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GENOTYPIC VARIATIONS IN SALT TOLERANCE OF SOME EGYPTIAN WILD BEET ACCESSIONS

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ABSTRACT

This study reports for the first time the eco-physiological responses of some Egyptian wild beet accessions (*WB1013*, *WB1021* and *WB1026*) under saline irrigation. The plants were exposed to five seawater salinities (0, 20, 30, 40 and 60% sws) for 6 weeks in a sandy culture in the greenhouse. Low salinity (20% sws) slightly enhanced the plant fresh weight of both *WB1013* and *WB1021* genotypes by 5% and 3% respectively, but significantly reduced that of *WB1026* genotype by about 10%. Higher seawater salinities, however, caused progressive growth reductions in all accessions, with maximum growth inhibition, being 59% in *WB1026* at 60% sws. Tolerance threshold was lowest (being at 20% sws) for *WB1026*, but highest (reached 40% sws) for both genotypes *WB1013* and *WB1021*. EC_{50} was at salinity level of 40 – 60% sws for *WB1026* genotype, but higher than 60% sws for genotypes *WB1013* and *WB1021*. These indicate that both accessions *WB1013* and *WB1021* are more salt-tolerant when compared with *WB1026*. The higher salt tolerance of *WB1013* and *WB1021* accessions is largely conferred by higher leaf K^+/Na^+ ratio, due to low Na^+ and Cl^- accumulation under saline conditions compared to *WB1026*. This was associated with lower dry weights and ion leakage, and with higher leaf area, chlorophyll readings, total soluble carbohydrates and Ca^{2+} concentrations when compared with *WB1026*. Both *WB1013* and *WB1021* accessions do not only offer the possibility of being an alternative promising cash crops under seawater irrigation, but also, through an understanding of its physiology, may provide possible routes to enhance salt tolerance in other beet crops.

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INTRODUCTION

Soil salinity is gaining great attention world over, due to its negative impacts on plant growth, crop yield and thus food security, particularly under arid climates **(Hussin et al 2017)**. Due to some estimates, 8% of the earth's surface and more than 30% of the global irrigated lands are salt-affected **(Hajiboland 2013)**. The widespread of soil salinization is becoming even more prevalent on the eve of the global climate changes **(Lavania et al 2015).** At present about 1×10^6 ha cultivated area in Egypt is salt-affected, due to improper irrigation and drainage practices **(Barbouchi et al 2015)**. Efforts to develop high yielding crops for salt-affected environments using conventional breeding and gene manipulation have been intensified over the past 25 years **(Flowers, 2004)**. The outcomes, although promising, remain not significant so far, due to the multi-genic nature of the trait and comparatively limited gene pool available **(Koyro et al 2013)**. The development of halophytes, which constitute about 1% of the world's flora **(Flowers & Colmer 2008)** to useful plants "cash crops" would be a feasible and efficient solution that fosters crop productivity on salt-hit areas **(Rozema & Flowers 2008)**. Owing to their high salt tolerance and diverse options of use, particularly for economic interests (food, fodder) or ecological reasons (soil desalinisation, dune fixation, CO_2-sequestration), cash crop halophytes has entered the realm of economic feasibility in the world's developed and developing countries **(Lieth & Mochtschenko 2002)**. Wild beet, *Beta vulgaris* ssp. *maritima* (L.), the halophyte ancestor of the existing beets (*B. vulgaris*) **(Biancardi et al 2012)**, can be an excellent cash crop with enormous potentials for marginal and salt-affected areas **(Koyro et al 2006)**. The first use of *B. maritima* goes back to prehistory, when the leaves and roots were harvested and

domesticated as raw vegetable or potherb **(von Boguslawski 1984)**. The leaves and roots have been also used against several ailments and diseases **(Biancardi et al 2012)**. Its center of origin is widely thought to be the Middle East, from where it spread west into the Mediterranean and north along the Atlantic seacoast and into the mountains of Turkey **(Biancardi et al 2012)**. Thought wild beet is closely related to the other cultivated forms of beet (i.e., table, leaf, fodder and sugar beet) **(Pink 1993)**, it is distinguished with specific allele composition, exhibiting potentially higher salt tolerance **(Daoud et al 2008 and Rozema et al 2015)**. A precondition for judicious utilisation of a promising candidate halophyte such as wild (sea) beet is precise and detailed information about its level of salinity tolerance and the various mechanisms enabling the plant to grow at (their natural) saline habitats **(Koyro et al 2006)**. Reportedly, salinity tolerance is a multifarious feature, governed by an array of interconnected physiological, morphological and biochemical mechanisms operating at cellular, organ and whole plant level **(Munns & Tester 2008 and Flowers et al 2015)**. Although the degree and the specific mechanism(s) of tolerance may vary from species to the other, all these mechanisms are largely connected to the major constraints of plant growth under saline conditions: (i) water deficit, (ii) restriction of CO_2 uptake, (iii) ion toxicity, and (iv) ion imbalance **(Eisa et al 2012 and Hussin et al 2017)**. A number of investigations have indicated that wild beet plants were able to adjust osmotically to maintain a positive water balance in response to water salinity **(Koyro & Huchzermeyer 1999 and Daoud et al 2008)**. Osmotic adjustment in sea beet was mainly achieved by massive accumulation of inorganic ions (Na^+ and Cl^-) in plant tissues, particularly in the leaves **(Koyro et al 2006 and Dauod et al 2008)**. In most cases, osmotic adjustment by salt inclusion was associated with a reduction in K^+, Ca^{2+} and Mg^{2+} contents, leading to extreme ion ratios within the plant tissues **(Koyro et al 2006 and Daoud et al 2008)**. In the tap root of salt-stressed wild beet, ion (Na^+, K^+, Ca^{2+} and Mg^{2+}) concentrations were found to be low to explain the low osmotic potential values **(Daoud et al 2008)**. The concentrations of organic osmolytes, mainly soluble carbohydrates and proline, were high enough to compensate the osmotic stress imposed by saline treatments **(Koyro et al 2006 and Daoud et al 2008)**. A considerable amount of evidence has accumulated recently, proving the existence of genetic diversity within *B. maritima*, which has evolved due to interactions of genetic, climatic, and soil factors **(Andrello et al 2015)**. This genetic variance is considered essential for survival at hostile environments **(de Vilmorin 1923)**, and might represent a valuable genetic resource within the goal of broadening sugar beet gene pool for biotic and abiotic stresses **(Panella & Lewellen 2007)**. Despite an apparent interest in using wild beet as a model system, a potential donor of salt tolerance genes, and even as a potential halophytic cash crop, very little is known about the differences in salt tolerance level and the mechanisms conferring this tolerance in the Egyptian wild beet accessions. Against this background, this study aims mainly at monitoring salt-induced responses of three Egyptian wild beet accessions to get precise insights into the salt tolerance threshold and individual adaptive mechanisms conferring tolerance differences (if any) in these closely related beet accessions. This may open prospects to select the most promising genotype(s) for more comprehensive field trials and point out physiological targets for breeding program aimed at improving salt tolerance of other beet crops.

MATERIALS AND METHODS

Plant materials, experimental design and growth conditions

This study was performed at the greenhouse of Agricultural Botany Dept., Fac. of Agric., Ain Shams Univ., Egypt. Two successive agricultural seasons were performed during 2014-2015 and 2015-2016 to investigate the eco-physiological responses of some Egyptian wild beet accessions to seawater irrigation. Three wild beet accessions (*Beta vulgaris* subsp. *maritima*) from different geographical and ecological zones were selected from twenty-six genotypes of Egyptian core collection. **Table (1)** shows the location, international database Beta number (IDBBNR) and international accession number (ID) for the selected accessions. The seeds of these accessions were kindly provided by Dr. Alan L. Hodgdon, USDA, ARS, USA. They were washed with distilled water to remove any germination inhibitor and sown (on December 25, 2014 for the first season and on December 31, 2015 for the second season) in black plastic pots (25 cm diameter), filled with washed quartz sand (12.5 kg each, dry weight base). The pots were kept on a bench in the greenhouse at ambient temperature of 20 ± 3ºC (daytime) and 13 ± 3ºC (night time), a photoperiod of 9 – 10 h, relative

humidity 45 - 70% and light intensity of 1500 – 2000 µmol m^{-2} s^{-1}. The pots were watered manually with tap water every 2-day intervals for 2 weeks. After the emergence of the true leaves (three weeks after germination), the seedlings were thinned to three seedlings of uniform size per pot. The plants were irrigated with Hoagland nutrient solution, modified by Johnson **(Johnson et al 1957)** and the soil water holding capacity was maintained at 50 – 60%. Salinity treatments started after 2 weeks by adding seawater to the nutrient solution step-wisely until the final concentrations were achieved to avoid salt shock injuries. Chemical analyses and mineral contents of the seawater used for irrigation in this study are presented in **Table (2)**. There were altogether five salinity treatments (five replicate pots each treatment): 1.4, 10, 15, 20, and 30 dS m^{-1} (equivalent to 0, 20, 30, 40 and 60% seawater salinity). Salinity treatments were performed for a total period of six weeks.

Harvest procedure and growth parameters measurement

Plants of each accession were destructively harvested (three replicates from each treatment). The plants were separated into roots and leaves. The root segments were gently cleaned from sand and washed with distilled water to remove excess of nutrients and salts. They were then blotted carefully with tissue paper to remove the surface water. The fresh weights of both roots (RFW) and leaves (LFW) were directly recorded. Salinity tolerance threshold (seawater salinity that caused an initial significant reduction in the maximum fresh weight; **Shannon & Grieve 1999)** and EC_{50} (seawater level that reduces maximum plant fresh by 50 %) were determined. The area of the uppermost three fully expanded mature leaves was recorded using LI-3100C area meter (LI-COR, Inc., Lincoln, NE, USA). For the determination of water contents, plant materials were dried in an oven at 70°C until constant weight was obtained.

Determination of electrolyte (ion) leakage

Electrolyte leakage was determined according to **Lutts et al (1996)**, with minor modification by cutting 1 cm diameter leaf disks from young leaves of each accession. Leaf discs were washed with distilled water to remove adhered electrolytes and then blotted carefully with tissue paper to remove the surface water. One gram of these washed leaf disks was immersed in 10 ml distilled water in stopped sigma tube, placed on orbital shaker with gently agitation (100 rpm) for 2 h at room temperature. Electrical conductivity of the agitated solution ($EC_{initial}$) was measured, before the samples were autoclaving. The electrical conductance of the solution was measured again after autoclaving (EC_{final}). Percentage of electrolyte (ion) leakage was then calculated using the following formula:

$$\textit{Electrolyte leakage (\%)} = (EC_{initial}/EC_{final}) \times 100$$

Determination of chlorophyll

Chlorophyll content of the young, but fully expanded leaves which grew completely under the treatment was determined with a chlorophyll meter SPAD-502Plus (Konica Minolta, Marunouchi, Japan).

Determination of root total soluble carbohydrates

Chopped dried roots were grinded in a stainless steel Wiley mill to a fine powder and used to determine root total soluble carbohydrate spectrophotometerically according to **Dubois et al (1956)**.

Determination of inorganic ion concentrations

Approximately 0.2 g of pulverized dried leaves were weighed and digested as described by **Wolf (1982)**. The cleared cool extracts were filtered and completed to a final volume of 50 ml with distilled water. Na^+ and K^+ concentrations in these extracts were measured using a Flame emission photometer (JENWAY, PFP-7, ELE Instrument Co. Ltd., UK). Ca^{2+} concentration was measured using an atomic absorption spectrophotometer (Perkin Elmer model PE 2100). Chloride (Cl^-) was determined in the dried tissue by titration against 0.05N silver nitrate using potassium chromate as an indicator as described by **Diatloff and Rengel (2001)**.

Statistical analysis

All data sets were subjected to a two-way-ANOVA analysis using the SPSS for Windows statistical data analysis package (SPSS Inc., 2002, release 16, Chicago, IL, USA) in order to determine if significant differences were existed between seawater salinity and accessions. Tukey's HSD multiple comparison post-hoc tests were employed to determine if significant ($P \leq 0.05$) differences occurred between individual treatments and accessions.

Table 1. Some information about the screened wild beet accessions. WB No, local wild beet number; IDBB NR, international database *Beta* number; Accession ID, international wild beet accession number

WB No	Location	Latitude	Longitude	IDBB NR	Accession ID
WB-1013	Alexandria	31.0N	30.2E	9742	PI 562591
WB-1021	Fayyum	29.2N	30.9E	9794	PI 562599
WB-1026	Luxor	25.0N	32.5E	9753	PI 562604

Table 2. Chemical analysis of seawater used for salinity treatments and tap water used for diluting seawater

Water source	pH	EC ($dS\ m^{-1}$)	meq/L							
			Cations				Anions			
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	SO_4^{--}	CO_3^{--}	HCO_3^{-}	Cl^{-}
Sea	7.68	62.19	93	147	635	15	122.5	0.21	3	508
Tap	6.57	0.448	3	4	1.15	0.2	0.42	Nil	1.0	2

RESULTS

Growth parameters

Fresh weight

Comparative growth responses of different Egyptian wild beet accessions (*WB1013*, *WB1021* and *WB1026*) to different seawater salinities (0, 20, 30, 40 and 60% sws) (6 weeks treatment) are illustrated in **Figure (1**). Under control conditions, the genotype *WB1013* exhibited relatively the highest fresh weight (216.6 g/plant), followed by *WB1021* (165.5 g/plant) and *WB1026* (126.5 g/plant) **(Fig. 1)**. Low seawater salinity (20% sws) slightly enhanced the plant fresh weight of both *WB1013* and *WB1021* genotypes by 5% and 3% respectively, compared to the relative controls. However, it led to a significant reduction of about 10% in the fresh weight of *WB1026* genotype compared to the controls. Higher seawater salinities, however, caused progressive growth reductions in the three wild beet accessions under evaluation, with maximum growth inhibition, being 59% in *WB1026* at 60% sws **(Fig. 1)**. The plants displayed conspicuous growth and continued to develop new leaves even at the highest salinity treatment, although with some symptoms of ion deficiency and/or toxicity, particularly in the genotype WB1026. Salinity tolerance threshold was lowest for *WB1026* (being at 20% sws), but highest for both genotypes *WB1013* and *WB1021* (reached 40% sws) **(Fig. 1)**. EC_{50} was at salinity level of 40 – 60% sws for *WB1026* genotype, but at salinity level higher than 60% sws for genotypes *WB1013* and *WB1021* **(Fig. 1)**.

Dry weight partitioning

The response of dry weight to seawater salinity differed greatly between under- and aboveground parts and between beet genotypes **(Fig. 2a and b)**. As a general trend, raising water salinity substantially declined the root dry weight of the three accessions under evaluation. Salt-induced reduction in root dry weight was greatest in *WB1026* (being 35.2%), followed by *WB1013* (30.5%) and *WB1021* (27.1%). In tendency, seawater salinity resulted in a significant increase in the leaf dry weight of WB1026, but did not significantly influence that of *WB1013* and *WB1021* accessions **(Fig. 2a and b)**. The roots and leaves of *WB1013 and WB1021* genotypes, however, showed significantly lower dry matter compared to the other genotype at the whole range of salinities ($P \leq 0.05$).

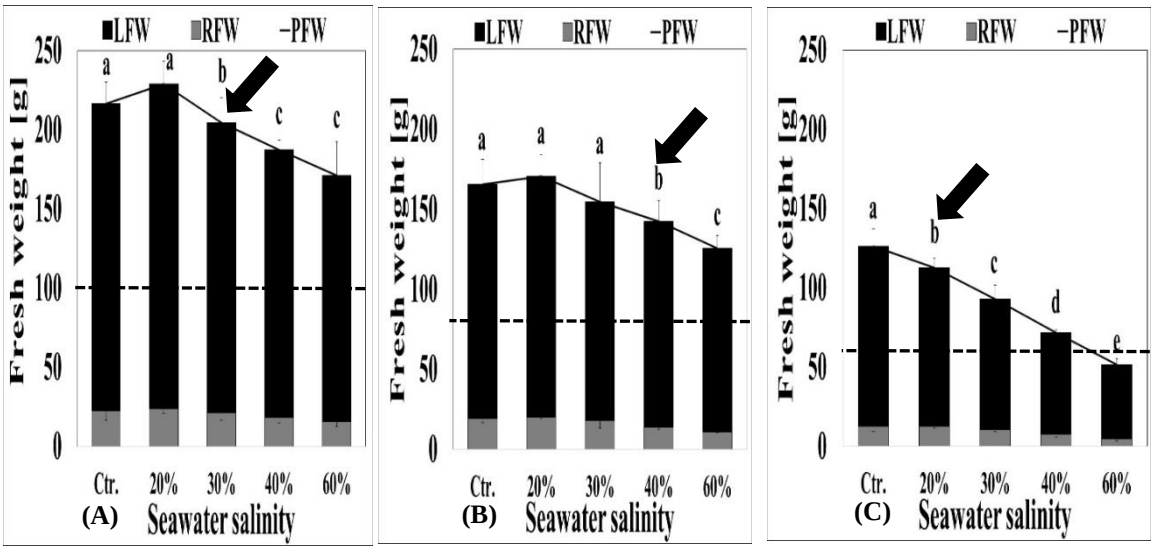

Fig. 1. Development and growth responses of the roots and leaves (expressed as fresh weights) of some Egyptian wild beet accessions upon exposure to elevating seawater salinity. (A) *WB1013*, (B) *WB1021* and (C) *WB1026*. LFW, leaf fresh weight; RFW, root fresh weight; PFW, plant fresh weight. Arrows mark the salinity resistance threshold, while the dotted lines mark EC50 values. Each column represents the mean values of six replicates ± SE. Columns with the same letter are not significantly different at $P \leq 0.05$, Tukey's HSD multiple comparison post-hoc tests.

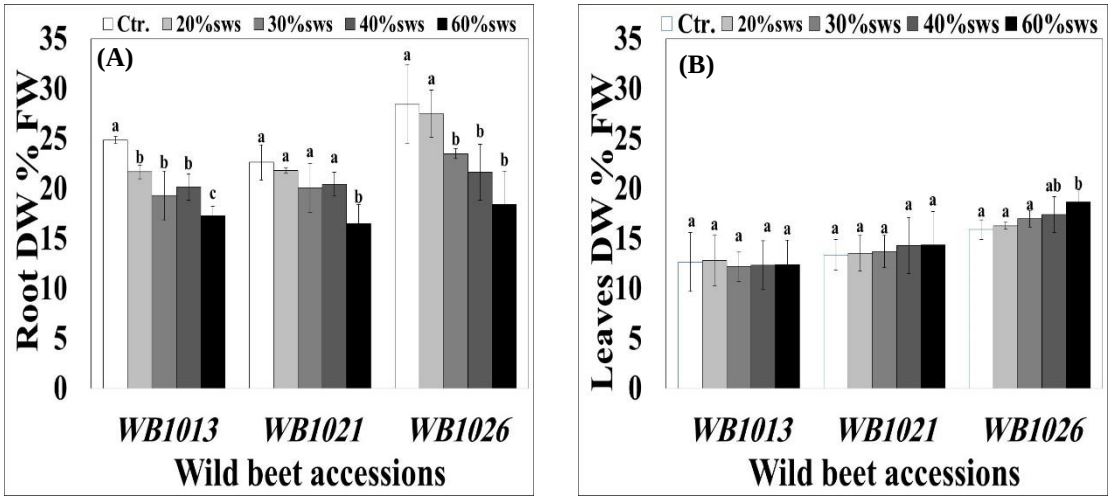

Fig. 2. Effect of seawater salinity level on the dry weight of the root (A) and leaves (B) of some Egyptian wild beet accessions. Each column represents the mean values of six replicates ± SE. Columns with the same letter are not significantly different at $P \leq 0.05$, Tukey's HSD multiple comparison post-hoc tests

Surface leaf area

Seawater substantially and significantly ($P \leq 0.05$) reduced the surface leaf area per leaf of all wild beet genotypes tested, with maximum reductions observed at the highest salinity treatment **(Fig. 3)**. Salt-induced reduction in leaf area was greatest in *WB1026* (48.8%), but lowest in *WB1013* (18.8%) genotype **(Fig. 3)**. At all salinity levels, leaf area was highest for accessions *WB1013* and *WB1021* **(Fig. 3)**.

Chlorophyll contents

SPAD readings were highest for *WB1013* and *WB1021* genotypes under control conditions **(Fig. 4)**. For all wild beet accessions, SPAD values were transiently increased in response to elevating seawater salinity, reached a maximum at seawater salinity of 40% sws **(Fig. 4)**. Thereafter, it declined significantly, although with less severe effects on the genotypes *WB1013* and *WB1021* **(Fig. 4)**.

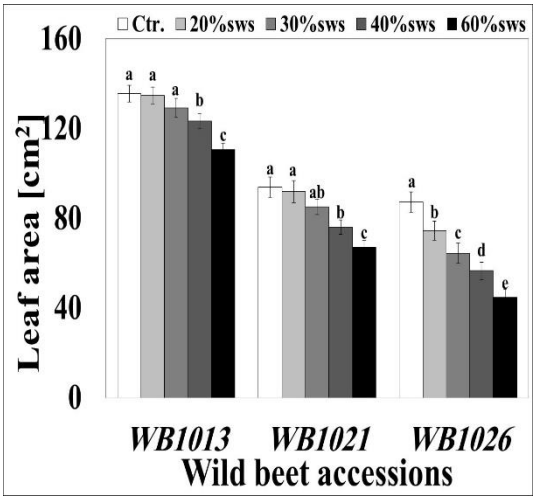

Fig. 3. Effect of seawater salinity level on the surface leaf area of some Egyptian wild beet accessions. Each column represents the mean values of six replicates ± SE. Columns with the same letter are not significantly different at $P \leq 0.05$, Tukey's HSD multiple comparison post-hoc tests

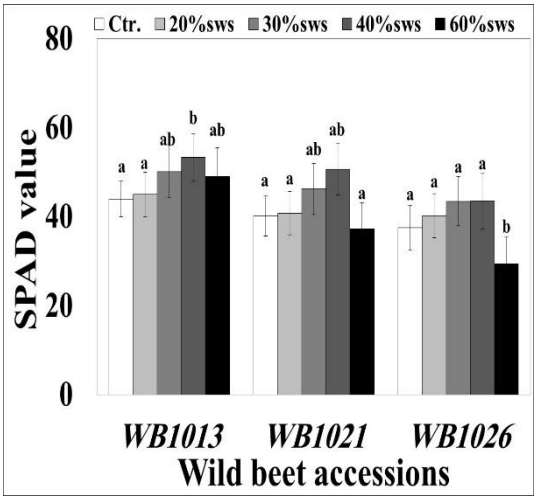

Fig. 4. Effect of seawater salinity level on leaf chlorophyll readings of some Egyptian wild beet accessions. Each column represents the mean values of six replicates ± SE. Columns with the same letter are not significantly different at $P \leq 0.05$, Tukey's HSD multiple comparison post-hoc tests.

Electrolyte (ion) leakage

Raising seawater salinity gradually and significantly ($P \leq 0.05$) increased electrolyte leakage of all wild beet accessions under the evaluation, with less adverse effect on both *WB1013* and *WB1021* **(Fig. 5)**. Wild beet accessions *WB1013* and *WB1021* exhibited the lowest ion leakage (being 76.2 and 77.8%, respectively), while *WB1026* genotype had the highest ion leakage (reached 89.1%) in response to high seawater salinity **(Fig. 5)**.

Root total soluble carbohydrates (TSC)

As a general trend, TSC increased significantly ($P \leq 0.05$) and gradually in all wild beet accessions under evaluation as seawater salinity rose **(Fig. 6)**. Again, the roots of both wild beet accessions *WB1013* and *WB1021* exhibited significantly the highest carbohydrate concentration, reached up to 30.5% and 28.2%, respectively, at the highest salinity treatment **(Fig. 6)**.

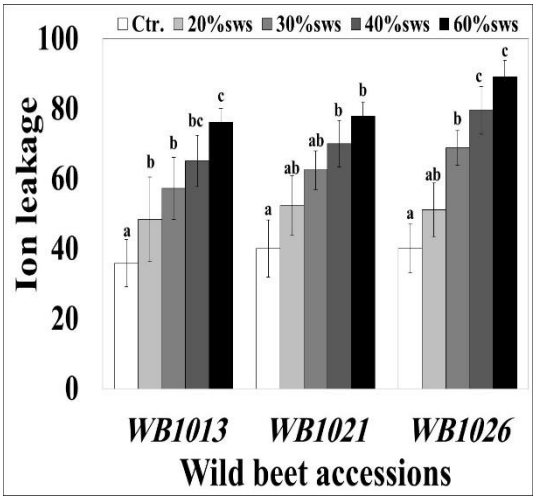

Fig. 5. Ion leakage of different wild beet accession in response to seawater salinity. Each column represents the mean values of six replicates ± SE. Columns with the same letter are not significantly different at $P \leq 0.05$, Tukey's HSD multiple comparison post-hoc tests.

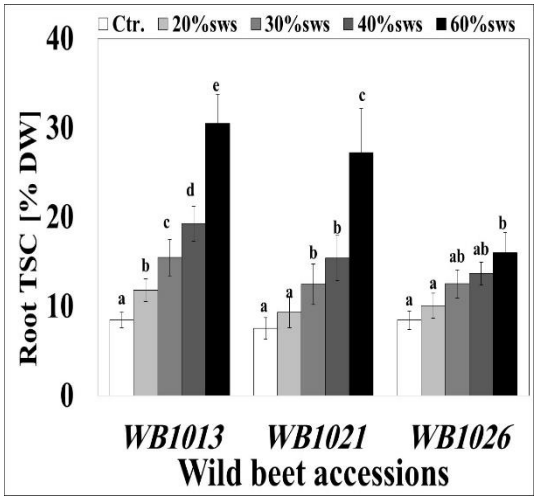

Fig. 6. Effect of seawater salinity level on the root TSC in some wild beet accessions. Each column represents the mean values of six replicates ± SE. Columns with the same letter are not significantly different at $P \leq 0.05$, Tukey's HSD multiple comparison post-hoc tests.

Leaf ion concentration

Analysis of variance revealed that leaf Na^+ concentrations were increased progressively and significantly ($P \leq 0.05$) in all wild beet accessions as seawater level increased **(Fig. 7a)**. High salinity level resulted in 9.1 – 9.4 fold increases in leaf Na^+ concentration relative to the respective controls, depending on the genotype. At this salinity level, the leaves of *WB1026* genotype had distinctly the highest Na^+ concentration (being 13.66%), followed by *WB1013* (12.52%) and *WB1021* (11.99%) **(Fig. 7a)**. The same pattern was also observed for Cl^-, i.e. it increased progressively as seawater salinity raised **(Fig. 7b)**. Salt-mediated increases in leaf Cl^- concentration were lowest (7.2 and 7.9 folds) for *WB1013* and *WB1021* genotypes, respectively, but highest (9.8 fold) for *WB1026* genotype **(Fig. 7b)**. Whatever the salinity treatment, plants of accessions *WB1013* and *WB1021* showed comparatively higher leaf K^+ concentrations **(Fig. 7c)**. Leaf K^+ concentrations decreased steadily and significantly ($P \leq 0.05$) as seawater salinity increased, reached their minimum at the highest salinity treatment **(Fig. 7c)**. This effect was much more pronounced for the plants of *WB1026* genotype, where high water salinity led to reduce leaf K^+ concentration by more than 85% compared to the respective controls **(Fig. 7c)**. Leaf Ca^{2+} concentrations were reduced upon exposure to seawater salinity in all wild beet accessions, with more adverse effect on *WB1026* genotype, where leaf Ca^{2+} reduced by 86%, relative to the controls, at the highest water salinity **(Fig. 7d)**. Salt-induced reductions in leaf Ca^{2+} were only 63% and 67% for *WB1013* and *WB1021* genotypes, respectively **(Fig. 7d)**.

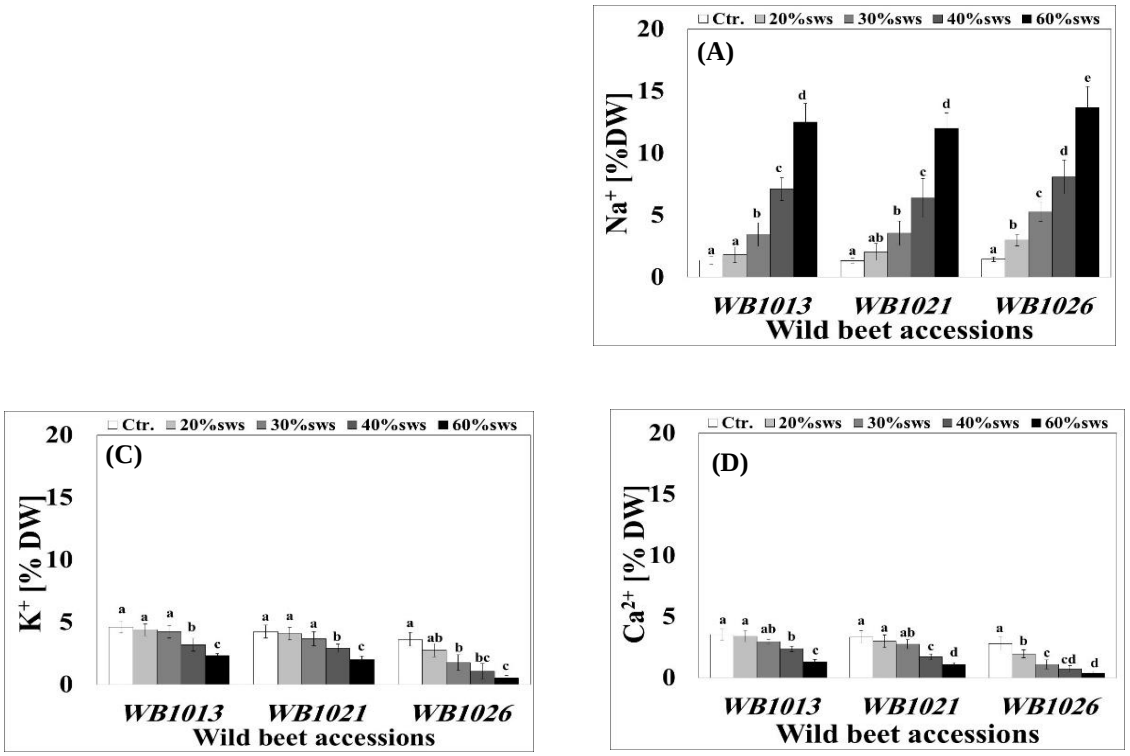

Fig. 7. Effect of seawater salinity levels on leaf Na^+ (A), Cl^- (B), K^+ (C) and Ca^{2+} (D) contents of different wild beet accessions. Each column represents the mean values of six replicates ± SE. Columns with the same letter are not significantly different at $P \leq 0.05$, Tukey's HSD multiple comparison post-hoc tests

DISCUSSION

Phenotypic variations in plant growth and biomass production were evident among sea beet accessions even when grown under non-saline conditions. Wild beet accessions *WB1013* and *WB1021* had comparatively higher fresh weight and leaf area per plant compared to *WB1026* accessions (Fig. 1 and 3). Similarly, significant differences in plant growth have been reported in *B. vulgaris*, and attributed to the wide genetic variability in this species **(Ghoulam et al 2002; Wu et al 2013 and Rozema et al 2015)**. Plant fresh weight was differentially influenced with raising seawater salinity. While low seawater salinity (20%) slightly stimulated the growth of both *WB1013* and *WB1021* genotypes, it significantly declined the fresh weight of *WB1026* genotype (Fig. 1). Salt-stimulated growth under moderate salinity has been also reported previously in sea beet **(Koyro et al 2006; Daoud et al 2008 and Rozema et al 2015)** and many other halophytic species **(Ramos et al 2004; Eisa et al 2012 and Hussin et al 2013)**. The mechanisms underlying growth promotion of halophytic species at low and moderate salinities remain unclear **(Flowers & Colmer 2008)**. Salt-induced growth stimulation in *WB1013* and *WB1021* genotypes might be largely the consequence of increased tissue water content as has been suggested for other halophytic species **(Eisa et al 2012 and Hussin et al 2013)**. High water salinity (60% sws), however, drastically reduced the fresh weight of all accessions, and again the screened accessions displayed considerable level of variability for salt tolerance. Plants of both *WB1013* and *WB1021* genotypes were least affected by high salinity treatments, exhibiting biomass reductions of about 20% and 21%, respectively, while those of *WB1026* were most sensitive, with reduction of about 59% **(Fig. 1)**. Interestingly, plants of all accessions showed conspicuous growth and developed new leaves even at the highest salinity treatment, albeit with some symptoms of ion deficiency and/or toxicity, particularly those of genotype *WB1026*. Reduced plant biomass in response to high substrate salinity is quite common in many halophytic species, including sea beet **(Koyro et al 2006 and Daoud et al 2008)**

and sugar beet **(Ghoulam et al 2002; Eisa et al 2011; Wu et al 2013 and Rozema et al 2015)**. As can be seen in **Figure (1)**, salt tolerance threshold was the lowest (at 20% sws) for *WB1026* accession, but highest (at 40% sws) for both *WB1013* and *WB1021* accessions **(Fig. 1)**. Furthermore, the EC_{50} value was lowest (at 40 – 60% sws) for *WB1026* genotype, but highest (at salinity level higher than 60% sws) for *WB1013* and *WB1021* genotypes **(Fig. 1)**. Taken together, the relative declines in biomass accumulation, EC_{50} and salinity tolerance threshold; these indicate that both accessions *WB1013* and *WB1021* are more salt-tolerant, while *WB1026* is more salt sensitive. These genotypic variations in biomass accumulation over the course of the experiment suggest that changes in plant fresh weight is a sensitive method for determining wild beet responses to salt stress. Several factors may function as a bottleneck for plant growth at high salinities **(Eisa et al 2012 and Hussin et al 2013)**. The initial detrimental effect of salinity is due to an osmotic constraint that the plant encounters in rooting medium **(Hussin et al 2017)**. Results of previous studies showed that sea beet could efficiently reduce the water potential below that of the substratum as a consequence of decreased tissue osmotic potential to maintain a positive water balance even under high saline conditions **(Koyro et al 2006 and Daoud et al 2008)**. Reportedly, salt-induced reductions in water and osmotic potentials is associated concurrently with a substantial accumulation of osmotically active solutes. Osmotic adjustment by massive accumulation of inorganic ions was amply reported in many dicotyledonous halophytic species **(Geissler et al 2009 and Hussin et al 2013)**. For the three wild beet accessions, elevating seawater salinity resulted in a substantial excessive accumulation of Na^+ and Cl^- and decreased K^+ and Ca^{2+} concentrations in the leaves **(Fig. 7a, b, c and d)**. Similar results have been reported in sea beet **(Daoud et al 2008 and Koyro et al 2006)**, sugar beet **(Daoud et al 2008; Eisa et al 2011 and Wu et al 2013)**. This implies that these wild beet accessions may behave – at the whole plant level- as salt includers, utilizing inorganic ions (less energy and carbon demanding compared to the adjustment by organic solutes) to adjust osmotically **(Koyro & Huchzermayer 1999 and Koyro et al 2006)**. This was further supported by the positive correlation between leaf dry weight and ion (Na^+ and Cl^-) accumulation in response to increasing seawater salinity. In our study, significant differences in Na^+ and Cl^- accumulation were observed between the three wild beet accessions. Among the screened accessions, *WB1013* and *WB1021* had comparatively the lowest accumulation of Na^+ (9.1 folds for both) and Cl^- (7.2 and 7.9 folds respectively). A possible explanation for the variation in ion accumulation could be due to genotypic difference in the rate of ion uptake and xylem loading between these accessions. In line with the results of **Shabala et al (2013)**, this allow to speculate that plants of *WB1013* and *WB1021* (more salt-tolerant) accessions exert more efficient mechanisms to control ion (Na^+ and Cl^-) uptake, translocation and sequestration at the whole plant level. High Na^+ and Cl^- accumulation in excess of what is needed for osmotic adjustment might lead to tissue dehydration and/or ion toxicity. This would result ultimately in growth reduction, inhibition of new leaf initiation and the formation of smaller ones, some with symptoms of nutrient disorders as observed at high salinity in *WB1026* accession (least salt-tolerant). Salt-induced increases in Na^+ concentrations were associated with drastic decreases in K^+ in all the three accessions **(Fig. 7c)**. This is in agreement with the findings from most of the published results **(Koyro et al 2006 and Daoud et al 2008)**. Such a response has been interpreted as a result of competition between K^+ and Na^+ uptake at the root level **(Hasegawa et al 2000)** or due to the changes in the membrane integrity caused by the displacement of Ca^{2+} by Na^+ **(Marschner 1995 and Tester & Davenport 2003)**. According to **Blumwald et al (2000)**, salt tolerance is, partially, related to the plant ability to avoid Na^+ accumulation and/or to maintain adequate levels of K^+ in the shoots. Intriguingly, plants of both genotypes *WB1013* and *WB1021* (most salt-tolerant) exhibited higher leaf K^+ concentrations (Fig. 7c). Consequently their leaves maintained comparatively higher K^+/Na^+ ratio (0.19 ± 0.08 and 0.18 ± 0.07), while those of *WB1026* exhibited low K^+/Na^+ ratio (0.03 ± 0.02) at the highest seawater salinity level. The substantial differences in Na^+ and K^+ accumulation between these wild beet accessions may attribute basically to the differences in selective ion uptake and transport capacities at root level as reported by **Wang et al (2002)**. Consistent with previous studies **(Blumwald et al 2000 and Munns & Tester 2008)**, this confirms that leaf K^+/Na^+ ratio could be a key physiological trait for salinity tolerance screening in wild beet. As has been previously assumed, Na^+ can replace K^+ to a certain degree in some cellular activities, especially in its osmotic functions **(Mäser et al 2002)**. This might explain, at least in part, why the growth of

both *WB1013* and *WB1021* genotypes was enhanced at low seawater salinities when K^+ concentrations reduced. Since K^+ has been implicated in turgor regulation and enzymes activation, including enzymes involved in chlorophyll biosynthesis and RubisCo **(Marschner 1995; Shabala 2003 and Munns & Tester 2008)**, severe reduction in K^+ concentrations in wild beet may have also contributed to the decreased leaf expansion, leaf area and hence plant fresh weight in seawater-treated plants. Results of the present study showed that the chlorophyll values were significantly reduced in all wild beet accessions upon exposure to high salinity treatment, although with less severe effects on the most salt-tolerant genotypes, i.e. *WB1013* and *WB1021* **(Fig. 4)**. Salt-mediated reductions in chlorophyll were widely reported under saline conditions **(Geissler et al 2009 and Eisa et al 2012)**. Ion deficiency, disturbance of chloroplast membrane, instability of the pigment protein complex and enhanced chlorophyllase activity might contribute to the reduction in chlorophyll values under saline condition **(Marschner 1995)**. Raising seawater salinity resulted also in a significant reduction in leaf Ca^{2+} concentrations in all sea beet accessions **(Fig. 7d)**. Similar observations were reported for sea beet **(Koyro & Huchzermeyer 1999; Koyro et al 2006 and Daoud et al 2008)**, sugar beet **(Wu et al 2013)** and many other halophyte species **(Wyn Jones & Gorham 2002)**. As mentioned before, high Na^+ concentration is thought to displace Ca^{2+} from the plasma membrane, causing a loss of membrane integrity and hence an increased ion leakage **(Tester & Davenport 2003)**. This was shown by the trends of electrolyte leakage **(Fig. 5)**, which increased markedly as the seawater salinity rose. Increasing electrolytes leakage due to elevating water salinity has been also reported in wild beet **(Bor et al 2003)**, sugar beet **(Ghoulam et al 2002)**, spinach plants **(Kaya et al 2001)** and rice **(Lutts et al 1996)**. Yet, the most salt tolerant wild beet accessions *WB1013* and *WB1021* exhibited clearly the lowest ion leakage, whereas *WB1026* genotype (least salt-tolerant) had the highest ion leakage in response to high seawater salinity **(Fig. 5)**, indicating severe disturbance in membrane permeability for the latter under salt stress. This conclusion is further confirmed by the fact that reductions in leaf K^+ and Ca^{2+} were lowest in *WB1013* and *WB1021* genotypes (most salt-tolerant) compared to *WB1026* genotype (least salt-tolerant) at the highest water salinity **(Fig. 7d)**. An important group of compatible solutes that was investigated in these beet accessions is the carbohydrates. Increasing seawater salinity was connected with a noticeable increase in root TSC in all accessions, albeit with significant differences between genotypes **(Fig. 6)**. Carbohydrate accumulation as a response to salinity has been reported previously in sea beet **(Koyro et al 2006 and Daoud et al 2008)** and sugar beet **(Eisa et al 2011)** and considered to play an important role in osmotic adjustment **(Koyro et al 2006)**. Several hypotheses have been mentioned to explain carbohydrates accumulation, even with suppressed photosynthesis, under salt stress. TSC accumulation is assumed to result initially from decreased export as a result of shortage of energy source (e.g. ATP) **(Munns & Termaat 1986)** or disturbed carbohydrate metabolism, regulated by various synthesizing and degrading enzymes that might be ion-specifically controlled **(Marschner 1995)**. In addition to their role as osmolytes, carbohydrates are presumed to be osmoprotectant involved in stabilizing cellular membranes, protecting proteins and enzymes, acting as a stress signal or as free radical scavengers **(Ashraf & Foolad 2007)**. These functions coincided with our results associating carbohydrate concentrations and membrane leakage in the most salt tolerant accessions *WB1013* and *WB1021*.

CONCLUSION

Results of the present study justified the potentials of wild beet as a highly salt tolerant subspecies (in terms of biomass production) able to grow, produce and reproduce at 60% sws. The ecophysiological responses of the screened wild beet accessions to seawater irrigation allow for the speculation that all of the considered parameters were affected by salinity with a varietal difference. *WB1026* was the less tolerant accession, whereas *WB1013* and *WB1021* were the more tolerant ones. Higher salt tolerance of *WB1013* and *WB1021* accessions is largely conferred by lower leaf K^+/Na^+ ratio, as a result of low Na^+ and Cl^- accumulation under saline conditions compared to *WB1026*. This was coincided with lower dry weight and ion leakage, but higher leaf area, chlorophyll values, TSC and Ca^{2+} concentrations when compared with *WB1026*. Finally yet importantly, both *WB1013* and *WB1021* accessions do not only offer the possibility of being an alternative promising cash crop under seawater irrigation, but also, through an understanding of its physiology, may provide possible routes to enhance salt tolerance in other beet crops.

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