical conductivity), NaCl EC and water fraction volume (WFV) values reached 0.04 dSm<sup>-1</sup>, 0.64 dSm<sup>-1</sup> and 15%, respectively. Growth, osmotic potential, ion concentration and stomatal conductance were determined. Fresh weight and dry weights were significantly affected by the combined stress. Fresh weight was abridged more than dry weight. The dry weights and leaf area per unit dry weights of treated plants were considerably reduced compared to unstressed, control plants. The osmotic potential of each genotype decreased in the combined-stressed plants compared to unstressed plants showing a variable trend regarding osmotic adjustment. Na<sup>+</sup> ions of each genotype increased significantly in the combined-stressed plants compared to unstressed plants. There were less K<sup>+</sup> and Ca<sup>++</sup> ions in the stress-treated plants than in the control, unstressed plants. Stomatal conductance of each genotype was retarded by the stress combination. Variable physiological responses were found among the genotypes showing slow- and fast-growing ecotypes.

**Keywords:** biomass, growth, ions, leaf area, salinity

## INTRODUCTION

Environmental stresses such as drought and high salinity are the major factors that limit plant growth and productivity by disturbing the intracellular water balance. Naturally, these stresses affect a crop plant when a combination of several stress factors like extreme conditions of light, humidity, temperature, salt and water deficit are applied. However, most of the studies have been devoted to assess the physiological response of plants in a single stress environment such as salinity (Kumar and Kumar 1990; Sinha 1991; Uma *et al.* 1992; Sharma and Gill 1994) or drought (Shinozaki and Yamaguchi-Shinozaki 1996, 2000). Further, studies on the physiological understanding under the combined drought and salinity are restricted to certain reports (Wang *et al.* 2003; Dudley and Shani 2003; Wang and Huang 2004; Jakab *et al.* 2005) even though they are not directly related to the combination of drought and salt stress.

*Brassica* species are considered to be a relatively moderately salt-sensitive genus (François 1994; Hayat *et al.* 2007) within which there is certain variability towards salt tolerance (Ashraf and Sharif 1997; Beltrao *et al.* 2000; Maggio *et al.* 2005). In contrast to the cultivated *Brassica*, the genetic diversity of its relatives may provide useful genes for improving this tolerance. For example, there are several wild relatives of *Cajanus cajan* (L.) (pigeonpea) Millse belonging to the genera *Atylosia*, *Dunbaria*, and *Rhynchosia* (Subbarao *et al.* 1991), which exhibit wide variation in their tolerance against salt stress and may represent genetic resources for the improvement of salt tolerance in cultivated *C. cajan* and *Lycopersicon pimpinellifolium* (Jusl.) Mill. (Foolad and Lin 1997). Unfortunately, the effects of combined drought and salt stress, which are supposed to be common prevalent factors in the field affect plant growth simultaneously, have not yet been studied in *Brassica* genotypes. In addition, the physiological response

of *Brassica* genotypes to drought stress has not been extensively studied thus far.

This study describes the physiological response of *Brassica napus* genotypes to a combination of drought and salt stress. This approach may be useful to identify physiologically tolerant genotypes for both salt and drought tolerance for further field experiments.

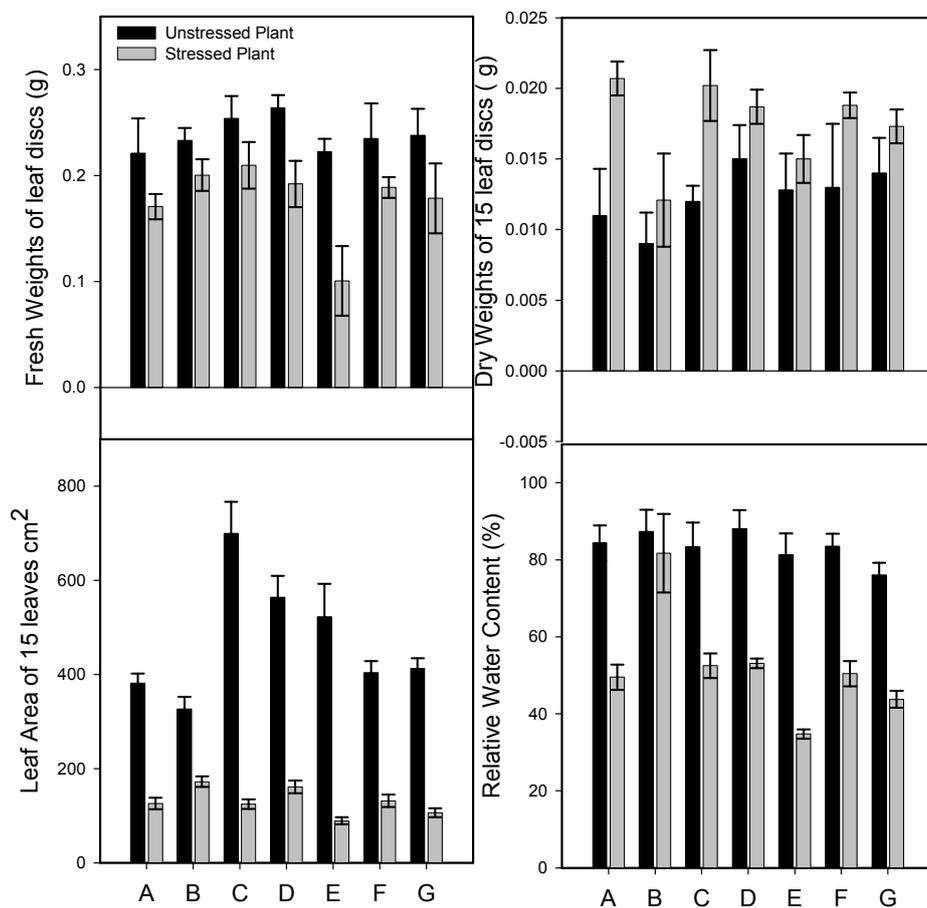
## MATERIALS AND METHODS

### Plant material

Seven germplasm accessions of *B. napus* i.e. 119463 (A), 31526 (B), 211363 (C), 167532 (D), 134983 (E), 147554 (F), 908979 (G) were collected from The Germplasm Center, the National Institute of Agricultural Biotechnology, RDA, Suwon, Korea.

### Treatment preparation

Seeds were sterilized with 50% Clorox for 10 min, then repeatedly washed with distilled water. The sterilized seeds were placed on wet paper in Petri dishes and kept at 25°C in the dark until germination. Germinating seeds were first transferred into small plastic pots (diameter 2.5 cm, height 4.5 cm) containing a commercial horticultural soil PARTNER (70%) composed of 23~35% peat moss, 40~50% coco peat, 10~16% perlite, 5~8% vermiculite and 8~11% zeolite manufactured by NangWoo Green-Tech Co., LTD. After 10 days, uniform size, young seedlings were transplanted one by one into the plug of a larger plastic tray (diameter and height was 6 cm). Plastic trays were kept in a growth chamber with a 13-h photoperiod (28°C, 600 ± 50 μmol m<sup>-2</sup> s<sup>-1</sup> PAR) and 11 h night (22°C) at 60% humidity. Plants were irrigated with 3 L of half-strength Hoagland's solution (Hoagland and Arnon 1950) until the plant developed five to six expanded leaves. Two sets of trays were prepared; one for the control, the other for the combined stress treatment. Each set had eight plants for each genotype.



**Fig. 1** Combined effect of salt and drought stress on fresh and dry weights, leaf area and relative water contents different *Brassica napus* genotypes. Symbols on Y-axis stand for the genotypes: A = 119463, B = 31526, C = 211363, D = 167532, E = 134983, F = 147554, G = 908979. Lines on bars represent the standard error of the mean.

Initially, soils were treated with 150 mM NaCl solution and soil electrical conductivity (EC), NaCl EC and water fraction volume (WFV) were regularly observed using a monitoring probe (Hydra Probe, Stevens Water Monitoring System Inc. USA).

In order to achieve concurrent drought and salt stress, watering of the nutrient solution + NaCl was stopped when the soils reached a soil EC of  $0.04 \text{ dSm}^{-1}$ , NaCl of  $0.64 \text{ dSm}^{-1}$  and a 15% WFV. While control plants were regularly irrigated with 2 L of half-strength Hoagland's solution up to one week. After three days 5-6 days of treatment when wilting was obvious, plants were harvested for physiological observation.

### Physiological measurements

Leaf area was measured with a leaf area meter (Li-3100, Li-COR Inc., USA). Upon sampling, fifteen leaf discs were taken from fully expanded young leaves using a cork borer ( $0.071 \text{ cm}^2$ ) for each genotype from each treatment. These leaf discs were used to determine relative water content (fresh weight, turgid weight and dry weights of leaf discs), osmotic potential, osmotic adjustment, and  $\text{Ca}^{++}$ ,  $\text{K}^+$  and  $\text{Na}^+$  ions. For osmotic potentials and ions, sampled leaf discs were placed in distilled water for 4 h until a fully turgid status was reached, then blotted and frozen in liquid. The frozen tissues were thawed at room temperature prior to the expression of tissue sap. Tissue sap was expressed using a centrifuge (Tomy Max 300 High Speed Ultra centrifuge USA) at 14,000 rpm for 20 min at  $4^\circ\text{C}$ . Osmotic potential of the expressed sap was determined using a vapor pressure osmometer (Wescor, Utah, USA). Relative water content was measured as:  $\{(\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight}) \times 100\}$ , and osmotic adjustment as:  $\text{OP}_{\text{turgid stressed plant}} - \text{OP}_{\text{turgid unstressed plant}}$  as described by Barr and Weatherley (1962) and Blum (1989), respectively.

Ions were analyzed with an inductively coupled plasma (ICP) emission spectrometer (Plasma 400, The Perkin Elmer Corp., USA) for  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{++}$  after diluting the expressed sap of turgid leaf tissues to 100 times using  $0.7\% \text{ HNO}_3$ .

Stomatal conductance was determined on the fully expanded leaves selected randomly using a leaf promoter (Model SC-1, Decagon, USA).

### Statistical analysis

Experiment was designed as randomized complete block and data sets were subjected to Paired "t" test between the stressed (treated) and unstressed (control) samples of each genotype. Each treatment and control was replicated four times.

### RESULTS

The application of stress significantly affected plant growth components such as FW and DW, and leaf area of *B. napus* genotypes (Fig. 1, Table 1). FW and DW of unit leaf area were significantly affected by the stress. However, the FW of unit leaf area was remarkably reduced due to the stress compared to DW where some non-significant increase was recorded. The FW of genotype 134983 showed the highest, significant reduction among the genotypes tested compared to the unstressed plants. Other genotypes also showed a significant reduction in FW following the same trend. DW of unit leaf area increased in the combined stress compared to the unstressed condition. However, the stress-induced maximum increase was recorded in genotypes 119463 and 211363.

Leaf area of each genotype was significantly reduced under the stress more than the unstressed plants (Table 1). However, maximum reduction was found in 211363 and 134983 genotypes compared to unstressed plant. Relative water contents were high in unstressed plants compared to stressed plants. However, treated genotype 31526 plants had relatively the same water content as unstressed plants.

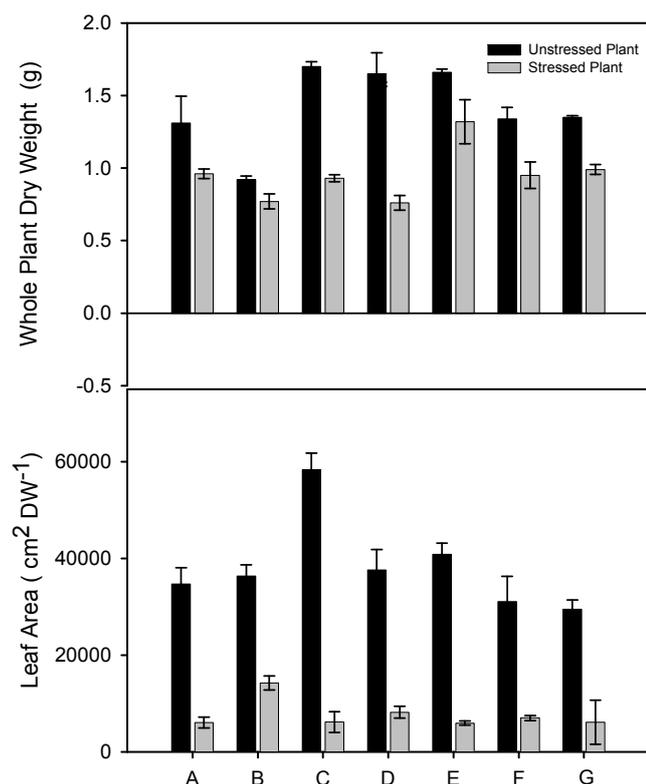
The stress reduced the dry weights of the whole plant and leaf area per unit dry weights (Fig. 2). Treated genotypes showed a significant reduction in dry weights except for genotypes 31526 in which insignificant reduction occurred (Table 1). Leaf area per unit dry weight decreased under the stress condition. However, genotype 31526 showed a significantly lower degree of reduction compared to other genotypes under the stress condition.

Imposing the stress significantly lowered the osmotic

**Table 1** Paired *t*-test values of unstressed and stressed plants of each genotype.

| Parameters                            | Genotype   |           |             |            |            |            |            |
|---------------------------------------|------------|-----------|-------------|------------|------------|------------|------------|
|                                       | A (119463) | B (31526) | C (2113661) | D (167532) | E (134983) | F (147534) | G (908479) |
| Fresh Weight                          | 7.69***    | 30.83***  | 125.29***   | 16.97***   | 16.44***   | 5.64**     | 21.03***   |
| Dry Weight                            | -14.41***  | -4.27*    | -16.56***   | -6.95***   | -1.43ns    | -4.55ns    | -0.92ns    |
| Leaf Area                             | 86.57***   | 30.98***  | 29.02***    | 36.02***   | 19.57***   | 69.27***   | 0.98ns     |
| RWC                                   | 78.92***   | 3.51*     | 28.13***    | 27.84***   | 29.95***   | 3.762*     | 7.77*      |
| WPDW                                  | 6.51***    | 15.71***  | 241.98***   | 26.59***   | 7.39**     | 78.79***   | 44.27***   |
| Leaf Area/DW                          | 36.82***   | 70.98***  | 111.42***   | 27.71***   | 52.66***   | 14.41***   | 25.12***   |
| Osmotic potential                     | 39.91***   | 8.42**    | 3.55*       | 70.77***   | 4.66*      | 12.13***   | 18.04***   |
| Na <sup>+</sup> Ions                  | -12.23***  | 34.69***  | -20.52***   | -12.68***  | 124.45***  | 0.68ns     | -33.38***  |
| K <sup>+</sup> Ions                   | 5.16**     | 41.27***  | 6.62**      | 5.48**     | 26.85***   | 3.04*      | 13.86***   |
| Ca <sup>++</sup> Ions                 | 7.29**     | 20.59***  | 30.73***    | 1.25ns     | 25.47***   | 3.80*      | 8.44**     |
| Na <sup>+</sup> /K <sup>+</sup> ratio | -10.03**   | -67.17*** | -21.06***   | -31.85***  | -56.57***  | -1.02ns    | -1.00ns    |
| Stomatal Conductance                  | 6.10*      | 21.70**   | 21.82**     | 11.78**    | 25.93***   | 30.04***   | 10.63**    |

Note: Asterisks stand for P<0.05 (\*), P<0.01 (\*\*), P<0.001 (\*\*\*). ns (non-significant) WPDW (whole plant dry weight), RWC (relative water content)

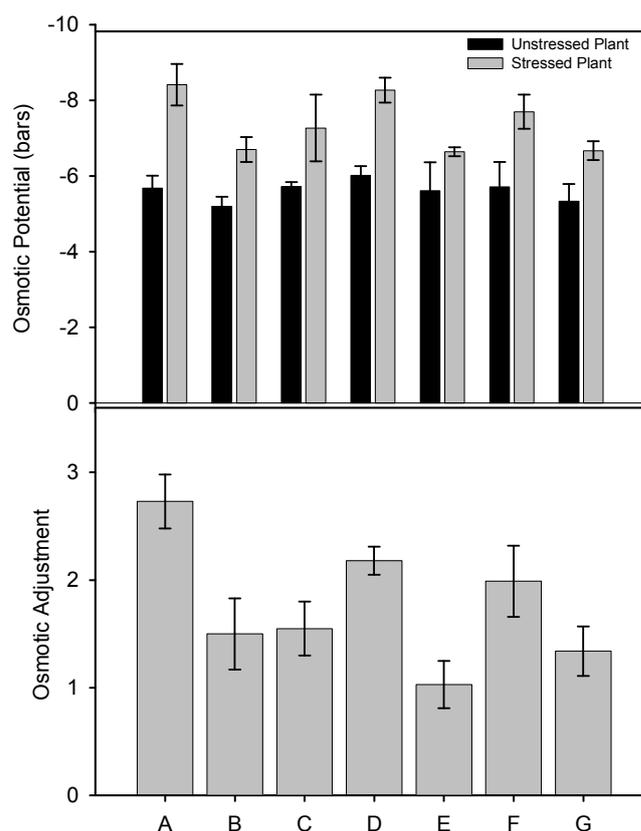


**Fig. 2** Combined effect of salt and drought stress on whole plant dry weights and leaf area per unit dry weights of *Brassica napus* genotypes. Symbols on Y-axis stand for the genotypes: A = 119463, B = 31526, C = 211363, D = 167532, E = 134983, F = 147554, G = 908979. Lines on bars represent the standard error of the mean.

potential of turgid tissues showing the capacity of osmotic adjustment (**Fig. 3, Table 1**). However, genotypes 119463, 167532, 147554 showed the lowest osmotic potential value and the greatest osmotic adjustment while genotypes 211868 and 167532 showed insignificant osmotic potential and osmotic adjustment values.

Ion (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>++</sup>) concentrations in the expressed sap were effected by the stress in all genotypes (**Fig. 4**). However, Na<sup>+</sup> ions of each genotype increased significantly in the stressed plants compared to unstressed plants (**Table 1**). Treated sample of genotypes 119463, 211363, 167532 and 908979 showed more Na<sup>+</sup> ions than unstressed plants. However, genotype 147554 showed statistically insignificant values to unstressed plants. There were fewer K<sup>+</sup> and Ca<sup>++</sup> ions in stressed genotypes than in unstressed controls, the lowest value shown by genotype 134983. A high Na<sup>+</sup>/K<sup>+</sup> ratio was found in 134983 genotype compared to the other genotypes.

Stomatal conductance of each genotype was reduced by the stress (**Fig. 5**). However, the greatest reduction by stress

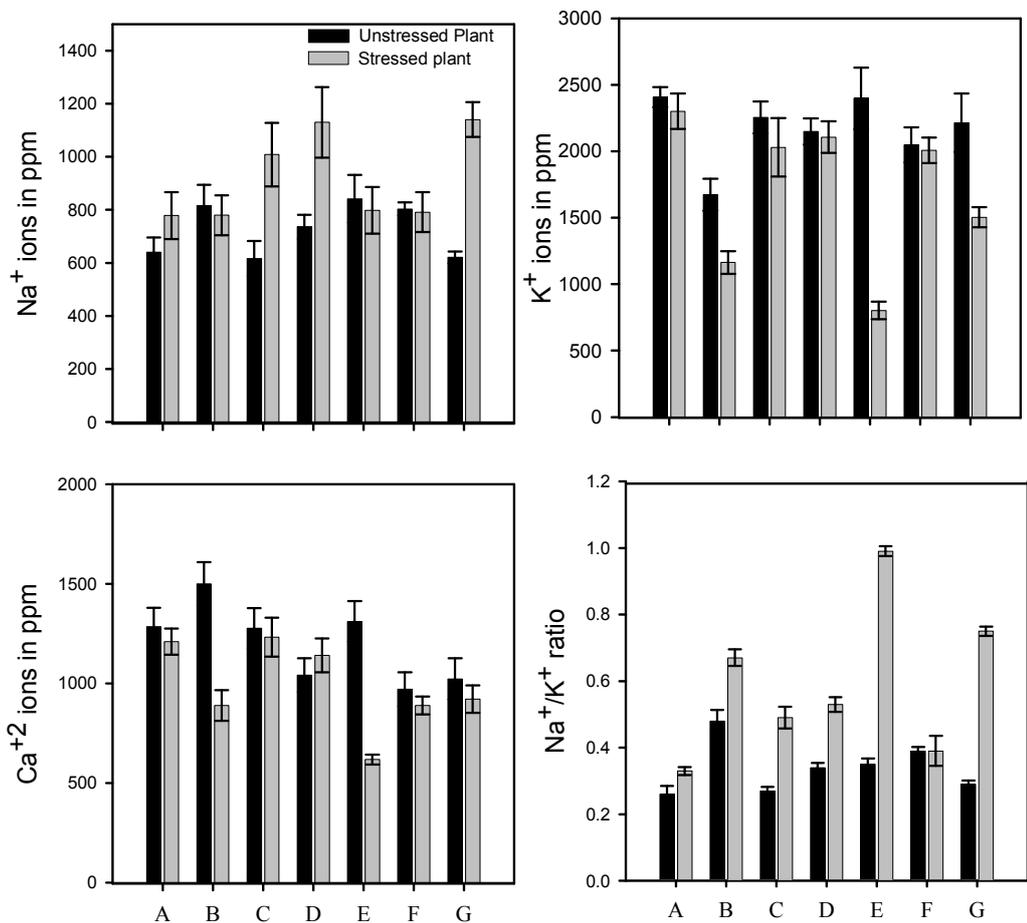


**Fig. 3** Combined effect of salt and drought stress on osmotic potential and osmotic adjustment of *Brassica napus* genotypes. Symbols on Y-axis stand for the genotypes: A = 119463, B = 31526, C = 211363, D = 167532, E = 134983, F = 147554, G = 908979. Lines on bars represent the standard error of the mean.

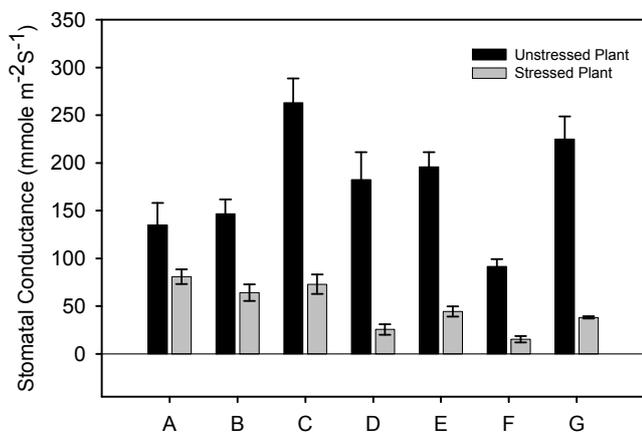
was found in genotypes 211868, 167532, 134983 and 908979 while genotypes 119463 and 31526 showed less inhibition.

## DISCUSSION

The physiological responses of *B. napus* genotypes were investigated under a drought and salt stress combination. The fresh weight and dry weights of 15 discs (1 disc = 0.71 cm<sup>2</sup>) of each genotype were significantly affected by combined stress (**Table 1**). Fresh weight of stressed plants was reduced but dry weight increased considerably compared to unstressed plant. It is presumed that the application of both drought and salt stress in combination contributed to the significant change in the water status of the soil (**Table 2**) the EC and WFV of which was decreased. As a result the water uptake was severely limited causing a reduction in fresh weight. In addition, an increase in dry weight may



**Fig. 4** Combined effect of salt and drought stress on K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup> and ions ratio of *Brassica napus* genotypes. Symbols on Y-axis stand for the genotypes: A = 119463, B = 31526, C = 211363, D = 167532, E = 134983, F = 147554, G = 908979. Lines on bars represent the standard error of the mean.



**Fig. 5** Combined effect of salt and drought stress on stomatal conductance of *Brassica napus* genotypes. Symbols on Y-axis stand for the genotypes: A = 119463, B = 31526, C = 211363, D = 167532, E = 134983, F = 147554, G = 908979. Lines on bars represent the standard error of the mean.

have been due to less amount of water in the leaf disc and more solute accumulation in a given measured area as compared to in the unstressed plant discs. However, the combined stresses of drought and salt caused a considerable reduction in the dry weights of whole plants and leaf area per unit dry weights.

There are several reports to show contradictory results on the effect of drought and salt stress on biomass accumulation (Pollock and Cairns 1991; Hendry and Wallace 1993; Pilon-Smits *et al.* 1995; Rezaei *et al.* 2006). Some studies have reported that biomass accumulation increased (Jones and Turner 1980; Munns and Weir 1981) while others have found that it decreased (Hanson and Hitz 1982) or remained unchanged (Morgan 1992) during stress conditions. In ad-

**Table 2** Change in soil water fraction, soil EC and NaCl EC after combined treatment.

| Date        | Time       | Water Fraction by Volume WFV (%) | Soil EC (Sm <sup>-1</sup> ) | NaCl EC (Sm <sup>-1</sup> ) |
|-------------|------------|----------------------------------|-----------------------------|-----------------------------|
| 3 Oct, 2007 | 2:30 p.m.  | 35                               | 0.15                        | 0.92                        |
|             | 3:30 p.m.  | 36                               | 0.145                       | 0.85                        |
|             | 4:30 p.m.  | 34                               | 0.145                       | 0.91                        |
| 4 Oct, 2007 | 10.05 a.m. | 28                               | 0.08                        | 0.73                        |
|             | 2:00 p.m.  | 23                               | 0.08                        | 0.74                        |
|             | 6:00 p.m.  | 15                               | 0.07                        | 0.74                        |
| 5 Oct, 2007 | 10.05 a.m. | 15                               | 0.04                        | 0.64                        |

Accuracy of the Hydra Probe was ± 0.003.

dition, Al Hakimi *et al.* (1995) and Kameli and Losel (1993) presented evidence that PEG-induced drought stress increased the water soluble carbohydrate concentrations in all soybean cultivars. Reports are also there showing more accumulation of water soluble biomass found in stress resistant than in stress sensitive genotype (Martin *et al.* 2002). In addition, accumulation of solute either actively or passively is an important adaptation mechanism for plants in response to osmotic stresses like water deficit and high salinity levels. In response to this condition, many organism synthesize solute that help to retain water within cells or protect cellular components from the injury caused by dehydration (Robinson and Jones 1986; Amtmann *et al.* 2001; Blumwald 2000; Hasegawa *et al.* 2000; Yang *et al.* 2003).

In present study we just studied the osmotic potential, ions determination and osmotic adjustment values to examine the physiological response and tolerance mechanism of *Brassica* genotypes. It was found that osmotic potential of each genotype decreased in stress plants compared to unstressed plants leading to corresponding osmotic adjustment values. However, low osmotic potential and corresponding high osmotic adjustment values were found in 119463 and

lowest in 134983 genotypes. Na<sup>+</sup> ion values were high in 119463, 211363, 167532 and 908979 indicating the role of certain genotype associated with Na<sup>+</sup> ions accumulation along with other compatible osmolytes. It was presumed that *Brassica* stress tolerance is associated with ions accumulation only (Ashraf and Sharif 1997; Beltrao *et al.* 2000; Maggio *et al.* 2005) but our results suggest that genotype response with regards to combined stresses like possible accumulation of organic compounds other than ions which contributes in increase in dry weight per leaf discs, osmotic adjustment and lowering of the osmotic potential values. We found high Na<sup>+</sup> ions values and high osmotic potential and less osmotic adjustment values in some genotypes such as 908979 and 134983 suggesting the genotype tolerance are varied and not always associated with ions accumulation. Perhaps, some genotypes adopt one or several physiological mechanism to synthesize organic compounds and accumulate ions to combat combined stress as was previously recorded (Shannon and Noble 1995; Ashraf 2001, 2002; Maggio 2005). In addition, Na<sup>+</sup> ions of each genotype were significantly increased in stress compared to unstressed plants but K<sup>+</sup> and Ca<sup>++</sup> ions were less in treated genotypes compared to untreated plants. It is suggested that selectivity of ions uptake, synthesis and accumulation of metabolites are varied among plants (Cramer *et al.* 1990; Cramer 1992; Huang and Redmann 1995b; Halperin *et al.* 1997; Sze 2000; Buchanan *et al.* 2001; Cramer 2002; Mann 2002; Chartzoulakis *et al.* 2006; Iqbal and Ashraf 2007; Valdez *et al.* 2007).

Stomatal conductance of each genotype was retarded by combined stress. Stresses like salinity and drought lowers down the rate of water uptake by the roots and this is the main strategy adopted by stress tolerance genotypes (Huang and Redmann 1995b). An imbalance between water uptake by roots and water loss by transpiration causes the plant to wilt (Lafitte 2002). Therefore, plant defends itself from water loss by closing the stomata. Closing the pores certainly helps the plant to avoid fast dehydration. However, closed pores also keep the plant from exchanging carbon dioxide and oxygen between its internal tissue and outside atmospheric air. Also, stopping (or drastically reducing) the flow of water through the plant reduces the uptake of nutrients. All factors described above causes the plant to reduce its metabolism to slow the speed in order to survive the under stressful conditions like salinity and drought periods and resume growth once moisture is no longer limited (Gimenez *et al.* 1992; Gunasekera and Berkowitz 1993; Lawlor 1995; Tezara *et al.* 1999; Cornic and Fresneau 2002; Tang *et al.* 2002).

In addition, comparisons of the phenotypic responses varied significantly in the degree of change over time in relation to both plant and stresses. Studies shows that variation in plasticity among traits is most pronounced under drought but it is more obvious when drought and salt stress are provided together. For instance, floral traits of drought-stressed plants showed significantly less plasticity than leaf water potentials showing average plasticity under drought, hinting at underlying variation in sensitivity to water availability (Carroll *et al.* 2001).

In conclusion, we found variable physiological responses among the genotypes showing slow and fast growth strategy. There may be three main strategies adopted by stress resistances *B. napus* genotypes 1) they are stress deferral, 2) stress tolerance and 3) stress escape. Usually, drought and salt stress deferral plant is able to function and keep hydrated while the water content in the surrounding environment is limited or more saline. A desiccation and salt tolerance plant are able to function under conditions of drought and saline medium. While stress escape plants does all of its growth and production during the unstressed season so that they do not have to worry about stress condition. In addition, the drought causes a physical reaction by decreasing the area of a leaf. A decrease in the leaves area would cause the leaf to be able to retain more water so it does not loose as much in transpiration. These three strate-

gies can exist in each genotype in the form of slight changes in the working of various physiological parameters. So that it can be suggested that genotypes 119463 may lie in category 2 and can be categorized as fast growing while 31526, 211868, 167532 and 147554 as slow growing category 1 and 134983 and 908979 in the category 3 respectively. Further, combination of two stresses like drought and salinity produce exclusive physiological effects which could be expressed and explained by unique gene expression as was expressed in rice (unpublished data).

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