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**SHORT COMMUNICATION**

# EFFECT OF SODIUM CHLORIDE SALINITY STRESS ON SEED GERMINATION, SEEDLING GROWTH AND PHYSIOLOGY OF SENNA (*CASSIA ANGUSTIFOLIA* VAHL.)

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**The objective of the present investigation was to study the impact of NaCl salinity stress on seed germination, seedling physiology and plant antioxidant systems in senna in order to evaluate the relative significance of these antioxidant systems in imparting tolerance to NaCl treatment. The impact of salt stress on seed germination, root and shoot length, fresh and dry biomass, carbohydrate, reducing sugar, starch, proteins, osmolytes like proline, phenols and antioxidant enzymes like polyphenol oxidase (PPO), peroxidase (POX), superoxide dismutase (SOD) activity was investigated in senna (*Cassia angustifolia*) at germination stage (7 DAS). Sodium chloride (NaCl) treatment resulted in a significant enhancement of proline and polyphenols along with stimulation in the activity of antioxidant enzymes like peroxidase, polyphenol oxidase, and superoxide dismutase. Seed germination, fresh and dry weight were decreased under increasing NaCl concentration.**

***Key words*: Phenols, POX, PPO, proline, salinity stress, senna, SOD**

Seed germination is one of the most important phases in the life cycle of a plant and is highly responsive to existing environment (Besma and Mounir 2010). Salinity is one of the major physical parameter of an environment, which determines the success or failure of a plants establishment. Alternatively, it is assumed that germination rate and the final seed germination decreases with the decrease of the water movement into the seeds during imbibitions.

Water stress causes stomatal closure, which reduces the $CO_2/O_2$ ratio in leaves and inhibits photosynthesis. These conditions increase the rate of production of reactive oxygen species (ROS) like superoxide radicals ($O^{\bullet-}$), hydrogen peroxide ($H_2O_2$) and hydroxyl radical ($OH^{\bullet}$) particularly in chloroplast and mitochondria (Neill *et al*. 2002) which lead to cellular damage via membrane peroxidation, protein denaturation and DNA damage. Therefore, antioxidant resistance mechanisms may provide this strategy to entrance salt tolerance. These mechanisms employ ROS scavenging enzymes which detoxify plant by scavenging oxygenic radical.

Superoxide dismutase is considered to be the first line of defence against ROS because it catalyses the dismutation of superoxide radical to generate $O_2$ and $H_2O_2$ and POX catalyse the conversion of $H_2O_2$ to $H_2O$ and $O_2$ ( Dia *et al*. 2009). Polyphenol oxidase (PPO) is one such small molecular antioxidant enzyme, which plays a key role in defence of plant against biotic and abiotic stress. Hence SOD, POX and PPO are the most important protective enzymes to remove reactive oxygen species.

*Cassia angustifolia* Vahl commonly known as senna is a medicinal plant, known for its sennoside content and

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is used to cure a large number of intestinal diseases (Singh *et al*. 2001).

*Plant material and treatment:* Authentic seeds of *Cassia angustifolia* Vahl were obtained from National Research Centre for Medicinal and Aromatic Plants, Boriavi, Gujarat. The seeds were surface sterilised with 0.1% $HgCl_2$ for five minutes and thoroughly rinsed with distilled water. Seeds were placed to germinate in 9 cm petridishes layered with filter paper for seven days. Five ml solutions NaCl (25, 50 and 100 mM) were added in different petri plates. The control contained 5 ml of sterile distilled water. Each treatment was maintained in triplicate along with control.

Fifteen seeds were put in each sterile petri plate for germination. The germination studies were carried out in seed germination chamber under controlled conditions. The germination percentage, the length of root and shoot, fresh and dry weight were measured after $7^{th}$ day of germination in all the seedlings. The seedling dry weight was determined after drying at 80°C for 24 h. Biochemical analysis like carbohydrate, reducing sugar, starch, proteins, osmolytes like proline, phenol contents and antioxidant enzymes like polyphenol oxidase (PPO), peroxidase (POX), superoxide dismutase (SOD) were activities estimated at $7^{th}$ day of germination.

*Determination of Carbohydrates:* The carbohydrate content was measured by Anthrone method (Hedge and Hofreiter 1962). 100 mg fresh (powdered) sample homogenisation and extraction of carbohydrate samples were hydrolysed by 2.5N HCl and neutralised with solid sodium carbonate. The volume was adjusted to 100 ml and 0.5 ml aliquot was adjusted to 1.0ml by adding distilled water. Anthrone reagent (4.0 ml) was added to the tubes and heated for 8 min and the intensity of the green colour developed was read at 630 nm.

*Determination of Starch:* Starch was measured by using Anthrone reagent as per the method described by (Thayumanavan and Sadasivam 1984). The sample (100 mg) was homogenised with hot 80% ethanol. In addition, 52% perchloric acid were added for hydrolysis of starch and final volume was made to 100 ml. 0.2 ml aliquot was adjusted to 1.0 ml. Anthrone reagent (4.0 ml) was added and heated for eight minutes. The mixture was recorded at 630 nm.

*Determination of reducing sugar:* Reducing sugars were estimated by using Dinitrosalicylic Acid (DNSA) method (Miller 1972). 100mg fresh sample was carried out by using 80% ethanol. The supernatant was collected and evaporated. Sugars were dissolved in 10 ml distilled water and 0.5 ml of it was taken in test tubes and the volume was equalised to 3.0 ml with distilled water. DNSA reagent (3.0 ml) and 1 ml of 40% Rochelle salt solution was added to it. The tubes were cooled and the intensity of dark red colour was read at 510 nm on UV-visible spectrophotometer (Shimadzu-1601).

*Determination of Protein:* Proteins were estimated by using Lowry *et al*. (1951) method. One gram whole seedling sample was homogenised in 10 ml of phosphate buffer pH, centrifuged and the supernatant was used to prepare the reaction mixture consisting of reagent- 'C' alkaline copper solution) and reagent-'D' (Folin-Cicalteau reagent). The absorbance of blue colour developed was recorded at 660 nm by using UV-visible spectrophotometer (Shimadzu- 1601).

*Determination of proline:* Proline content was estimated by the methods of Bates *et al*. (1973). 0.5 g of sample was homogenised in 10 ml of 3% sulphosalicylic acid and centrifuged at 10000 g for 20 min. The mixture of 2 ml of supernatant, 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid was boiled at 100°C in a water bath for 30 min, reaction was stopped in an ice bath and then 4ml of toluene was added to each sample. The toluene (upper) layer was read at 520 nm on a UV visible spectrophotometer (Shimadzu-1601).

*Determination of phenol:* The total phenolic contents were measured by Folin-Ciocalteu method (Malick and Singh 1980). Fresh samples (1.0 g) were homogenised in 80% ethanol repetitively and final volume was made up to 10.0 ml. The mixtures were sonicated for 15min for complete extraction and centrifuged at 9000 rpm for 10min.

*Determination of SOD activity:* The activity of superoxide dismutase (SOD) was assayed according to Dhindsa *et al*. (1981). 1.0 g of sample estimation of phenol with 10.0 ml of chilled 50mM potassium phosphate buffer, pH 7.8 and was centrifuged in a refrigerated centrifuge. The supernatant was used as an enzyme source. The reaction mixture contained 50 mM potassium phosphate buffer, pH 7.8, 13 mM methionine, 0.1 mM EDTA, 75 µM nitroblue tetrazolium chloride (NBT) and 50 µL of enzyme extract. Reaction was started by adding 2 µM riboflavin and by placing the tubes below 15W fluorescent lamps for 15min. The reaction was stopped by switching off the light and covering the tubes with black cloth. Absorbance was recorded at 560 nm.

*Determination of POX activity:* The peroxidase activity was assayed by Vidyasekharan and Durairaj (1973) method. 1.0 g of sample was homogenised in 0.1M phosphate buffer (pH 7.0). The extract was cold centrifuged. The assay mixture 0.1M phosphate buffer (pH 7.0), 10 mM Guaicol, enzyme extract and 12.3 mM $H_2O_2$. Initial optical density was read at 430nm and then increase in optical density was noted after every 30 seconds intervals on UV-visible spectrophotometer (Shimadzu- 1601).

*Determination of PPO activity:* The polyphenol oxidase activity was assayed by Vidyasekharan and Durairaj (1973) method. One gram of the seedling sample was homogenised 0.1M phosphate buffer (pH 6.00). The extract was centrifuged in cold temperature centrifuge at rpm. The oxidation of catechol was measured from the reaction mixture containing phosphate buffer (pH 6.5), enzyme extract and+ 0.01M catechol. Initial absorbance was read at 495 nm and then absorbance was measured at every 30s interval on UV-visible spectrophotometer (Shimadzu- 1601).

The data was presented as an arithmetic mean of three replicates ±standard deviation. The significance of the mean differences was explored through one-way-ANOVA statistics followed by DMRT (Duncan's multiple range test) at p=0.05 as a post hoc test. SPSS for Windows ver. 11.5 and Microsoft Excel 2003 were used to carry out statistical analyses and graphical data presentations.

The NaCl treatments resulted in a significant decrease in senna seed germination percentage over control at concentration 50mM and more (Fig. 1A). At the highest concentration (100 mM) the germination percentage reduced by 56.7% as compared to unstressed seedlings, which was followed by NaCl 50 mM (33.4%) and NaCl 25 mM (10.3%).

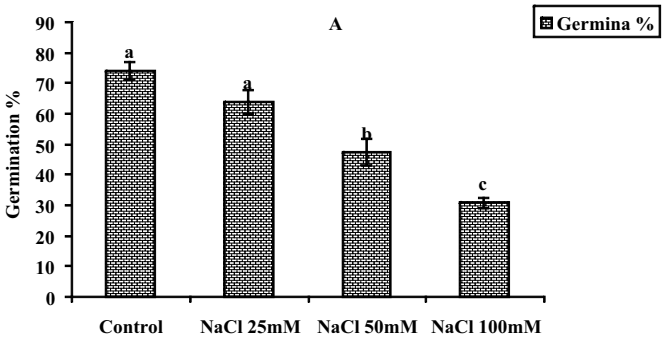


**Fig. 1(A). Effect of sodium chloride salinity stress on, germination percentage of senna seedlings (7 DAS)**

The salt stress resulted in to significant reduction in the root and shoot length of seedling at 7 DAS (Fig. 1B). Maximum decrease in root and shoot length (80.34% and 84.7%) was recorded at higher concentration of NaCl (100 mM). The data illustrated in Fig. 1C for fresh and dry biomass exhibited a similar trend. Fresh biomass of seedlings gradually decreased with an increase in NaCl concentration. The reduction was more pronounced at 100 mM (64.6%). The dry biomass was also affected by NaCl treatment. At low concentration of NaCl (25 mM) the dry weight increased (8.7%) but with further increase in NaCl concentration i.e. at 50 mM and 100

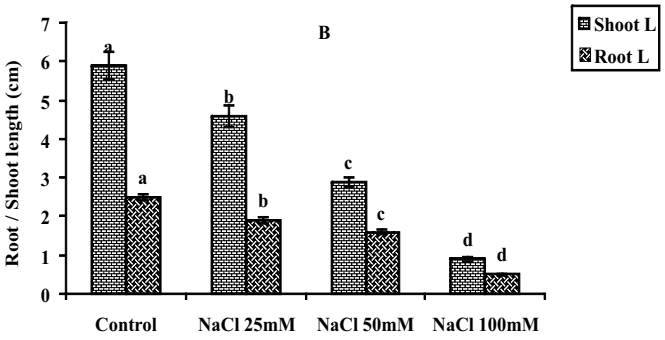


**Fig. 1(B). Effect of chloride salinity stress on, shoot and root length of senna seedlings (7 DAS)**

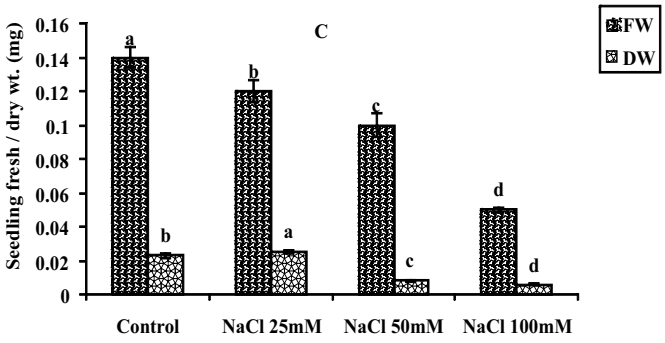


**Fig. 1(C). Effect of chloride salinity stress on, fresh and dry weight of senna seedlings (7 DAS)**

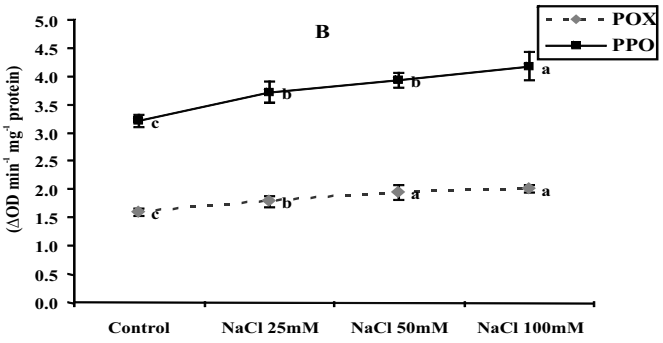


**Fig. 2(B). Effect on chloride salinity stress on polyphenol oxidase and peroxidase activity of senna seedlings (7 DAS)**

mM NaCl, the dry weight reduced by 65.2% and 73.9% respectively over control.

The result presented in Fig. 2B exhibited the effect of chloride salinity stress on biochemical constituents of senna seedlings at 7 DAS. Carbohydrate content increased at NaCl 25 mM concentration by 14% over control. Higher concentration of NaCl (100 mM) showed decrease in carbohydrate content by 15% over control. NaCl 25 mM and 50 mM enhanced the reducing sugar content by 7.7% and 11.9% over control. The starch content increased by NaCl 25 mM (5%), however the highest concentrations of NaCl (100 mM) treatment the starch content decreased by 13% over control. NaCl 25 mM enhanced the protein content by 19.5% over control, while at 100 mM concentration protein content decreased by 41.35% over control.

The NaCl treatment exhibited 48% increase at 100 mM concentration while 50mM NaCl increased phenol content by 35% over control (Fig. 2A). Proline content was also increased significantly by increasing NaCl concentrations (Fig 2A). The proline content was increased by 30.1% at 25 mM concentration while at 50 mM and 100mM concentrations it was increased by 61.7% and 98.3%, respectively over control. Phenols content was the highest (34.5%) at 50 mM NaCl.

SOD activity increased significantly by increasing NaCl concentration (Fig. 2C). At 25 mM and 100mM NaCl concentration the SOD activity increased by 6.9% and 26.1% over control. POX and PPO activity significantly increased with increasing salinity stress. At 25 mM NaCl the activity of PPO and POX were increased by 15.9 % and 11.3% while at 100 mM NaCl it was increased by 30.2 % and 25.6%, respectively over control. Thus, salinity (NaCl) adversely affected the seedling growth parameters as well as biochemical constituents of senna seedlings.

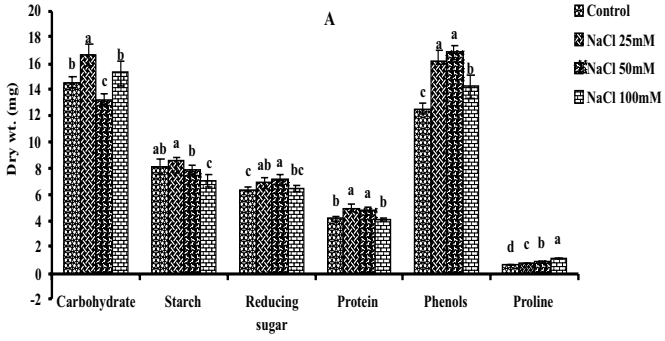


**Fig. 2(A). Effect on chloride salinity stress on biochemical constituents of senna seedlings (7 DAS)**

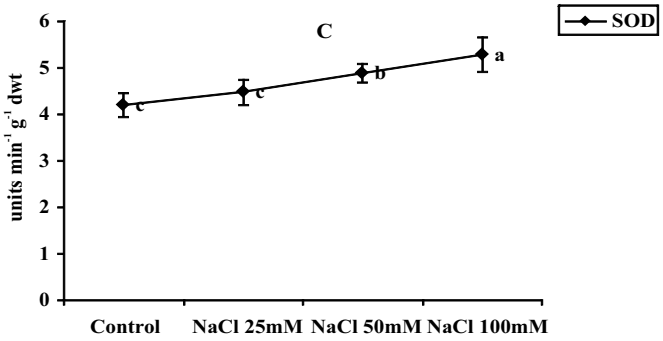


**Fig. 2(C). Effect on chloride salinity stress on superoxide dismutase activity of senna seedlings (7 DAS)**

Thakur and Sharma (2005) and Besma and Mounir (2010) have reported decrease in germination particularly under salt stress. It has been reported that salinity delays germination of several species but does not appreciably reduce the final germination percentage (Ayers and Westcot 1985). Some studies have reported that biomass accumulation increased (Jones and Turner 1980, Munns and Weir 1981) while others have found that it decreased (Hanson and Hitz 1982) or remained unchanged (Morgan 1992) during stress conditions.

The results of the present investigation were similar to Agarwal and Pandey (2004). They also have reported that salinity adversely affected the seedling growth parameters (germination, fresh and dry biomass, root and shoot length) of senna seedlings. Similar were the reports of Dantas *et al*. (2005) in cowpea, Khan *et al*. (2004) in *Ceratoides lanata*, Aliakbar and Maghsoudi (2008) in wheat seedlings, Khalid *et al.* (2009) in *Cassia absus* L. Muhammad and Hussain (2010) revealed that the germination of *Lepidium sativum* L., *Linum usitatissimum* L., *Nigella sativa* L., *Plantago ovata Forssk,* and *Trigonella foenum-graecum* L. was strongly affected by all salt treatments.

NaCl stress also had a significant influence on seedling physiology. Ebru *et al*. (2004) reported that under salt stress plants exhibit a remarkable increase in proline content. Supporting findings were reported from other plants, alfalfa, soybean and pea (Tramontano and Jouve 1997) and sugarbeet, where salt stress resulted in extensive proline accumulation. Singh (2004), found that tolerant genotypes of chickpea (*Cicer arietinum*) showed a higher level of total phenols, whereas a significant reduction was observed in susceptible genotypes which is the same as the results of Dostanova *et al*. (1979) and Latha *et al*. (1989). The same authors also confirmed that phenol accumulation in tolerant genotypes could be a cellular adaptative mechanism for scavenging the free radicals of oxygen and preventing subcellular damage during stress.

Similar results were reported by Dash and Panda (2001) in *Phaeseolus mungo* and sugar beet. Under salinity stress the activity of antioxidant enzymes was increased in wheat shoot reported by Meneguzzo *et al*. (1999) and pea Hernandez *et al*. (1999). Demir and Kocaliskan (2001) reported that PPO activity increased under salt stress in tobacco. With increase in NaCl concentration and duration of stress, proline content increase in wheat seedlings (El-Shintinawy and El-Showbagy 2001) and *Ceriops roxburghiana*. SOD activity increased with increasing salt stress levels in wheat seedlings reported by Ezatollah *et al*. (2007). Similar results were reported in sugar beet cultivars, in rice (Lutts *et al*. 1996) and in *Sorghum bicolor* (Colmer *et al*. 1996). In conclusion, the result of this experiment indicates that NaCl disturbed the mechanism of germination in senna seeds and altered biochemical composition of seedlings.

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