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ARTICLE

Seedling, early vegetative, and adult plant growth of oilseed rapes (*Brassica napus* L.) under saline stress

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Abstract: Salinity is a major limiting factor for early crop establishment and yield. In this study, 131 *Brassica napus* genotypes were evaluated for germination and early seedling growth in Murashige & Skoog medium supplemented with NaCl. Selected genotypes were then evaluated for tolerance to salinity at the vegetative and reproductive stages in greenhouse and semi-hydroponic systems using 1.4, 5, 10, 15, 20, and 28 dS m^{-1} salt stress. Relative salt tolerance (RST) was calculated and compared with genotype performance under no salt stress (control). The area under the germination progress curve (AUGPC) varied from 53 to 90 in the control and from 6 to 89 under 200 mmol L^{-1} NaCl stress. The seedling vigor index (SVI) ranged from 200 to 1606 and 10 to 736 in the control and 200 mmol L^{-1} salt stress treatments, respectively. The RST for germination, root length, shoot length, and SVI ranged from 8% to 97.7%, 2% to 98.3%, 6.5% to 70.8%, and 1.9% to 83%, respectively. Root length was most severely affected by saline conditions, followed by shoot length and AUGPC, when RST percentages of these traits were compared among responses of the 131 genotypes. Genotypes showed varying levels of proline and glucosinolate accumulation under different levels of saline stress. Greater accumulation of proline and glucosinolates was recorded with increased salinity level. This study indicates that variation exists in seedling and adult plant responses to saline stress in *B. napus* genotypes and that improvement for salinity tolerance requires selection at the seedling, vegetative, and reproductive plant stages.

Key words: Brassica napus, canola, glucosinolate, oilseed rape, proline, salinity, seedling vigor, tolerance.

Résumé : La salinité est un important facteur qui nuit à une implantation rapide et au rendement des cultures. Dans le cadre de cette étude, les auteurs ont évalué la germination et la croissance des plantules de 131 génotypes de *B. napus* sur un milieu de culture Murashige Skoog enrichi de NaCl. Ensuite, ils ont déterminé la tolérance au sel des génotypes retenus aux stades végétatif et reproductif, en serre et avec un système semi-hydroponique engendrant un stress salin de 1,4, 5, 10, 15, 20 ou 28 dS par mètre. Les chercheurs ont calculé la tolérance relative au sel (TRS), puis l'ont comparée à la performance des génotypes non soumis à un stress salin (témoins). La surface sous la courbe représentant l'évolution de la germination (AUGPC) varie de 53 à 90 po pour les témoins et de 6 à 89 po pour les plantules soumises à un stress de 200 mM de NaCl. L'indice de la vigueur des plantules (IVP) varie respectivement de 200 à 1606 et de 10 à 736 pour les témoins et les plantules assujetties au stress salin. La TRS à la germination, la longueur des racines, la longueur de la pousse et l'IVP varient respectivement de 8% à 97,7%, de 2% à 98,3%, de 6,5% à 70,8% et de 1,9% à 83%. Quand on compare la TRS des 131 génotypes pour les différents paramètres, on constate que la longueur des racines est la plus affectée par la salinité. Viennent ensuite la longueur de la pousse et l'AUGPC. Les génotypes accumulent une quantité variable de proline et de glucosinolates, selon l'ampleur du stress salin. Plus ce dernier s'intensifie et plus la quantité de proline et de glucosinolates augmente. Ces résultats indiquent que la réaction au stress salin varie chez les plantules et les plants adultes des génotypes de *B. napus* et qu'on pourrait

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améliorer la tolérance au sel en procédant à une sélection au niveau de la plantule, ainsi que des stades végétatif et reproductif. [Traduit par la Rédaction]

Mots-clés : Brassica napus, canola, glucosinolate, colza, proline, salinité, vigueur des plantules, tolérance.

Introduction

Canola or oilseed rape (*Brassica napus* L.) is third most important oilseed crop worldwide after soybean and cotton, and accounts for 12% of the total annual global oil production (FAOSTAT 2014). Various biotic and abiotic stresses limit successful cultivation of canola, with salinity being one of the major abiotic factors limiting production (Puppala et al. 1999; Ashraf 2001; Qasim et al. 2003). Over 800 million ha of land is under salinity stress, accounting for 6% of the total cultivated land on earth (Arzani 2008; Munns and Tester 2008). It has been reported that 20%–27% of the world's irrigated land is also under some level of salinity stress (Ghassemi et al. 1995). Saskatchewan accounts for approximately 40% of Canada's total arable land, with approximately 15 M ha sown to field crops; however, approximately 250 000 ha are considered to be agriculturally non-productive due to high salinity, with a much larger area being unproductive due to some degree of salinity (Government of Saskatchewan). The majority of the Canadian canola crop is also grown in Saskatchewan, approximately 5 M ha annually (Statistics Canada 2017); however, saline soil and saline irrigation water pose potential hazards to canola production (Puppala et al. 1999).

Salt tolerance is a complex phenomenon in plants, as various mechanisms and their interactions regulate salt tolerance at the cellular, tissue, organ, and whole-plant levels. Generally, germination and seedling vigor are considered to be highly sensitive to saline conditions, which lead to a considerable reduction of yield and biomass production (Hamdy et al. 1993). Indeed, salt stress reduces germination and establishment of seedlings in many plant species (Fowler 1991; Khan and Ungar 1999; Guma et al. 2010; Jamila et al. 2010; Zivdar et al. 2011), including *B. napus* (Puppala et al. 1999; Ashraf 2001; Qasim et al. 2003). *Brassica napus* seed germination decreases sharply under salinity stress, from 87% at 0 dS m^{-1} to 0.8% at 26 dSm^{-1} (Puppala et al. 1999). Generally, genotypes possessing better germination and seedling growth (seedling vigor) under salinity stress are also more tolerant to salinity in later growth stages (Ashraf 2001). Ashraf (2001) showed genotypic differences in shoot biomass among different Brassica species, whereas Qasim et al. (2003) reported salt-induced changes in canola cultivars, with Dunkeld being salt-tolerant and Cyclone being salt-sensitive in terms of shoot biomass and seed production. A positive correlation between salinity tolerance at the seedling and reproductive plant stages in *B. napus* has been reported (Ashraf 2001; Ashraf and Ali 2008). Therefore, the assessment of salinity tolerance at the seedling stage, specifically germination and seedling growth (root and shoot growth), may reflect the ability of genotypes to tolerate salinity at later growth stages. However, evidence from other species indicates that salt tolerance is a developmentally regulated and growth stage specific phenomenon (Shannon 1986). The objective of the current study was to identify salinity-tolerant *B. napus* genotypes from a world collection at multiple developmental stages (seedling, vegetative, and adult plant).

Materials and Methods

Plant material

Initially, approximately 200 *B. napus* accessions from the Plant Gene Resources of Canada (PGRC), Saskatoon, SK, were selected from widely different geographical regions, countries, and continents. Each line was selfed once and seed from a single descendant line was used in this study to increase uniformity. Lines were grown in a greenhouse with a 16 h light–8 h dark photoperiod at 20 °C ± 2 °C day/16 °C ± 2 °C night conditions. All genotypes were bagged to avoid any cross pollination. Seeds were stored in a controlled-environment seed storage facility at 4 °C at PGRC and used within 12–24 mo. A total of 131 genotypes were used for the salinity tolerance study, as these produced a sufficient quantity of seed. The list of genotypes and their origins are available in Supplementary Table S1.[1]

Plate assay

Initially, *B. napus* 'Westar DH101' and 'DH12075' were tested for salinity stress tolerance on Petri dishes containing Murashige and Skoog (1962) (MS) medium at one-half ingredient concentration (3.32 g MS powder, 1.6 g MES buffer, 15 g sucrose, and 10.5 g agar per 1500 mL water) supplemented with 0, 50, 100, 150, 200, 250, 300, and 350 mmol L^{-1} NaCl. Approximately 300 seeds from each *B. napus* genotype were surface-sterilized in 20 mL of 6% sodium hypochlorite solution supplemented with two drops of Tween-20 and agitated on a Classic C1 rotary shaker (New Brunswick Scientific, Enfield, CT) at 80 rev min^{-1} for 15 min. Immediately afterward, each sample was rinsed with a continuous flow of 400 mL sterile distilled water, dried overnight in a laminar flow hood and then kept sterile until use. Ten seeds were placed on each Petri dish (three replicates)

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjps-2019-0023.

and the dishes were placed in an incubator (Adaptis A1000, Conviron, Winnipeg, MB) set at 21 °C ± 1 °C and a 16 h light–8 h dark photoperiod provided by white fluorescent light of 225 μmol m^{-2} s^{-1}. Seed germination was recorded every 24 h for 10 d, while root and shoot lengths (cm) were recorded on 10-d-old seedlings. A seed was deemed to have germinated if the radical was 2 mm long, while seedlings were considered abnormal if they had a short, thick, or spiral hypocotyl or the primary root was absent or stunted (ISTA 2003). Root and shoot lengths were measured 10 d after seeding using a standard scale. Based on this data, no (0 mmol L^{-1} NaCl; no salinity stress) and 200 mmol L^{-1} NaCl (high salinity stress) salinity levels were selected for assessment of salinity tolerance in the other *B. napus* genotypes. The experiment was conducted in a split-plot arrangement of treatments with salinity stress (0 and 200 mmol L^{-1} NaCl) as the main plot and canola genotypes ($n = 131$) as the subplot.

The germination percentage, area under the germination progress curve (AUGPC), seedling vigor index (SVI), and relative salt tolerance percentage (RST%) were calculated according to Sun et al. (2015) as follows:

$$\text{Germination \%} = \frac{\text{Seeds germinated at final count}}{\text{Total seeds plated for germination test}} \times 100$$

$$\text{AUGPC} = \sum_{i=1}^{n} \left[(G_{i+1} + G_i)/2 \right] \left[(t_{i+1} - t_i) \right]$$

where G_i is the germination percentage on the ith day, t_i is the time in days at the ith observation, and n is the total number of observations.

$$\text{SVI} = \text{AUGPC} \times (\text{root length} + \text{shoot length})$$

$$\text{RST\%} = \frac{\text{Variable recorded in 200 mmol L}^{-1}\text{ NaCl}}{\text{Variable recorded in 0 mmol L}^{-1}\text{ NaCl}} \times 100$$

where higher AUGPC values indicate higher germination rates, while higher SVI values indicate better seedling growth and vigor. Similarly, higher RST% values indicate higher salinity tolerance relative to the control without salt stress.

Based on the results of the plate assays, 11 genotypes exhibiting diverse RST% (ranging from 2% to 61%) were further assessed at five different salinity levels (0, 50, 100, 200, and 300 mmol L^{-1} NaCl). The genotypes included Kuju 29 (South Korea), Kuju 32 (South Korea), Dong Hae 12 (South Korea), Dong Hae 20 (South Korea), Dong Buk (South Korea), Zhong You 821 (China), Ashi Natane (Japan), Surpass 400 (Australia), Av. Sapphire (Australia), Westar DH101 (Canada), and DH12075 (Canada). The experiment was conducted in a randomized complete block design with three replications and factorial arrangement of treatments (split-plot design), where salinity was applied as the main factor and genotype as the subplot factor. The measurements of germination, AUGPC, root length, shoot length, and salinity tolerance were carried out as above.

Potted plant greenhouse assay

The genotypes that were identified as being salt sensitive at the seedling stage (Westar DH101, DH12075, and Dong Hae 20) or relatively salt tolerant (Dong Buk, Zhong You 821, Ashi Natane, Dong Hae 12, and PAK85869) were examined throughout the entire growing period in a greenhouse (Ashraf 2001; Steppuhn et al. 2001). Seeds were germinated and seedlings grown on half strength MS medium for 10 d. After 10 d, seedlings were transplanted into 5 cm wide × 5 cm long × 7 cm deep pots filled with ground brick granules (Turface Athletics MVP, Buffalo Grove, IL) soaked in water. Brick granules were used as they are easily separated from the root by washing under water to allow recovery of nearly all root biomass. Eight pots in three replicates were placed in a flat rectangular pan in a complete randomized design and transferred to a greenhouse. The pots were continuously immersed to two-thirds of the pot height in Hoagland solution [7 mmol L^{-1} $Ca(NO_3)_2$, 5 mmol L^{-1} KNO_3, 2 mmol L^{-1} KH_2PO_4, 2 mmol L^{-1} $MgSO_4$, trace elements and FeEDTA, and pH 7.5] for 9 d. Thereafter, pots were immersed in Hoagland solution with 0, 50, 100, 200, or 300 mmol L^{-1} NaCl. The experiment was conducted in a randomized complete block design with a split-plot arrangement of treatments where salt level was considered the main-plot factor and genotypes as the subplot. Hoagland solution supplemented with different salt levels was replaced twice a week. To avoid algal growth, brick granules in individual pots were covered with aluminum foil. The experiment was conducted in a greenhouse at 22 °C ± 1 °C during the day and 18 °C ± 1 °C at night. The greenhouse was supplemented with light to provide a 16 h day–8 h night cycle. After 19 d, the brick particles were washed from the roots with tap water and blotted on paper towels to remove excess moisture. Fresh root and shoot biomasses were recorded immediately after harvest. Dry biomass was determined after incubation in an oven at 55 °C for 8–10 d until a constant weight was achieved. The RST% for fresh and dry biomasses were calculated as explained above.

Semi-hydroponic chamber greenhouse assay

The Agriculture and Agri-Food Canada Salt Tolerance Testing Laboratory in Swift Current, SK, is equipped with a programmable logic controller for temperature, radiation, supply of nutrient solutions and fertilizer, and root zone salinity (Steppuhn and Wall 1999). In brief, the experiment was conducted in an environmentally-controlled greenhouse with plants grown in plastic tanks filled with washed silica sand (99.8% pure) with

an average bulk density of 1.5 mg m^{-3}. Natural greenhouse day length was extended during the growing period (simulated according to canola growing conditions on the Canadian Prairies) using 475 W sodium lamps positioned 1.5 m above the sand surface. The greenhouse temperature was maintained at 22 °C ± 1 °C during the day and 18 °C ± 1 °C at night. The testing solution (modified Hoagland solution with different levels of salinity) was prepared and applied according to Steppuhn et al. (2001) and Steppuhn and Wall (1999). Six solute concentrations were maintained at solution electrical conductivities (EC_{sol}) averaging 1.4 dS m^{-1} for the control and 5, 10, 15, 20, and 28 dS m^{-1} for the increasing salt stresses. The salinity of the control Hoagland nutrient solution was similar to the salt level (1.4 dS m^{-1}) most prevalent in prairie soils of the upper Midwest United States and Canada (Steppuhn and Wall 1999), while the higher salinity levels tested reflect the range in this region where crops are grown (Steppuhn and Wall 1999; Steppuhn et al. 2001). Each tank was supplied with nutrient solution for 5 min to allow the silica sand to saturate completely. The solution was then drained to a 612 L reservoir (one per each different salinity level) and held for the next round of supply. The tanks were flushed four times daily at 0100hr, 0900hr, 1300hr, and 1700hr. Eight *B. napus* genotypes, namely Dong Buk, Kuju 32, Kuju 29, Zhong You 821, Surpass 400, Av. Sapphire, Westar DH101, and DH12075, were tested. These genotypes exhibited a wide range of RST% (2%–62%) at the seedling (plate assays) and vegetative stages (greenhouse assay). The experiment was laid out in a factorial arrangement of genotypes and EC_{sol} in 60 pots with five replicates, in a total of 10 blocks. Each pot contained four genotypes positioned at the northwest (NW), northeast (NE), southeast (SE), and southwest (SW) quadrants. The factor classifications genotypes and EC_{sol} were orthogonal and balanced, while the factors' classifications with blocks or with positions were unbalanced; however, effort was made at the design stage to keep this to a minimum. Plant height and leaf counts were measured multiple times at different growth stages (GS; GS 39, stem elongation; GS 49, vegetative; and GS 69, flowering) while branching, pod number, and total shoot dry biomass were recorded at the mature stage (GS 69, flowering; and GS 79, development of seed) before harvesting. Proline and glucosinolate levels were also measured at the adult plant stage (silique formation) by sampling 250–300 g of fresh leaf biomass.

Proline and glucosinolate measurement

For each genotype, 250–300 mg of leaf tissue was sampled from each treatment at GS 35–39 (50%–80% stem elongation) in the greenhouse experiment to assay free proline and glucosinolate content. Samples were frozen immediately in liquid nitrogen until the assays were performed. Proline content was determined using freeze-dried leaf tissues according to Bates et al. (1973). Calibration was done with L-proline (Sigma Aldrich, St. Louis, MO) as a standard. The proline content was calculated according to the following formula: [(mL toluene) × (μg proline mL^{-1})]/(g sample × 2/3) = μg proline g^{-1} tissue weight. Three independent measurements for each salinity treatment were done. Leaf samples from various plants were analyzed for their glucosinolate content by High Performance Liquid Chromatography with benzyl glucosinolate as the internal standard (Daun and McGregor 1981). A diode array detector at wavelength 229 nm was used with an Agilent Poroshell 120 EC-C18 4.6 mm × 100 mm 2.7 micron column.

Statistical analysis

For the initial plate assay experiment, the analysis of variance (ANOVA) for germination and growth parameters was carried out in randomized block design to detect genotypic differences using data for each salinity level separately. This analysis allowed experimental error variance to vary with salinity level. To detect genotype × salinity interaction, the full dataset was analyzed under a split-plot design structure with the two salinity levels as the main plot and 131 *B. napus* genotypes as subplots. Fisher's protected least significant difference was calculated to separate means of treatment effects under each salinity level used in the experiment. Pearson's correlation coefficients were calculated for germination, root and shoot lengths, and seedling vigor for both the control (0 mmol L^{-1} NaCl) and salt stress (200 mmol L^{-1} NaCl).

For the plate and greenhouse assays with multiple salinity levels, the data were analyzed using ANOVA for a split-plot design structure with five salinity levels as the main plot and genotypes as subplots. Logarithmic transformation of proline content in fresh weight was used to normalise the data, an assumption for ANOVA.

In the semi-hydroponic experiment, the factors included in the model were EC_{sol}, genotypes, position (direction), blocks, and pots within blocks, and their suitable interactions. As the data collected were unbalanced, the mixed model was fitted to the data to estimate the variance components and predict means (in terms of best linear unbiased predictor). The assumed model was composed of random terms, blocks, EC_{sol} × blocks interaction, and plots within blocks, and the fixed terms fitted were EC_{sol}, position, genotypes, EC_{sol} × position, EC_{sol} × genotypes, position × genotypes, and EC_{sol} × position × genotypes. Variables recorded in the semi-hydroponic experiment were analyzed on square root-transformed data except the number of leaves. Main effects and interactions were assessed using the Wald test provided in Genstat software (Payne 2013). Furthermore, the agronomic traits and biochemical measurements were analyzed using data from the first five levels of EC_{sol} (1.4–20 dS m^{-1}), while proline and glucosinolate content data were analyzed with only the

first four levels of EC_{sol} (1.4–15 dS m^{-1}), as some genotypes did not produce enough biomass for measurements at higher salinity levels, and these were left out of the analysis. The *P* values to assess statistical significance, means, standard errors, and least significant difference at 5% were tabulated for all the experiments. The computations were done using appropriate directives for model descriptions and output options in Genstat software.

Results

In this study, we investigated the effects of salinity on early vigor (germination and seedling development), as well as growth during the vegetative and reproductive plant stages in a diverse set of *B. napus* genotypes. We demonstrated that variation existed in the response of these genotypes to salinity stress and in the accumulation of proline and glucosinolate content in leaves.

Plate assay

Initially, a world collection of 131 *B. napus* genotypes was assessed for early seedling growth parameters and RST% in plate assays (Table 1). The mean and standard deviation for each growth parameter at 0 mmol L^{-1} NaCl (control) and 200 mmol L^{-1} NaCl (salt stress) are shown in Supplementary Tables S1 and S2,[1] respectively. The ANOVA of early seedling tolerance to salinity, using AUGPC, root and shoot length, and seedling vigor index (SVI), indicated a significant ($P < 0.001$) effect of genotype and salinity, and an interaction of genotype × salinity (Supplementary Table S3[1]). The box-whisker plots of AUGPC, root and shoot lengths, and SVI are shown in Fig. 1 and suggest highly diverse responses to salinity stress. The *B. napus* genotypes showed high variation for AUGPC at 200 mmol L^{-1} NaCl compared with 0 mmol L^{-1}, suggesting that the germination of some genotypes was significantly reduced by salinity stress. Similarly, the SVI and average root and shoot lengths were reduced at 200 mmol L^{-1} NaCl. High SVI variation of among the genotypes indicated that the collection was diverse in germination, root and shoot length responses. The *B. napus* genotypes that showed a superior salinity tolerance level (top 20% of genotypes) for early seedling parameters, specifically SVI, and their rankings are shown in Supplementary Table S4.[1] The correlation coefficients for early seedling parameters in control and saline conditions are presented in Supplementary Table S5.[1]

In plate assays, the most salinity-tolerant genotypes were Ag-Outback, Ag-Spectrum, Azumasho, Kraphouser, Barplina, Bronowski, Buk Wuk, Buk Wuk 13, Buk Wuk 16, Buk Wuk 3, Chikuzen Natane, Cresor, Dae Chosen, Dong Hae 6, Dong Hae 9, Kuju 13, Kuju 27, Kuju 29, SRS3728, Kuju 32, Kuju 36, Kuju 4, Kuju 7, Liho, Linetta, Optima, and Russian No. 6 (Tables 1 and Supplementary Table S4[1]). These genotypes showed the highest tolerance to salinity for AUGPC, root and shoot growth, and SVI, and ranked among the top 20% of the 131 genotypes based on RST% of SVI. Three of these genotypes, namely Kraphouser, Ag-Spectrum, and Kuju 36, showed higher levels of salinity tolerance for root and shoot growth, but their AUGPC under the control condition and at 200 mmol L^{-1} NaCl was poor. Genotypes Chikuzen Natane, Buk Wuk 16, and Liho had poor root growth under saline conditions, but exhibited higher levels of tolerance when AUGPC, shoot growth, and SVI index were considered. Kuju 29, Russian No. 6, Cresor, and Azumasho showed moderate salinity tolerance based on all parameters measured, and the overall SVI of these genotypes was comparable to the tolerant genotypes. Several of these genotypes showed a moderate level of seedling vigor in the absence of salinity stress, were less affected by 200 mmol L^{-1} salinity stress with respect to seedling vigor, and had the greatest RST values (ranging from 40% to 80%). This group, namely Buk Wuk 3, Buk Wuk 13, Buk Wuk 16, Buk Wuk 24, Dae Chosen, Kuju 26, Kuju 27, Kuju 29 SRS3728, Kuju 32, Kuju 33, and Russian No 6, can be considered as highly tolerant to salinity for seedling vigor.

Eleven *B. napus* genotypes representing a wide range of responses to salinity stress at the 200 mmol L^{-1} NaCl level above were evaluated under five salinity levels at the seedling stage in plate assays. Analysis of variance indicated that salinity, genotype, and genotype × salinity interaction had highly significant ($P < 0.0001$) effects on early seedling growth parameters and SVI (Supplementary Table S6[1]). The effects of salinity on AUGPC, root and shoot length, and SVI were highly variable (Fig. 2). AUGPC (germination rate) was more effective in discriminating *B. napus* genotypes compared with germination % alone. Zhong You 821, Dong Buck, and Kuju 29 were more tolerant than Westar DH101, Av. Sapphire, Surpass 400, Ashi Natane, Dong Hae 12, and Dong Hae 20. Germination and root and shoot growth for DH12075 were severely affected by increased salinity; therefore, SVI declined sharply as salinity increased from 0 to 300 mmol L^{-1} NaCl. Av. Sapphire and Surpass 400 (Australian genotypes) responded positively to germination and root and shoot growth when salinity increased from 0 to 50 mmol L^{-1}.

Potted plant greenhouse assay

Eight of the more salt-sensitive and salt-tolerant *B. napus* genotypes were evaluated under five salinity levels at the seedling stage in pots grown in the greenhouse. The effects of salinity and genotype on root and shoot biomass and proline accumulation in leaves are presented in Supplementary Table S7.[1] The mean values for dry root and shoot biomass, as well as proline accumulation for genotype × salinity interactions, are presented in Table 2. Salinity significantly ($P < 0.05$) affected root and shoot biomass production, as well as proline accumulation in leaf tissues. The genotype × salinity interaction was not significant for root and

Table 1. Early seedling growth parameters and relative salt tolerance (RST) determined for a world collection of 131 *Brassica napus*.

	AUGPC		Root length (cM)		Shoot length (cm)		Seedling vigor index (SVI)	
Genotype	MS	RST%[a]	MS	RST	MS	RST	MS	RST
Abukuma natane	88	88	9.8	30	2.1	60	1052	32
Ag-Outback	86	91	3.5	93	2.2	68	495	76
Ag-Spectrum	75	74	2.6	99	1.1	91	273	73
Ashi natane SRS1624	87	98	10.8	15	1.4	57	1064	20
Atlas NGB 13611	86	88	7.2	16	2.2	29	807	17
Av. Sapphire	58	10	3.7	37	1.2	74	286	6
Azumasho-SRS3598	87	99	4.9	50	1.5	65	552	53
Bansai SRS3600	83	85	3.8	45	1.7	55	456	40
Barplina-SRS3601	86	99	4.8	79	1.9	82	577	79
Bronowski CN31304	83	90	6.2	65	2.2	69	690	61
Buk Wuk 13-SRS3608	90	97	7.5	77	2.2	59	875	70
Buk Wuk 14-SRS3609	85	88	5.7	48	2.1	64	664	46
Buk Wuk 15O020097	90	95	9.8	54	2.5	58	1106	52
Buk Wuk 16-SRS3611	90	81	6.6	82	1.9	88	769	67
Buk Wuk 20-SRS3613	88	76	5.9	14	2.4	35	731	15
Buk Wuk 21-SRS3614	89	81	4.8	15	2.6	33	653	17
Buk Wuk 23-SRS3615	90	83	9.9	31	2.2	52	1086	29
Buk Wuk 24-SRS3616	90	94	9	42	2	71	984	44
Buk Wuk 26-SRS3617	81	58	5.2	19	1.6	65	548	18
Buk Wuk 27-SRS3618	87	95	6	15	2	47	695	22
Buk Wuk 3-SRS3604	87	98	6.8	68	2.2	71	782	68
Buk Wuk 4	89	88	9.7	14	1.1	77	958	18
Buk Wuk 7	86	77	10.5	19	1.3	86	1011	20
Canard CN 35437	90	94	12	6	2.6	23	1302	8
Ceska-CN31394	87	98	8.9	40	2.2	61	962	43
Chikuzen Natane-15O0200055	85	87	3.7	78	1.7	79	461	68
Cresor CN31417	84	97	6.1	52	1.9	69	675	55
Dae Chosen-SRS1721	84	94	6.4	65	2.3	72	720	62
DH12075	83	55	11.7	10	2.4	32	1163	7
Dong Buk SRS3625	88	94	11.7	19	2.4	52	1247	23
Dong Hae 12	88	93	9.8	9	3	32	1137	14
Dong Hae 1-SRS3627	79	75	7.4	17	2	57	739	19
Dong Hae 20	80	57	6.4	8	1.4	49	627	8
Dong Hae 3-SRS3629	87	93	8.1	31	2.1	75	884	37
Dong Hae 4	88	90	12.5	4	1	83	1193	9
Dong Hae 6-SRS3631	86	93	6.8	52	1.7	71	736	52
Dong Hae 9 15O0200098	75	93	12.4	47	2.6	67	1113	47
Dong Hae-SRS3626	70	29	5.9	21	1.4	79	502	9
Drakkar-15O0200106	82	96	12.6	25	2.7	60	1265	30
Erglu-CN 34519	84	69	5.6	26	3.2	58	739	26
Gan You 4 SRS2076	77	79	7.8	11	0.7	73	664	12
Global CN46333	89	68	13.4	11	2.8	32	1434	10
Gulle NGB 585	83	69	9.6	13	3.9	26	1122	12
Hisinchu3	89	54	10.5	20	2.5	43	1157	14
IR22 SRS153	84	57	6.6	10	1.9	42	713	10
Jet Neuf	89	87	14.4	11	1.7	51	1437	13
Jumbo-CN 87033	78	84	4.6	42	2.4	67	565	44
Kinki 18	87	57	3.4	52	2.3	47	497	30
Kinki 20	84	60	5.4	27	2.1	41	631	20
Kinki 21 SRS3702	88	64	11.5	23	1.8	49	1178	17
Kinki 28-SRS3704	87	93	6.9	37	2.2	63	785	40
Kinki 29	85	70	3.6	32	2.1	62	486	30
Kinki 30-SRS3706	87	90	7.3	27	2.5	46	848	29
Kinki SRS3700	85	78	4.2	33	2.1	48	529	30

Table 1. (*continued*).

	AUGPC		Root length (cM)		Shoot length (cm)		Seedling vigor index (SVI)	
Genotype	MS	RST%[a]	MS	RST	MS	RST	MS	RST
Kinki Wase-SRS3707	66	88	4.6	27	1.7	61	411	32
Kovakevskij-15O0200092	82	67	7	46	2.5	57	783	33
Kraphouser-SRS3709	75	96	3.2	91	1.8	71	372	80
Kuju 11	88	30	6.6	12	2.8	33	820	6
Kuju 13-SRS3717	87	94	3.6	63	2.6	47	534	53
Kuju 16-SRS3719	84	85	5.4	55	2.5	58	664	47
Kuju 19	88	72	7.3	34	1.9	52	811	27
Kuju 22	89	87	10	31	1.1	70	991	30
Kuju 24	89	92	11.7	6	1.3	65	1163	11
Kuju 25	84	90	12.5	9	2.5	42	1265	13
Kuju 26-SRS3726	85	91	9.2	40	2.4	66	977	42
Kuju 27-SRS3727	86	99	8.1	89	2.3	68	903	83
Kuju 29	86	69	11.9	15	2.7	34	1265	14
Kuju 29-SRS3728	86	96	5.9	63	2.7	65	735	61
Kuju 32-SRS3729	86	94	7.8	67	2.8	63	905	62
Kuju 33-SRS3730	79	84	9.4	46	2.5	61	939	41
Kuju 35	88	56	8.9	23	3.2	43	1069	16
Kuju 36-SRS3732	78	10	4.4	83	2.7	56	545	71
Kuju 37 SRS3733	87	83	10.4	12	2.8	40	1149	15
Kuju 4-SRS3712	86	98	2.9	98	2.6	71	475	83
Kuju 7-SRS3713	87	97	6.9	54	2.3	73	803	57
Kuju 8-SRS3714	88	73	9	40	2.3	53	989	32
Kuju 9-SRS3715	90	99	7.8	32	1.9	69	869	38
Liho-CN 101876	86	88	4.1	82	2.8	67	595	67
Line CN43834	81	23	7.3	11	2.7	28	805	4
Linetta	85	78	6.4	25	2	52	713	25
Linetta-CN101877	84	83	5.8	51	2.4	87	680	51
Marjanovskij K 4611	90	99	10.9	32	2.5	67	1203	37
MLCP 49-15O0200178	80	98	5.4	44	1.7	61	570	46
MO-83-5	90	81	9.6	30	2.8	47	1112	27
No name SRS1630	86	83	5.3	24	2.3	41	648	23
No name SRS1631	89	71	7.6	30	2.2	46	872	26
No name SRS2211	86	89	7.1	12	2.7	30	834	16
No name SRS471	89	90	13.2	6	3	44	1444	12
No name SRS1304	86	94	9.6	32	2.5	60	1034	35
No name SRS905	75	97	5.8	40	2.5	53	626	43
No name SRS442	82	91	8.1	26	2.8	56	894	30
Nolza 531 SRS2630	84	82	10.2	28	2.4	62	1060	28
Nolza 541 SRS2629 (P1)[b]	84	87	5.5	38	1.4	81	582	41
Nolza 541 SRS2629 (P2)[b]	81	15	5.3	40	1.5	67	547	7
Nora-NGB 1796	84	37	3.6	59	2.1	68	481	22
Norin 25-SRS1727	80	77	6.4	48	2.5	57	716	39
Nosovskijg-SRS441	88	80	9.6	48	2.8	58	1086	39
Oac Summit CN 525168	84	84	4.5	49	1.9	42	536	39
Olimpiade	84	76	13.3	17	1.5	43	1249	15
Optima-CN43969	80	83	5.6	62	1.7	71	592	53
ORO SRS6	84	89	9	31	3.2	45	1020	31
Pactol 15O02001133	80	8	4.9	22	2.2	25	573	2
PAK85484	89	81	9.9	22	1.6	62	1023	23
PAK85487	88	93	10.1	21	2.6	41	1114	24
PAK85489	85	85	10.2	32	3.1	40	1132	28
PAK85841	86	68	13.3	26	3.2	42	1421	20
PAK85854	87	93	5.5	42	2	56	647	42
PAK85864	62	68	6.6	25	2.7	58	570	25
PAK85869	87	98	15.5	17	2.9	51	1606	21

Table 1. (*concluded*).

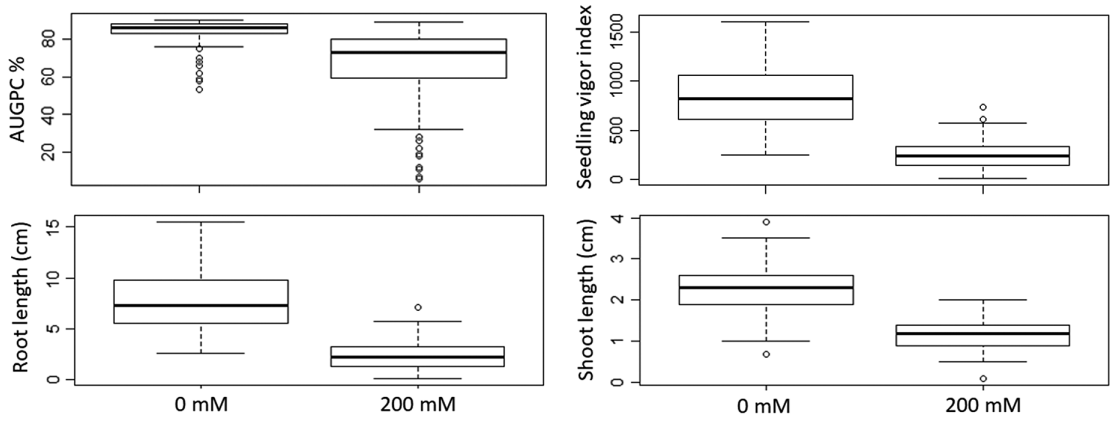
	AUGPC		Root length (cM)		Shoot length (cm)		Seedling vigor index (SVI)	
Genotype	MS	RST%[a]	MS	RST	MS	RST	MS	RST
PAK85880	77	88	8	10	2.6	40	819	15
PAK85893	87	86	10.5	26	2.1	40	1090	25
PAK85903	90	94	8.5	12	2.3	44	966	18
PAK85908	87	83	8.2	22	3.5	33	1012	21
PAK85912	89	88	5.9	24	3.5	33	841	24
Petranova-CN35599	59	91	4.6	29	2.3	68	425	41
Rivette-SCDC	68	28	5.8	32	2.1	41	541	10
Russian No. 6-15O0200053	88	96	6	58	2.3	62	724	57
Shang You SRS1639	88	85	8.5	14	1.7	51	903	17
Surpass 400	78	69	4.3	2	1.6	7	458	2
Tanto-15O02000131	86	81	12.4	19	3.5	51	1363	21
Tapidor DH	90	90	10.4	39	2.5	58	1159	38
Tatyoon CN43824	77	62	4.8	65	2.1	69	530	41
Tokiwa Natane-15O0200054	70	41	4.4	20	1.6	65	427	13
Topas DH	86	69	9	25	2.5	52	975	21
Toro CN31404	53	21	3.1	40	1.5	75	244	13
Valecovska-CN 31406	87	79	9.3	40	2.5	61	1030	37
Vasilkovskij-15O0200074	87	94	8.2	25	2.4	51	921	29
Vastaceno Sibirskij-15O0200070	85	92	8.3	44	2.5	57	915	43
Wesreo SRS1195	85	94	7.2	33	2.1	54	780	35
Westar DH101	76	78	11	17	3	39	1061	17
Zhong You 821	90	95	12.2	30	1.8	35	1259	29
±SE	2.82	5.72	0.64	0.33	0.16	0.1	67.5	38.4
$LSD_{0.05}$	7.85	15.93	1.79	0.93	0.438	0.28	187.8	156.9

Note: AUGPC, area under germination progress curve; SVI, seedling vigor index; MS, Murashige and Skoog growth medium; SE, standard error.

[a]RST, relative salt tolerance (RST) % calculated as salinity tolerance at 200 mmol L^{-1} NaCl salinity stress relative to control at 0 mmol L^{-1} NaCl. The higher the value of RST%, the more salt-tolerant the genotype.

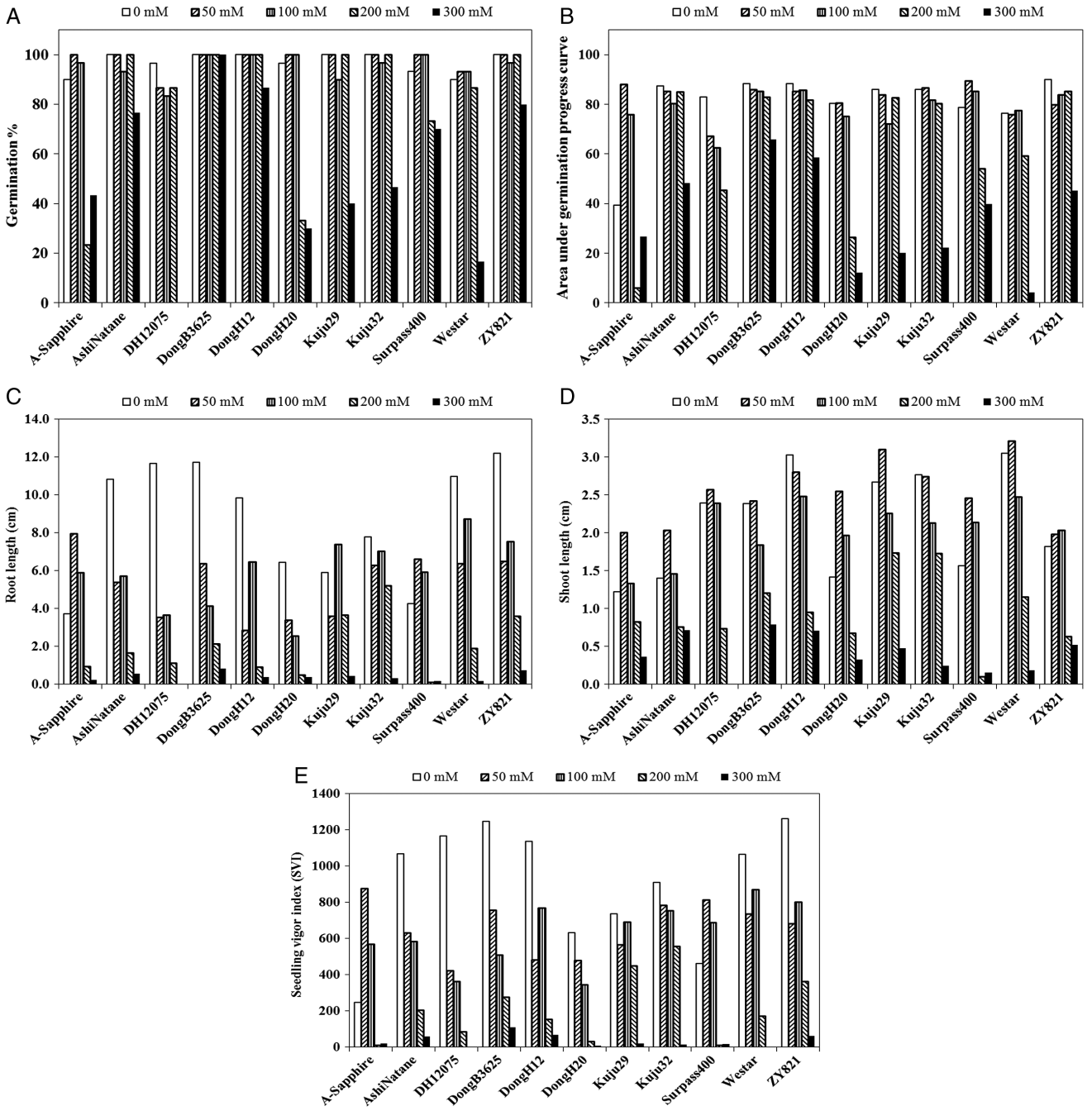
[b]Genotypes with the same name that showed different phenotypes or special growth habits, therefore both genotypes were reported.

Fig. 1. Box-whisker plots of AUGPC, root length, shoot length, and seedling vigor under 0 and 200 mmol L^{-1} NaCl.



shoot biomass production, while proline accumulation was significantly ($P < 0.05$) affected. In general, higher salinity level reduced root and shoot biomass production, while proline accumulation increased. In the absence of NaCl, proline accumulation was 0.128 mg g^{-1} of fresh leaf tissue on average, while it reached 16.33 mg g^{-1} of fresh leaf tissue (127-fold increase) at 300 mmol L^{-1} NaCl. All genotypes showed the highest proline accumulation at 300 mmol L^{-1} salinity compared with lower levels of salinity; however, there was a

Fig. 2. Response of *B. napus* genotypes to different salinity levels. (A) Germination %, (B) area under germination progress curve (AUGPC), (C) root length, (D) shoot length, and (E) seedling vigor index (SVI).



significant difference in the amount of proline produced at different levels of salinity (Table 3). DH12075 accumulated the greatest amount of proline, followed by PAK85869, Dong Hae 12, and Zhong You 821. These genotypes also had the highest root and shoot biomass production under salinity stress. Ashi Natane and Dong Buk accumulated the least amount of proline, and these genotypes also had the lowest biomass production.

Semi-hydroponic chamber assay

Eight *B. napus* genotypes exhibiting a range of RST% (2%–62%) at the seedling and vegetative stages were also evaluated under five salinity levels through a full developmental cycle in semi-hydroponic chambers in the greenhouse. The means of plant height, dry biomass, number of branches, number of siliques, and proline and glucosinolate accumulation are presented in Table 4, while a comparison of the proline and

Table 2. Root and shoot weights and proline content means for the five salinity levels and for the eight *B. napus* genotypes grown in the greenhouse.

	Root		Shoot		Proline[a]
Salinity	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)	Fresh weight (mg g^{-1})
0 mmol L^{-1}	2.9	0.126	20.8	1.34	0.128
50 mmol L^{-1}	2.6	0.111	17.5	1.13	0.258
100 mmol L^{-1}	1.8	0.095	10.7	0.75	0.546
200 mmol L^{-1}	1.5	0.076	6.7	0.53	6.354
300 mmol L^{-1}	1.4	0.075	5.0	0.42	16.333
SE	0.29	0.013	1.02	0.08	—
$LSD_{0.05}$	0.95	0.043	3.2	0.36	—
Genotypes					
Ashi Natane	1.11	0.044	9.7	0.597	2.64
DH12075	2.46	0.103	12.2	0.833	6.787
Dong Buk	1.62	0.88	10.1	0.676	3.44
Dong Hae 12	2.36	0.096	13.3	0.946	5.267
Dong Hae 20	1.86	0.078	12.5	0.811	4.48
PAK85869	1.85	0.100	10.0	0.767	5.593
Westar DH101	2.41	0.134	15.3	1.091	4.553
Zhong You 821	2.75	0.132	13.9	0.992	5.047
SE	0.26	0.011	1.15	0.091	—
$LSD_{0.05}$	0.72	0.031	3.24	0.257	—

[a]Proline estimated in mg g^{-1} of fresh weight leaf tissue according to Bates et al. (1973). The analysis was carried out on a logarithmic transformation of proline content. Only unadjusted means are given. Standard error (SE) will vary with the mean and, therefore, are not given.

Table 3. Proline content means (mg g^{-1} fresh weight) in leaves for the combinations of five salinity levels and eight *B. napus* genotypes grown in the greenhouse.

	Genotypes							
Salinity (mmol L^{-1})	DH12075	Westar DH101	Ashi Natane 1624	Dong Hae 12	Dong Buk SRS3625	Zhong You 821	Dong Hae 20	PAK 85869
0	0.0	0.1	0.4	0.0	0.0	0.3	0.0	0.2
50	0.6	0.1	0.2	0.2	0.3	0.3	0.2	0.3
100	0.7	0.4	0.3	0.7	0.3	0.7	0.3	1.0
200	8.1	3.9	1.4	7.2	2.8	6.7	6.5	14.3
300	24.5	18.4	11.1	18.1	13.7	17.3	15.3	12.2

Note: The proline content was measured in mg g^{-1} fresh weight of leaf tissue. The analysis was carried out on a logarithmic transformation of proline content. Only unadjusted means are given. Standard error will vary with the mean and, therefore, are not given.

glucosinolate accumulation is shown in Fig. 3. We found significant effects ($P < 0.01$) of salinity, genotype, and genotype × salinity interactions for all parameters (Supplementary Table S8).[1] Salinity adversely affected plant height and biomass production, indicating that salinity has similar effects on plant growth at the reproductive stage as it did at the seedling and vegetative stages. All genotypes showed reduced plant development at increased salinity levels, with the exception of Westar DH101 and Surpass 400, where a positive effect was observed for number of branches and siliques per plant at 5 dS m^{-1}. In general, proline and glucosinolate accumulation in leaves increased with elevated salinity level and genotypes recording higher biomass production generally accumulated more proline in leaves. This was especially noticeable for Kuju 29 and Kuju 32, which had the highest biomass production under salinity stress and accumulated the most proline compared with other genotypes. Interestingly, these two genotypes showed less effects on vegetative growth, but were the most severely affected for number of branches and siliques which indicates these genotypes were highly sensitive to salinity at the reproductive stage.

A general trend toward increasing glucosinolate content in leaves was observed under increasing salinity

Table 4. Best linear unbiased predicted means of *B. napus* genotypes grown under different salinity levels at the adult plant stage for agronomic traits and proline and glucosinolate levels using a mixed model.

Salinity (EC_{sol})	Av. Sapphire	DH12075	Dong Buk	Kuju 29	Kuju 32	Surpass 400	Westar DH101	Zhong You 821
Plant height (cm)								
1.4	1467	1267.6	633.6	1119.4	1652.2	1820.2	1426.6	1701.4
5	1316.6	1220.8	639.4	1069.8	1372.4	1739.2	1441.8	1410.2
10	616.8	862.4	565	678.2	1119	1505.8	1301.4	1136.6
15	280.8	553.8	394	513.4	588.4	1208.8	989.6	537.8
20	133.6	0	211.6	171.2	124	101.2	412.4	68.2
Total dry shoot biomass (g)								
1.4	45.62	18.3	24.61	62.57	64.87	47.74	30.15	52.29
5	32.14	21.5	22.04	45.54	54.44	45.21	31.7	25.6
10	7.44	9.73	14	33.08	23.35	29.26	11.57	18.05
15	1.84	4.39	5.5	13.91	11.12	10.97	7.16	5.35
20	0.49	0	1.08	3.16	0.22	0.23	1.03	0.11
Number of branches bearing siliques[a]								
1.4	22	8.6	0	6.2	25.4	25.6	27.6	21
5	16.8	16	0	6	17	29	34.2	9.6
10	1.6	2.8	0	0	7.6	20.2	16.2	5.6
15	0	0.6	0	0	0	9.2	9.4	0.6
20	0	0	0	0	0	0	1.4	0
Number of siliques								
1.4	282	116	0	9.2	172.8	644.6	519	437
5	217.8	259.2	0	35	91.8	705	580.4	202.4
10	8.8	34.4	0	0	54.6	454.2	296	93.4
15	0	0.6	0	0	0	180.6	152.2	6.6
20	0	0	0	0	0	0.6	14.2	0
Proline (μmol g^{-1} fresh leaf tissue)[b,c]								
1.4	0.00844	0.00761	0.00873	0.01439	0.00857	0.00617	0.00619	0.00826
5	0.0063	0.00725	0.01111	0.10931	0.00823	0.00653	0.00615	0.00741
10	0.03286	0.04868	0.0114	0.23503	0.06915	0.0079	0.01168	0.01007
15	0.09398	0.25912	0.10088	0.44469	0.13446	0.04086	0.08733	0.0595
Glucosinolate (μmol g^{-1} fresh leaf tissue)[b,c]								
1.4	0.0251	0.0102	0.6878	0.2455	0.1302	0.0104	0.0321	0.3011
5	0.0226	0.0195	0.0876	0.3441	0.0942	0.008	0.0123	0.1927
10	0.0305	0.0189	0.1277	0.7897	0.2477	0.01	0.013	0.167
15	0.0615	0.092	0.3766	0.849	0.2557	0.0108	0.0233	0.2116

Note: Variables (plant height, total dry shoot biomass, number of branches bearing siliques, number of siliques, proline, and glucosinolate) were subject to square-root transformation for statistical analysis.

[a]Branch-bearing fertile silique; 0 indicates a single stem (no lateral branch-bearing silique was recorded).

[b]Target electrical conductivity of the hydroponic solutions (EC_{sol}) maintained in dS m^{-1}. Many genotypes did not survive at 28 dS m^{-1}; therefore, the data for 28 dS m^{-1} are not presented.

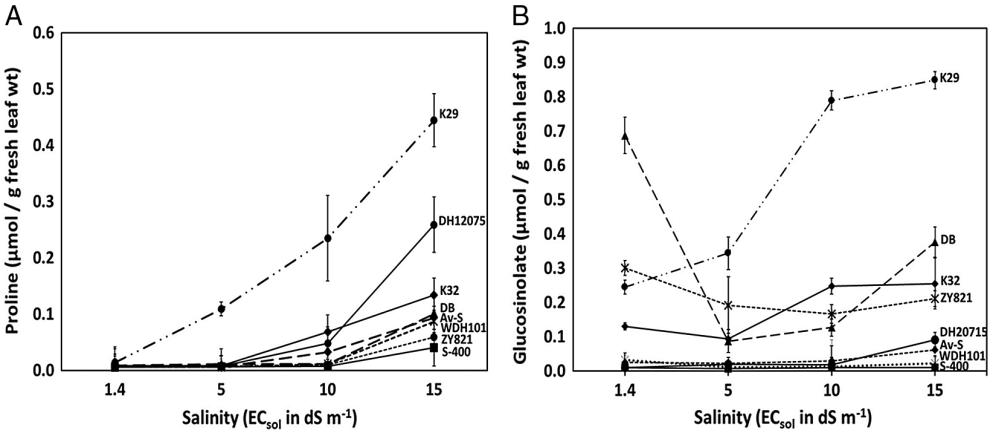
[c]Proline and glucosinolate content not analyzed due to an inadequate amount of tissue being available for genotypes at 20 dS m^{-1}.

(Table 4 and Fig. 3). However, the correlation between glucosinolate increase and salinity was not as strong in more contemporary *B. napus* cultivars, such as Surpass 400 (Australia), Av. Sapphire (Australia), Westar DH101 (Canada), and DH12075 (Canada), whereas Kuju 29 (an old Korean cultivar) showed a sharp increase (3.5-fold) under elevated salinity stress. In the case of Dong Buk, a decrease in leaf glucosinolates was observed at low salinity levels, but glucosinolates increased sharply at higher salinity levels.

Discussion

The adverse effects of abiotic stresses, including increased soil salinity on plant growth and development has been widely documented (Ashraf and McNeilly 2004; Munns and Tester 2008; Khan et al. 2009). Growth and development are an outcome of the coordination of the main biological processes in plants (Vassilev et al. 1998); abiotic stress disrupts this coordination and is, therefore, negatively correlated with growth in plants (Marschner 2002), including *B. napus* (Stumpf et al. 1986;

Fig. 3. Best linear unbiased predicted means of salinity levels and eight *B. napus* genotypes at the adult plant stage for (A) proline and (B) glucosinolate contents. Av-S, Av. Sapphire; DB, Dong Buk; DH12075; K29, Kuju 29; K32, Kuju 32; S-400, Surpass 400; ZY821, Zhong You 821; WDH101, Westar DH101.



Ashraf and Foolad 2005). *Brassica* oilseed species are the third-most important source of vegetable oil (Ashraf and McNeilly 2004). Gutierrez Boem et al. (1994) reported that seed germination slowed, and root and shoot growth was adversely affected, in canola with increasing salinity levels. Increasing salinity also adversely affects canola silique formation, seed filling, plant height, pod number, and seed number per plant. However, the crop has considerable potential to grow in salt-affected areas (Francois 1994).

High salinity reduces germination, seedling emergence, and ultimately crop establishment (Stumpf et al. 1986; Fowler 1991; Khan and Ungar 1999; Puppala et al. 1999; Ashraf 2001; Qasim et al. 2003; Ashraf and Foolad 2005; Guma et al. 2010; Jamila et al. 2010; Zivdar et al. 2011). Ahamad et al. (2012) reported salinity stress significantly affected the rate of germination, root and shoot growth of four canola cultivars, while Long et al. (2015) reported that root growth of canola is affected by salinity stress as early as 12 h post-exposure. Our study confirmed that several parameters need to be considered when examining salinity tolerance, and that no single measure is sufficient when selecting a salinity-tolerant genotype. Ashraf et al. (2010) suggested that elevated salinity retards cell division and cell elongation due to reduced nutrient uptake, cell membrane disruption, loss of cell turgidity, increased reactive oxygen species (ROS), and disrupted hormonal balance, which ultimately affect plant growth and development. Several studies have suggested that a positive correlation exists between salinity tolerance at the seedling and adult plant stages in *B. napus* (Ashraf 2001; Ashraf and Ali 2008), while others have indicated that the correlation is poor (Greenway and Munns 1980; Minhas et al. 1990; Mahmoodzadeh 2008). Minhas et al. (1990) and Mahmoodzadeh (2008) argued that individual stages (germination, emergence, seedling survival, vegetative growth, and reproduction) should be evaluated separately for the assessment of salinity tolerance and identification of useful material. Mahmoodzadeh (2008) reported that in the later stages of development, particularly at flowering and seed filling, oilseed rape is more sensitive to high salinity than during germination and seedling growth. Johnson et al. (1992) also reported that the salinity tolerance is growth-stage dependent in alfalfa. The current study examined salinity tolerance of *B. napus* genotypes at different growth stages. The data suggested that some genotypes showed consistent salinity tolerance across different developmental stages, while in other genotypes, salinity tolerance was growth stage dependent. Zhong You 821 showed consistent salinity tolerance throughout all developmental stages (germination, vegetative, and reproductive). Westar DH101 showed poor salinity tolerance at the seedling stage, but exhibited the highest tolerance to salinity during the reproductive stage, based on the number of siliques and branches bearing siliques among the genotypes tested. Conversely, Kuju 29 and Kuju 32 showed higher levels of salinity tolerance at the seedling and vegetative stages, resulting in higher SVI, good plant height, and higher biomass production at the adult plant stage. This was coupled with higher proline accumulation in these two genotypes; however, salinity (beyond 10 dS m^{-1}) severely affected the number of branches and silique production in both genotypes at the adult stage. In contrast, DH12075 was severely affected by increased salinity at the seedling stage (germination, root and shoot growth, and SVI) and failed to produce fertile branches and siliques beyond 10 dS m^{-1}. However, vegetative growth of this genotype was not as affected by increased salinity compared with the reproductive stage. In comparison, Av. Sapphire was highly sensitive to salinity, resulting in poor performance during the seedling, vegetative, and reproductive stages. Although both Surpass 400 and Westar DH101 showed moderate susceptibility to salinity at the early seedling

stage, their salinity tolerance improved tremendously in the later growth stages, resulting in the least effect at the reproductive stage among the genotypes. Therefore, these data suggest that salinity tolerance is a developmentally dependent phenomenon, and identification of salinity tolerance at different stages will offer the best opportunity to combine genetic factors responsible for overall salt tolerance.

In all assays, including the plate assay at the seedling stage, the greenhouse assay at the vegetative stage, and the semi-hydroponic assay at the reproductive stage, Surpass 400 showed positive effects of increased salinity on germination rate, root and shoot growth, biomass production, number of branches, and silique production at 50 mmol L^{-1} NaCl or 5 dS m^{-1} salinity. A similar response was also observed in Westar DH101, but only at the adult plant stage at 5 dS m^{-1} salinity. These results agree with findings of Ahamad et al. (2012) where Abasyn-95 consistently showed positive effects of 50 mmol L^{-1} NaCl on germination, root and shoot length, and fresh biomass. Our data suggests that, in at least two Australian genotypes, namely Surpass 400 and Av. Sapphire, low levels of salinity stress (50–100 mmol L^{-1} NaCl or ≤5 dS m^{-1}) may have positive effects on development. Mahmoodzadeh (2008) reported that lower levels of NaCl at the early seedling stage have positive effects on germination because an optimal amount of ions is required for metabolic activities. It may, therefore, be possible to breed new cultivars with positive effects on plant growth and development under low-level salinity stress and reduced negative effects at higher salinity levels.

Ashrafijou et al. (2010) and Bybordi (2010) reported higher proline accumulation in *B. napus* under saline conditions, while Sakr et al. (2012) reported that an exogenous supply of proline significantly reduced the negative effects of salinity on growth and development. Qasim et al. (2003) observed a sharp increase in proline content in leaves of two canola cultivars, Dunkeld and Cyclone under salinity stress (4, 8, and 12 dS m^{-1}) compared with low salinity stress (2 dS m^{-1}). Long et al. (2015) showed that proline dehydrogenase (ProDH) and Δ1-pyrroline-5-carboxylate synthase (P5CS) were differentially expressed in *B. napus* under salt stress. Both these enzymes are involved in proline metabolism in plants. Our results corroborate these previous findings and indicate that proline accumulation has significant effects on salinity tolerance, specifically at the early growth and vegetative stages. At the adult plant stage, proline accumulation increased with increasing salinity levels; however, proline accumulation failed to contribute to salinity tolerance at the reproductive stage in some genotypes, and as a result, Dong Buk and Kuju 29 exhibited poor branching and lower silique counts. Av. Sapphire, Kuju 32, and DH12075 failed to produce branches bearing siliques beyond the 10 dSm^{-1} salinity level even though proline accumulation steadily increased.

Glucosinolate levels have been shown to increase when salinity stress is present above certain tolerance levels (Qasim et al. 2003). In canola, Qasim et al. (2003) reported that glucosinolate levels increased in seed meal with increasing salinity stress in two canola cultivars, Dunkeld and Cyclone. Yuan et al. (2011) found total glucosinolate content increased in radish sprouts treated with 100 mmol L^{-1} NaCl, while Pang et al. (2012) reported salt-induced stress increases glucosinolate content in leaves of *Thellungiella salsuginea* (Pall.) Schulz. Further investigation is required to assess the effects of salinity on glucosinolate content in canola seed.

Conclusions

Salinity tolerance is a global problem for many crops, including *B. napus*. The salinity-tolerant genotypes identified in this study represent valuable genetic resources for *B. napus* improvement and sustainable production of canola under salinity stress. These results suggest that salinity tolerance is a developmental phenomenon; therefore, careful selection of genotypes at different development stages is required for successful improvement of *B. napus* for salinity tolerance. Several *B. napus* genotypes exhibited higher levels of salinity tolerance at the seedling stage, while Zhong You 821, Westar DH101, and Surpass 400 were identified as good genetic materials for improving salinity tolerance at the adult plant stage. It should be noted that most assessments of salinity tolerance use only NaCl, which is necessary for comparative purposes. However, salinity in natural soils is due to a combination of elements that may generate similar electrical conductivities but are handled very differently by the plant. Experiments using simulated salt solutions based on local soil conditions or field trials are necessary to determine the translatability of findings in this and similar studies.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

Ahamad, I., Ibrar, M., Muhammad, Z., and Ullah, B. 2012. Effect of salinity on four cultivars of canola (*Brassica napus* L.) under laboratory conditions. Pak. J. Plant Sci. **18**(1): 85–91.

Arzani, A. 2008. Improving salinity tolerance in crop plants: a biotechnological view. In vitro Cell. Dev. Biol. Plant, **44**(5): 373–383. doi:10.1007/s11627-008-9157-7.

Ashraf, M. 2001. Relationships between growth and gas exchange characteristics in some salt-tolerant amphidiploid Brassica species in relation to their diploid parents. Environ. Exp. Bot. **45**(2): 155–163. doi:10.1016/s0098-8472(00)00090-3. PMID:11275223.

Ashraf, M., and McNeilly, T. 2004. Salinity tolerance in some Brassica oilseeds. Crit. Rev. Plant Sci. **23**: 154–174. doi:10.1080/07352680490433286.

Ashraf, M., and Foolad, M.R. 2005. Pre-sowing seed treatment-a shotgun approach to improve germination, plant growth, and crop yield under saline and nonsaline conditions. Adv. Agron. **88**: 223–271. doi:10.1016/S0065-2113(05)88006-X.

Ashraf, M., and Ali, Q. 2008. Relative membrane permeability and activities of some antioxidant enzymes as the key determinants of salt tolerance in canola (*Brassica napus* L.). Environ. Exp. Bot. **63**(1–3): 266–273. doi:10.1016/j.envexpbot.2007.11.008.

Ashraf, M., Akram, N.A., Arteca, R.N., and Foolad, M.R. 2010. The physiological, biochemical and molecular roles of brassinosteroids and salicylic acid in plant processes and salt tolerance. Crit. Rev. Plant Sci. **29**(3): 162–190. doi:10.1080/07352689.2010.483580.

Ashrafijou, M., Sadat-Noori, S.A., Izadi-Darbandi, A., and Saghafi, S. 2010. Effect of salinity and radiation on proline accumulation in seeds of canola (*Brassica napus* L.). Plant Soil Environ. **56**(7): 312–317.

Bates, L.S., Waldren, R.P., and Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. Plant Soil, **39**: 205–207. doi:10.1007/BF00018060.

Bybordi, A. 2010. The influence of salt stress on seed germination, growth and yield of canola cultivars. Not. Bot. Hort. Agrobot. Cluj. **38**(1): 128–133. doi:10.15835/nbha3813572.

Daun, J.K., and McGregor, D.I. 1981. Glucosinolate analysis of rapeseed (Canola). Anal. Methods, **2**: 301–416.

FAOSTAT. 2014. Oilcrop primary data. FAO Statistics Division, Rome, Italy. http://www.fao.org/faostat/en/#data/QC [verified 8 June 2017].

Fowler, J.L. 1991. Interaction of salinity and temperature on the germination of Crambe. Agron. J. **83**(1): 169–172. doi:10.2134/agronj1991.00021962008300010039x.

Francois, L.E. 1994. Growth, seed yield and oil contents of canola grown under saline conditions. Agron. J. **86**: 233–237. doi:10.2134/agronj1994.00021962008600020004x.

Ghassemi, F., Jakeman, A.J., and Nik, H.A. 1995. Salinisation of land and water resources. Human causes, extent, management and case studies. University of New South Wales Press, Sydney, Australia.

Greenway, H.A., and Munns, R. 1980. Mechanisms of salt tolerance in nonhalophytes. Annu. Rev. Plant Biol. **31**(4): 149–190. doi:10.1146/annurev.pp.31.060180.001053.

Government of Saskatchewan. Nature and management of salt-affected land in Saskatchewan. Government of Saskatchewan, Saskatoon, SK. http://www.publications.gov.sk.ca/redirect.cfm?p=75823&i=84797 [accessed 05 Aug. 2019].

Guma, I.R., Padrón-Mederos, M.A., Santos-Guerra, A., and Reyes-Betancort, J.A. 2010. Effect of temperature and salinity on germination of *Salsola vermiculata* L. (Chenopodiaceae) from Canary Islands. J. Arid Environ. **74**(6): 708–711. doi:10.1016/j.jaridenv.2009.10.001.

Gutierrez Boem, F.H., Scheiner, J.D., and Lavado, R.S. 1994. Some effects of soil salinity on growth, development and yield of rapeseed (*Brassica napus* L.). J. Agron. Crop. Sci. **172**: 182–187. doi:10.1111/j.1439-037X.1994.tb00164.x.

Hamdy, A., Abdul-Dayem, S., and Abu-Zeid, M. 1993. Saline water management for optimum crop production. Agric. Water Manage. **24**(3): 189–203. doi:10.1016/0378-3774(93)90023-4.

ISTA. 2003. ISTA handbook on seedling evaluation. 3rd ed. International Seed Testing Association.

Jamila, Z., Arbi, G., and Mohamed, N. 2010. Germination responses of *Spartidium saharae* (Coss. & Dur.) Pomel (Fabaceae) to temperature and salinity. African J. Ecol. **48**(1): 37–44. doi:10.1111/j.1365-2028.2009.01070.x.

Johnson, D.W., Smith, S.E., and Dobrenz, A.K. 1992. Genetic and phenotypic relationships in response to NaCl at different developmental stages in alfalfa. Theor. Appl. Genet. **83**(6–7): 833–838. doi:10.1007/BF00226705. PMID:24202934.

Khan, M.A., and Ungar, I.A. 1999. Effect of salinity on seed germination of *Triglochin maritima* under various temperature regimes. Great Basin Nat. **59**(2): 144–150.

Khan, N.A., Nazar, R., and Anjum, N.A. 2009. Growth, photosynthesis and antioxidant metabolism in mustard (*Brassica juncea* L.) cultivars differing in ATP-sulfurylase activity under salinity stress. Sci. Hort. **122**: 455–460. doi:10.1016/j.scienta.2009.05.020.

Long, W., Zou, X., and Zhang, X. 2015. Transcriptome analysis of canola (*Brassica napus*) under salt stress at the germination stage. PLoS ONE, **10**(2): e0116217. doi:10.1371/journal.pone.0116217. PMID:25679513.

Mahmoodzadeh, H. 2008. Comparative study of tolerant and sensitive cultivars of *Brassica napus* in response to salt conditions. Asian J. Plant Sci. **7**(6): 594–598. doi:10.3923/ajps.2008.594.598.

Marschner, H. 2002. Mineral nutrition of higher plants. 2nd ed. Academic Press, London, UK.

Minhas, P.S., Sharam, D.R., and Khosla, B.K. 1990. Effect of alleviation of salinity stress at different growth stages of Indian mustard (*Brassica juncea*). Indian J. Agric. Sci. **60**(5): 343–346.

Munns, R., and Tester, M. 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. **59**(1): 651–681. doi:10.1146/annurev.arplant.59.032607.092911.

Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. **15**(3): 473–497. doi:10.1111/j.1399-3054.1962.tb08052.x.

Pang, Q., Guo, J., Chen, S., Chen, Y., Zhang, L., Fei, M., Jin, S., Li, M., Wang, Y., and Yan, X. 2012. Effect of salt treatment on the glucosinolate-myrosinase system in *Thellungiella salsuginea*. Plant Soil, **355**: 363–374.

Payne, R.W. 2013. The guide to the GenStat® command language (Release 16). Part 2: Statistics. Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden, Herts, United Kingdom.

Puppala, N., Fowler, J.L., Poindexter, L., and Bhardwaj, H.L. 1999. Evaluation of salinity tolerance of canola germination. Pages 251–253 in J. Janick, ed. Perspectives on new crops and new uses. ASHS Press, Alexandria, VA.

Qasim, M., Ashraf, M., Ashraf, M.Y., Rehman, S.U., and Rha, E.S. 2003. Salt-induced changes in two canola cultivars differing in salt tolerance. Biol. Plant. **46**(4): 629–632. doi:10.1023/a:1024844402000.

Sakr, M.T., El-Sarkassy, N.M., and Fuller, M.P. 2012. Osmoregulators proline and glycine betaine counteract salinity stress in canola. Agron. Sustain. Dev. **32**: 747–754. doi:10.1007/s13593-011-0076-3.

Shannon, M.C. 1986. New insights in plant breeding efforts for improved salt tolerance. Hort. Technol. **6**: 96–99.

Statistics Canada. 2017. Farm and farm operator data. Release 95-640-X. Statistics Canada, Ottawa, ON. https://www150.statcan.gc.ca/n1/pub/95-640-x/95-640-x2016001-eng.htm [accessed 5 Aug. 2019].

Steppuhn, H., and Wall, K.G. 1999. Canada's salt testing laboratory. Can. Agric. Eng. **40**: 241–247.

Steppuhn, H., Volkmar, K.M., and Miller, P.R. 2001. Comparing canola, field pea, dry bean, and durum wheat crops grwon in saline media. Crop Sci. **41**: 1827–1833. doi:10.2135/cropsci2001.1827.

Stumpf, D.K., Prisco, J.T., Weeks, J.R., Lindley, V.A., and O'Leary, J.W. 1986. Salinity and *Salicornia bigelovii* Torr. seedling

establishment. Water relations. J. Exp. Bot. **37**(2): 160–169. doi:10.1093/jxb/37.2.160.

Sun, Y., Niu, G., Wallace, R., Masabni, J., and Gu, M. 2015. Relative salt tolerance of seven strawberry cultivars. Horticulturae, **1**: 27–43. doi:10.3390/horticulturae1010027.

Yuan, G., Wang, X., Guo, R., and Wang, Q. 2011. Effect of salt stress on phenolic compounds, glucosinolates, myrosinase and antioxidant activity in radish sprouts. Food Chem. **121**: 1014–1019. doi:10.1016/j.foodchem.2010.01.040.

Vassilev, A., Yordanov, I., and Tsonev, T. 1998. Physiological responses of two barley cultivars to soil pollution with cadmium. Environ. Pollut. **100**: 1–7.

Zivdar, S., Khaleghi, E., and Dehkordi, F.S. 2011. Effect of salinity and temperature on seed germination indices of *Zinnia elegans* L. J. Appl. Hort. **13**(1): 48–51.