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Comparative Physiological and Morphological Characterization of Salt Tolerance in *Raphanus sativus* L.

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Abstract

Salt stress is one of the main constraints for successful crop production throughout the world. Different physiological and morphological plant attributes help in prediction of plant growth and yield under salt stress. The core objective of current research was to characterize physiological and morphological parameters of different varieties of radish (*Raphanus sativus*) involved in salt stress resistance. Our results exhibited that morphological and physiological parameters including total soluble proteins, total soluble sugars, root length and leaf area had strong correlation with salt stress. The reduced quantity of morphological and physiological features excluding reducing sugars exhibited low coefficient of variation along with positive and significant correlation with yield of the plants. These morphological and physiological characteristics of crop may be used to assume salt stress tolerance in crop.

Keywords: Morphological; Physiological; Radish; *Raphanus sativus*; Salt stress

Introduction

Radish (*Raphanus sativus L.*) is an annual herbaceous plant which belongs to the family Brassicaceae. It is commonly used as vegetable crop. All parts of this plant have nutritious and medicinal value. It has an enormous potential to cure respiratory, liver, cardiovascular and gastrointestinal disorders [1,2]. Crop production is negatively affected by abiotic stress [3]. Salt stress reduces growth and yield of the crops cultivated in affected areas [4]. Different kinds of plants growing over 900 million hectares of area in arid and semiarid regions are severely affected by salt stress. During current scenario salt stress has become an alarming constraint for successful crop cultivation in all parts of the world including Pakistan. There is no sufficient research on breeding of salt resistant radish varieties in Pakistan.

The biotic stress reduces growth, yield and modifies the morphological and physiological attributes in subjected plants [5]. Reduced dry matter production has been observed in plants under stress [6]. Stress also hinders formation of chlorophyll contents and rate of photosynthesis in plants [7]. Researchers have also found improved quantity of soluble proteins, sugars, protein and reactive oxygen species in plants growing under abiotic stress [8]. In general, the susceptible plant varieties exhibit poor growth and biomass production under stress [9].

It is crucial to investigate different biological markers which depict salt stress resistance in radish. Therefore, our current research unveils the physiological and morphological attributes of salt stress resistance in different varieties of radish.

Materials and Methods

Different cultivars of radish were obtained from vegetable seed importers of Lahore, Pakistan (Table 1). Seeds were sterilized with sodium hypochlorite and sown under greenhouse conditions. These seeds were sown in sterilized salt affected beds (6 ft long) arranged in Randomized Complete Block Design (RCBD). Row to row distance was maintained at 3 ft and there were three replicates for each variety. The experimental beds were irrigated fortnightly. After harvesting, the morpho-agronomic characteristics of plants such as leaf area, root diameter, root length and root weight (yield) were analyzed. Moreover, physiological characteristics including total soluble proteins, total sugar contents, reducing and non-reducing sugars were estimated colorimetrically with the help of spectrophotometer. The prewashed root and leaf samples (1 g) were homogenized independently with phosphate buffer (10 ml) at pH 7 by using ice chilled mortar and pestle. The supernatants acquired after centrifugation of homogenates at 14000 rpm for 15 min was placed falcon tubes. The total soluble proteins, total sugar contents and reducing sugars were evaluated

S No	Varieties	Origin/Source
1	April Cross	Europe
2	Bunny Tail	Italy
3	Cherry Belle	North America
4	Champion	Europe
5	Red King	USA
6	Sicily Giant	Italy
7	Snow Ball radish	Mediterranean region
8	White Icicle	Asia
9	French Breakfast	Asia
10	Plum Purple	France/USA
11	Rood bol	Netherlands
12	Easter Egg	Greece and Egypt
13	Black Spanish	Europe
14	Daikon	Asia
15	Masato Red	India
16	Masato Green	South Asia
17	Sakurajima radish	Japan
18	Chinese radish	China
19	Misnomer	Singapore

Table 1: List of radish varieties used during present study.

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according to methodology of Roensen and Johnson [10], Yemm and Willis [11], Muhammad and Tabassum [12] respectively. Data obtained was analyzed with the help of statistical software (Statistix 8.1 and SPSS Statistics 20).

Results

During current study, results of descriptive statistics showing overall mean, minimum, maximum, standard deviation, standard error and coefficient of variation (CV %) for all the traits were observed (Table 2). The CV% of observed characteristics is a useful statistical tool for evaluating stress relating parameters. It was found that CV% ranged from 1.5% to 132.8% and 1.6% to 136.3% under control and salt stress respectively (Table 2).

The highest value of CV% was found in root weight (132.8), while lowest value for reducing sugars (1.5) was observed under control. Similar trend of CV% value for all the studied traits was shown under drought stress, whereas the highest CV was 136.3% in root weight and lowest (1.6%) in reducing sugars. Results of the current research demonstrate that parameters having lowest CV% values were more common among different varieties in contrast to those having highest CV% values. Thus, more common traits may be employed as more dependable markers for prediction of success among various varieties but the highest CV% value of root weight and total soluble protein contents confirmed the genetic diversity which exists among the wild and cultivated varieties as shown in Table 2.

During present trial, it was studied that root diameter has positive and significant correlation with root weight (r=0.961), total sugars (r=0.583), total leaf area plant-1 (r=0.563) and total soluble protein contents (r=0.765) however it is negatively and non-significantly correlated with reducing sugars (r=-0.151) as shown in Table 3. Similarly, positive and significant correlation of root weight in comparison with total leaf area plant-1 (r=0.592), root length (r=0.615), total sugars (r=0.635) and total soluble protein contents (r=0.827) was observed nevertheless negative and non-significant correlation was found in reducing sugar (r=-0.263) (Table 3). Data shown in Table 3 demonstrates that root length have positive and highly significant correlation with leaf area (r=0.892), total soluble proteins (r=0.674) and total sugars (r=0.974), however it reveals negative and non-significant correlation with reducing sugars (r=-0.356).

During current research, it was observed that leaf area exhibits negative and non-significant correlation with respect to reducing sugars (r=-0.398) but it shows positive and highly significant correlation in relation to total sugars (r=0.951) and total soluble protein contents (r=0.648). Moreover, the results of current study shown in Table 3 demonstrate that the correlation of total sugar contents depicts positive and highly significant relation with total soluble proteins (r=0.697) and negative and non-significant with reducing sugars (r=-0.374).

Results presented in Table 4 exhibits the analysis of variance for the observed traits of wild radish varieties. Analysis of variance proves that both growth and biochemical characteristics differ from variety to variety.

Discussion

The current study exhibited that most of the radish varieties showed low CV for their morphological and physiological characteristics. However, higher CV value for total soluble protein and root weight verify difference in genetic makeup of different varieties (Table 2). Nevertheless, it was tried to find some other morphological and physiological parameters for screening of salt resistant varieties of radish. Different metabolic activities of plants may be used for screening of plant varieties with desirable characteristics [13]. The correlation of these enviable metabolic activities is a valuable tool in genetic manipulation of varieties with desirable agro-economical characteristics [14]. Plants subjected to environmental stress may trigger certain metabolic activities involving synthesis of proteins and sugars which enables it to cope up with the issue [15]. The results also showed that observed parameters (except reducing sugars) had significant correlation in all varieties under trial (Table 3). The researchers have found that foliage size of the different varieties have a direct interaction with root length [16].

Stress causing reduced water utilization may reduce leaf area, transpiration rate and photosynthetic activity of the plants [17]. The Plants having higher leaf area exhibit higher photosynthetic activity and subsequent increased root length, root weight, carbohydrates/ sugar production and overall growth [18,19]. The poor plant growth may be a result of decreased growth parameters such as leaf area and sugar contents under stress [20]. Similarly, the plants under stress show reduced sugars production, photosynthetic activity, growth

Attributes	Mean		Minimum		Maximum		SD		SE	CV%		
	T_0	T_1	T_0	T_1	T_0	T_1	T_0	T_1	T_0	T_1	T_0	T_1
RD (cm)	0.45	0.43	0.0122	0.012	1.86	1.78	0.37	0.34	0.085	0.082	79.5	82.6
RL (cm)	16.4	16.68	11.37	11.5	20.19	21.3	2.57	2.61	0.57	0.55	14.8	18.1
RW (gm)	2.29	2.25	0.224	0.166	14.28	13.23	3.08	2.86	0.69	0.67	132.8	136.3
LA (cm^2)	387.9	375.8	295.12	287	462.75	450.2	53.64	54.71	11.68	11.85	12.6	15.8
RS (mg gm^{-1} of tissue)	3.52	3.54	3.45	3.46	3.53	3.58	0.061	0.058	0.014	0.015	1.5	1.6
TS (mg gm^{-1} of tissue	4.09	4.15	3.43	3.49	4.86	4.88	0.504	0.51	0.12	0.118	11.4	16.1
TSP (mg gm^{-1} of tissue)	11.35	11.45	3.65	3.68	44.25	44.3	12.65	12.59	2.81	2.74	106.4	104.35

T_0=Control, T_1=Treatment, SE=Standard Error, SD=Standard Deviation, CV=Coefficient of Variation. RD, RL, RW, LA, RS, TS and TSP Indicate Root diameter, Root length, Root weight, Leaf area, Reducing sugars, Total sugars and Total Soluble Protein Contents respectively.

Table 2: Data represents the descriptive statistics of the effects of treatment for evaluated attributes of radish varieties.

Traits	RD	RW	RL	LA	TS	RS	TSP
RD	1	0.961**	0.582**	0.563*	0.583*	-0.151	0.765**
RW		1	0.615**	0.592**	0.635**	-0.263	0.827**
RL			1	0.892**	0.947**	-0.356	0.674**
LA				1	0.951**	-0.398	0.648**
TS					1	-0.374	0.697**
RS						1	-0.332
TSP							1

Each r value superscript by asterisk/s demonstrates significant correlation: *, ** shows significance at 0.05 and 0.01% level of probability, respectively.

Table 3: Pearson Correlation between observed attributes of radish varieties under salt stress.

SOV	DF	SS	MS	F
Between	7	2166507	360987	915***
Within	129	49943	397	
Total	135	2215764		

*=Significant (p<0.05), **=Significant (p<0.01), ***=Highly Significant (p<0.001)

Table 4: Analysis of variance for the observed attributes of radish varieties.

and biomass production [21]. The susceptible plants subjected to salt stress exhibit reduced stomatal and photosynthetic activity resulting in poor growth and biomass production. The soluble sugars have a positive role in plant resistance and rejuvenation. The sugar contents also help to depict the extent of photosynthesis and production of reactive oxidative species [3,22]. Our results showed increased sugar and protein contents in resistant radish varieties. These findings are in accordance with Thalmann et al. [23] and Rascio et al. [24] who demonstrated that, sugar contents help plants to survive under water scarcity by prevention of protein degradation during stress.

The difference in morphological and physiological activities of different radish varieties reveals the potential of these parameters for genetic manipulation of radish cultivars having higher yield and better salt resistance (Table 4).

Conclusion

The current study shows that growth parameters such as leaf area and root weight in conjunction with total soluble protein and sugar contents may help in depiction of salt stress in different varieties of radish. The observed morphological and physiological traits will help in genetic manipulation of salt tolerant radish cultivars through genetic engineering.

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