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Physiological and biochemical characterization of sugar beet against salt-stress

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Salt stress is a major problem at the global level and it is very important to utilize salt-affected soils using appropriate techniques. Growing halophytes on saline-sodic soil is a win-win strategy for getting economic crop production and conservation of soil resources. However, it is prerequisite to identify the cultivars which can be recommended for salt-affected soils considering physiological and biochemical responses under salt-stress. Therefore, sugar beet cultivars were characterized at seedling stage based on growth, physiological and biochemical parameters under different levels of salt stress. A hydroponic experiment was conducted using eight sugar beet cultivars (AMALDI, GELLERT, VANGELIS, VEDA, WYNNYK, SERENADA, CALIFORNIA and ALBINA VEREDUANA) and two salt stress levels (60 and 120 mM NaCl) in comparison with that of an untreated control. The plants were harvested ten days after giving salt stress. Cluster dendrogram and principal component analysis were the most appropriate methods for screening the suitable cultivar. Most of the sugar beet cultivars responded as resistant against salt stress, however, VEDA cultivar performed best against it. Thus, VEDA cultivar acquired highest position at 60 mM NaCl showing 6.4% plant biomass improvement as compared to control, while 2.1% reduction was recorded at 120 mM NaCl as compared to 60 mM NaCl. Additionally, this cultivar showed highest growth attributes, antioxidant activity with higher K^+/Na^+ ratio, hydrogen peroxide, phenolic and malondialdehyde content, indicating strong defense mechanism against salt induced oxidative and osmotic stress mainly owing to the best K^+ and Na^+ balance. It is concluded from this study that VEDA is the best salt-resistant cultivar with great potential to be grown on salt-affected soils and can be proceeded for field trials at various salt-stress levels.

Keywords: salinity, sugar beet, Na^+/K^+ ratio, antioxidants, protein contents.

INTRODUCTION

Salt-affected soils are increasing day by day due to low water availability leading to use of low quality water and is considered as a threat to global food security (Ahammed *et al.*, 2018). It is reported that one billion hectares remain barren due to water scarcity and salinity in arid and semi-arid areas of the world, while secondary salts are affecting 20% area of irrigated land (Sahab *et al.,* 2021). Similarly, an average of 3.5 tons salt ha^{-1} per year is added to the irrigated areas of Pakistan. Currently, the magnitude of salty areas is increasing about 4.5 Mha due to arid conditions (Qureshi and Perry, 2021). It has been extended by 8% all over the world leading to a reduction in agriculture production. It is estimated that 27.3 USD billion annual agriculture reduction is calculated due to salinity/sodicity (Rengasamy, 2010). Salt and drought stresses are further increasing as forecasted in climate change scenario (Arzani and Ashraf, 2016). There is a dire need to utilize salt-affected soils for sustainable agriculture under such circumstances to feed the rising population. Plants are harmed through salts mainly due to direct toxic effect of ions such as Na^+, and reduction in water potential leading to hindrance in water and nutrient uptake (Wakeel *et al.*, 2010; Khan *et al.*, 2020). Many agronomic and physiological parameters of plants are affected under salt stress conditions such as photosynthetic activity, ionic imbalance and oxidative damage to protein (Muchate *et al.*, 2016; Zhang *et al.*, 2018). Seed germination is an important growth phase, affected by salinity due to imposition of water deficiency to plants reducing water uptake (Wang *et al.*, 2011; Kiran *et al.*, 2021). Under salt stress conditions, plants also undergo cellular injury especially in transpiring leaves due to accumulation of salts (Khan *et al.*, 1995).

Halophytes are salt-resistant plants playing an effective role for production under various levels of salt stress (Ozgur *et al.*, 2013). Such salt resistant-crop plants exhibit very complex physiological mechanism for scavenging the toxic effects of salts including vascular compartmentalization of Na^+ and hormone maintenance through extrusion, reactive oxygen species (ROS) scavenging enzymes production and some

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other anatomical adaptations (Stevanato *et al.*, 2013; Wang *et al.*, 2017a). Plant growth rate is directly related to stomatal conductance (Yang *et al.*, 2012) and higher stomatal conductance improves plant growth owing to more CO_2 fixation and energy production. Furthermore, CO_2 fixation reduces due to ROS production species under salt stress conditions (Sharma *et al.*, 2012; Hossain *et al.*, 2017; Cheng *et al.*, 2020).

The sequestration of Na^+ in salt resistant crop plants via Na^+/H^+ antiporter in vacuole is an important mechanism (Pehlivan *et al.*, 2016; Wu *et al.*, 2019). It reduces the concentration of Na^+ in cytoplasm during osmotic adjustments (Maathuis, 2014). High concentration of Na^+ disrupts metabolic activities in plant cell like photosynthesis generating ionic imbalance such as reduced Ca^{2+} concentration. This reduction in Ca^{2+} ions weaken plasma membrane stability (Munns and Tester, 2008; Saito and Uozumi, 2020). Thus, salt sensitive plants, which are not able to reduce Na^+ concentration in cytosol, are more affected by Na^+ stress (Peng *et al.*, 2016; Bouras *et al.*, 2021).

Different organic compounds like DNA, RNA, proteins and lipids are oxidized by reactive oxygen species (ROS) produced under salt stress, and the plants undergo severe damage (Miller *et al.*, 2010). In order to regulate under such conditions, the plants have an adaptive and defense system against antioxidants production (Romano *et al.*, 2013; Zou *et al.*, 2019). For instance, superoxide dismutase (SOD) production is increased to scavenge ROS by converting O^{-2} into O_2 and H_2O_2 in plant roots (Ahmad *et al.*, 2019). The reduction reactions convert H_2O_2 into H_2O and O_2 via catalase pathway (CAT) in peroxisomes organelle. Ascorbate peroxidase pathway produces peroxidase (POD), which is heame-containing glycoprotein to catalyze H_2O_2 using electron donors like phenolic compounds and secondary metabolites in reduction reaction (Ghaffari *et al.*, 2019). Malondialdehyde (MDA) content being an indicator of plant under stress is enhanced with increasing of lipoxygenase (LOX) activity, it acts as a catalyst for fatty acids reduction (Morales and Munné-Bosch, 2019; Namjoyan *et al.*, 2020). In general, SOD and CAT enzymatic activity is lower in salt sensitive plants than salt resistant genotype (Zhou *et al.*, 2008; Wang *et al.*, 2019). Salt resistant sugar beet cultivars exhibit greater SOD activities, POD, ascorbate and glutathione reductase with low CAT activity (Tahjib-UI-Arif *et al.*, 2019; Zhang *et al.*, 2020). Sugar beet plants perform well at 3 mM NaCl as compared to control (with no Na^+ application) (Peng *et al.*, 2014), and sugar beet can perform well in soil containing 85-140 mM NaCl salts (Li *et al.*, 2007). In sugar beet like plants, K^+ is substituted by Na^+ without reducing the yield (Wakeel *et al.*, 2010). The water availability is very crucial at this stage and salinity affects lower water intake by seed that leads to poor germination (Sadeghian and Yavari, 2004). Nevertheless, mild salinity does not affect sugar beet germination and yield. Growing of sugar beet under saline condition is an effective approach to overcome this problem. Therefore, it was hypothesized that mild NaCl application promotes salt resistance in sugar beet seedlings growth and development and different sugar beet cultivars have different capacities to resist against the salt stress. Therefore, it is worth working to identify the most salt resistant cultivars to characterize their abilities to perform under salt stress.

This study was also planned and executed to explain antioxidants production for scavenging salt stress with the aim to characterize most salt resistant sugar beet cultivars against different degrees of salt stress based on growth, physiological and biochemical changes in plants.

MATERIALS AND METHODS

Seeds of eight sugar beet cultivars were taken and tested for various degrees of salt stress under controlled conditions. Among these, five cultivars (AMALDI, GELLERT, VANGELIS, VEDA and WYNNYK) were supplied by seed production company Strube Germany. Two sugar beet cultivars (SERENADA and CALIFORNIA) were provided by KWS Germany and one (ALBINA VEREDUANA) was acquired from United Kingdom.

***Plant material and growth conditions*:** A hydroponic experiment was conducted at growth chamber at Department of Botany, University of Agriculture Faisalabad Pakistan to characterize the sugar beet cultivars for salt resistance. Sugar beet seeds were soaked overnight in water to loosen the seed coat and nursery was developed in sand filled trays. The watering was continued on a regular basis to maintain optimummoisture. The uniform seedlings were transferred to dark walled 10 L water tank at 3 leaf stage, one week after germination. The solution contained all nutrients i.e. modified Hoagland solution (Epstein and Bloom, 2006) having following recipe: [N (5.3 mM), P (4.0 mM), K (0.3 mM), Ca (2.0 mM), Mg (0.5 mM), Cu (0.2 µM), Zn (0.1 µM), Mn (0.5 µM), B (10.0 µM), Mo (0.01 µM) and Fe (10.0 µM). The plants were placed in growth chamber at 25/20 ºC, 70±5% relative humidity with 13/11 h light and dark cycle and quantum flux density was ~400 µmol m^{-2} s^{-1} (Wakeel *et al.*, 2011a). At the time of transplantation, the strength of nutrient solution was kept half and was increased to full concentration after 24 h. The nutrient solution was changed on 3rd day after transplanting the plants to containers. The nutrient solution pH was adjusted at 6.0 with 0.1 N KOH (when needed) and was checked regularly.

Overall, the arrangement of the experiment was two-way factorial (salt stress and sugar beet cultivar) and 24 treatment combinations were made. Three salt stress levels i.e. Control (without salt stress), 60 mM (S_{60}) and 120 mM (S_{120}) NaCl were quadruplicated using 10 L water tank for each replication. Salt stress was applied after establishing the plants in container for four weeks (October 17, 2019 to November 14, 2019). The salt stress was applied in splits (30

mM NaCl per 12 h) to avoid the sudden shock/injury to plants. Nutrient solution in the water tank was well aerated with air pump.

***Growth parameters*:** One week after applying the full salt stress to the highest salt treatment, plants were harvested at the age of 28 days after germination. The agronomic parameters like fresh shoot and root weight were recorded immediately after harvesting the crop. Plant dry matter was recorded after drying the plant sample at 65 ºC in a forced air oven (Red line by BINDER) to attain constant weight (Tayyab *et al.*, 2022).

***Physiological parameters*:** One plant leaf from each replicate was separated and leaf fresh weight (LFW) was measured. The fresh leaves were placed in petri plates dipped in deionized water and after covering it was placed for 48 hours in dark. The leaves weight was taken for calculation of leaf turgid weight (LTW). The samples were placed in an oven at 65 ºC for calculation of leaf dry weight (LDW) to attain constant weight. The relative leaf water content wascalculated by the following formula (Barrs and Weatherley, 1962).

$$\text{Relative water contents of leaf } (\%) = \frac{LFW-LDW}{LTW-LDW} \times 100$$

The chlorophyll contents were determined one week after applying salt stress using *SPAD* 502 Plus chlorophyll meter.

***Ascorbate peroxidase activity*:** Ascorbate peroxidase activity (APX) activity was measured using biochemical test as followed by Asada (1987). Sugar beet leaves were ground, homogenized and centrifuged at 10,000 rpm for 15 minutes. Reaction mixture (1600 µL) was comprised of potassium phosphate buffer (50 mM) ascorbic acid (0.5 mM), H_2O_2 (0.1 mM) and enzyme extract (400 µL). Declining oxidation rate of ascorbate was measured by monitoring the absorbance at 290 nm at 25ºC using spectrophotometer (Cecil Aquarius CE 7400S).

***Catalase activity*:** Sugar beet leaves were emulsified in 50 mM potassium phosphate buffer (pH 7.0) and 1 mM dithiothreitol for estimation of catalase activity followed by the method explained by Aebi (1984). Reaction mixture for CAT activity contained 50 mM potassium phosphate buffer (pH 7.0), H_2O_2 (59 mM) and sample extract (0.1 mL). The decreasing pattern in absorbance was recorded at 240 nm after adding sample in the reaction mixture. The absorbance reading was taken after every 20 s at 240 nm using spectrophotometer. One unit of CAT activity was altered as change in absorbance 0.01 min^{-1}.

***Malondialdehyde contents*:** Malondialdehyde contents (MDA), a lipid from lipid peroxidation was assessed in sugar beet leaves by thiobarbituric acid (TBA) method as reported by Dhindsa *et al.* (1981). Supernatant extract sample (1 mL) along with mixture of 1 mL trichloro acetic acid, and 2 mL of thiobarbuturic acid was determined at wavelength of 532 nm using spectrophotometer.

***Hydrogen peroxide contents*:** The preserved sample in buffer (0.1 mL), trichloro acetic acid (0.1 mL) and potassium iodide (0.1 mL) were mixed and determined hydrogen peroxide contents at 390 nm wavelength using spectrophotometer (Velikova *et al.*, 2000).

***Total soluble proteins*:** To determine total soluble proteins from preserved sample in potassium phosphate buffer, 1.0 mL Barford reagent and 0.25 mL sample were mixed and incubated it for 15-20 minutes. The absorbance was taken to calculate total soluble proteins using spectrophotometer at 595 nm wavelength followed by Bradford (1976) method.

***Ions (Na^+ and K^+) analyses*:** Fine, ground and homogenized 0.5 g dry plant sample was taken in 50 mL digestion flasks. A mixture of nitric and perchloric acid (2:1 ratio) 5 mL was added to each flask and let these for overnight covering with aluminum foil. The sample was heated at 280 ºC on a hot plate. Continuous heating was done until digested material converted into transparent solution. Samples were diluted with distilled water to make 50 mL volume in volumetric flasks after cooling. The digested material (50 mL) was filtered through Whatsman filter paper No. 1 and filtrate was collected and stored in plastic bottles for ionic (Na^+ and K^+) analyses. Sodium and K^+ concentration was determined in root and shoot samples using flame photometer (Model; Jenway PFP 7) following method (Chapman and Pratt, 1961).

***Statistical analysis*:** All the data were subjected to evaluate the significant difference among treatment means by applying two-way factorial analysis of variance (*ANOVA*) at probability level of 5% according to least significant difference (*LSD*) test with the help of Ri386 4.1.1 software using "agricolae" packages. The bar graphs were constructed using GraphPad Prim 9, principal component analysis (PCA) and hierarchical cluster dendrogram were performed using R i386 4.1.1 software with the help of "factoextra" and "ggforce" packages (Lê *et al.*, 2008; Wickham, 2016).

RESULTS

***Sugar beet growth and salt stress*:** Sugar beet growth parameters fresh shoot weight (FSW), fresh root weight (FRW), dry shoot weight (DSW), dry root weight (DRW) (Table 1) and total plant biomass have shown significant variations between cultivars under various salt stress levels. Application of Na^+ (S_{60}), as NaCl, improved total plant biomass in all sugar beet cultivars as compared to control where no salt was added (Fig. 1C). However, the reduction trend in total plant biomass in all sugar beet cultivars was recorded at higher salinity level i.e. S_{120} compared to S_{60} and control. Nevertheless, the improvement in plant biomass was the highest in VEDA at S_{60}. The increase in total biomass was due to a significant increase in shoot and root weight. In addition, total biomass was also improved significantly in GELLERT and SERENADA beet cultivars at S_{60} as compared to all other beet cultivars. The significant reduced plant bio-

Table 1. Response of various sugar beet cultivars as influenced by different application of salt stress. FSW=Fresh shoot weight, FRW=Fresh root weight, DSW=Dry shoot weight, DRW=Dry root weight.

Sugar beet cultivars	Treatments	FSW (g)	FRW (g)	DSW (g)	DRW (g)
Amaldi	Control	27.45±0.32 ef	09.40±0.30 ef	4.57±0.05 ef	1.55±0.03 de
	S_{60}	27.55±0.17 de	09.53±0.16 e	4.59±0.02 de	1.56±0.01 d
	S_{120}	20.31±0.20 m	05.28±0.18 l	3.38±0.03 m	0.86±0.04 j
Gellert	Control	26.29±0.13 gh	08.27±0.11 gh	4.38±0.02 gh	1.35±0.03 f
	S_{60}	28.64±0.40 bc	10.59±0.38 bc	4.77±0.06 bc	1.76±0.06 b
	S_{120}	21.18±0.27 kl	06.16±0.25 jk	3.53±0.04 kl	1.02±0.03 hi
Vangelis	Control	24.01±0.28 i	05.99±0.30 k	4.00±0.04 i	1.01±0.04 hi
	S_{60}	27.35±0.09 ef	09.33±0.07 ef	4.55±0.01 ef	1.54±0.02 de
	S_{120}	21.01±0.27 l	05.96±0.28 k	3.50±0.04 l	0.99±0.03 hi
Veda	Control	29.13±0.12 b	11.10±0.14 b	4.85±0.02 b	1.83±0.02 b
	S_{60}	30.34±0.09 a	12.32±0.08 a	5.05±0.01 a	2.05±0.01 a
	S_{120}	22.45±0.20 j	07.43±0.19 i	3.74±0.03 j	1.21±0.02 g
Wynnyk	Control	26.91±0.22 fg	08.88±0.20 fg	4.48±0.03 fg	1.46±0.02 e
	S_{60}	27.59±0.04 de	09.56±0.03 de	4.59±0.00 de	1.62±0.02 cd
	S_{120}	20.95±0.03 lm	05.93±0.05 k	3.49±0.00 lm	0.97±0.02 i
California	Control	24.30±0.23 i	06.27±0.22 jk	4.05±0.03 i	1.08±0.02 h
	S_{60}	27.72±0.21 de	09.67±0.19 de	4.62±0.03 de	1.61±0.03 cd
	S_{120}	21.66±0.29 k	06.63±0.30 j	3.61±0.04 k	1.04±0.03 hi
Serenada	Control	26.16±0.12 h	08.14±0.10 h	4.36±0.02 h	1.35±0.01 f
	S_{60}	28.18±0.37 cd	10.15±0.35 cd	4.69±0.06 cd	1.67±0.04 c
	S_{120}	20.31±0.10 m	05.28±0.08 l	3.38±0.01 m	0.86±0.01 j
Albina vereduna	Control	25.84±0.04 h	07.82±0.05 hi	4.30±0.00 h	1.30±0.00 fg
	S_{60}	27.43±0.31 ef	09.38±0.32 ef	4.57±0.05 ef	1.54±0.05 de
	S_{120}	19.33±0.23 n	04.31±0.21 m	3.22±0.03 n	0.73±0.02 k
	LSD value	*0.642*	*0.616*	*0.107*	*0.093*

Means in a column (± SE) followed by same letters are not significant at α=0.05 by Least significant difference test.

mass of ALBINA VEREDUANA was recorded at S_{120} as compared to other cultivars in respective treatments.

***Physiological parameters*:** Physiological parameters like chlorophyll contents (*SPAD*) and leaf relative water contents (%) were determined (Fig. 1A and B). In general, *SPAD* value as well as LRWC were improved significantly with increment of salt up to S_{60} in all sugar beet cultivars. In contrast, the reduction trend was noticed in S_{120} as compared to control and S_{60}. VEDA, sugar beet cultivar performed well in control and S_{60} as compared to all other cultivars. The physiological parameters of ALBINA VEREDUANA were significantly reduced in S_{120} as compared to control.

***Ionic (Na^+ and K^+) parameters*:** It was determined that Na^+ concentration in sugar beet shoot cultivars was increased with increment of salt stress as compared to control (Fig. 2A). No significant difference for shoot Na^+ was determined among the beet cultivars in control and S_{60}. The significant reduced shoot Na^+ concentration was determined in VEDA cultivar as compared to all other beet cultivars at S_{120}.

The beet cultivars showed non-significant trend regarding root Na^+ concentration in control where no salt was added. Surprisingly, the significant reduced concentration of Na^+ in VEDA root was determined as compared to all other beet cultivars at S_{120} (Fig. 2C). High root Na^+ concentration was determined in AMALDI, GELLERT, VENGELIS, CALIFORNIA and ALBINA VEREDUANA at S_{120} as compared to control.

Generally, shoot and root K^+ concentration in beet was determined negatively correlated to increment of salt stress (Fig. 2B and D). Shoot and root K^+ concentration was improved significantly in VEDA beet cultivar in control treatment than S_{60} and S_{120} for same cultivar. The significant improvement shoots K^+ concentration was recorded in VEDA beet cultivar as compared to other beet cultivars at S_{60}. The significant reduced shoot K^+ concentration was recorded in AMALDI, CALIFORNIA and SERENADA as compared to VEDA at S_{120}. Similarly, the significant reduced root K^+ concentration was observed in AMALDI, VANGELIS, CALIFORNIA and SERENADA as compared to VEDA cultivar at $S_{120.}$

K^+/Na^+ ratio per plant was calculated (Fig. 1D). The significant K^+/Na^+ improvement was recoded in VEDA cultivar as compared to all other beet cultivars in control treatment where no salt was added. Similarly, K^+/Na^+ improved in VEDA cultivar as compared to GALLERT, WYNNYK and ALBINA VEREDUANA at S_{60}. K^+/Na^+ also increased in VEDA as compared to AMALDI, GELLERT, VANGELIS and CALIFORNIA at S_{120}. The negative correlation was observed between K^+/Na^+ ratio and salt increment.

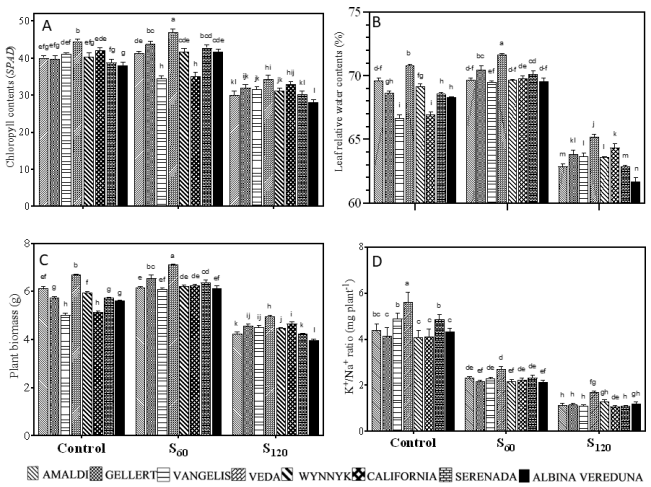

Figure 1. Effect of different salinity levels on growth related parameters of various sugar beet seedlings (A) chlorophyll contents (B) leaf relative water contents (C) plant biomass and (D) K^+/Na^+ ratio. Treatments were applied as control (without salinity), S_{60}= 60 mM NaCl and S_{120}=120 mM NaCl under controlled conditions. The column shows meansof four replications while error bars show standard error.Means sharing the similar letter (s) do not differ significantly at α=0.05 according to *LSD* test. *LSD value* 2.29, 0.57, 0.19 and 0.52 were recorded for above mentioned parameters respectively.

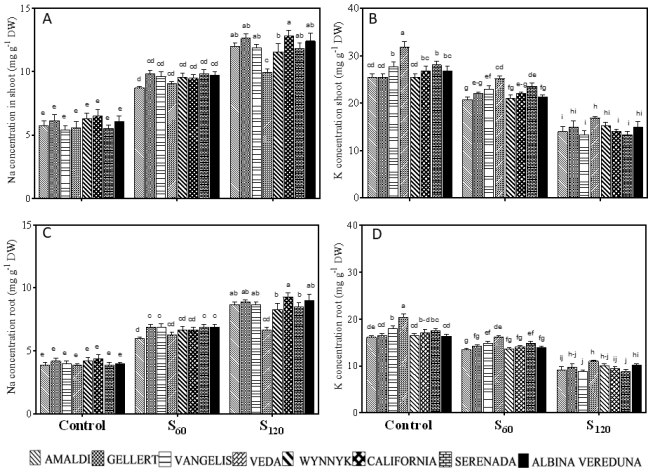

Figure 2. Effect of different salinity levels on ionic parameters of various sugar beet seedlings (A) Na^+ concentration in shoot (B) K^+ concentration in shoot (C) Na^+ concentration in root and (D) K^+ concentration in root. Treatments were applied as control (without salinity), S_{60}=60 mM NaCl and S_{120}=120 mM NaCl under controlled conditions. The column shows means of four replications while error bars show standard error. Means sharing the similar letter (s) do not differ significantly at α= 0.05 according to *LSD* test. *LSD value* 1.09, 2.22, 0.73 and 1.23 were recorded respectively for above mentioned parameters.

The data regarding K^+ uptake by plant was calculated as shown in heat map and hierarchical cluster dendrogram (Fig.4). The allocation of green color represented significant K^+ uptake in VEDA, beet cultivar in control (no salt added) followed by the same cultivar at S_{60} and S_{120} as compared to other beet cultivars. A negative relationship was found between K^+ uptake by plant and salt stress increment.

***Antioxidant/plant stress parameters*:** The antioxidant parameters including protein, catalase, phenolic contents and malondialdehyde (MDA) in shoot as well as in root of sugar beet cultivars were determined after applying salt stress. It was found that salt increment application improved shoot protein at S_{60} as compared to control but shoot protein production decreased at S_{120} in all beet cultivars (data not represented). Protein production improved in VEDA shoot at S_{60} as compared to all beet cultivars in control and S_{120}.

It was found that root protein was improved with salt increment up to S_{60} but the reverse relation was found upon further salt acclimation in sugar beet cultivars. Significant enhancement of root protein in VEDA cultivar was found at S_{60} saline conditions as compared to control and S_{120}. Low root protein production was found in GELLERT at S_{120} followed by CALIFORNIA in control treatment (data not represented). Shoot catalase activity increased upon salt application in various beet cultivars. VEDA, beet cultivar showed significant improvement response at S_{60} as compared to control. Catalase activity in root was improved significantly upon salt increment up to specific level. However, the response wasreduced gradually at S_{120} as compared to S_{60}. Veda cultivarperformed well at S_{60} as compared to control. However, nosignificant effect was observed at S_{120} and control. rootcatalase activity was reduced in AMALDI in control as comparedto other treatments (data not represented).

A positive correlation was found between shoot phenol and salt acclimation. low shoot phenolic contents were observed in VEDA cultivar in control treatment where no salt was addedas compared to other beet cultivars as well as salt stress levels(S_{60} and S_{120}). The phenolic contents in roots of beet cultivars were increased gradually as salt increment application. Less root phenol was produced at S_{60} as compared to control in VEDA cultivar (data not represented). Furthermore, root phenolwas produced high at S_{120}.

Shoot MDA contents (µg mL^{-1}) were increased gradually upon salt increment. Low shoot MDAcontents were produced in VEDA and WYNNYK cultivars as compared to other beet cultivars in control where no salt wasadded. Similarly, VEDA and CALIFORNIA cultivars performed wellat s_{60} as compared to other cultivars. High shoot MDA contentswere observed in GELLERT cultivar in all treatments. Low root MDA contents were produced in VEDA cultivar in control and S_{60}. Generally, root MDA contents in beet cultivars were increased upon salt increment. High MDA contents were produced in GELLERT cultivar at S_{120}.

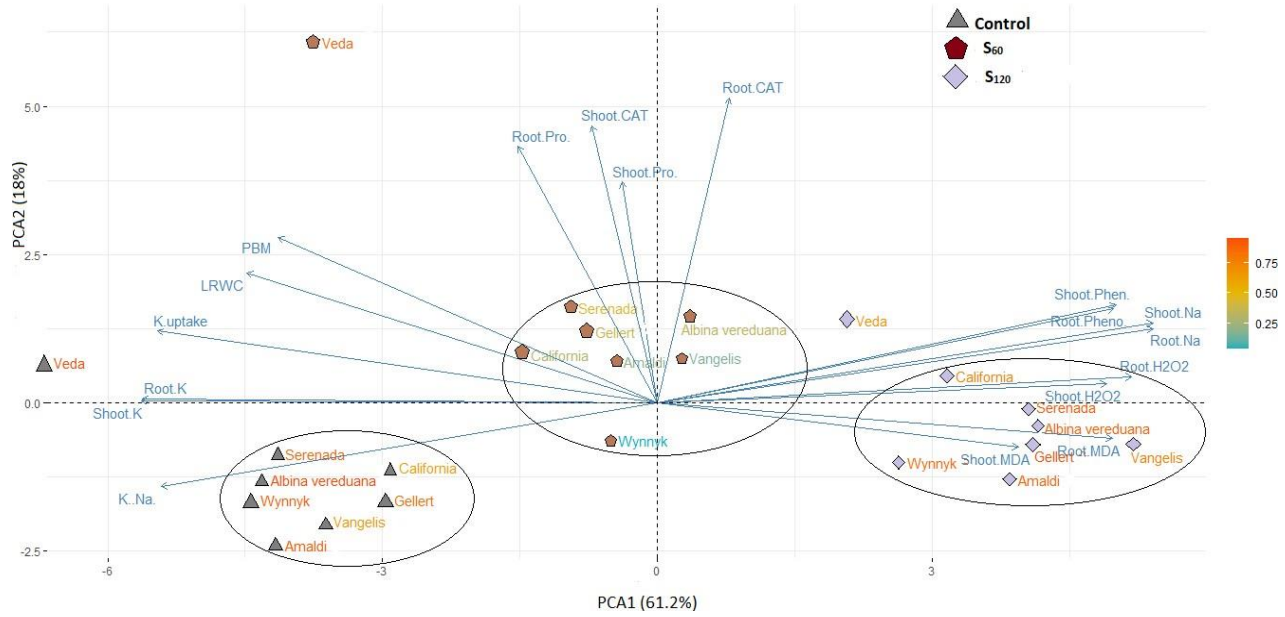

Figure 3. Cumulative principal component analysis (PCA) for the first two components (PCA1 and PCA2), describing the classification of eight sugar beet cultivars (*n* = 4) under different salinity levels (S_{60}=60 mM NaCl, S_{120}=120 mM NaCl) along with control based on agronomic, ionic and biochemical parameters of 10 days salt treated sugar beet seedlings. Sugar beet cultivars responded alike under specific treatment conditions for different parameters mentioned in circles, while VEDA cultivar responded significantly different in S_{60} followed by control and S_{120} as compared to other cultivars. The lines originating from the central point shows positive or negative correlations of different variables, their closeness indicates correlation strength and are considered for plant growth controlling factors.

cultivars and growth performance were low in that cultivar (data not represented).

Multivariable analysis/principal component analysis: The principal component analysis described 79% variability among beet cultivars in two levels of salt stress along with control by two principal components (Prin). More specifically, the first principal component (Prin 1) explained 61.2% variability and was characterized by positive and high impact factors coefficients of shoot and root Na^+ concentration, H_2O_2 contents, phenolic contents, MDA contents, catalase activity and protein production. Hence this component was representative of ionic and biochemical traits of sugar beet cultivars. This showed that sugar beet plants established effectively under salt stress conditions. The second principal component (Prin 2) explained 18% variability among the sugar beet cultivars under various salt stress conditions showing positive and negative (loading) coefficients impact factors for PBM, LRWC, K^+ uptake per plant, root and shoot K^+ concentration and K^+/Na^+ ratio (Fig. 3).

Additionally, the principal component analysis was further divided into six major components (F1, F2, F3, F4, F5 and F6). In F1 (variance: 61.2%) was identified in PBM, root and shoot Na^+, K^+, protein, catalase activity, H_2O_2, MDA, phenolic contents respectively along with LRWC, K^+ uptake per plant and K^+/Na^+ ratio as a major contributor. Meanwhile F2 (variance: 18%) was identified for above-mentioned variables as a major contributor (Table 2). In F1 all the selected factors (sugar beet cultivars and salt stress levels) were major contributors for control and S_{120} except for S_{60} (Table 3)

Most of the sugar beet cultivars fall in F1 in control and S_{120}, which indicates F1 explains the database more than F2, F3, and F4. Values in bold correspond for each cultivar to the factor for which the squared cosine is the largest.

Cluster/dendrogram analysis was performed using R software to evaluate the strong relationship between dependent variables (PBM, root and shoot Na^+, K^+, protein, catalase activity, H_2O_2, MDA, phenolic contents respectively along with LRWC, K^+ uptake per plant and K^+/Na^+ ratio) and salt stress levels (S_{60} and S_{120}) along with control. The light green color in data represented for cluster analysis showed strong positive correlation among the variables and treatments followed by dark green. Similarly, light red color showed strong negative correlation followed by dark red color. VEDA cultivar performed well at S_{60} as compared to other beet cultivars at S_{120} and control (Fig. 4).

Table 2. Values of squared cosines for PBM, K^+ uptake and K^+/Na^+ ratio in plant, LRWC and Na^+, K^+, protein, catalase activity, H_2O_2, MDA and phenolic contents in shoot and well as in root of 8 sugar beet cultivars.

Variables	F1	F2	F3	F4	F5	F6
PBM	**0.5197**	0.2362	0.0753	0.1163	0.0374	0.0074
Shoot Na^+	**0.8895**	0.0555	0.0022	0.0020	0.0201	0.0012
Root Na^+	**0.8889**	0.0477	0.0004	0.0011	0.0158	0.0016
Shoot K^+	**0.9645**	0.0000	0.0030	0.0048	0.0142	0.0007
Root K^+	**0.9599**	0.0002	0.0025	0.0048	0.0150	0.0004
Shoot Pro.	0.0045	0.4201	**0.5124**	0.0002	0.0075	0.0298
Root Pro.	0.0704	**0.5665**	0.2605	0.0113	0.0223	0.0325
Shoot CAT	0.0154	**0.6616**	0.0819	0.0736	0.1322	0.0035
Root CAT	0.0187	**0.8013**	0.0108	0.0244	0.0807	0.0139
Shoot H_2O_2	**0.7301**	0.0031	0.0033	0.0322	0.0857	0.0082
Root H_2O_2	**0.8139**	0.0059	0.0016	0.0135	0.0025	0.1212
Shoot MDA	**0.4728**	0.0170	0.0500	0.3073	0.1035	0.0029
Root MDA	**0.7482**	0.0111	0.0157	0.0736	0.0360	0.0147
Shoot Pheno.	**0.7615**	0.0828	0.0608	0.0083	0.0221	0.0075
Root Pheno.	**0.7528**	0.0760	0.1190	0.0178	0.0166	0.0073
LRWC	**0.6095**	0.1455	0.0844	0.1205	0.0247	0.0067
K uptake	**0.9024**	0.0444	0.0151	0.0270	0.0000	0.0029
K^+/Na^+	**0.8902**	0.0606	0.0075	0.0016	0.0283	0.0014
Eigenvalue	11.0129	3.2355	1.3064	0.8401	0.6649	0.2638
Variability (%)	61.1830	17.9750	7.2577	4.6673	3.6937	1.4655
Cumulative %	61.1830	79.1580	86.4156	91.0829	94.7766	96.2421

In F1, the distribution of PBM, Na^+, K^+, H_2O_2, MDA, phenolic in beet cultivars are the major contributors, meanwhile in F2 catalase activity in plant and protein in shoot sugar beet cultivars are the major contributor, In F3, shoot protein in beet cultivars is the major contributor. Whereas: PBM, plant biomass; Pro., protein; CAT, catalase activity; MDA, malondialdehyde; Pheno., phenolic; LRWC, leaf relative water contents. Values in bold correspond for each variable to the factor for which the squared cosine is the largest.

Table 3. Values of squared cosines of 8 sugar beet cultivars along with salinity levels (S_{60}= 60 mM NaCl, S_{120}= 120 Mm NaCl) along with control under hydroponic culture.

Sugar beet cultivars	Treatments	F1	F2	F3	F4	F5	F6
Amaldi	Control	**0.6376**	0.2538	0.0100	0.0123	0.0241	0.0041
Gellert	Control	**0.6626**	0.1701	0.1258	0.0023	0.0162	0.0076
Vangelis	Control	**0.5017**	0.1533	0.2700	0.0034	0.0432	0.0044
Veda	Control	**0.9297**	0.0190	0.0010	0.0132	0.0168	0.0023
Wynnyk	Control	**0.7311**	0.1643	0.0103	0.0043	0.0556	0.0058
California	Control	**0.5178**	0.1158	0.0649	0.1051	0.0887	0.0595
Serenada	Control	**0.7907**	0.0745	0.0124	0.0019	0.0409	0.0009
Albina vereduana	Control	**0.7744**	0.1752	0.0000	0.0090	0.0020	0.0000
Amaldi	S_{60}	0.0104	0.1894	0.0395	**0.2954**	0.0718	0.1620
Gellert	S_{60}	0.0027	**0.3711**	0.3329	0.1172	0.0704	0.0002
Vangelis	S_{60}	0.0136	0.1430	0.2296	**0.2721**	0.1849	0.0003
Veda	S_{60}	0.1745	**0.6948**	0.1123	0.0024	0.0135	0.0008
Wynnyk	S_{60}	0.0068	0.0368	**0.4249**	0.1662	0.2026	0.0070
California	S_{60}	0.1032	0.1952	**0.4075**	0.0056	0.0429	0.1220
Serenada	S_{60}	0.0091	**0.4466**	0.1355	0.1884	0.0037	0.1106
Albina vereduana	S_{60}	0.0919	0.2420	**0.2713**	0.1129	0.0218	0.0848
Amaldi	S_{120}	**0.7622**	0.1068	0.0385	0.0049	0.0743	0.0062
Gellert	S_{120}	**0.8776**	0.0596	0.0013	0.0037	0.0210	0.0233
Vangelis	S_{120}	**0.7413**	0.0138	0.0825	0.1250	0.0295	0.0006
Veda	S_{120}	**0.4989**	0.1758	0.0117	0.0638	0.1624	0.0027
Wynnyk	S_{120}	**0.8090**	0.0486	0.0003	0.0424	0.0237	0.0008
California	S_{120}	**0.7348**	0.0197	0.0103	0.1612	0.0063	0.0544
Serenada	S_{120}	**0.8992**	0.0008	0.0069	0.0355	0.0140	0.0050
Albina vereduana	S_{120}	**0.8672**	0.0116	0.0288	0.0182	0.0060	0.0279

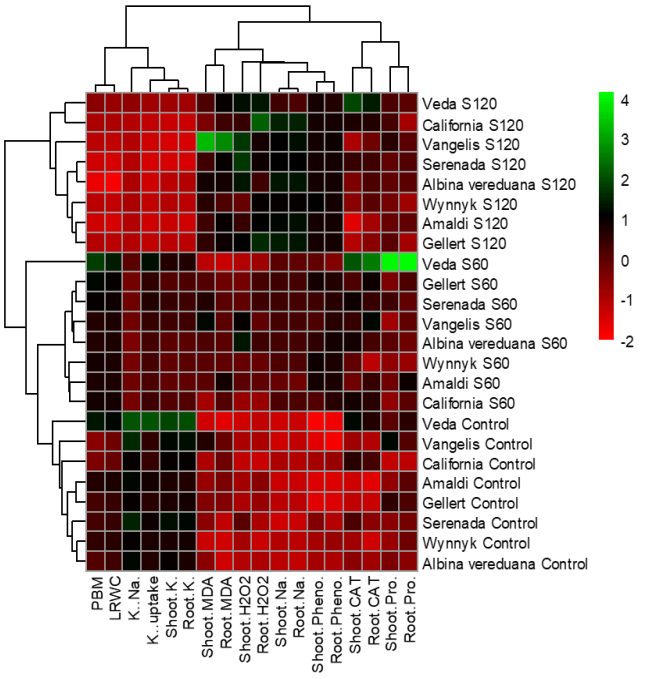

Figure 4. Hierarchical clustering analysis and heat map of agronomic, ionic and biochemical components of 8 sugar beet cultivars under different salinity levels (S_{60}= 60mM NaCl, S_{120}=120 mM NaCl) along with control

DISCUSSION

beet plants growth was improved under sodic conditions, and it acted as an osmoticum (Hussain *et al.*, 2014; Khodadadi *et al.*, 2020) In addition Na^+ played an important role for cell expansion regardless disturbing the metabolic functions in halophytes (Wang *et al.*, 2012; Wakeel, 2013). Our result was corroborated with above-mentioned studies and Na^+ might be involved in improvement of osmotic adjustment and cell expansion. Furthermore, the growth was reduced under low concentration of Na^+. In contrast it replaced K^+ functions (non-specific) under K^+ deficiency (Wakeel *et al.*, 2011b; Ali *et al.*, 2021) Although, plant growth is reduced under salt stress conditions owing to disturbing the physiological processes in plant bodyespecially photosynthesis (Müller *et al.*, 2014; Chen *et al.*, 2021). Disturbance in photosynthetic process is directly related to chlorophyll contents reduction (Ferroni *et al.*, 2020;Zhu *et al.*, 2020; Ishfaq *et al.*, 2022). Although salinityhampers the chlorophyll contents in plants depends on plant salt tolerance level. In current study the chlorophyll contents were significantly improved in sugar beet cultivars at S_{60} but the reduction trend was also observed at S_{120} salineconditions. Similar type of an experiment was conducted by (Tahjib-Ul-Arif *et al.*, 2019). They applied salinity as 75, 100,150 and 250 mM NaCl for cultivating the beet cultivars and observed that chlorophyll contents were significantly improved under 75 mM NaCl. This study was supported by the findings on many other plants (Boestfleisch and Papenbrock, 2017; Zhang *et al.*, 2017). The results showed that sugar beet has the capacity to tolerate salts due to its halophytic ancestors' sea beet The experiment was conducted to characterize the salt resistant sugar beet cultivars under various degrees of salt stress based on agronomic, physiological, ionic and stress parameters. Additionally, all the parameters were taken after analytical analysis. The relationship among these based on different concentration of salt treatments were analyzed to evaluate the performance of beet cultivars.

Halophytes require low Na^+ concentration for optimum growth and development, which are considered as salt tolerantplants (Maathuis, 2014; Zelm *et al.*, 2020). Salt tolerant plantshave unique mechanism for Na^+ extrusion from cytosol to external environment and compartmentalization of Na^+ in vacuole of cell via tonoplast Na^+/H^+ antiporters (Almeida *et al.*, 2017; Wu, 2018). However, some plants may continue metabolic functions with that element, not essential but chemically and physically is very similar to essential nutrient.Therefore, Na^+ and K^+ may replace with each other in specific plants for non-specific metabolic functions (Maathuis, 2014; Keisham *et al.*, 2018). In the current study, the result was very interesting for sugar beet plant growth under saline conditions. Na^+ played a significant role for plant biomass production at S_{60} as compared to S_{120} and control. It was observed that Na^+ played a vital role for osmotic adjustment of plant cell and improved the succulence of plant leaves without reducing the dry matter. It was evidence that sugar (Zachow *et al.*, 2014; Skorupa *et al.*, 2019) and well explains the mechanism under low andmild saline conditions. Chlorophyll contents improvement after mild salt exposure in sugar beet (Fig. 1A) might be the result of enhancement of chloroplasts in leaves and low chlorophyll contents were recorded at S_{120} owing to induced pigments degradation of its structure by ROS production(Hossain *et al.*, 2017; Wang *et al.*, 2019). Leaf relative watercontents were also improved at S_{60} in this study. High salt concentration affected hydraulic root resistance in plants to avoid loss of water by closing the stomata and sustained LRWC as reported by Farkhondeh *et al.* (2012). Nutrient balance is very crucial for optimum plant growth under saline stress conditions (Gupta and Huang, 2014; Zaman *et al.*, 2015). However, Na^+ accumulation in aerial parts of the plant is more common resulting cytosolic Na^+ accumulation than roots under saline conditions. This leads todisturb the photosynthetic machinery and other metabolic processes occurring in cytosol and ultimately reduction in plant growth is observed in most plants (Wang *et al.*, 2017, 2011). On contrary, the response of sugar beet is different under moderate saline conditions due to halophytic in nature.Higher Na^+ concentration in plants parts upregulates Na^+/H^+ mechanism to sequester cytosolic Na^+ in vacuole than exclusion in sugar beet. Plant growth was improved at S_{60} as

compared to S_{120} and control. Similar findings were identified and showed no drastic effects under moderate saline conditions (70 mM NaCl) as reported by Wang *et al.* (2017). The growth improvement in mild salinity might be due to significant involvement of Na^+ as an osmoticum in sugar beets. Furthermore, Na^+ might be involved to support nonspecific functions like osmotic adjustments in halophytes (Blumwald *et al.*, 2000; Flowers *et al.*, 2015; Xu *et al.*, 2016). Sugar beet plants showed significant growth improvement in current study at S_{60}.

Under stress conditions, ROS is produced in response to β-oxidation and photorespiration of fatty acids that are considered as normal metabolic functions. On the other hand, lethal ROS production generates and damages plasma membrane structure integrity, enzymatic dysfunctions and reduces CO_2 assimilation in plants in high salinity (You and Chan, 2015; Choudhury *et al.*, 2017; Waszczak *et al.*, 2018). Enzymatic and non-enzymatic antioxidants production take place in plant cells to protect the cell damage by scavenging excessive ROS (Birben *et al.*, 2012). A lot of studies have been conducted to improve antioxidant enzymatic activity under saline environments in different plants (Gupta *et al.*, 2018; Ahmad *et al.*, 2019). In plant cell, CAT activity is responsible to neutralize the drastic effects of H_2O_2 and phenolic contents resulting protein integrity under salt stress (Birben *et al.*, 2012). CAT activity was increased upon salt increment at S_{60} while it was decreased at S_{120} (data not represented). In addition, shoot-root H_2O_2 contents were increased with salt increment and lower contents were observed in control (Fig. 4). Different response patterns of salt tolerance were observed in each treatment. CAT and protein production might be responsible for resistance against S_{60} and S_{120} and it was found that accumulation of H_2O_2 and phenolic contents were lesser in control as compared to S_{60} and S_{120}. On contrary, high accumulation of H_2O_2 and phenolic contents under salt stress conditions might be responsible and displayed least salt resistance (data not represented). Similar results were found in rice, olive tree (*Olea europaea*) (Ahmed *et al.*, 2010), sugar beet (Zou *et al.*, 2018), soybean (*Glycine max*) (Weisany *et al.*, 2012), citrus (Arikan, 2022) and maize (*Zea mays*) (AbdElgawad *et al.*, 2016) under various degrees of salt stress. Sugar beet var. HI-0473 growth and physiological processes were significantly reduced under high concertation of salts.

In electron transport chain of plant tissue, ROS level increases owing to anomalies and increases the photo reducing power under various abiotic stresses. The extra electrochemical energy can be dissipated via the Mehler reaction producing ROS and damage the cell membrane, showed elevated MDA level (Choudhury *et al.*, 2013; Calderón *et al.*, 2018). The current study showed that MDA level was increased upon salt increment, but low MDA level was observed in control followed by S_{60} and S_{120} (data not represented) which reflects the growth performance of sugar beet. Same results (Increment of H_2O_2 and MDA) were observed in some plants (You and Chan, 2015; Nxele *et al.*, 2017; Zou *et al.*, 2018). Elevated levels of MDA and H_2O_2 might be due to photosynthesis impairment and low activity of enzymatic antioxidants.

In last, the whole dataset was analyzed to validate the effect of NaCl concentration using PCA and hierarchal cluster method (Figs. 3 and 4). The results showed that growth, physiological, chemical and stress parameters were positively correlated at S_{60} in all beet cultivars especially in VEDA, whereas at S_{120} negatively correlated to growth parameters and positively correlated to stress parameters. These findings suggest that S_{60} leads to sugar beet growth improvement while the retardation may occur under S_{120} owing to ROS production.

***Conclusion*:** High salt stress (S_{120}) reduced normal growth of sugar beet cultivars with low plant biomass production at seedling stage resulting in oxidative damage and disrupting ionic (K^+/Na^+) homeostatic balance. However, VEDA cultivar exhibited best growth performance based on agronomic and physiological parameters at S_{60}. It is concluded that VEDA is the best salt-resistant cultivar with great potential to be grown on salt-affected soils and can be proceeded for field trials at various salt-stress levels.

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***Author's Contribution Statements*:** MT executed the experiment and prepared the first draft AW conceived the idea, planned and supervised the work, and helped in manuscript preparation. MS supervised the work and helped to interpret the data, SMAB supervised the work from planning to execution.

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