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Evaluation of 10 Genotypes of Rapeseed (*Brassica napus* L.) Under NaCl Salinity Tolerance

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ABSTRACT

Soil salinity is the serious abiotic stress that affects plant growth attributes along with physiology and biochemical processes through osmotic stress, which consequently reduced biomass production. This adverse effect of salt stress appears on all growth stages including germination, seedling, vegetative and maturity. However, tolerance to salt stress at different plant developmental stages varies from species to species. The present research work was conducted to explore the impact of salts stress on ten varieties of rapeseed (*Sandal canola, Rainbow, Legend, Punjab canola, Dunkled, Oscar, AC Excel, Super canola, Shiralee and Faisal canola*) and to screen the best salt tolerant variety of Rapeseed. Seeds were sown in plastic trays of (70x50cm) to check their germination under salt stress in Lab experiment. Four treatments were prepared with control (0 mM) and three NaCl treatments (50, 100 and 150 mM). The results showed that at 150Mm levels of salinity germination occurred, but at low level as compared to other treatments (0, 50 and 100 mM). Different parameters such as germination percentage, shoot and root length and shoot and root weights (both fresh and dry) showed considerable variations. Germination percentage, root and shoot length and root and shoot weights decreased in all varieties with the increase of salt concentration. However, Shiralee and Super canola showed the highest germination and well growth in all parameters considered as resistant to salt stress while Punjab canola and Oscar showed less germination regarded as sensitive to salinity. Other varieties showed intermediate values in terms of germination and shoot and root fresh and dry weights.

INTRODUCTION

Any extraneous biotic (herbivore, parasite, pathogen, etc.) or abiotic (salinity, heat, water, etc.) barrier that decelerates photosynthesis and makes it intricate for plants to convert energy into biomass is referred to as stress[1]. Due to its impact on agricultural and plant yield, environmental stress has become a significant scientific concern. There are several environmental pressures that plants face during their life cycle including cold, heat, drought, salinity and nutrient inadequacy[2]. Many of the world's most important crops may suffer a drop in production of up to and even more than 50% as a result of these pressures[3]. Salinity is categorized into two forms, i.e., primary, which is naturally occurring, and secondary, which is human-imposed (dry land and irrigated land salinity). Primary salinity is the gradual deposition of salt for an extended period due to natural phenomena, while secondary salinity is the deposition of salt as a result of improper management of natural resources. Most saline soils have developed as a result of natural processes like salt deposition over extended periods of time in arid and semi-arid regions[4]. This is due to the fact that the parent rock from which it developed contains salts primarily sodium, calcium, and magnesium chlorides, as well as sulfates and carbonates to some extent. Another main origin of salt is seawater along the coast. A considerable amount of cultivated land has turned salty as a result of land clearing or irrigation in addition to natural salinity[4]. Reduced agricultural productivity is a global issue caused by soil salinity. Water stress, cytotoxicity from an excess of ions like sodium (Na+) and chloride (Cl-), and nutritional imbalance all contribute to the negative effects of salinity on plant growth and development. Salinity also triggers oxidative stress because it produces reactive oxygen species (ROS). Various factors contribute to an individual's resistance to both types of stress, including osmotic tolerance, ionic tolerance, and tissue tolerance. To save water, stomatal conductance quickly decreases as osmotic tolerance builds. These systems often use rapid long-distance (root to shoot) transmission channels and they do not distinguish between osmotic effects mediated by NaCl, KCl, mannitol, or polyethylene glycol[5]. The chief topics of study on plants' ability to tolerate salinity include water relationships, photosynthesis, and the deposition of different inorganic ions and molecules. Moreover, due to

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the intricate nature of the process governing salt tolerance, these factors differ between species to species[6]. In some reviews it is claim that there is still a lack of understanding of the metabolic areas where salt stress harms plants, and that there are no well-defined physiological or biochemical selection criteria for plants that may be utilized to increase plants' capacity to withstand salt in crops[6]. A number of studies, however, illustrated that the intra-specific genetic variability for salt resistance may be evaluated by inspecting a large range of cultivars under high salinity, employing traits like growth, photosynthetic capacity, osmotic adjustment, ion homeostasis, antioxidant enzymes and cell membrane integrity. It is widely observed that salt tolerant plants screened in greenhouse circumstances also exhibited salt tolerance in field conditions, despite the fact that selection criteria for salt tolerance should be based on field performance of plants over the whole growing season[7]. All the Brassica species including Indian mustard (*Brassica juncea* L.), Turnip rape (*Brassica rapa* L.), canola (*Brassica napus* L.) and cabbage (*Brassica oleracea* L.) are affected by salinity in both qualitative and quantitative parameters, but *Brassica rapa* is more susceptible as comparison with B. napus (canola) [8]. It is vital to find and describe better genotypes against these different environmental conditions since the high salt content in irrigation water and soil reduces the germination rate of nearly all Brassica species, but occasionally plant germination might occur with stunted growth and poor development[8]. Brassica's genetic makeup possesses inter- & intraspecific variability, which can be employed to improve plant's susceptibility in selection and breeding[9]. Rapeseed (*Brassica napus* L.) is an allotetraploid crop that falls under the family Brassicaceae, which has 419 genera and 4130 species. The crop was created by the natural hybridization and pairing of Brassica rapa and Brassica oleracea around 7500 years ago[10]. Following soybean and palm, rapeseed is the third-highest source of edible oil[11]. Its seeds are composed mainly of protein (25%), with a small amount of oil[12]. About 30.6 to 48.3 percent of the dry weight of rapeseed is made up of oil, and the oil status of rapeseed includes important fatty acids like oleic (56.80 to 64.92 percent), palmitic (4.18 to 5.01 percent), linoleic (17.11 to 20.92 percent) acids and 13.22 percent to 40.01 percent α-tocopherol[13]. The world's dry and semiarid regions, where salinity is or will be a major problem, are frequently where canola is farmed. Due to its evolutionary history and complex genome, rapeseed is a promising genome for plant breeding research[13]. Because of its incredible history, it plays a leading role among crops that have been exposed to substantial breeding and genetic modification. Additionally, it is acknowledged as a genetically engineered crop on a global scale[14]. Because canola oil is edible, it is regarded as the third most important crop after soybeans and palm oil. As a result, canola production has expanded to keep up with demand, but the majority of the land is suffering from salinity[15]. Soil irrigated with saline water has been caused economic consequences for farmer and consumers by declining the growth and production of this essential oilseed crop[15]. Salinity is a significant global issue for the production of rapeseed despite the fact that it is a crop that tolerates some salt and is regarded as semi-salt tolerant. Although some rapeseed genotypes exhibit excellent saline resistance, others are salt-sensitive[16]. By boosting the salt tolerance in field crops and by leaching the salt from soil, it is helpful to gain the yield that this major abiotic stress (salinity) has reduced. *B. napus* is also thought to grow potentially well in salt-affected locations, although further research is needed to determine the extent of the species' genetic diversity with regard to resistance to salinity[17]. Brassica's germination is seriously affected by the upward mobility of soil fluids pursued by evaporation, which also causes salt buildup in leaves results in leaf mortality before maturity[9]. Because of its high economic worth, rapeseed is frequently grown for its oil, leafy greens, spice, fodder, and green manure[18]. Abiotic stressors (restricted moisture availability, excessive transpiration, and persistently high temperatures) have recently exacerbated soil salinization and further impeded rapeseed development in the Ukraine. The majority of earlier studies on Brassica species were primarily concerned with comparing the morphology, physiology, and gene expression of various cultivars in response to salt stress[19], while very little research has been done on the morphological and physiological processes of rapeseed's various tissues' response to salt stress. Therefore, we sought to learn more about the processes underlying morphological adaptation and the impacts of antioxidant enzymes in the roots and shoots of salinity-exposed rapeseed seedlings. Different tissue adaptations help us understand the process of salt tolerance and will help future breeding efforts improve how plants can respond to stress. The present study was designed to access phenotypic responses of different rapeseed cultivars under salt stress and to compare the performance of selected varieties of rapeseed under saline conditions.

MATERIAL AND METHODS

Experimental Setup

At the Bahauddin Zakariya University Multan's Institute of Pure & Applied Biology, the experiment was carried out in a laboratory. Ten varieties of rapeseed (Sandal canola, Rainbow, Legend, Punjab canola, Dunkled, Oscar, AC Excel, Super canola, Shiralee and Faisal canola) were obtained from Ayub Research Center Faisalabad, Pakistan. Plastic trays of size 70x50cm containing three filter paper's layers are taken, and Hoagland solution containing micronutrient was added and salt (NaCl) treatment of various concentrations (50mM, 100mM, and 150mM) were applied to the seeds of each variety to promote salinity stress and the preservation of consistent osmotic potentials. One was a control that simply utilized the Hoagland solution (Non-saline). On October 6th, 2024, twenty seeds from every variety of rapeseed were planted in that plastic tray that had been lined with filter paper. The experiment was executed under laboratory circumstances at (33+3°C) using a completely randomized design (CRD). In plastic trays, 15 ml of Hoagland solution with and without NaCl stress were given.

Meta-Analyses Plants

After 56 days of seedling development, the harvesting of plants was done, and data was collected for various measurements. In this study, shoot length, root length,

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shoot fresh weight, shoot dry weight, root fresh weight and root dry weight were explored by following the method adopted by reference[20,21].

Statistical Analyses

Analysis of variance was performed to evaluate the influence chromium stress in the presence of biochar on the growth parameters of bottle gourds and the accumulation of metals in plant tissues. Analysis of variance method used and each treatment was with four replications. For statistical analysis of data, Minitab (Version 17) computer program was used. The differences among the means were compared using the Duncan multiple range test (DMRT) ($p < 0.05$).

RESULTS

Shoot Length

Treatment had influenced the shoot length in all ten varieties of rapeseed (Table 1). Replicate had non-significant difference between them. Control (non-saline) plants had shown more shoot length as compared to other three salinity treatments (T2, T3 and T4). Shoot length obtained by ten varieties was between 8.59cm and 9.18cm under non-saline condition, whereas Shiralee showed the highest and Punjab canola showed the lowest shoot length. In 50mM salt stress treatment (T2) shoot length obtained by various varieties of rapeseed was between 6.98cm and 9.24cm, Super canola showed highest shoot length. Ten varieties of rapeseed showed shoot length in between 6.08cm and 8.56cm under 100mM NaCl treatment (T3). The lowest shoot length was observed in 150mM salt stress treatment (T4) which was in between 5.37cm and 8.43cm. Among all ten varieties Super canola and Shiralee (V4) showed highest shoot length and Punjab canola and Oscar showed lowest Shoot length (Fig 1).

Table 1

Analysis of Variance (Anova) Exhibiting Morphometric Attributes of Shoot Length, Root Length, Shoot Fresh Weight, Shoot Dry Weight, and Root Fresh Weight & Root Dry Weight in Ten Varieties of Brassica Napus under Salt Stress Treatments.

Variables		Shoot length	Root length	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight
Variety (9)	*F-Value*	117.64	279.62	27.89	37.94	82.07	20.63
	P-value	0.000	0.000	0.000	0.000	0.000	0.000
Treatment (3)	*F-Value*	367.66	359.69	115.73	85.61	125.82	32.28
	P-value	0.000	0.000	0.000	0.000	0.000	0.000
Treatments* variety (27)	*F-Value*	10.99	11.47	18.85	1.50	117.64	0.77
	P-value	0.000	0.000	0.000	0.072	0.000	0.785
Total (799)							

Root Length

Treatment had influenced the shoot length in all ten varieties of rapeseed (Table 1). Replicate had non-significant difference between them. Control (non-saline) plants had shown more shoot length as compared to other three salinity treatments (T2, T3 and T4). Shoot length obtained by ten varieties was between 4.67cm and 6.515cm under non-saline condition, whereas Shiralee showed the highest and Punjab canola showed the lowest shoot length. In 50mM salt stress treatment (T2) shoot length obtained by various varieties of rapeseed was between 3.31cm and 5.85cm (Fig 2). Ten varieties of rapeseed showed shoot length in between 2.895cm and 5.69cm under 100mM NaCl treatment (T3).

Shoot Fresh Weight

Treatment had influenced the shoot fresh weight in all ten varieties of rapeseed (Table 1). Replicate had non-significant difference between them. Control (non-saline) plants had shown more shoot fresh weight as compared to other three salinity treatments (T2, T3 and T4). Shoot fresh weight obtained by ten varieties was between 0.271g and 3.543g under non- saline condition, whereas Legend showed the lowest shoot fresh weight. In 50mM salt stress treatment (T2) shoot fresh weight obtained by various varieties of rapeseed was between 0.2004g and 0.3222g. Ten varieties of rapeseed showed shoot fresh weight in between 0.1648g and 0.29945g under 100mM NaCl treatment (T3). The lowest shoot fresh weight was observed in 150mM salt stress treatment (T4) which was in between 0.1355g and 0.2718g. Among all ten varieties Super canola and Shiralee showed highest shoot fresh weight and Punjab canola and Oscar showed lowest Shoot fresh weight (Fig 3).

Shoot Dry Weight

Treatment had influenced the shoot dry weight in all ten varieties of rapeseed (Table 1). Replicate had non-significant difference between them. Control (non-saline) plants had shown more shoot dry weight as compared to other three salinity treatments (T2, T3 and T4). In non-saline condition (T1) shoot dry weight obtained by ten varieties was between 0.0308g and 0.0597g. In 50mM salt stress treatment (T2) there was reduction in shoot dry weight of various varieties of rapeseed and was between 0.0275g and 0.05125g. In 100mM NaCl treatment (T3) ten varieties of rapeseed showed shoot dry weight in between 0.025g and 0.048g. The lowest shoot dry weight was observed in 150mM salt stress treatment (T4) which was in between 0.02355g and 0.0402g. Among all ten varieties Shiralee, Faisal canola and Super canola showed higher shoot dry weight than other varieties and Legend, Punjab canola and Oscar showed lower shoot dry weight than other varieties (Fig 4).

Root Fresh Weight

All four varieties differed significantly in the root fresh weight. Higher root fresh weight was 14g which was obtained from Super canola (V3) and lowest fresh weight was obtained from Punjab canola (V1) which was 9.5g under no salt treatment (Table 1; Fig 7).

Root Dry Weight

Treatment had significantly influenced the dry weight of root in rapeseed (Table 1). Replicate had non-significant difference between them. Control (non-saline) plants had shown more root dry weight as compared to other three salinity treatment's (T2, T3 and T4) plants. Root dry weight obtained by four varieties was between 1.4g and 3.7575g under non- saline condition. Under 50mM salt stress treatment (T2) root dry weight obtained by various varieties of rapeseed was between 1.3025g and 2.5625g. Four varieties of rapeseed showed root dry weight in between 1.2075g and 1.7525 g under 100mM NaCl treatment (T3). The lowest root dry weight was observed

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in 150mM salt stress treatment (T4). In all treatments Punjab canola (V1) showed lowest root dry weight (Fig 8). The lowest root dry weight was observed in 150mM salt stress treatment (T4). Among all four varieties Shiralee (V4) showed highest root dry weight which was 3.7575g and Punjab canola (V1) showed lowest weight which was 1.4g in non-saline condition. Oscar (V2) had highest root dry weight in saline conditions T2 and T3 which were 2.5625g and 2.1875g. Under highest 150mM NaCl treatment highest weight was observed in Super canola (V3) which was 1.7525g. In all treatments Punjab canola (V1) showed lowest root dry weight (Fig 10).

Figure 1

Response of Salt Stress on Shoot Length of Ten Varieties of Brassica Napus.

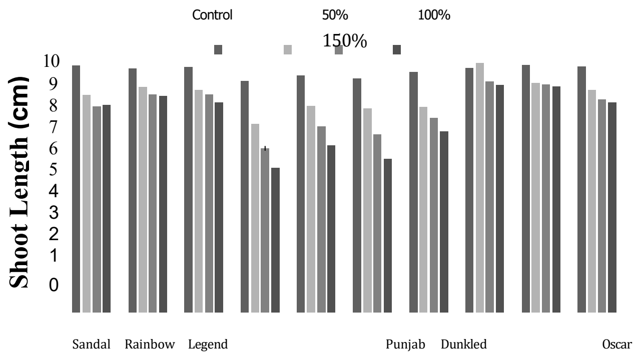


Figure 2

Response of Salt Stress on Root Length of Ten Varieties of Brassica Napus.

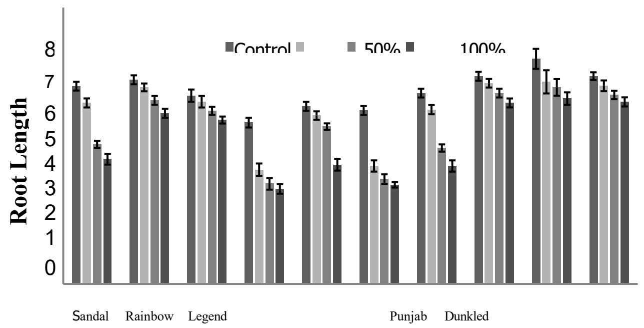


Figure 3

Response of Salt Stress on Shoot Fresh Weight of Ten Varieties of Brassica Napus.

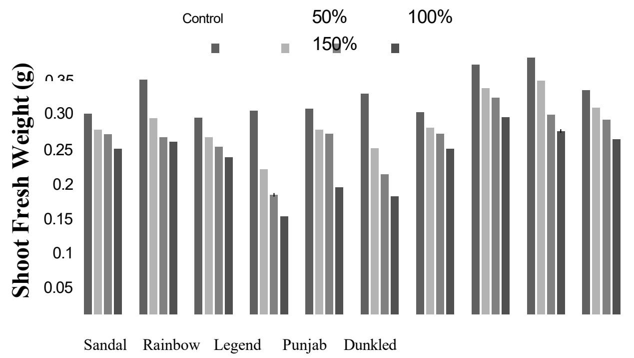


Figure 4

Response of Salt Stress on Shoot Dry Weight of Ten Varieties of Brassica Napus.

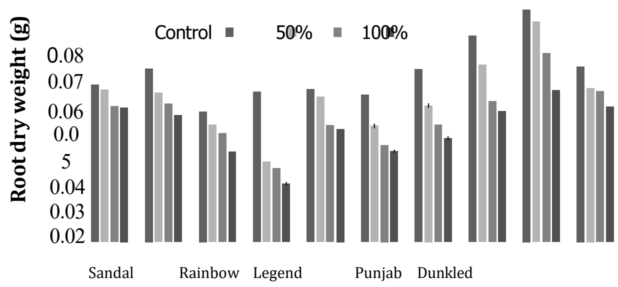


Fig 5

Response of Salt Stress on Root Fresh Weight of Ten Varieties of Brassica Napus.

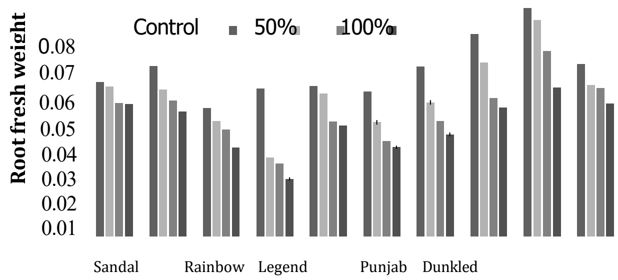
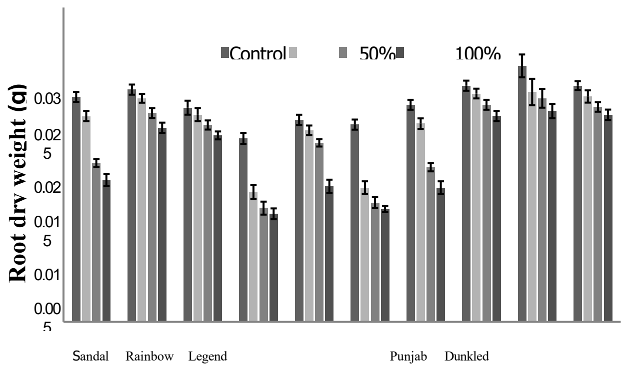


Figure 6

Response of Salt Stress on Root Dry Weight of Ten Varieties of Brassica Napus.



DISCUSSION

The adult trial in the current investigation likewise revealed a decrease in fresh and dry matters. Due to harmful ions, osmotic impacts, and unbalanced hormone release and nutrient distribution, salinity reduced the amount of fresh and dry weight of plants[22]. Reference[20] observed that salinity trigger reduction in the dry and fresh masses of rapeseed. Salinity affects both the roots

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and the upper sections of canola because it results in a loss of fresh and dry masses, was documented by reference[22] in Brassica napus. A decrease in rhizosphere water potential inhibits both root and shoots development when this circumstance exists. Reference[23] in *Brassica juncea* and reference[22] in *Brassica napus* showed the same results for plant height. The osmotic effect, which disrupts the water balance and causes water deficits, photosynthetic reductions, and, ultimately, a decrease in seedling development under salt stress, may be to blame for the reduced seedling growth under salt stress[24]. Most canola cultivars demonstrated a high level of resistance to salt stress management. Compared to the other two rapeseed kinds, the two used in this research were salt-tolerant to a greater extent. Several other plant species have also been shown to suffer from a reduction in growth due to salt stress. Plants were unable to grow and develop because of a metabolic abnormality. Metabolism, according to some researchers, was adversely affected by an increase in ion buildup via altered membrane permeability[25]. Saline circumstances have a negative impact on the growth of most crops. Water stress, ion toxicities, ion imbalance, or a combination of these mechanisms have all been implicated in the harmful effects of salinity[25]. However, numerous studies reported that crop plants belong to *Brassicaceae* growing under salinity stress, growth attributes encounter by physiological adaptation. Nitrogenase activity decreased in response to rising salinity, according to the latest research. This may be due to a reduction in water demand by the plant, which reduces the amount of leaf area that grows compared to the amount of root growth, enabling the soil to retain moisture and prevent salt levels from rising over safe levels[26]. Salt stress, on the other hand, may result in an ion imbalance in plant cells, resulting in oxidative stress and a reduction in plant development[26]. During the plant's early development phases, the impacts of salt treatment were more pronounced. When it comes to plant development, salt affects both the plant's osmotic pressure and the concentration of certain ions in the water. In plant growth, the total number and activity of photosynthetic pigments were critical. As a result of a decline in photosynthetic characteristics, crop growth and yields have dropped[27]. When Brassica napus seedlings were exposed to relative salt, they suffered a variety of metabolic problems[21]. Salinity-induced oxidative stress is likely to be to blame for these decreases, since various researchers have shown that proteins are more susceptible to proteolysis and chlorophyll depletion when exposed to salt stress[28].

CONCLUSION

Salinity showed a negative impact on the germination percentage, growth, physiology, and biochemical characteristics of all rapeseed cultivars. Salt stress has a deleterious impact on the whole plant, affecting it from germination through seedling development, vegetative growth, and flowering. It was concluded that germination percentage, shoot and root length, dry and fresh weights in experiment one was declined. Salt stress had varying degrees of influence on the 10 varieties, yet all ten varieties germinated regardless of salt treatment. Salinity had a greater impact on Punjab canola and Oscar than on any other variety thus regarded as salt sensitive. Least impact of salt stress was seen on Super canola and Shiralee which were considered as salt tolerant. All the result had shown that Super canola and Shiralee were recommended for salty soil due to their better defense mechanisms against salt stress, but for Punjab canola and Oscar saline soil is not suitable. Great progress has been made in the last decades but yet many of the basic processes that contribute to tolerance are only partially understood. Further studies are urgently needed to unravel the details of Na+, and especially Cl−, uptake mechanisms. There should be techniques to develop salt resistance in salt sensitive varieties of rapeseed in order to get more yields to fulfill the requirements of the world for oil. Future research requires further attention on the biochemical, molecular and genetic mechanisms crucial for salt stress resistance requires further attention for future research. Moreover, genetic modification should be combined with marker-assisted breeding programs with stress-related genes, and ultimately, the different strategies should be integrated, and genes representing distinctive approaches should be combined to substantially increase plant stress tolerance.

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