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APPRAISAL OF PHYSIOLOGICAL AND BIOCHEMICAL SELECTION CRITERIA FOR EVALUATION OF SALT TOLERANCE IN CANOLA (*BRASSICA NAPUS* L.)

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Abstract

Of *Brassica* species, canola (*Brassica napus* L.) is potentially important due to its good quality edible oil and potential to grow on salt affected areas. A greenhouse experiment was conducted to screen 34 local and exotic accessions of canola (*Brassica napus* L.) for salt tolerance and to identify suitable traits as selection criteria. Six week-old hydroponically grown plants of canola cultivars were subjected to 0 or 150 m*M* NaCl for five weeks. Various physiological and biochemical traits such as net CO_2 assimilation rate, stomatal conductance, transpiration rate, water use efficiency, leaf proline, leaf glycinebetaine, leaf Na^+, leaf K^+ and leaf K^+/Na^+ ratio, leaf osmotic potential and leaf relative water content were measured. All canola cultivars were ranked on the basis of relative salt tolerance using various physiological and biochemical attributes and then correlated with plant salt tolerance (plant growth) to identify the suitable selection criteria. Thus, the 34 lines were possible to categorize into three groups, i.e., salt sensitive, moderately salt tolerant, and salt tolerant. Dunkeld followed by Con-II, Rainbow were highly salt tolerant, while Westar, Balero, Oscar, RGS 003, Option-500 and Cyclone were salt sensitive. However, cvs BLN-877, Haanza, Goliath, and Olga were also considered potential candidates as salt tolerant cultivars. According to the analysis of linear regression of the scores of the physiological traits against those of plant growth, except leaf K^+, leaf osmotic potential and RWC, all physiological and biochemical traits were positively related with their salt tolerance. However, A and g_s were found as the most suitable determinants. Overall, photosynthetic capacity, proline and GB accumulation ability, and ion discrimination can be used as potential biochemical or physiological selection criteria for salt tolerance in canola. Although leaf Na^+, leaf K^+/Na^+ ratio, proline and GB accumulation were positively related with salt tolerance, the strength of relationship was week.

Introduction

A great deal of research has provided a lot of information on salinity tolerance of plants with the main focus on water relations, photosynthesis and accumulation of various inorganic ions and organic metabolites (Munns, 2002; 2005; Ashraf, 2004). However, these determinants of salt tolerance vary amongst species and even among cultivars due to complex nature of the mechanism of salt tolerance (Ashraf, 1994; Flowers, 2004; Munns, 2007). In some comprehensive reviews Ashraf (2004) and Ashraf & Harris (2004) reported that metabolic sites at which salt stress damages plants are still not well understood and there are no well-defined plant physiological or biochemical selection criteria that could be used for improvement of salt tolerance in crops. However, there are a number of studies which show that intra-specific genetic variability for salt tolerance can be assessed by screening large number of lines/cultivars in saline conditions,

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using attributes such as growth, photosynthetic capacity, osmotic adjustment, ion homeostasis, antioxidant enzymes, cell membrane stability etc. (Ashraf, 2004; Munns, 2002; 2007; Ashraf *et al*., 2006; Cuartero *et al*., 2006). Although the selection criteria for salt tolerance should be based on field performance of plants during full growing season (Sammons *et al*., 1978), it is well evidenced that salt tolerant plants tested under greenhouse conditions also exhibit salt tolerance in field conditions. Furthermore, because in field conditions soil salinity is more heterogeneous and occurs in patches, it is more suitable to screen plants in greenhouse conditions where saline conditions are reasonably uniform (Munns *et al*., 2003).

Rape seeds (*Brassica campestris* L. and *B. napus* L.) and mustards [*B. juncea* (L.) Czern. and Coss, and *B. carinata*] are commercially grown throughout the world as an important source of vegetable oil (Ashraf & McNeilly, 2004). Of these *Brassica* species, canola (*Brassica napus* L.) is potentially important due to low erucic acid in its oil, which makes it a good quality edible oil. However, the growth and yield of canola is adversely affected due to high salinities. Although the crop is ranked among the moderately salt tolerant crops (Francois *et al*., 1994), detailed information about genetic variability for salt tolerance is still lacking in the literature. Keeping in view all this information, an experiment was conducted to examine the genetic variability for salinity tolerance in the available germplasm of canola using a number of biochemical and physiological attributes. Furthermore, it was also assessed that whether these biochemical and physiological attributes could be used as perspective selection criteria to evaluate/screen canola cultivars for salt tolerance.

Materials and Methods

The study was carried out in the wire house of old Botanic Garden of the Department of Botany, University of Agriculture Faisalabad, Pakistan (latitude 31°30 N, longitude 73°10 E and altitude 213 m), with 10/14 light/dark period at 800-1570 μmol $m^{-2}s^{-1}$ PPFD, a day/night temperature cycle of 28/13°C and relative humidity 68±4%. The experiment was conducted in a completely randomized (CRD) factorial arrangement with three replications. Seeds of 34 canola cultivars/accessions were obtained from the National Agricultural Research Centre (NARC), Islamabad. Two hundred seeds of each canola cultivar/accession were surface sterilized in 5% sodium hypochlorite solution for 10 minutes and then thoroughly rinsed with distilled water. Seeds of each cultivar were allowed to germinate in Petri plates double lined with filter paper moistened with 10 mL of Hoagland's nutrient solution. After 8 days of germination, eight seedlings per replicate of each cultivar were grown in plastic container (45 x 66 x 23 cm) filled with continuously aerated Hoagland's nutrient solution. Six weeks after transplanting, the plants were subjected to 0 or 150 mM NaCl in Hoagland's nutrient solution. The salt level (150 m*M* NaCl) was developed in aliquots of 50 m*M* on alternate days. Nutrient solutions in all containers were replaced after every week. Five weeks after exposure to salinity, two plants per replicate were harvested and separated into shoots and roots. Plants were washed with distilled water and fresh weights recorded. Then they were oven-dried at 65°C for one week and their dry weights recorded. Before harvest, following physiological attributes were measured:

Gas exchange parameters: All gas exchange measurements such as net CO_2 assimilation rate (A), transpiration rate (E), water use efficiency (A/E), stomatal conductance (g_s), and sub-stomatal CO_2 concentration (C_i) were made using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddeson, England). These measurements were made on 3rd leaf from top of each plant from 10:00 to 14.00 hours with the following specifications/adjustments of the leaf chamber: leaf surface area 6.25 cm^2, ambient CO_2 concentration (C_{ref}) 356 µmol mol^{-1}, temperature of leaf chamber (Tch) varied from 27.2 to 34.9°C, leaf chamber volume gas flow rate (v) 296 mL min^{-1}, leaf chamber molar gas flow rate (U) 257 µmol s^{-1}, ambient pressure (P) 97.95 kPa, molar flow of air per unit leaf area (Us) 221.06 mol m^{-2} s^{-1}, *PAR* (Q leaf) at leaf surface maximum up to 1570 µmol m^{-2} s^{-1}.

Relative water contents: A fully developed and young leaf from each plant was excised and fresh weight recorded. All the samples were immersed in distilled water for 10 h and turgid weight of each leaf recorded. Then all the samples were oven dried at 70 °C for measuring dry weights. Then RWC (relative water content) was calculated using the following equation:

$$\text{Relative water content (\%)} = \frac{\text{Leaf fresh weight - Leaf dry weight}}{\text{Leaf turgid weight - Leaf dry weight}} \times 100$$

Leaf osmotic potential: A proportion of the leaf used for RWC measurements, was frozen into 2 cm^3 polypropylene tubes for two weeks at -40°C in an ultra-low freezer, thawed and the frozen sap was extracted by crushing the material with a glass rod. The sap was used directly for the determination of osmotic potential in a vapor pressure osmometer (Vapro, 5520). Leaf osmotic potential values were corrected for the dilution of the symplastic sap by apoplastic water, which occurs when the sap is expressed. Apoplastic water was considered 10% following Wilson *et al.* (1980).

Determination of proline: Proline in the leaves was determined according to the method of Bates *et al*. (1973) after extraction at room temperature with 3% 5-sulfosalicylic acid solution. The proline concentration was determined from a standard curve and calculated on fresh weight basis.

Determination of glycinebetaine: Leaf glycinebetaine was determined following Grieve and Grattan (1983). Leaf glycinebetaine was extracted from the dry leaf material with warm distilled water (70°C). The extract (0.25 ml) was mixed with 0.25 ml of 2*N* HCl and 0.2 ml of potassium tri-iodide solution. The contents were shaken and cooled in an ice bath for 90 min. Then 2.0 ml of ice cooled distilled water and 20 ml of 1-2 dichloromethane (cooled at -10°C) were added to the mixture. The two layers were formed in the mixture. The upper aqueous layer was discarded and optical density of the organic layer was measured at 365 nm. The concentrations of glycinebetaine were calculated on fresh weight basis.

Determination of mineral elements in plant tissues: The dried ground shoot material (0.1 g) was digested with sulphuric acid and hydrogen peroxide mixture according to the method of Wolf (1982). The volume of each digest was made up to 50 mL with distilled water, filtered and used for the determination of mineral elements. Na^+ and K^+ ions were determined with a flame photometer (Jenway, PFP-7).

Statistical analysis of data and ranking of canola cultivars: The data for each variable was subjected to analysis of variance using the COSTAT v 6.3, statistical software (Cohort Software, Berkeley, California). The mean values were compared with the least significance difference test following Snedecor & Cochran (1980). In order to assess suitability of various physiological and biochemical attributes as selection criteria for salt tolerance, polynomial regressions at 3rd order were applied to mean values of all parameters of salt stressed plants using MS-Excel-2003. In addition, cultivars were also ranked based on each parameter. Before ranking canola cultivars, salt tolerance indices were measured, i.e., means of each parameter of salt stressed plants divided by the means of their respective controls. Then all cultivars were ranked in 6 classes. Usually, number of groups/classes and class interval set based on range of observations and general trend of data. However, this method is only useful when number of cultivars is low in number and screening based on single parameter. Furthermore, this process becomes inaccurate when large number of cultivars (more than 10) must be screened based on multiple parameters. In the present study, all canola cultivars were grouped into six classes according to the formula: number of classes = 1.0+3.3 log10*n*, where *n* is the number of tested canola cultivars (Josef, 1985). Canola cultivars were classified into five classes. Class intervals were determined as the difference between high and low salt tolerance indices divided by the number of classes. Scores were assigned to classes and ranked into groups following Khrais *et al*. (1988) and Jolliffe *et al*. (1989) and. Classes of canola cultivars were assigned scores (1 to 6) from highest to lowest in shoot fresh and dry weight, A, g_s, C_i, WUE, RWC, proline, GB, leaf K^+, leaf K^+/Na^+ ratio, while classes having lowest to highest salt tolerance indices scores (1-6) were assigned for the parameters such as leaf Na^+, leaf osmotic potential, and transpiration (E). Relationships between the scores of shoot dry weight and the scores of various biochemical, physiological and nutrient relation parameters were analyzed by simple linear regression by using MS-Excel-2003 to assess the suitability of various biochemical and physiological parameters to use as selection criteria for salt tolerance. Furthermore, canola cultivars were grouped and ranked using JMP ver. 6, 2005 release software (SAS Institute Inc., SAS Campus Drive, Cary, NC, USA) based on Ward's minimum variance cluster analysis of the averages of the salt tolerance indices for all parameters examined in this study.

Results

A significant inter-cultivar variation for shoot fresh and dry weights has also been observed among the set of canola cultivars examined in the present study when grown under both normal and saline conditions (Fig. 1). Cultivar Duckeld, Con-I, Con-II, Hanza, BLN-877, Wild cat, and Sponsor excelled the other cultivars in fresh and dry biomass under saline conditions (Fig. 1). Although cultivar Rainbow, Olga and Goliath produced lower fresh and dry biomass compared with the above mentioned cultivars, they also had lower shoot fresh and dry biomass under normal conditions. Thus, to assess relative salt tolerance of canola cultivars, salt tolerance indices were calculated. Ranking of some cultivars remained unchanged due to this mode of evaluation. For example, on the basis of dry weight under saline conditions moderately tolerant cvs Ranibow, Olga, and Goliath were salt tolerant. Similarly, salt tolerant Con-I, Wild Cat and Sponsor were ranked as moderately salt tolerant. These results again showed a tremendous genetic variability in these growth attributes. In addition, cvs Cyclone, #2952, Option-500, Kirstina, RGS 003, Balero and Westar were ranked as salt sensitive cultivars.

Table 1. Scores for the relative salt tolerance of 34 canola cultivars on growth, gas exchange, biochemical attribute and ion contents in leaves at 150 m*M* NaCl for 5 weeks.

Canola cultivars	**Gas exchange attributes**					**Growth (Reference indicator)**		**Ranking**
	A	E	g_s	C_i	**WUE**	**Sht Fwt**	**Sht Dwt**	
Dunkled	1	1	1	1	1	1	1	Tolerant
CON-II	1	1	2	2	3	1	1	Tolerant
Rainbow	1	1	1	2	2	1	1	Tolerant
Olga	1	3	3	3	3	2	2	Tolerant
Goliath	3	4	3	3	5	2	2	Tolerant
BLN-877	2	2	3	3	4	2	2	Tolerant
Haanza	3	2	3	2	4	2	2	Tolerant
Wild cat	2	4	1	1	5	3	3	Moderate
Norseman	3	3	2	3	5	3	3	Moderate
RW 008911	4	3	2	4	5	2	3	Moderate
Rabel	3	3	5	4	4	2	3	Moderate
CON-I	2	3	3	1	4	3	3	Moderate
Sponsor	5	6	5	4	6	4	4	Moderate
Shiralee	4	2	4	5	5	4	4	Moderate
19-H	4	4	4	5	5	4	4	Moderate
Heros	5	4	3	5	6	4	4	Moderate
Hybridol	3	4	4	3	5	4	4	Moderate
Crusher	4	3	5	3	5	4	4	Moderate
20-E	4	3	4	3	5	4	4	Moderate
Profit	4	5	4	4	6	4	4	Moderate
LG-3295	4	6	4	5	6	4	4	Moderate
RAS 3/89	4	2	3	5	5	4	4	Moderate
CCS-01	5	5	6	4	5	5	5	Sensitive
Mozeri	4	5	5	4	5	5	5	Sensitive
Cyclone	5	6	6	5	6	5	5	Sensitive
#29-52	4	5	5	4	5	5	5	Sensitive
Oscar	5	5	5	4	6	5	5	Sensitive
Excel	5	5	6	4	6	5	5	Sensitive
Quest	5	4	5	6	5	5	5	Sensitive
Option-500	5	5	5	6	6	5	5	Sensitive
Kristina	6	6	6	5	6	6	6	Sensitive
RGS 003	6	6	5	5	6	5	6	Sensitive
Balero	5	6	6	5	6	6	6	Sensitive
Westar	6	6	6	6	6	6	6	Sensitive

Net CO_2 assimilation rate (A) of all cultivars reduced significantly ($P \leq 0.001$) due to imposition of salt stress. Cultivars differed significantly in this attribute but most of them were similar in behavior under saline conditions (Fig. 2) except a few where Dunkeld followed by Rainbow, Con-II, and Con-I again exhibited maximum photosynthetic rate. Furthermore, minimum photosynthetic rate under saline conditions was found in Cyclone followed by Excel, Option-500 and RGS 003. However, on the basis of salt tolerance indices, cvs Dunkled, Con-II, Rainbow, Olga, BLN-877, Wild Cat and Con-I were ranked as salt tolerant, while Cyclone, Option-500, RGS 003, and Westar ranked as salt sensitive (Table 1).

Table 2. Scores for the relative salt tolerance of 34 canola cultivars on growth, gas exchange, biochemical attribute and ion contents in leaves at 150 m*M* NaCl for 5 weeks.

Canola Cultivars	Gas exchange attributes					Proline	GB	Growth	
	Na^+	K^+	K^+/Na^+ ratio	RWC	OP			Sht dwt	Ranking
Dunkled	1	1	1	1	3	2	1	1	Tolerant
CON-II	2	1	3	5	4	2	1	1	Tolerant
Rainbow	2	2	3	2	3	3	2	1	Tolerant
Olga	2	4	4	5	4	3	4	2	Tolerant
Goliath	3	6	6	3	4	1	3	2	Tolerant
BLN-877	3	2	5	5	2	3	3	2	Tolerant
Haanza	1	3	3	1	4	1	3	2	Tolerant
Wild cat	3	1	4	5	4	5	1	3	Moderate
Norseman	3	4	5	5	1	2	3	3	Moderate
RW 008911	2	6	5	2	5	2	4	3	Moderate
Rabel	3	6	6	6	4	4	2	3	Moderate
CON-I	1	1	2	1	5	3	2	3	Moderate
Sponsor	3	1	3	4	6	4	2	4	Moderate
Shiralee	4	3	5	6	3	3	3	4	Moderate
19-H	1	6	5	4	1	5	2	4	Moderate
Heros	2	5	5	3	4	4	5	4	Moderate
Hybridol	3	4	5	6	5	4	2	4	Moderate
Crusher	3	2	4	2	5	6	5	4	Moderate
20-E	2	4	4	1	2	4	5	4	Moderate
Profit	3	5	6	5	1	5	5	4	Moderate
LG-3295	5	3	6	4	4	5	1	4	Moderate
RAS 3/89	2	6	5	6	6	5	5	4	Moderate
CCS-01	3	2	4	6	1	3	3	5	Sensitive
Mozeri	3	5	5	6	2	6	6	5	Sensitive
Cyclone	6	2	6	5	6	6	4	5	Sensitive
#29-52	4	6	6	4	3	6	4	5	Sensitive
Oscar	4	5	6	4	1	5	6	5	Sensitive
Excel	4	2	5	5	4	5	6	5	Sensitive
Quest	2	5	5	4	3	4	4	5	Sensitive
Option-500	6	6	6	6	2	4	6	5	Sensitive
Kristina	5	5	6	3	5	5	3	6	Sensitive
RGS 003	4	5	6	4	3	4	6	6	Sensitive
Balero	5	6	6	6	4	5	5	6	Sensitive
Westar	5	5	6	6	6	4	4	6	Sensitive

Maximum values of stomatal conductance were found in the salt stressed plants of Dunkeld, Con-II, Con-I, Rainbow, and Wild Cat, while the lowest stomatal conductance was observed in Cyclone followed by Oscar and Kirstina. Furthermore, almost similar pattern of salt tolerance was observed among canola cultivars when cultivars were ranked on the basis of stomatal conductance (Table 1).

There was consistent pattern of increase or decrease in *E* with the change in salt tolerance ranking. However, cvs Dunkled, Con-II, Rainbow and Shiralee had higher transpiration indices than the other cultivars, while cvs Cyclone, Oscar and RGS 003 had lower transpiration indices.

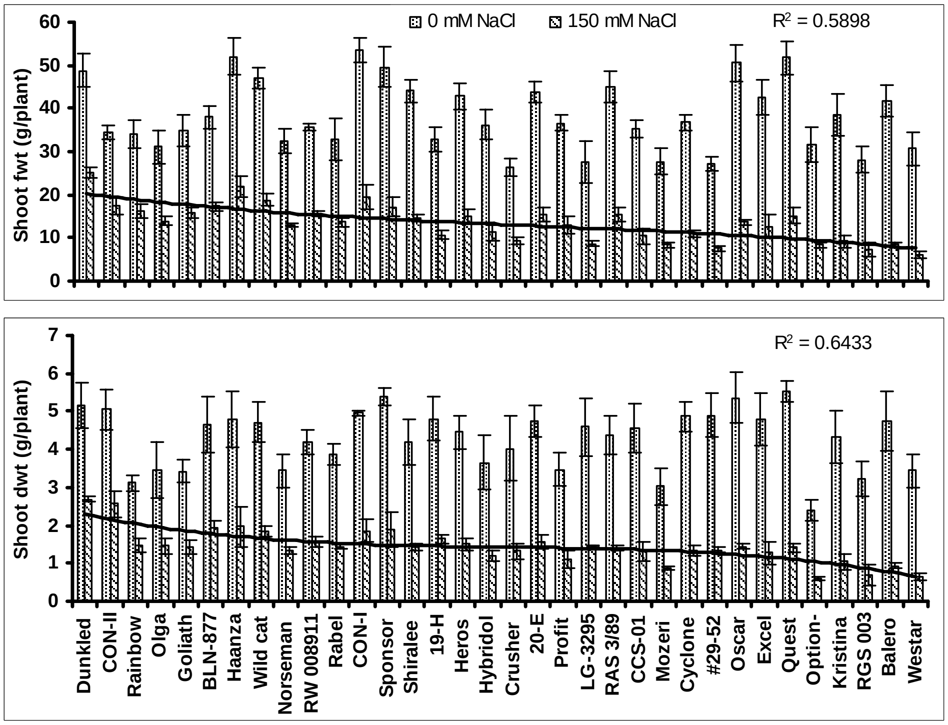

Fig. 1 Shoot fresh and dry weight (g/plant) of 34 canola cultivars when grown in nutrient solution containing 0 or 150 m*M* NaCl, for 5 weeks (n = 3).

Maximum value for sub-stomatal CO_2 concentration tolerance index was observed in Dunkeld followed by Con-I and Wild Cat, while the lowest value was observed in Quest, Option-500 and Westar.

Although cv. Con-II showed maximum WUE (*A/E*) in salt stressed plants compared with all other cultivars (Fig. 2), Dunkeld followed by Rainbow exhibited maximum WUE tolerance index. While most of the cultivars exhibited lower WUE tolerance index (Table 1).

Salt stress caused increase in leaf Na^+ with a concomitant decrease in leaf K^+ of all cultivars. Cultivars also differed significantly in these attributes. In salt stressed plants, maximum Na^+ accumulation in leaves was found in Westar, while minimum Na^+ accumulated in Dunkeld, Con-I, Crusher and Profit (Fig. 4). However, on the basis of Na^+ tolerance index, Dunkeld, Con-I, Haanza and 19-H were ranked as tolerant, while those of Option-500 and Cyclone ranked as sensitive (Table 2). In contrast, maximum leaf K^+ was observed in Con-I followed by Dunkled and CON-II under saline conditions, while that in 19-H leaf K^+ was minimum. In addition, Dunkeld, Con-I, Con-II, Wild Cat, and Sponsor ranked as tolerant, while Option-500, #29-52, and Balero ranked as sensitive (Table 2).

Maximum leaf K^+/Na^+ ratio was observed in Dunkeld followed by Con-I, Wild Cat and Crusher, while low leaf K^+/Na^+ ratio was observed in Option-500, Oscar and Cyclone. In addition, on the basis of leaf K^+/Na^+ ratio tolerance index Dunkeld followed by Con-I was ranked as tolerant, while most of the cultivars were ranked as sensitive (Table 2).

Table 3. Equations of linear regression, slopes and regression coefficients between the scores on shoot dry matter and the scores on physiological, biochemical and ion contents in the leaves at 150 m*M* NaCl for 5 weeks.

Physiological/ biochemical/ nutrient accumulation attribute	Regression equation	Slope	r^2
Shoot fwt	y = 0.9925x - 0.0597	0.992	0.962***
Net assimilation rate (*A*)	y = 0.9245x + 0.2297	0.925	0.840***
Stomatal conductance (g_s)	y = 0.8997x + 0.5307	0.899	0.727***
Transpiration rate (*E*)	y = 0.9088x + 0.437	0.908	0.691***
Water use efficiency (*A*/*E* = WUE)	y = 0.6833x + 2.2993	0.683	0.679***
Sub-stomatal CO_2 (C_i)	y = 0.7297x + 1.0041	0.729	0.576***
Leaf proline	y = 0.641x + 1.461	0.641	0.450**
Leaf Na^+	y = 0.6136x + 0.7421	0.613	0.425**
Leaf K^+/Na^+ ratio	y = 0.5721x + 2.5771	0.572	0.413**
Leaf glycinebetaine (GB)	y = 0.6534x + 1.0605	0.653	0.350*
Leaf K^+	y = 0.5348x + 1.7786	0.534	0.182ns
Leaf relative water content (RWC)	y = 0.4494x + 2.4287	0.449	0.152ns
Leaf osmotic potential	y = 0.0307x + 3.4121	0.031	0.0008ns

In plants grown in saline solution, highest leaf RWC was found in Dunkeld, Con-I, while CCS-01 and RAS 3/89 were lowest in RWC. However, on the basis of RWC tolerance index, Dunkeld, Con-I and Haanza were ranked as tolerant, while Westar, Balero and Option-500 ranked as sensitive. In contrast, for leaf osmotic potential Noreseman, 19-H, Profit, CCS-01 were ranked as tolerant, while Sponsor, RAS 3/89, and Westar as sensitive (Table 2; Fig. 4).

Both proline and glycinebetaine accumulation increased significantly in the leaves of all cultivars under saline conditions. Cultivars also differed significantly in both these biochemical attributes. Under saline conditions, highest proline accumulation was found in Dunkled followed by CON-II and Rainbow, while Crusher and Mozeri accumulated minimum proline. In contrast, on the basis of proline tolerance index, Goliath and Haanza ranked as tolerant, while Crusher, Mozeri, Cyclone and #29-52 ranked as sensitive. However, accumulation of GB was maximum in Con-I, Wild Cat, Sponsor and Dunkeld, while Oscar followed by Excel and 20-E had lower accumulation of GB. In addition, Dunkeld, Con-II, Wild Cat, and LG 32-95 were ranked as tolerant, and Mozeri, Oscar, Excel, Option-500 and RGS 003 as sensitive. All other cultivars were intermediate in response.

The suitability of various physiological, biochemical and ion accumulation parameters as selection criteria for salt tolerance in canola was further assessed by drawing relationships between the scores of physiological traits and shoot dry biomass using linear regression (Table 3; Fig. 5). The slope of equation reveals the degree of genetic variation for a given parameter. Thus, if the regression coefficient is significant with greater variation for a given parameter, it could be used as a selection criterion. From the results of the present study, the scores of *A*, g_s, C_i, *E*,and WUE were significantly correlated with the scores of shoot dry biomass (Table 3). However, proline, leaf Na^+, K^+, and K^+/Na^+ ratio and GB, RWC, and leaf osmotic potential in the leaves were not significantly correlated with shoot dry biomass.

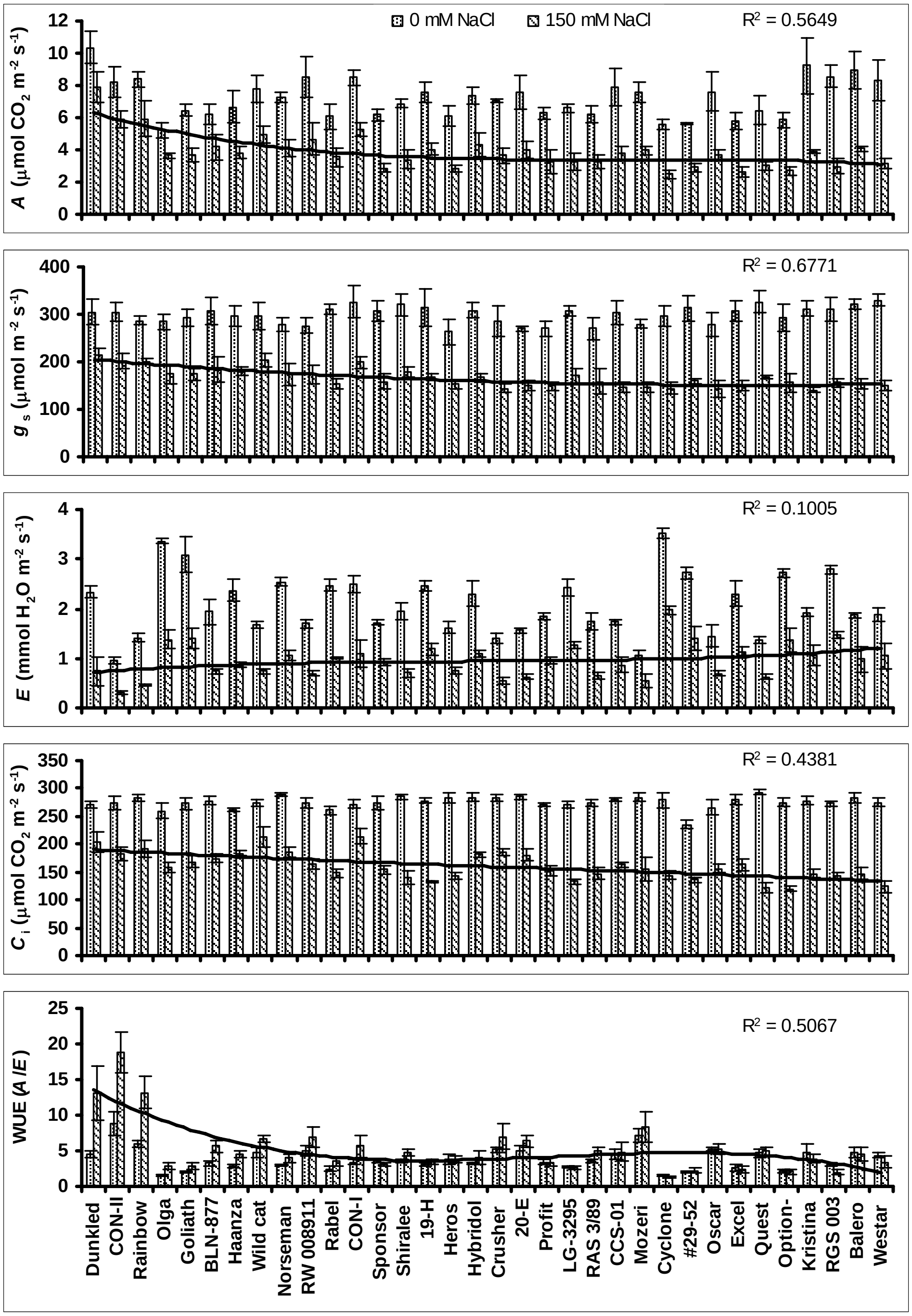

Fig. 2. Gas exchange attributes of 34 canola cultivars when grown in nutrient solution containing 0 or 150 m*M* NaCl, for 5 weeks (n = 3).

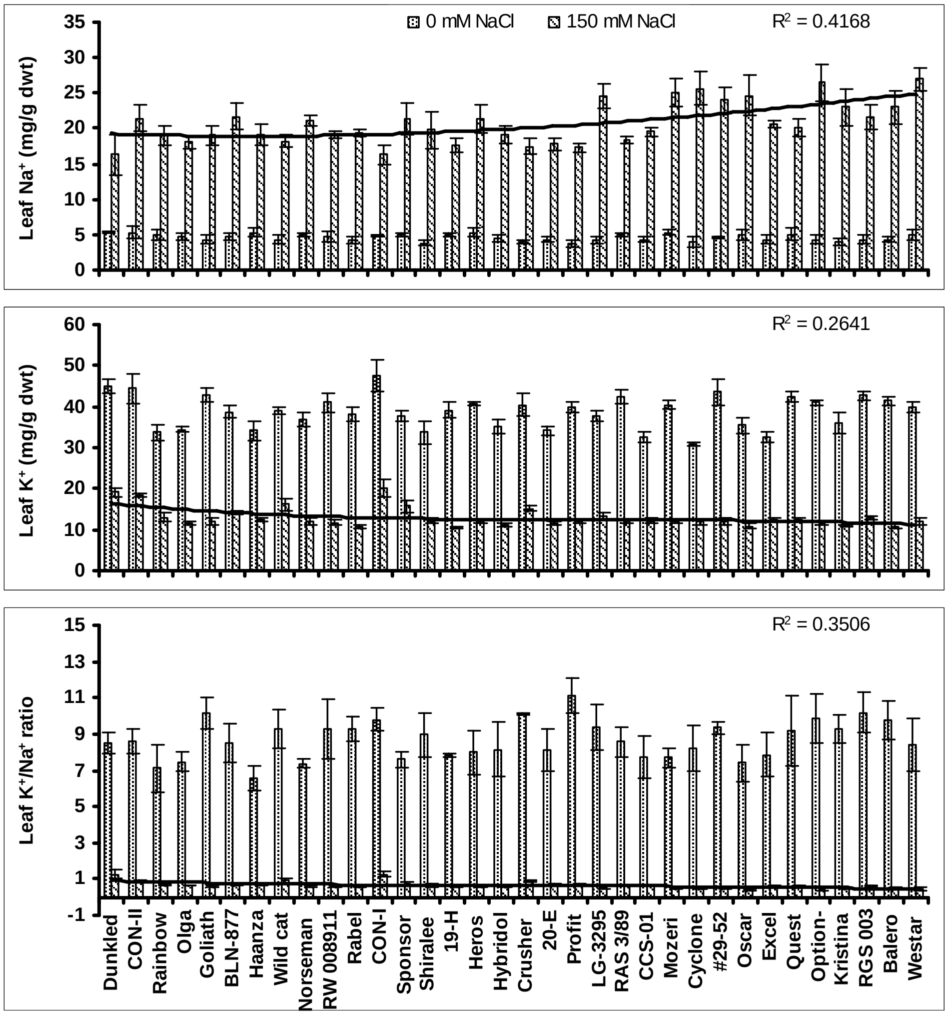

Fig. 3. Leaf ion accumulation of 34 canola cultivars when grown in nutrient solution containing 0 or 150 m*M* NaCl, for 5 weeks (n = 3).

Discussions

In the present study first genetic variation for salt tolerance in canola cultivars was assessed and the cultivars were ranked on the basis of relative shoot dry biomass production under saline conditions. Then suitability of any biochemical or physiological indicator for salt tolerance was assessed with reference to shoot biomass production, because it has been widely accepted that plant growth determines crop yield. From the results of the present study, salt stress reduced the plant growth of all canola cultivars but a considerable variation for salt tolerance was observed in the canola cultivars. For example, it was found that Dunkeld had maximum shoot fresh and dry weights under

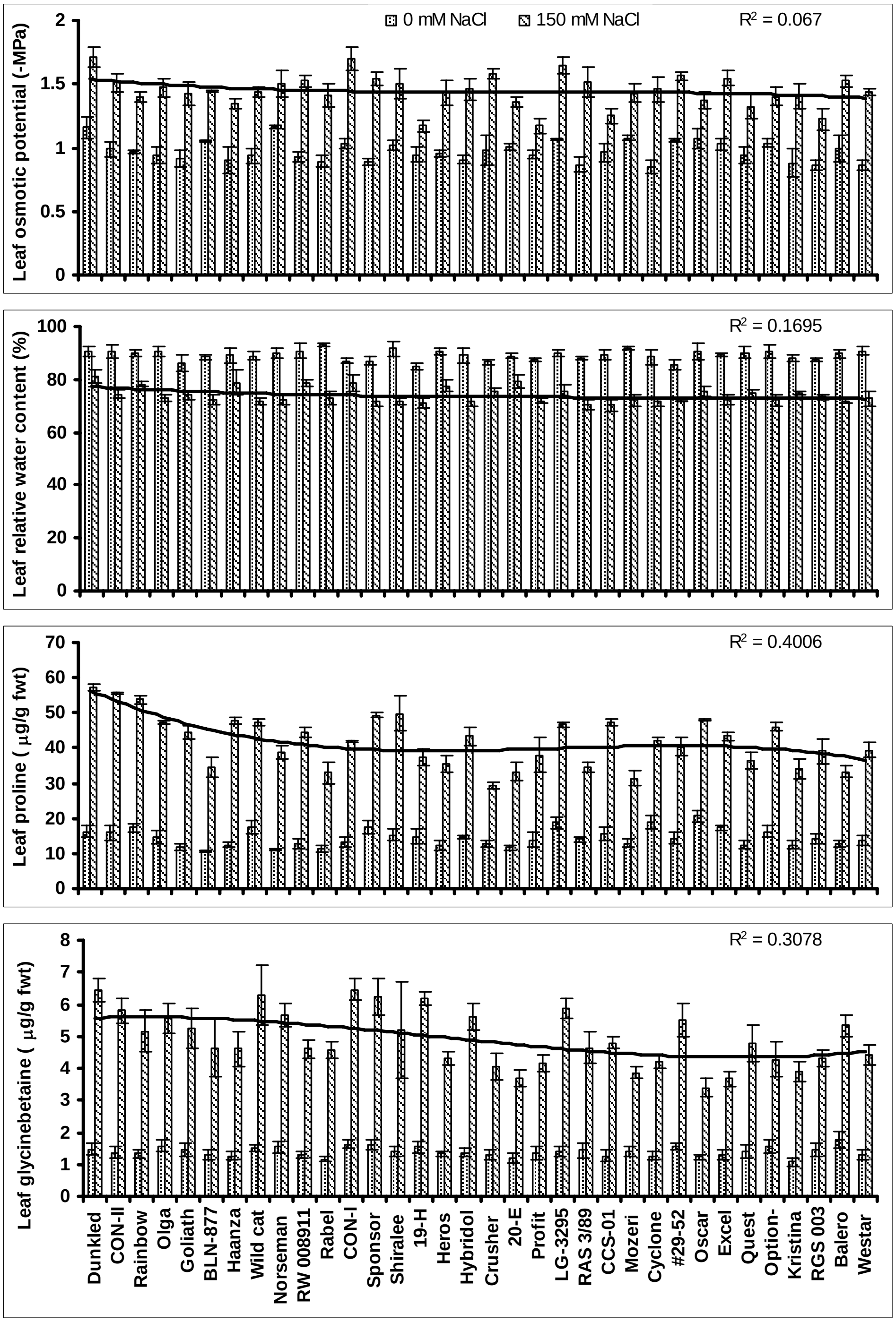

Fig. 4. Leaf water relations and accumulation of compatible solute capacity of 34 canola cultivars when grown in nutrient solution containing 0 or 150 m*M* NaCl, for 5 weeks (n = 3).

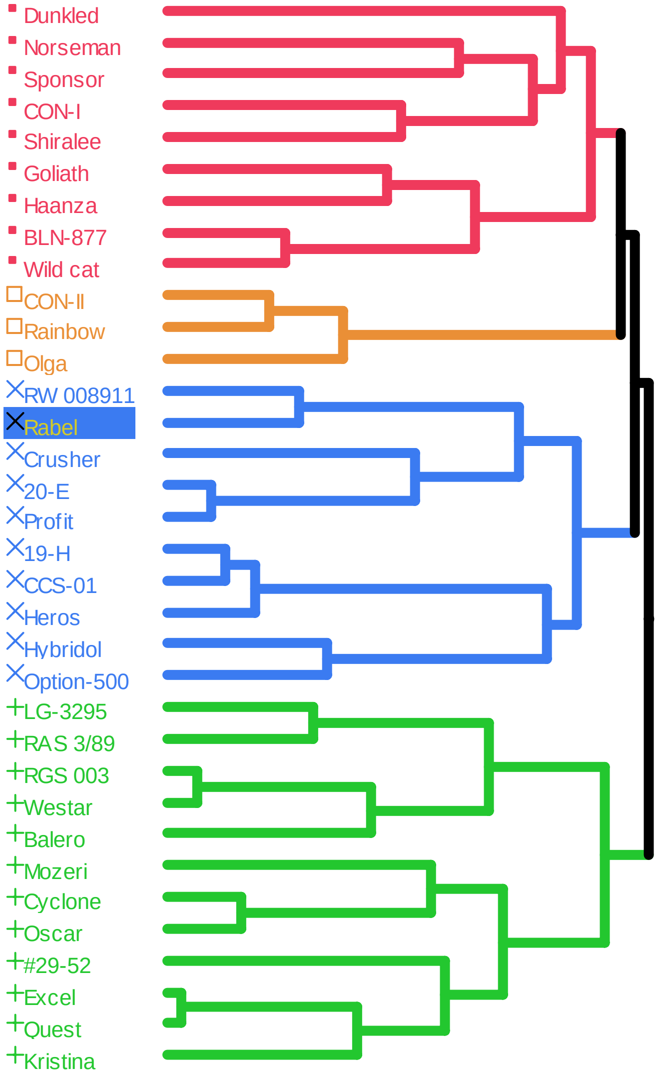

Fig. 5. Cluster analysis of the averages of salt tolerance indices of canola cultivars.

saline conditions and thus it could be used as a reference cultivar for salt tolerance in canola (Fig. 1; Table 1). Furthermore, cvs. Con-II and Rainbow were also ranked as salt tolerant compared with other canola cultivars. However, cvs BLN-877, Haanza, Goliath, and Olga were also considered potential candidates as salt tolerant cultivars (Table 1). While Cyclone, #29-52, Option-500, Oscar, Kistina, RGS 003, Balero and Westar were ranked as salt sensitive (Table 1).

A considerable genetic variation for salt tolerance in canola cultivars observed in the present study might have been due to variation in a number of biochemical or physiological traits under saline conditions such as photosynthesis, nutrient imbalance, accumulation of compatible solutes, enzyme activities, hormonal imbalance etc., which mainly contribute in crop growth and productivity. In view of this information available in the literature, generally Na^+ exclusion, K^+/Na^+ ratio, leaf water relations, accumulation of compatible solutes and photosynthesis were used for screening germplasms for salinity tolerance (Munns & James, 2003; Ashraf, 2004). Although lack of effective biochemical or physiological indicators for salt tolerance is one of reasons for limited success in enhancing crop yield under saline conditions through breeding programs, it is still necessary to identify crop specific biochemical or physiological indicators for salt tolerance, which should be reliable and economically viable (Ashraf & Harris, 2004; Juan *et al*., 2005). Of various physiological attributes, assessment of photosynthetic capacity is very important as it directly contributes to plant productivity (Lawlor, 2002). Furthermore, photosynthetic rate, stomatal conductance, and transpiration rate in leaves can be measured by a non-destructive, rapid and easy technique using “infra red gas analysis”. In the present study, all gas exchange attributes were positively correlated with salt tolerance in terms of plant growth. A significant genetic variation in all gas exchange attributes was also observed (Table 3). However, positive relationship between A and $g_{s,}$ and salt tolerance were higher than the other gas exchange attributes examined. In addition, genetic variation for A and g_s among canola cultivars was also greater than the other gas exchange attributes. Highly significant correlation between A and g_s or C_i also emphasizes that A and g_s could be used as effective selection criteria for salt tolerance in canola. This view was further supported by some earlier studies in which it has been found that salt tolerant wheat cultivar had higher photosynthetic rate than the salt sensitive cultivar (Raza *et al*., 2006; 2007; Arfan *et al*., 2007). While working with modern and obsolete cotton cultivars, Faver *et al*. (1997) suggested that improvements in cotton yield may be achieved through enhanced assimilatory process in modern cultivars. Similarly, Faville *et al*. (1999) found that rate of photosynthesis had a positive association with the crop yield of *Asparagus*. In view of the above-mentioned reports and the results of the present study, it is obvious that crop improvement against salt stress can be achieved by selecting cultivars with higher photosynthetic rate.

It is now well evident that glycophytes respond to salt stress by partial exclusion of ions and the synthesis of organic osmotica for osmotic adjustment. Therefore, in a number of crop species, Na^+ exclusion, and leaf K^+/Na^+ ratios have been suggested to be reliable traits for selecting salt tolerant crops (Munns & James 2003; Ashraf, 2004; Poustini & Siosemardeh 2004; Chen *et al*., 2005). However, for different crop species selection criteria for salt tolerance will be different (Ashraf, 2004). Therefore, it is necessary to identify those traits that are associated with salinity tolerance in canola. In the present study, leaf Na^+ and K^+/Na^+ ratio appeared to be more closely related with shoot dry biomass ($r^2 = 0.425^*$; 0.413^* respectively). These results suggest that plants with better ability to exclude Na^+ from the leaves had highest leaf K^+/Na^+ ratio. It can be inferred from the findings of the present study that regulation of Na^+ with respect to K^+ uptake could be one of the possible mechanism of salt tolerance. These results can be related to the findings of Haq *et al*. (2002) who found in a field experiment conducted on naturally salt affected soils that the degree of salt tolerance in four *Brassica* species (relatively salt tolerant, *B. napus* and *B. carinata*; salt sensitive, B. *campestris* and *B. juncea*) was associated with the ability to exclude both Na^+ and Cl^-. Although leaf Na^+ and leaf K^+/Na^+ ratio are positively related with plant growth, the magnitude of this relationship (value of r^2) is low compared with those of photosynthetic attributes (Table

3). Secondly, genetic variation in these attributes are low as reflected from their slope values compared with those of photosynthetic attributes (Table 3). Furthermore, several mechanisms including net Na^+ uptake at root level, net Na^+ loading in xylem etc. may control leaf Na^+ accumulation in salt tolerant cultivars (Tester & Davenport, 2003). However, in some instances K^+/Na^+ ratio is more important than simply maintaining low leaf Na^+ (Tester & Davenport, 2003; Munns *et al.*, 2006). For example, a salt sensitive cultivar Quest had no relationship with the accumulation of Na^+ in leaves, while its salt sensitivity was strongly associated with leaf K^+/Na^+ ratio (Table 3). In addition, although the transpiration rate is one of the important controlling factors for the accumulation of salt ions in shoots (Tester & Davenport, 2003), the degree of association between the scores on leaf transpiration rate and plant growth among the tested canola cultivars was low compared with those of A and g_s, as has earlier been observed in leaf Na^+ and leaf K^+. A few years back, in a comprehensive review on suitability of physiological selection criteria for salt tolerance Ashraf (2004) suggested that a physiological trait could only be used as marker if strength of relationship between marker and plant response to salinity is high. Therefore, these ionic relation parameters could be used as selection criteria for salt tolerance, but with some caution. Furthermore, because both these traits are controlled by a number of genes (Munns, 2005), it should be further investigated to identify accurate selection criteria for salt tolerance related to ionic relations.

From the results of the present study, it is clear that there is no significant relationship between salt tolerance and RWC or leaf osmotic potential. However, leaf proline and leaf glycinebetaine accumulation appeared to be positively correlated ($P \leq 0.05$) with salt tolerance. Furthermore, genetic variation among cultivars for these attributes is appreciably significant. However, the strength of relationship among these biochemical attributes and salt tolerance. Thus, leaf proline or leaf GB accumulation can also be considered as selection criteria.

In conclusion, all physiological and biochemical attributes examined in the present study, except leaf K^+, RWC, and leaf osmotic potential, showed a substantial amount of genetic variation indicating that these traits may possible be used as selection criteria. However, of all selection criteria, the scores of A and g_s are closer to their plant growth compared with other physiological or biochemical traits, suggesting that A and g_s are more reliable screening criteria. Furthermore, measurements of A and g_s on a large scale is also non-destructive, repeatable, and economically viable strategy.

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References

Arfan, M., H.R. Athar and M. Ashraf. 2007. Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress? *J. Plant Physiol.*, doi:10.1016/jplph.2006.0510.010.

Ashraf, M. and P.J.C. Harris. 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci*., 166: 3-16.

Ashraf, M. 1994. Breeding of salinity tolerance in plants. *Crit. Rev. Plant Sci*., 13: 17-42.

Ashraf, M. 2004. Some important physiological selection criteria for salt tolerance in plants. *Flora*, 199: 361-376.

Ashraf, M. and T. McNeilly. 2004. Salinity tolerance in *Brassica* oilseeds. *Crit. Rev. Plant Sci.*, 23 (2): 157-174.

Ashraf, M., Q. Ali, and Z. Iqbal. 2006. Effect of nitrogen application rate on the content and composition of essential oil and minerals in black cumin (*Nigella sativa* L.) seeds. *J. Sci. Food Agric*., 86(6): 871-876.

Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water stress studies. *Plant Soil,* 39: 205-207.

Chen, Z., I. Newman, M. Zhou, N. Mendham, G. Zhang and S. Shabala. 2005. Screening plants for salt tolerance by measuring K^+ flux: a case study for barley. *Plant Cell Environ*., 28: 1230-1246.

Cuartero, J., M.C. Bolarin, M.J. Asins and V. Moreno. 2006. Increasing salt tolerance in the tomato. *J. Exp. Bot*., 57: 1045–1058.

Faver, K.L., T.J. Gerik and R.G. Percy. 1997. Modern Pima cotton cultivars have higher photosynthetic capacity than obsolete varieties. In: The proceedings of Beltwide cotton conference, New Orleans, LA, USA, 2: 1499–502.

Faville, M.J., W.B. Silvester, T.G. Allan Green and W.A. Jermyn. 1999. Photosynthetic characteristics of three asparagus cultivars differing in yield. *Crop Sci*., 39: 1070-1077.

Flowers, T.J. 2004. Improving crop salt tolerance. *J. Exp. Bot*., 55: 307-319.

Francois, L.E., T.J. Donovan, E.V. Mass, and S.M. Lesch. 1994. Time of salt stress affects growth and yield components of irrigated wheat. *Agron. J*., 86: 100-107.

Grieve, C.M. and S.R. Grattan. 1983. Rapid assay for determination of water-soluble quaternary-amino compounds. *Plant Soil*, 70: 303-307.

Haq, T.U., J. Akhtar, A.U. Haq and M. Hussain. 2002. Effect of soil salinity on the concentration of Na^+, K^+ and Cl^- in the leaf sap of the four *Brassica* species. *Int. J. Agric. Biol*., 4: 385-388.

Jollife, I.T., O.B. Allen and B.R. Christie. 1989. Comparison of variety means using cluster analysis and dendrograms. *Exp. Agric*., 25: 259–269.

Josef, E.V. 1985. Deskriptive Statistik mit einem Anhang Zur Bev¨olkerungs- und Erwerbsstatistik. Eckart Bomsdorf., durchgesehene Auflage-Lohmar, K¨oln (In German).

Khrais, T., Y. Leclerc and D.J. Donnelly. 1988. Relative salinity tolerance of potato cultivars assessed by in vitro screening. Am. J. Potato Res., 75: 207–210.

Lawlor, D.W. 2002. Limitation to photosynthesis in water stressed leaves: stomata versus metabolism and the role of ATP. *Ann. Bot*., 89: 1-15.

Juan, M., R.M. Rivero, L. Romero and J.M. Ruíz. 2005. Evaluation of some nutritional and biochemical indicators in selecting salt-resistant tomato cultivars. *Env. Exp. Bot*., 54: 193-201.

Munns, R., R.A. James and A. Lauchli. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot*., 5(57): 1025–1043.

Munns, R. 2002. Comparative physiology of salt and water stress. *Plant Cell Environ*., 25: 239-250.

Munns, R. 2005. Genes and salt tolerance: bringing them together. *New Phytol*., 167(3): 645.

Munns, R. and R.A. James. 2003. Screening methods for salinity tolerance: a case study with tetraploid wheat. *Plant Soil*, 59: 1-18.

Munns, R., 2007. Utilizing genetic resources to enhance productivity of salt-prone land. CAB Rev.: Perspectives in Agric. Veterinary Sci. Nutr. Nat. Res., 2. No. 009.

Munns, R., G.J. Rebetzke, S. Hussain, R.A. James and R.A. Hare. 2003. Genetic control of sodium exclusion in durum wheat. *Aust. J. Agric. Res*., 54: 627-635.

Poustini, K., and A. Siosemardeh. 2004. Ion distribution in wheat cultivars in response to salinity stress. *Field Crops Res*., 85: 125-133.

Raza, S.H., H.R. Athar and M. Ashraf. 2006. Influence of exogenously applied glycinebetaine on the photosynthetic capacity of two differently adapted wheat cultivars under salt stress. *Pak. J. Bot*., 38(2): 341-351.

Raza, S.H., H.R. Athar, M. Ashraf and A. Hameed. 2007. Glycinebetaine-induced modulation of antioxidant enzymes activities and ion accumulation in two wheat cultivars differing in salt tolerance. *Env. Exp. Bot*., 60: 368-376.

Sammons D.J., D.B. Peters, D.B., T. Hymowitz. 1978. Screening soybeans for drought resistance. I. Growth chamber procedure. *Crop Sci.*, 18: 1050-1055.

Snedecor, G.W. and W.G. Cochran. 1980. Statistical methods. 7th Edition Iowa State University Press, AMES, Iowa.

Tester, M. and R. Davenport. 2003. Na^+ tolerance and Na^+ transport in higher plants. *Ann. Bot.,* 91(5): 503-527.

Wilson, J.R., M.M. Ludlow, M.J. Fisher and E.D. Schulze. 1980. Adaptation to water stress of the leaf water relations of four tropical forage species. *Aust. J. Plant Physiol*., 7: 207–220.

Wolf, B., 1982. A comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. *Commun. Soil Sci. Plant Anal*., 13: 1035-1059.

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