ABSTRACT

Salinity is among the major stresses prevailing throughout the world that severely limits crop establishment and production. Every crop has an intra-specific genetic variation that enables it to cope with variable environmental conditions around the globe in various seasons. Hence, this genetic variability is a good tool to exploit germplasms while looking for salt-tolerant genotypes that can be utilized to grow on salt-affected areas. Further, the selected cultivars can be effectively used by plant breeders and molecular biologists for the improvement of salinity tolerance in a number of important agricultural crops. In the present study, it was planned to evaluate some effective physiological, biochemical, and molecular attributes in canola (*Brassica napus* L.) crop which can be employed for development of salt-tolerant crops with high commercial values. For this purpose, a number of experiments were carried out. Initially, screening experiments were carried out at germination and early vegetative growth to screen out salt sensitive and salt tolerant canola cultivars. Experiments were carried according to completely randomized design (CRD) with 2 factors (cultivars and salt stress) and 3-8 replicates according to the need of the experiment and parameter under study. Screened out salt tolerant and salt-sensitive cultivars were subjected to 200 mM NaCl stress in another set of experiments, to evaluate their performance in terms of growth, ion uptake, water relations, gas exchange attributes, chlorophyll fluorescence studies, and differential expression of genes. Response of all the cultivars was observed to vary under the influence of salt stress with a consistent reduction in germination percentage with an increase in salinity stress. In all canola cultivars, a consistent delay was observed in mean emergence time with the increase in salinity level. Canola cultivars differed significantly under control and saline conditions as regards seedling length, root length, seedling fresh weight, seedling dry weight, and seedling vigor index. Imposition of salt stress caused a significant reduction in shoot fresh weight at the vegetative growth stage. Proline, glycine betaine, and total soluble protein contents were also observed to be higher in plants grown under salt stress as compared to those grown under non-saline conditions. There was a marked increase in shoot Na^+ contents in all canola cultivars evaluated, when grown under salt stress. Similarly, salt stress significantly reduced shoot K^+ contents in the cultivars under study. There was an overall decrease in shoot Ca^{2+} concentration. Shoot Cl^- concentration was also observed to increase significantly in all the cultivars, on the imposition of salt stress in the rooting media. Shoot K^+/Na^+ decreased significantly under the influence of salt stress. Ca^{2+}/Na^+ consistently decreased in all cultivars, under the effect of 200 mM NaCl

treatment. Nutrient utilization efficiency decreased significantly in all the cultivars on the imposition of salt stress in the rooting medium. There was a highly significant impact of salt stress on chlorophyll contents in plants grown under saline rooting medium. In response to salinity in the rooting medium, the net CO_2 assimilation rate was observed to reduce. Saline rooting medium affected transpiration rate, significantly, and caused an overall reduction in transpiration rate in all cultivars. Water use efficiency of all the cultivars decreased under the influence of salinity in the rooting medium. In plants grown under saline condition, the highest water use efficiency was observed in Dunkled, while the lowest water use efficiency was observed in Cyclone and Oscar. Stomatal conductance also decreased under the influence of salinity in the external environment. Sub-stomatal CO_2 concentration was also significantly affected by salt stress and the values were lower in plants grown in saline nutrient solution as compared to those grown in non-saline condition. Imposition of salt stress on canola cultivars caused significant variation in H_2O_2 contents examined in leaves. The levels of H_2O_2 contents were lower in cultivars considered tolerant to NaCl stress (Faisal Canola, DGL, Dunkled and CON-II) when grown under 200 mM NaCl level. However, the level of H_2O_2 contents was remarkably higher in cultivars considered sensitive to NaCl stress (Legend and Cyclone) when grown under 200 mM NaCl level. Catalase contents consistently increased in plants grown under salt stress as compared to those grown under non-saline conditions. Peroxidase contents also increased in plants grown under salt stress as compared to those grown under non-saline conditions. Similarly, superoxide dismutase contents also consistently increased in plants grown under salt stress as compared to those grown under control. Salt stress was observed to cause photo-damages to the thylakoid membranes of chloroplast and reduce efficiency of the light-dependent reactions of photosynthesis. In the present study, it was observed after the OJIP test that Fo decreased significantly in all four cultivars under study, on the imposition of salinity stress while Fm decreased significantly in cultivars DGL, Legend, and Oscar, and there was no statistical difference in maximal fluorescence in the cultivar Dunkled. Fv/Fo and Fv/Fm decreased significantly in all four cultivars under study, on the imposition of salinity stress. Efficiency index expressed as the density of reaction centers per chlorophyll complex increased significantly in all four cultivars under study, on the imposition of salinity stress. Similarly, absorption flux per active reaction center and the ratio of active to inactive reaction centers decreased significantly in all four cultivars. Energy flux trapped by one active reaction center also decreased significantly. These findings depicted that there is some photo-damage caused by applied salinity that might have been caused by

oxidative stress as depicted by antioxidant level and H_2O_2 contents. Fluorescence studies using DualPAM-100 also revealed that the quantum yield of PSII was observed to decrease under salt stress. ETR II was also observed to decrease in cultivars DGL, Legend, and Oscar. However, ETR I was observed to increase in all the cultivars on the imposition of salt stress showing that cyclic ETR has also been contributed. Cyclic ETR and NPQ were observed to increase under salt stress. In conclusion, salt tolerance of canola was found to be associated with relative growth, photosynthetic capacity, and high Ca^{2+} and accumulation of osmotica. Differentially expressed genes (DEGs) supported the data for physiological and biochemical attributes. The differentially expressed genes (DEG) were observed to be involved in the regulation of ionic concentration, activation of RuBisCO, formation of antioxidant enzymes, cell signaling or transcription factors acting for the regulation of other pathways, and even gene regulation. Significant DEGs included sodium hydrogen exchanger (NHX), sodium transporter (HKT), potassium transporter (POT), Na-K-Cl co-transporter (NCKK1), cyclic nucleotide-gated ion channel 1 (CNGC1), mechanosensitive ion channel (MSL), potassium channel (KOR), chloride channel (ClCa), calmodulin (Calm), calmodulin binding transcriptional activator (CBTA), calcium transporting ATPase (Ca-ATPase), vacuolar ATPase (V-ATPase), heat shock proteins (HSP), late embryogenesis abundant proteins (LEA), Fe-superoxide dismutase (Fe-SOD), Cu-superoxide dismutase (Cu-SOD), Mn-superoxide dismutase (Mn-SOD), catalase (CAT), peroxidase (POD), tonoplast intrinsic protein (TIP), plasma-membrane intrinsic protein (PIP), nucleoplasm intrinsic protein (NIP), expansins (EXP), cell wall integrity and stress response component (WSC), NAC domain containing transcription factor (NAC), ethylene responsive transcription factor (ERF), MYB domain containing transcription factor (MYB), bZIP transcription factor (bZIP), 1-aminocyclopropane-1- carboxylic acid transcription factor (ACC), heat shock transcription factor (HSP). The differentially expressed genes (DEGs) were observed to be involved in the regulation of ionic concentration, activation of RuBisCO, formation of antioxidant enzymes, cell signaling or transcription factors acting for the regulation of other pathways, and even gene regulation. It is concluded that genes expressed in the cultivar Dunkled provide supporting evidence for the documented tolerance of the cultivar and relates to the regulation of ionic concentration, activation of RuBisCO, formation of antioxidant enzymes, cell signaling or transcription factors acting for the regulation of other pathways and even gene regulation under salt stress.