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Research Article

Morphological, Photosynthetic and Antioxidant Response of Rapeseed (*Brassica napus* L.) Seedlings Grown Under Elevating Levels of Salinity Stress

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Abstract

High salinity is a common abiotic stress factor that seriously affects crop production in some parts of the world, particularly in arid and semi-arid regions. The edible oil production in worldwide is highly dependent on the screening and development of salinity-resistant rapeseed cultivars especially in saline stress areas. An investigation was conducted to study the response of growth, chlorophyll components and antioxidants on rapeseed and their effect on germination under salinity levels. The impact of salt stress (0 (control), 20, 40 and 100 mM) on rapeseed was conducted at germination and early seedling stages. The seeds were germinated in Petri dishes with double filter paper in distilled water under different levels of salinity for 14 days. The results indicated that elevating levels of sodium chloride in the medium causes significant decrease in pant height, plant fresh weight, and plant dry weight and chlorophyll contents in the leaves of rapeseed. Moreover, activities of antioxidative enzymes (superoxide dismutase and peroxidase) were initially increased with the exposure of salinity level of 20 and 40 mM, but decreased by further increasing in the sodium chloride (100mM), which also indicating the oxidative stress which is manifested by high Malondialdehyde (MDA) and proline contents also. It is concluded that antioxidative defense system and photosynthetic pigments are major components of salt tolerance in rapeseed in addition to salt exclusion. In addition, physiological studies complemented with proteomics will help in understanding detailed mechanism of salt tolerance.

Keywords: Rapeseed; Antioxidants; Oxidative stress; Salinity; Growth; Proline

Introduction

Salinity stress adversely affects crop productivity worldwide. The problem of salinity is consistently becoming worst as the extent of salinized land is increasing all over the world. However, to judiciously utilize salt affected lands, development of salt tolerant cereals and oil-yielding crops seems to be very plausible approach [1]. It is often associated with irrigation, which is practiced to combat drought. The main causes of soil salinity are incorrect irrigation management and the use of unconventional waters for irrigating, which have recently become more common. World agriculture is facing many challenges like producing 70% more food for the growing population and the productivity of crops is not increasing in parallel with the food demand. The lower productivity in most of the cases is attributed to various abiotic stresses. Curtailing crop losses due to various environmental stressors is a major area of concern to cope with the increasing food requirements [2]. The major abiotic stresses like high salinity, drought, cold, and heat negatively influence the survival, biomass production, and yield of staple food crops up to 70%. It has been shown that soil salinity subsisted long before humans and agriculture; however, the problem has been arisen by agricultural practices such as irrigation. Salt stress is one of the most serious limiting factors for crop growth and production [3].

Salinity is reported to increase the activity of enzymes such as, glucose-6-phosphate dehydrogenase and peroxidase in the leaf of plants grown in polluted soil. Sodium and chloride ions play an important role in the antioxidant network, as these are essential cofactors of most antioxidant enzymes [4]. Salinity is involved in the direct or indirect generation of Free Radicals (FR) and Reactive Oxygen Species (ROS) in the following ways: 1. Direct transfer of electron in single electron reduction; 2. Disturbance of metabolic pathways resulting in an increase in the rate of FR and ROS formation; 3. Inactivation and down regulation of the enzymes of the antioxidative defense system and 4. Depletion of low molecular weight antioxidants [5,6]. The ROS produced in leaf cells are removed by complex enzymes Superoxide Dismutase (SOD) and Peroxidase (POD) of antioxidant systems [7].

Among the oil crops, rapeseed (*Brassica napus* L.) together with soybean and oil palm contribute largely to oil production. More than 60 million tons of rapeseed is produced per annum though; its yield in response to salinity stress greatly reduced [8]. Among Brassica species, rapeseed has been found relatively more tolerant to salinity than other Brassica species possibly due to its specific physiological and biochemical activities. Thus, it can be an ideal candidate to grow in saline soil to get rid of oil crop [9].

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The uniqueness of rapeseed due to its high biomass production and tolerance towards salinity can be valuable traits for germination capability; however, sufficient information is not available regarding salinity stress on growth and antioxidative defense system, when grown as oil seed crop. Therefore, the present study was planned to investigate the effects of different levels of salinity on growth, lipid peroxidation, and antioxidant enzymatic activities in rapeseed. Findings from the present study will add to our understanding the mechanism of salinity tolerance in rapeseed.

Materials and Methods

Plant growth conditions

Rapeseed seeds were used for the petri dish experiments. Seeds of each rapeseed were disinfected by incubating seeds in 1% sodium hypochloride solution for 10 minutes. Seeds were incubated in distilled water for 5 minutes and rinsed with water. Healthy seeds (40) of rapeseed were sown in petri dish. Seeds of Faisal canola were undergoing under different levels of salinity 0 (control), 20, 50 and 100 mM that were prepared in pure distilled water in laboratory by using sodium chloride. Salinity level was gradually increased in aliquots of 50molm^{-3} on alternate day until desired salinity stress level attained. Forty seeds were placed on double filter paper in each petri dish and 5ml of solution was used as prepared above. The experiment was complete randomized design with three replications of each treatment. Two liters of Hoagland's nutrient solution without NaCl salinity was added to each petri dish on weekly basis to avoid any nutrient deficiency. Plants were grown further for two weeks under control and saline conditions and then data for the following attributes was obtained. The petri dishes were monitored daily for fungal and other type of infections. The plants were harvested after 14 days of seed sowing and sampling was took for enzymatic study of rapeseed. The growth parameters like plant height, plant fresh weight, and plant dry weight and chlorophyll contents were measured after 14 days of seed sowing.

Sampling and data collection

Plant height was measured from root to shoot tip of the plants. In addition, the total weight was measured from weight balance (Shimadzu AY-220) by measuring the total fresh weight of the plant. Then plants were over dried at 105°C for 1h and 65°C for 72h. Then total dry weight and shoot dry weight was measured with the help of weighting balance (Shimadzu AY-220). For the analysis of chlorophyll contents, 0.1g of fresh leaf sample was extracted with 8 mL 95% acetone for 24 h at 4°C in darkness. The absorbance was measured by a spectrophotometer (Shimadzu UV-2550, Kyoto, Japan) at 646.6, 663.6, and 450 nm. For the analysis of chlorophyll contents, 0.1g of fresh leaf sample was extracted with 8mL 95% acetone for 24h at 4°C in darkness. The absorbance was measured by a spectrophotometer (Shimadzu UV-2550, Kyoto, Japan) at 646.6, 663.6, and 450nm. Chlorophyll contents were calculated by the standard method of Arnon [10] and expressed in mg g-1 Fresh Weight (FW).

Lipid peroxidation, proline content and antioxidants

The degree of lipid peroxidation was evaluated as Malondialdehyde (MDA) contents. 0.1g of frozen leaves was ground at 4°C in a mortar with 25mL of 50mM phosphate buffer solution (pH 7.8) containing 1% Polyethene Pyrrole (PVP). The homogenate was centrifuged at 10,000×g at 4°C for 15 min. The mixtures were heated at 100°C for 15-30 min and then quickly cooled in an ice bath. The absorbance of the supernatant was recorded by using spectrophotometer (xMarkTM microplate absorbance spectrophotometer, BIO-RAD, USA) at the wavelengths of 532, 600, and 450 nm. Lipid peroxidation was expressed as l molg^{-1} using the following formula: 6.45 (A532 - A600) - 0.56 A450. The method was followed by Halliwell and Gutteridge [11] and expressed in mg g^{-1} Fresh Weight (FW).

Proline contents were measured by using (0.1g) homogenate in 3% of aqueous sulphosalicylic acid and distilled water. The proline content was assessed by the technique described by Bates et al. [12] and expressed in mg g^{-1} Fresh Weight (FW).

In order to check enzymes activities, fresh leaves (0.5g) were homogenized in liquid nitrogen and 5mL of 50mmol sodium phosphate buffer (pH 7.0) including 0.5mmol EDTA and 0.15mol NaCl. The homogenate was centrifuged at 12000×g for 10 min at 4°C and the supernatant was used for measurement of SOD and POD activities.

The SOD activity was assayed in 3mL reaction mixture containing 50mM sodium phosphate buffer (pH 7), 56mM Nitroblue Tetrazolium (NBT), 1.17mM riboflavin, 10mM methionine, and 100µL enzyme extract. Finally, reading was taken by using spectrophotometer (xMarkTM micro plate absorbance spectrophotometer, BIO-RAD, USA). The method was followed by Chen and Pan [13] and expressed in mg g^{-1} Fresh Weight (FW).

POD activity in leaves was estimated using the method of Sakharov and Ardila [14] and was assayed using guaiacol as the substrate. The reaction mixture (3mL) contained 0.05mL of enzyme extract, 2.75ml of 50mM phosphate buffer (pH 7.0), 0.1mL of 1 % H_2O_2, and 0.1mL of 4% guaiacol solution. The increase in the absorbance at 470nm due to guaiacol oxidation was recorded for 2 min. One unit of enzyme activity was defined as the amount of the enzyme causing a change in absorbance of 0.01 per minute. The specific POD activity was expressed as U g^{-1} FW min^{-1}.

Statistical analysis

All the results were given as arithmetic means with standard deviations except otherwise defined. Data were tested with one-way ANOVA, followed by HSD tests using Statistix 8.1. The significance level was set at $P<0.05$ or $P<0.01$. Graphical presentation was carried out using SigmaPlot 12.

Results and Discussion

Effect of different levels of salinity on plant growth and chlorophyll contents

Salinity is a serious abiotic stress factor limiting crop growth and agricultural productivity. Increasing level of salt stress reduced the plant fresh and dry weights, germination rate plant length, and root dry weight, rate of photosynthesis, lipids and energy production [1]. Chlorophyll concentration is an important parameter for the evaluation of plant stress. The present study investigated the morphological changes of rapeseed grown under different levels of salinity (Table1). Therefore, a preliminary experiment was conducted on germination of rapeseed seedling under salinity stress. Results regarding the plant growth and chlorophyll contents are showed

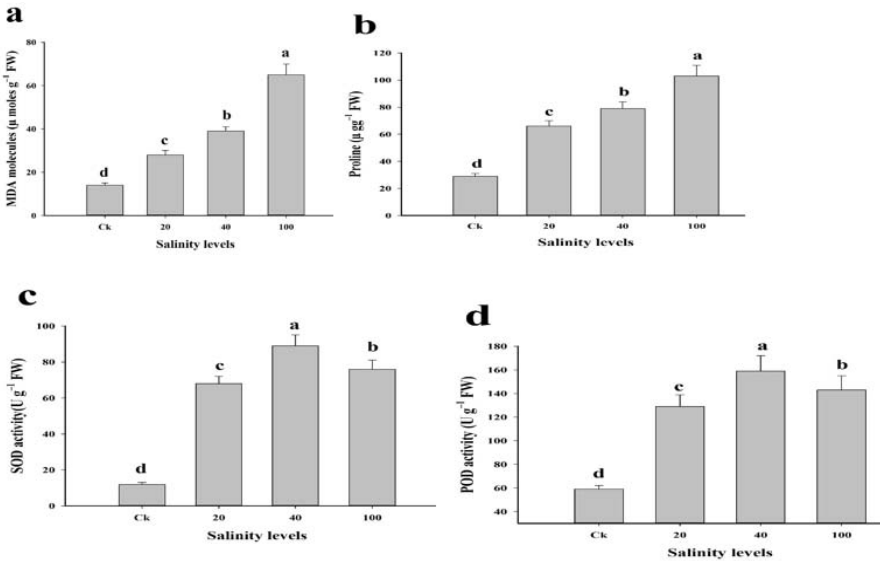

Figure 1: Effect of different levels of salinity on MDA (a), proline (b), SOD (c) and POD (d) in the laves of rapeseed. Values in the figures is just one harvest. Mean ± SD (*n*=3). Different letters within a column indicate significant difference between the treatments (*P*<0.05). Relative radiance of plastic filter used: Ck, 20, 40 and 100 mM.

Tables 1: Effect of different levels of salinity on plant height (cm), plant fresh weight (g), plant dry weight (g) and total chlorophyll contents (mgg^{-1} FW) in rapeseed seedlings.

Salinity levels	Plant height	Plant fresh weight	Plant dry weight	Total chlorophyll
Ck	18±0.8 a	9±0.4 a	4.5±0.08 a	2.7±0.08 a
20 mM	16±0.6 b	8±0.6 b	4.1±0.09 b	2.3±0.06 b
40 mM	13±0.2 c	7.5±0.1 c	3.4±0.1 c	1.9±0.06 c
100 mM	10±0.4 d	6±0.1 d	2±0.1 d	1.4±0.07 d

Values in the figures is table is just one harvest. Mean ± SD (*n*=3). Different letters within a column indicate significant difference between the treatments (*P*<0.05). Relative radiance of plastic filter used: Ck, 20, 40 and 100 mM.

in the table. All growth parameters were taken after 14 days of seed sowing. All the data taken of seedling growth was greatly affected by different levels of salinity stress. Results showed that increase in the salinity stress continuously decreases the plant height, fresh and dry biomass. It was observed that plant height, plant fresh and dry biomass and contents of chlorophyll decreased by 67%, 45%, 21% and 54% respectively in the seedlings grown under salinity level of 100mM compared to the control. High concentration of salinity in the medium affected plant growth and biomass has previously showed by many studies [1,5]. Salt in soil water inhibits plant growth for two reasons. First, it reduces the plant's ability to take up water and this leads to slower growth. This is the osmotic or water deficit effect of salinity. Second, it may enter the transpiration stream and eventually injure cells in the transpiring leaves, further reducing growth. This is the salt-specific or ion-excess effect of salinity. The excessive salt concentration correspondingly increases the osmotic potential of the soil that restricts the water uptake by plants. The Na^+ and Cl^- ions are the major ions that produce many physiological disorders and detrimental effects on plants. However, Na^+ is the primary ion as it interferes with the uptake of potassium (K^+) ion and disturbs stomatal regulation that ultimately causes water loss while the Cl^- ion disturbs the chlorophyll production and causes chlorotic toxicity. However, Cl^- is more dangerous than Na^+ [15]. These findings are similar to the findings of Zafar et al. [1]. The appropriate ion ratios provide a tool to the physiological response of a plant in relation to its growth and development. The reduction in chlorophyll pigments in plants under salt stress is mainly due to the reduction in water potential. Photosynthesis is also inhibited when high concentrations of Na^+ and/or Cl^- are accumulated in the chloroplasts and chlorophyll being important content of photosynthesis directly correlates to the healthiness of plant [16].

Effect of different levels of salinity on lipid peroxidation, proline content and antioxidants

Stress conditions can disturb the dynamic equilibrium of Reactive Oxygen Species (ROS) production and elimination under normal growth in plants, which promotes ROS accumulation, membrane lipid peroxidation, and disrupt the structure and function of cell membrane system. It was reported that excess of NaCl can increase lipid peroxidation and MDA, an oxidized product of membrane lipids, indicating the prevalence of oxidative stress and membrane damage. NaCl-mediated lipid peroxidation could support ion leakage by plasma lemma membrane and cell turgor loss [17,18]. Environmental stress can trigger ROS production in plants, which results in oxidative damage. However, plants possess an efficient antioxidative defense system to detoxify the ROS generation. SOD is a metalloprotein also involved in ROS metabolism by dismutation of superoxide anion to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) thus maintains superoxide radicals in a steady state. POD is a heme protein that decomposes H_2O_2 by oxidation of co-substrates [5,6]. In the present study, high concentration of NaCl in the medium increased MDA contents, which induced that salinity, induced oxidative damage in the leaves of rapeseed (Figure 1). In this study, content of MDA and proline increased by 264% and 354% in the plant grown under the level of 100mM compared to the plants grown without concentration of salinity. It was also observed that under high concentration of NaCl, the activities of antioxidants increased while at highest level of salinity the activities of antioxidants start decline. Highest activities of SOD

and POD were observed at 40mM, which were increased by 116% and 267% compared to the control. The increase in lipid peroxidation and oxidative stress might be due to the progressive increase in soil NaCl contents, which is a stress factor triggering physiological responses in plants. The MDA content of saline-stressed plants was significantly higher than control. In fact, MDA content showed a dose-dependent increase with the concentration of NaCl in the medium [19]. Proline accumulation in plant tissue/organs is a response to salinity stress, which might be associated with signal transduction and prevents membrane distortion. The dose-dependent increase in proline concentration with salinity stress might be due to the disturbance in osmoregulatory solutes [20]. The variable antioxidant response under different NaCl stress conditions might be due to changes in gene expression and function of the protein in various plant tissues. These results are coincide with the findings showed by Zafar et al. [5] when they studied wheat under different concentration of NaCl and noticed that salinity stress induced oxidative damage while antioxidants comes into play to reduce the metal toxicity. In the present study, significant increase in antioxidant enzyme activities can be considered as an indicator of increased ROS production and mitigation. Production of antioxidant enzymes (SOD and POD) in rapeseed, consequently, serves as an approach to strengthen cell antioxidant system and overcome the risk of ROS production due to salinity stress [21]. In the present study, better growth of rapeseed at low Cu concentration might be linked with a better defense system.

Conclusion

Based on the findings of the present study, it can be concluded that rapeseed has a considerable potential to cope with salinity stress due to an antioxidative defense mechanism. However, the concentration of NaCl plays a significant role in altering growth, antioxidant enzymatic activities, and MDA and proline contents in leaves of rapeseed. This study provides a direction for future research and may support the use of NaCl in terms of oil seed crop or animal feed. However, future research is needed on the effects of salinity on quality of both fiber and fodder from rapeseed. Moreover, potential for rapeseed in tolerance with high concentration of NaCl in the soil should be tested on field experiment.

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