

Effects of Salinity and Water Stress Factors on Seed Germination, Early Seedling Growth and Proline Content in an Oil Crop, Black Sesame (*Sesamum indicum* L.)

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Present study aims to evaluate the impacts of salinity and water stress factors on seed germination and early seedling growth under *ex-vitro* conditions in an oil crop, black sesame (*Sesamum indicum* L., cv. TMV3). During salinity stress, various concentrations of NaCl-solutions (10mM, 25mM, 50mM, 100mM, and 250mM) were employed while during water stress treatments, mannitol and sorbitol solutions in equal concentrations (10mM, 25mM, 50mM, 100mM, 250mM, and 500mM) and polyethylene glycol (PEG) solution (5%, 10%, 15%, 25%, and 50%) were considered. Furthermore, after 3-days and 7-days of stress treatments, observations were recorded as partial and full germination respectively. Results indicate that with 100mM of NaCl solution, sesame seed was found to germinate without root development ($80\pm 0.0\%$) after 7-days of treatments in comparison to control experiment ($100\pm 1.0\%$) and further high concentration (250mM) of NaCl solution was proved to be completely toxic for sesame seed germination. Additionally, seedling growth was also affected with NaCl concentrations and seedling's height was recorded as minimum with root and shoot lengths ($0.5\pm 0.21\text{cm}/2.1\pm 0.78\text{cm}$) in seeds growing with NaCl solution (50mM) while further high concentration (100mM) was proved to be strongly inhibitory for root development in germinated seeds. During water stress treatments, results indicated that mannitol (100mM) turns out to be relatively strong inhibitor for seed germination ($50\pm 0.5\%$) than sorbitol ($60\pm 1.0\%$) with (250mM) solution. However, both mannitol and sorbitol solutions (500mM) were proved to be fully toxic for seed germination. Furthermore, during PEG treatments, PEG solution (25%) was found to be significantly inhibitory and germination frequency ($40\pm 0.0\%$) was recorded while further increase in PEG concentration (50%) was proved to be lethal. Moreover, during early seedling growth, mannitol (100mM) was turned out to be strongly inhibitory for root development and root-shoot length ratio was recorded as ($0.0\text{cm}/0.25\pm 0.41\text{cm}$) while sorbitol (100mM) was proved to be slightly weak inhibitor ($0.71\pm 0.27\text{cm}/0.93\pm 0.32\text{cm}$). Significantly high concentration of mannitol (250mM) was turned out to be toxic for seedling growth while even very high concentration of sorbitol (500mM) was failed to suppress seedling growth completely. During PEG treatments, the minimum seedling height ($0.79\pm 0.31\text{cm}/1.43\pm 0.32\text{cm}$) was recorded with PEG (15%) solution while PEG (25%) solution was found to suppress root formation completely. Furthermore, during endogenous proline content estimation in tissues growing with NaCl salt solutions, results indicated that proline content gradually increases with the increase in NaCl concentrations and was found to be maximum ($128.3\times 10^{-3}\text{g}^{-1}$) in tissues growing with very high concentration of NaCl (100mM) solution than the tissues growing with very low concentration (10mM) of NaCl salt solution ($10.94\times 10^{-3}\text{g}^{-1}$).

Key words: Proline, Seed germination, Salinity, Seedling, Sesame, Water stress

Sesame (*Sesamum indicum* L.) is one of the world's oldest oil seed crops and also the sixth most important oil seed crop grown in India. It is widely used in food nutraceutical and pharmaceutical industries in many countries because of its high oil protein and antioxidant contents (Sumathi and Muralidharan, 2009; Kamaraj *et al.*, 2017). Additionally, sesame is also rich in various bioactive compounds including phytosterols, tocopherols and lignans such as sesamin, sesamolin and sesaminol, which are known to play an important role in providing stability against oxidation of oil and help as anti-oxidative activity (Al-Yemeni *et al.*, 2000; El Harfi *et al.*, 2016).

Sesame is being grown in arid and semi-arid areas where high temperatures, high levels of solar radiation, high evaporation rate and drought climates are very common which negatively impact the productivity at large scale (Witcombe *et al.*, 2007; Hassanzadeh *et al.*, 2009; Dossa *et al.*, 2019). All these combined factors generally cause an increase in soil salinity which is a serious abiotic stress factor that restricts the production of sesame crop.

Moreover, seed germination is a crucial phase in plant life that plays important roles in seedling establishment and subsequent growth of seedlings (Bewley, 1997). Although, stress affects all growth stages of a plant but for most plant species, seed germination and seedling growth stages are known to be more sensitive to stress factors (Cuartero *et al.*, 2006) and seed germination has been reported to decline with increasing stress levels (Houle *et al.*, 2001).

Salinity tolerance is a complex phenomenon in which primary reaction is to cope up the osmotic and ionic stresses. Moreover, glycophytes are considered to play special roles in mechanisms for salt tolerance (Levitt, 1980; Hasegawa *et al.*, 2000; Munns, 2002). It is argued that poor seed germination in saline soils leads to poor crop establishment and hence causes low productivity. Germination and early seedling growth of many crop plants are the most sensitive stages to environmental stresses and increase in salinity stress reduces water uptake by seeds, thereby inhibiting germination and root

elongation (Begum *et al.* 2013).

Although studies reveal that the sesame crops are sensitive to salinity (Rhoades *et al.*, 2000; Suassuna, 2013); however, sesame seeds could be also proved moderately tolerant to salt stress (Abbasdokht *et al.*, 2012; Bahrami and Razmjoo, 2012). Moreover, salt stress is known to increase the intake of toxic ions that may have altered certain enzymatic or hormonal activity in seeds during germination (Smith and Comb, 1991; Begum *et al.*, 2013).

Drought stress is equally known to show adverse influence on water relations (Babu and Rao, 1983), photosynthesis (Bhagsari *et al.*, 1976), mineral nutrition, metabolism, growth and yield (Suther and Patel, 1992; Kambiranda *et al.*, 2011). Compared to other crops, sesame has better drought tolerance; however, it is particularly sensitive to drought occurring during germination and seedling stages (Orruno and Morgan, 2007; Boureima *et al.*, 2011).

Significantly, very limited studies have been carried out on the effect of salt and water stress on sesame seed germination indicating that germination and early seedling growth parameters are negatively affected and the effect severity varied depending on the salt and water stress levels and the sesame cultivar. Hence, in present study, attempts have been made to evaluate black sesame (cv. TMV3) for its sensitivity to salinity and drought stress factors during seed germination and early seedling growth stage under laboratory conditions.

MATERIALS AND METHODS

Seed Collection and Sterilization

Seeds of black sesame (*Sesamum indicum* L., cv. TMV3) cultivars were collected from Tamil Nadu Agriculture University, TNAU, Coimbatore (India). Healthy and uniform seeds were selected and washed thoroughly with teepol-20 for 15-20 min and further were surface sterilized with ethanol (70%) for 1-3 minutes followed by HgCl₂ (0.1%) treatments for 8-10 minutes.

Furthermore, sterilized seeds were washed 3-4 times with distilled water and were soaked in the respective stress solutions for 3hrs. The soaked seeds were then transferred to sterile petridishes (9.0cm diameter) lined with two sterile filter papers added with 5ml of distilled

water or the respective stress solutions.

Stress Treatments

During present study, sesame seeds were treated with salinity and water stress inducing agents at different concentrations. Moreover, responses of these stress treatments were observed and recorded after 3rd day and 7th day of the treatments.

Salinity Stress: Sterilized sesame seeds were treated with NaCl salt solutions in various concentrations (10mM, 25mM, 50mM, 100mM and 250mM) to understand the response on seed germination and also during early seedling growth.

Water Stress: Sterilized seeds were also treated with water stress inducing agents mannitol and sorbitol in equal concentrations (10mM, 25mM, 50mM, 100mM, 250mM, and 500mM) and PEG-6000 (5%, 10%, 15%, 25%, and 50%) solutions.

During stress treatments, 15-20 seeds per petridish were employed and three replicates were performed in each treatment. Germination tests were conducted under dark condition at normal room temperature (25-30°C) in laboratory conditions. A seed was considered partially germinated when radicle was 2mm long and complete seed germination was considered when both radical and plumule were emerged out from the seed. Moreover, a control experiment was maintained with distilled water.

Statistical Analysis

The germination mean percentage was determined based on counting the number of germinated seeds on the 3rd and 7th day of the treatments. Statistical data were performed after first count (3rd day after treatments as partial seed germination) and final count (7th day after treatments as complete seed germination). Moreover, germination percentage (GP) and germination rate (GR) was calculated by using the following formula (Ruan *et al.*, 2002).

GP = Number of Total Germinated seeds / Total number of seeds tested x 100

$$GR = \frac{\text{Number of Germinated seeds}}{\text{3rd Day of Count}} + \frac{\text{Number of Germinated Seeds}}{\text{7th Day of Count}}$$

Moreover, responses of salinity and water stresses

during early seedling growth were measured and calculated in terms of lengths of root and shoot of the seedlings after the stress treatments.

Root length: - Length of the tap root of all germinated seedlings from each replication was measured and from these values, the mean was calculated as the root length (cm).

Shoot length: - From the same seedlings which were used for measuring the root length, the shoot lengths of all germinated seedlings were calculated. Moreover, from these values, the mean was taken as the shoot length (cm).

Determination of Endogenous Proline Content

For biochemical analysis, SDW treated control and NaCl treated germinating seeds of sesame after 7 days of treatments were separated and used for biochemical analysis of the NaCl stress induced proline accumulation in the germinating seeds.

Free proline content was estimated based on the protocol proposed by Bates *et al.* (1973). Fresh germinated seeds (0.5gm) of sesame were homogenized in 5ml of sulphosalicylic acid (3%) by using a mortar and pestle. About 2ml of extract was taken in test tube and 2ml of glacial acetic acid followed by 2ml of ninhydrin reagent were added in it. The reaction mixture was boiled in a water bath at 100°C for 30min. After cooling the reaction mixture, 4ml of toluene was added.

After thorough mixing, the chromophore containing toluene was separated and absorbance of red colour developed was read at 520nm against toluene black on UV-visible spectrophotometer. Finally, proline concentration was determined using calibration curve prepared from known concentrations of proline solutions and quantification of proline was expressed as mg/g fresh weight of tissue.

RESULTS

All the observations during salinity and water stress treatments were recorded at the end of 3rd day and 7th day of the treatments as the partial or incomplete seed germination and full or complete seed germination respectively. Moreover, observations during early seedling growth were also recorded in terms of shoot

and root lengths of the seedlings at the end of 7th day of the treatments.

Effects of Salinity Stress

Germination and seedling growth of black sesame seeds were significantly affected by salinity stress induced by various concentrations of NaCl (10mM, 25mM, 50mM, 100mM and 250mM) solutions. Significantly, results were found to be based on concentrations of stressors and also on durations of treatments.

Effects on NaCl Stress on Seed Germination

During control and lower concentrations (10mM, 25mM, and 50mM) of NaCl-solutions treatments, seeds exhibited the indication of germination next day of the treatments but the complete emergence of radicles could be seen only at the end of 3rd day of treatments while with higher concentrations of NaCl (100mM and 250mM) solutions, sesame seed germination was observed as either very slow or completely lacking at the end of 3rd day of treatments.

Furthermore, at the end of 7th day of treatments, the full germination with complete seedling development was observed in control experiment (**Figure 1A**) whereas similar responses were also seen with the seeds that were treated with lower concentrations (10mM, 25mM and 50mM) of NaCl solutions (**Figure 1B, C & D**) respectively. Significantly, lower concentrations of NaCl (10mM and 25mM) were seen to be less effective to inhibit germination response (**Figure 1B & C**) respectively while further higher concentration (50mM) of NaCl solution relatively was proved to be slightly inhibitory (**Figure 1D**) and the seed germination was found to be slow and suppressed in comparison to control treatment and other lower concentrations (10mM and 25mM) of NaCl-solutions.

Interestingly, very high concentration (100mM) of NaCl-solution was turned out to be significantly inhibitory for seed germination (**Figure 1E**) while with very high concentration (250mM) of NaCl solution; seed germination was completely lacking and was not visible even after 10-days of treatments (**Figure 1F**). Thus, NaCl (250mM) solution was proved to be highly toxic level for sesame seed germination.

Effects of NaCl- Stress on Rate of Seed Germination

Similar to control treatment, germination percentage was also found to be (100±1.0%) in seeds that were treated with lower concentrations (10mM, 25mM, and 50mM) of NaCl-solutions at the end of 3rd day of the treatments. However, with further higher concentration (100mM) of NaCl-solution, the frequency was found to decline and recorded as (80±0.0%) while with 250mM of NaCl solution; the frequency of sesame seed germination was obtained as zero (**Table 1**).

Furthermore, after 7th day of treatments, the maximum frequency (100±1.0%) of seed germination was obtained in case of control treatments while even with the increase in NaCl concentrations, similar response in terms of germination frequency (100±0.5%) was recorded with lower concentrations of NaCl (10mM, 25mM, and 50mM) solutions. However, further increase in NaCl concentration (100mM), frequency of seed germination was declined and recorded as (80±0.0%). Moreover, high concentration (250mM) of NaCl solution was turned out to be lethal and toxic completely for seed germination.

Effects of NaCl- Stress on Early Seedling Growth

Seedling height in terms of shoot and root elongations were found to decrease with increase in salinity stress caused by concentrations of NaCl-solutions. Moreover, with lower concentrations of NaCl (10mM, 25mM and 50mM) solutions, sesame seeds were found to show maximum tolerance to salinity stress and seedlings growth were slightly affected with the increase in NaCl concentrations (**Figure 2A & B**) respectively.

However, seedlings lengths were found to decline with the increase in NaCl concentrations in the treated solutions and therefore, seedlings growing with 100mM of NaCl- solution, were exhibited the minimal seedling length and hence, root-shoot length ratio was recorded as (0.0/0.12±0.08cm) in comparison to control seedling (2.07±0.13/3.31±0.17cm) (**Table 1**). Moreover, this concentration of NaCl (100mM) was proved to be strongly inhibitory for root development in germinated seeds.

Furthermore, NaCl solutions treated seedlings were

transferred to paper cups soil after 7 days of treatments and also seedlings were irrigated with respective concentrations of NaCl solutions. Significantly, there was much influence of salinity stress on seedling growths and seedlings were found to be shorter in heights (**Figure 2C & D**) with the increased concentrations of NaCl solutions (10mM, 50mM, and 100mM) respectively. However, poorly germinated seeds without roots growing with NaCl (100mM) solution were failed to grow into complete seedlings.

Effects of Water Stress

In order to evaluate the effects of water stress during seed germination and early seedling growth in black sesame seeds, equal concentrations (10mM, 25mM, 50mM, 100mM, 250mM and 500mM) of mannitol and sorbitol solutions and PEG solutions (5%, 10%, 15%, 25% and 50%) were employed during the experiments. Moreover, results were influenced with the concentration of stressors and also on duration of treatments.

Effect of Mannitol- Stress on Seed Germination

At the end of 3rd day of treatments, seeds in control experiment and with lower concentrations of mannitol solutions (10mM and 25mM) started to show fast germination without any distinguished indication of germination inhibition caused by mannitol solutions, while with further higher concentrations (50mM and 100mM), seed germination was found to be relatively slow and delayed. Moreover, with very high concentrations of mannitol (250mM and 500mM) solutions, seed germination was completely lacking up to 3rd day of treatments.

Further, at the end of 7th day of mannitol treatments, the growth of seedlings growing with low concentration of mannitol (10mM) solution was found to be almost similar to the seedlings growing under control treatments (**Figure 3A & B**) respectively but with further high concentration of mannitol (25mM), stress inhibition was recorded in terms of seedling growth (**Figure 3C**). Moreover, with further higher concentrations (50mM and 100mM) of mannitol solutions, a significant water-stress inhibitions could be observed (**Figure 3D & E**) than the seedlings that were grown with mannitol concentration (25mM) solution (**Figure 3C**). Further, very high concentrations of mannitol (250mM and 500mM)

solutions were proved to be toxic and seeds were completely failed to germinate even after 7-days of the treatments. However, merely emergence of proliferated embryos could be seen in few seeds treated with mannitol (250mM) solution (**Figure 3F**).

Effects of Mannitol- Stress on Rate of Seed Germination

During mannitol treatments, germination frequency was observed to remain constant even with increase in mannitol concentrations in the treatment solutions. Moreover, similar to the control experiment (100±0.5%), even (50mM) of mannitol solution was found to be ineffective to cause water stress inhibition and, the germination frequency (100±1.0%) was recorded at the end of 7-days of treatments (**Table 2**).

However, further increase of mannitol concentration in the solution (100mM) was exhibited significant inhibitions in the rate of germination and therefore, frequency of seed germination was found to be (50±0.5%). Significantly, sesame seeds were found to show strong sensitivity to water stress on treatments with mannitol solutions (250mM and 500mM) and seed germination was completely inhibited even after 10-days of treatments and thus, proved to be toxic and lethal (**Table 2**).

Effects of Mannitol- Stress during Early Seedling Growth

Seedling heights in terms of shoot and root elongations were found to decrease with increase in osmotic stress caused by mannitol solutions. Even very low concentration of mannitol (10mM) solution was found to show strong sensitivity to induce water stress and seedlings heights (1.56±0.25cm/2.96±0.11cm) were found to be much suppressed in comparison to the control seedlings (3.11±1.04cm/3.77±0.19cm) (**Figure 4A**) respectively (**Table 2**).

Similarly, seedlings grown with further high concentration of mannitol (25mM) solution were appeared to be very much inhibited (**Figure 4B**) in comparison to seedlings grown with 10mM of mannitol solution (**Figure 4A**). Seedlings lengths were found to decline with the increase in mannitol concentrations in treated solutions and therefore, with high concentration

(100mM) of mannitol solution, germinated seed was found to lacking the root formation completely, therefore, root-shoot length ratio was recorded as (0.0cm/0.25±0.41cm) in comparison to control seedling (3.11±1.04cm/3.77±0.19cm) (**Table 2**).

Furthermore, after 7th day of treatments, seedlings were transferred to the paper cups and were treated with respective concentrations of mannitol solutions. Results indicate that after 10-days of mannitol-treatments, seedlings growth were affected with the mannitol solutions (10mM and 25mM) and found to be gradually suppressed in comparison to the control seedlings (**Figure 4C & D**) respectively.

Effect of Sorbitol-Stress on Seed Germination

Similar to mannitol solution response, seeds could to be seen to show quick germination without any indication of germination inhibition caused by sorbitol-solutions (10mM and 25mM). Moreover, slight inhibitions in sesame seed germination could be observed when seeds were treated with increased concentrations (50mM, 100mM, and 250mM) of sorbitol solutions at the end of 3rd day of treatment.

After 7th day of sorbitol (10mM and 25mM) solution treatments, similar to control seedlings the complete seed germination was followed by the growth of young seedlings (**Figure 5 A, B, & C**) respectively but with higher concentrations of sorbitol solutions (50mM and 100mM), seed germination was found to be significantly inhibited leading to suppressed seedling growth (**Figure 5D & E**) respectively.

However, further increase of sorbitol concentration (250mM) unlike mannitol solution response, it was proved to be relatively less inhibitory but significant inhibitions were also noticed (**Figure 5F**). Moreover, with very high concentration (500mM) of sorbitol solution treatment, sesame seed germination was strongly inhibited but this solution was failed to be completely toxic like mannitol solution (500mM).

Effect of Sorbitol Stress on Rate of Seed Germination

The maximum germination rate (100±0.5%) was recorded with (10mM and 25mM) of sorbitol concentrations similar to control experiments after 7th

day of treatments (**Table 3**). However, gradual reduction in seed germination and complete seedlings formation could be recorded as (90±1.5%, 70±0.5%, and 60±1.0%) with concentrations (50mM, 100mM and 250mM) of sorbitol solutions respectively.

Moreover, very low frequency (10±0.5%) of seed germination was also obtained with very high concentration (500mM) of sorbitol solution treatments indicating that in comparison to mannitol solution response, sorbitol solution (500mM) was proved to be a weak water stress causing agent.

Effects of Sorbitol- Stress during Early Seedling Growth

In sesame, generally seedlings height in terms of shoot and root elongations were seen to decrease with increase in water stress caused by sorbitol concentrations. With low concentration of sorbitol (10mM) solution treatment, sesame seedlings showed maximum tolerance to water stress and seedlings heights were observed almost similar to the control seedlings (**Figure 6A**). However, seedlings grown at higher sorbitol stress levels (100mM and 250mM) showed maximum difference in all aspects of seedlings (**Figure 6B**).

Seedlings heights were found to decline with the increase in sorbitol concentrations in treatment solutions and therefore, with 250mM of sorbitol solution treatments, seedling length was found to be the minimal and root-shoot length ratio was recorded as (0.38±0.14cm/0.44±0.16cm) in comparison to control seedling (3.5±1.63cm/4.04±1.06cm) (**Table 3**).

Moreover, seedlings that were grown with various concentrations of sorbitol solutions were transferred to paper cup soil (**Figure 6 C & D**) after 7-days of treatments and were irrigated with respective sorbitol solutions. Significantly, after 15th day of treatments, inhibitions in seedlings growth caused by water stress induced by sorbitol solutions were recorded.

Effects of PEG Stress on Seed Germination

In comparison to control experiment, seed germination was also observed to be initiated at the end of 3rd day of treatments with (5%, 10%, and 15%) of PEG solutions; however, with high concentration (25%)

of PEG solution, seed germination was observed to be very slow and delayed. Moreover, with further high concentration (50%) of PEG solution, symptom of seed germination was completely lacking till 3rd day of treatments.

After 7th day of PEG- solution treatments, similar to control experiment (**Figure 7A**) sesame seeds were found to show tolerance and complete seed germination with low concentration (5%) of PEG solution (**Figure 7B**) treatments. However, with further increased concentrations (10% and 15%) of PEG solutions, sesame seed germination was found to be relatively slow and delayed leading to developments of suppressed seedlings (**Figure 7 C & D**) respectively.

Moreover, suppression in seed germination and seedling growth was observed to be more prominent with the seeds that were treated with (25%) of PEG solutions (**Figure 7E**). Furthermore, seed germination was found to be completely lacking if seeds were treated with very high concentration (50%) of PEG solutions and there was complete inhibition of sesame seed germination (**Figure 7F**) at the end of 7th day of treatments.

Effects of PEG- Stress on Rate of Seed Germination

The rate of seed germination was found to decrease with the increase in PEG concentrations in the treatment solutions. In control experiment, the maximum frequency of seed germination ($100 \pm 0.5\%$) was recorded after 7th day of treatments and similar response in rate of seed germination ($100 \pm 2.0\%$) was also recorded with 5% of PEG solution treatments (**Table 4**).

However, with further increased concentrations (10%, 15%, and 25%) of PEG solutions, seed germination rate was gradually decreased and recorded as ($90 \pm 2.0\%$, $70 \pm 0.5\%$, and $40 \pm 0.0\%$) respectively. Moreover, PEG concentration (50%) was proved to be strong inhibitor for sesame seed germination and seeds were failed to germinate even after 7th day of treatments.

Effects of PEG Stress during Early Seedling Growth

Height and length of differently PEG solution treated sesame seedlings was significantly affected by water stress caused by PEG solutions. For the survival tendency and growth of the seedlings, the maximum

length as root/shoot length ratio was observed in the seedlings growing under control condition ($3.2 \pm 0.57\text{cm}/3.33 \pm 1.69\text{cm}$) than the PEG treated seedlings.

During PEG (5%) solution treatments, germinated seeds were observed to exhibit normal seedlings growth after 7th day of treatments and appeared similar to the control seedlings (**Figure 8A**). Seedlings lengths were found to gradually decrease with the increase in PEG concentrations (15%) in the treatment solutions (**Figure 8B**). However, with 25% of PEG solution treatment, seedling length was recorded to be the minimal and thus, root-shoot length ratio was recorded as ($0.0\text{cm}/0.78 \pm 0.34\text{cm}$) in comparison to the control seedling ($3.2 \pm 0.57\text{cm}/3.33 \pm 1.69\text{cm}$) (**Table 4**). Moreover, results reveal that 25% of PEG solution proves to be toxic for root development during seed germination.

Remarkably, root length was found to be more elongated even in PEG-treated seedlings (5% and 15%) than control seedlings (**Figure 8A & B**) respectively. Furthermore, seedlings were shifted to the paper cup soil stage after 7 days of treatments followed by irrigation with respective concentrations of PEG-solutions.

Significantly, seedlings grown with low concentration (5%) of PEG solution were found to be healthy and similar to the control seedlings (**Figure 8C**) while seedlings that were treated with high PEG solution (15%) were observed to be much suppressed in terms of overall growth in comparison to control seedlings (**Figure 8D**).

Effect of NaCl Stress on Endogenous Proline Content

Results indicated that a gradual increase in proline content was recorded in NaCl treated tissues that were growing with the increased concentrations of NaCl solutions. The control treated samples were found to exhibit the minimum quantity of proline content ($7.22 \times 10^{-3}\text{g}^{-1}$) whereas the maximum quantity of proline content ($128.3 \times 10^{-3}\text{g}^{-1}$) was estimated in the samples that were extracted from the tissues growing under high concentration (100mM) of NaCl solution (**Table 1**).

Moreover, minimum quantity ($10.94 \times 10^{-3} \text{g}^{-1}$) of proline content was obtained in tissues that were extracted from the seeds germinated with low concentration (10mM) of NaCl solution. Hence, results reveal that concentration

of endogenous proline accumulation in germinated tissues increases with the increase in NaCl concentrations in the treatment solutions.

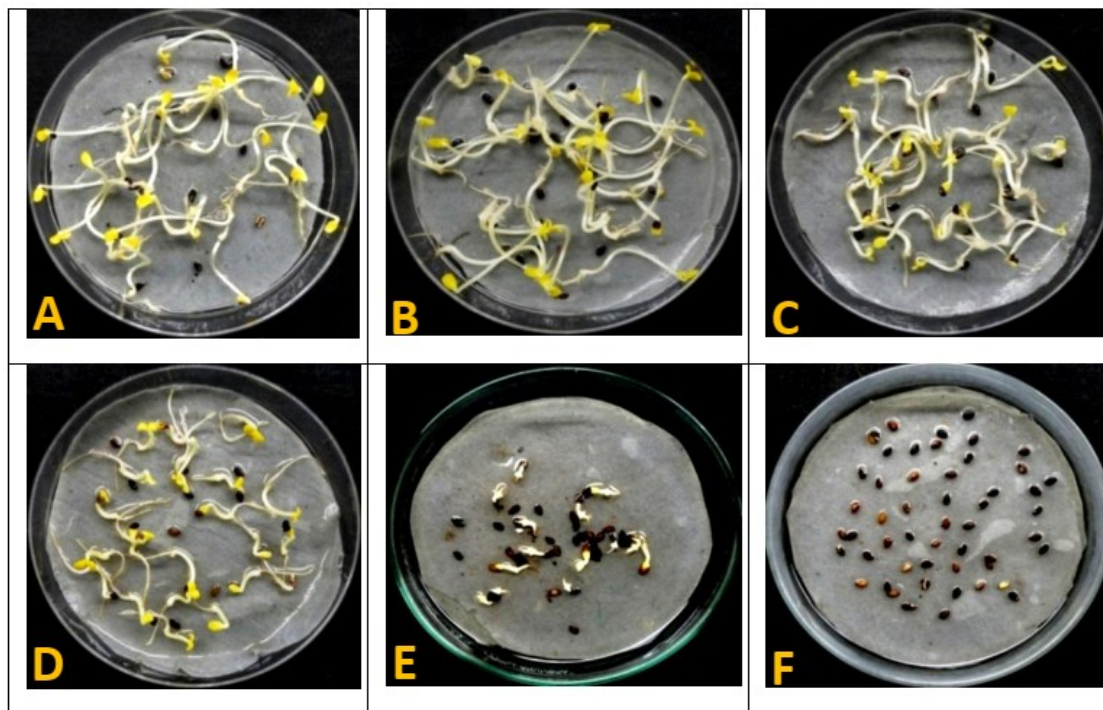


Figure 1. (A-F) *Sesamum indicum* L., NaCl (Salinity Stress) Treatments - (A) Control (B) 10mM (C) 25mM (D) 50mM (E) 100mM (F) 250mM of NaCl solutions (after 7 days of treatments).

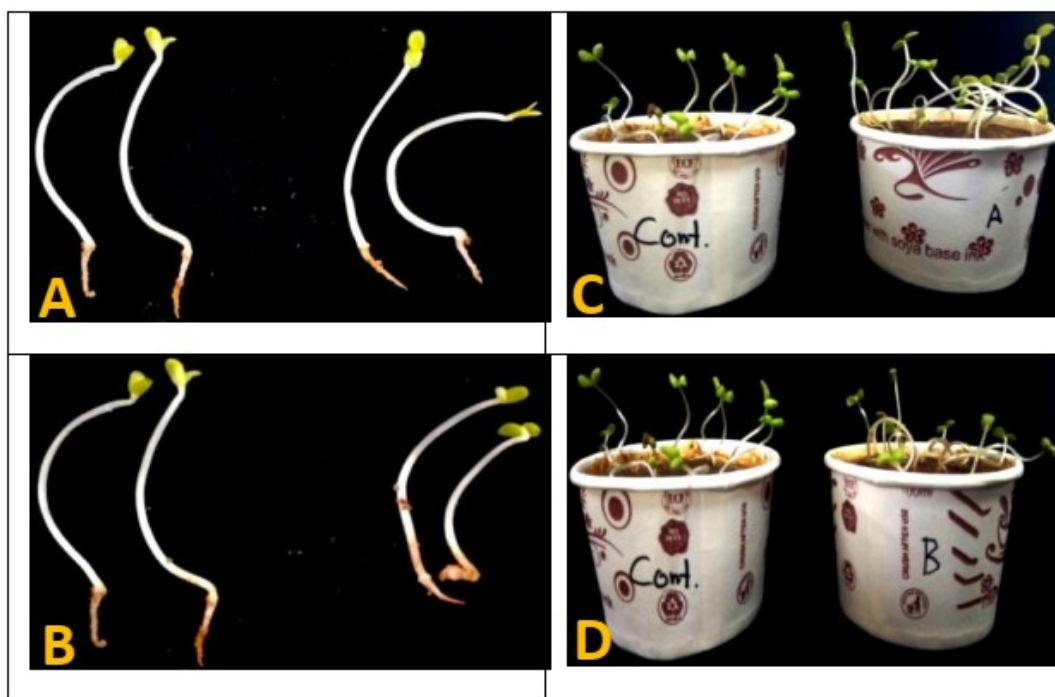


Figure 2. (A-D) *Sesamum indicum* L., NaCl (Salinity Stress) Treatments – (A) Control + 10mM (B) Control + 50mM of NaCl solutions (after 7 days of treatments) (C) Control + 10mM (D) Control + 50mM of NaCl solutions (Seedlings were transferred on paper cups after 12 days of treatment).

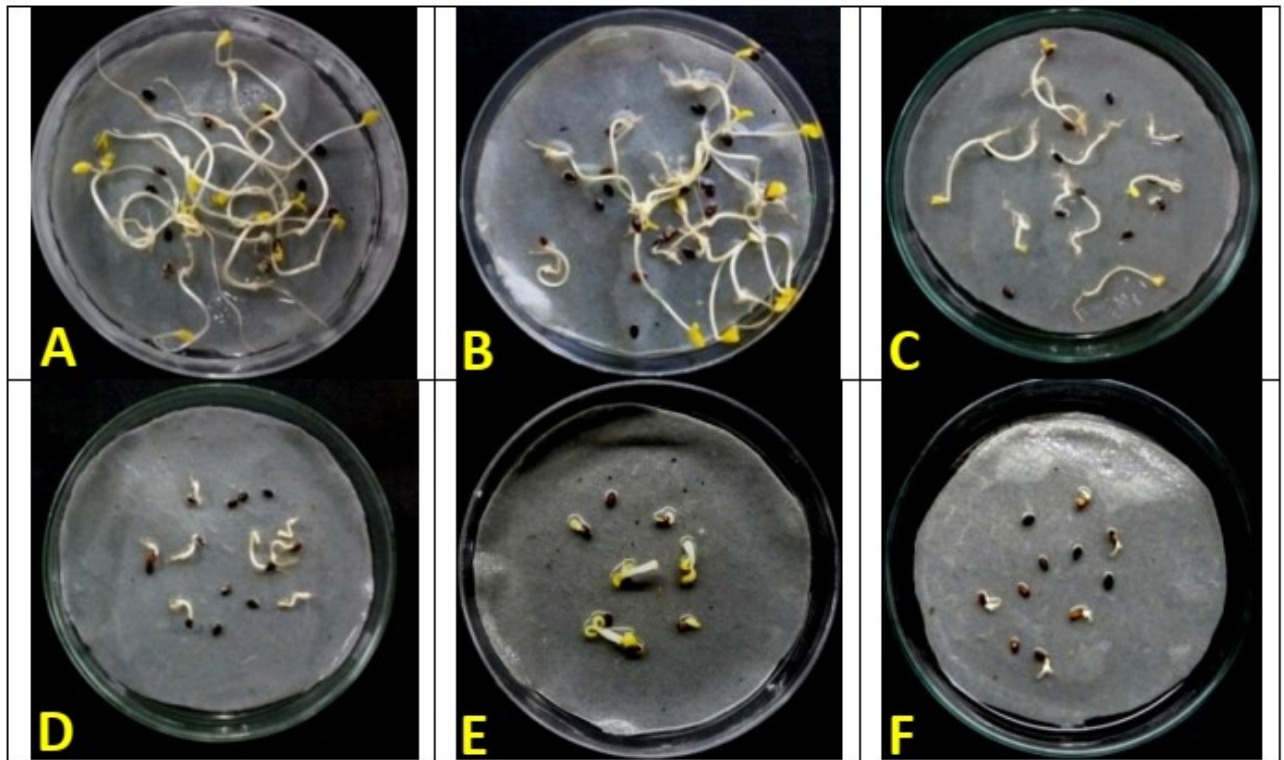


Figure 3. (A-F) *Sesamum indicum* L., Mannitol (Water Stress) Treatments – (A) Control (B) 10mM (C) 25mM (D) 50mM (E) 100mM (F) 250mM of mannitol solutions – after 7 days of treatments.

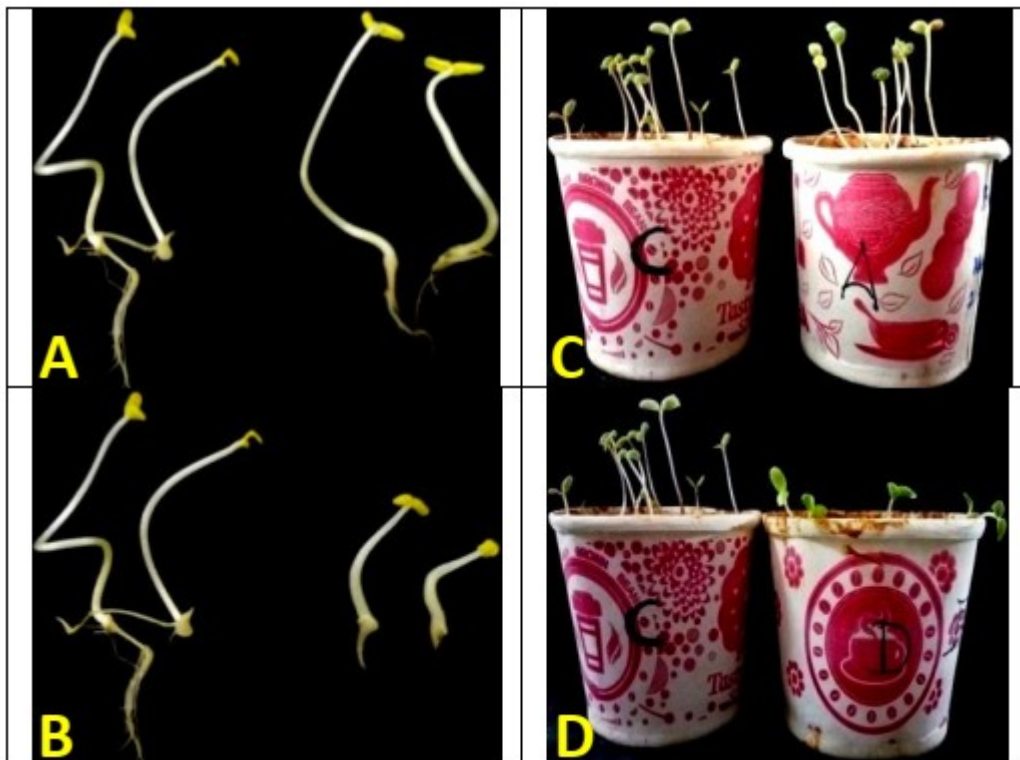


Figure 4. (A-D) *Sesamum indicum* L., Mannitol (Water Stress) Treatments – (A) Control+10mM (B) Control+25mM- seedlings after 7-days of treatments and (C) Control + 10mM (D) Control+25mM of mannitol solution (Seedlings were transferred on paper cups after 10 days of treatment).

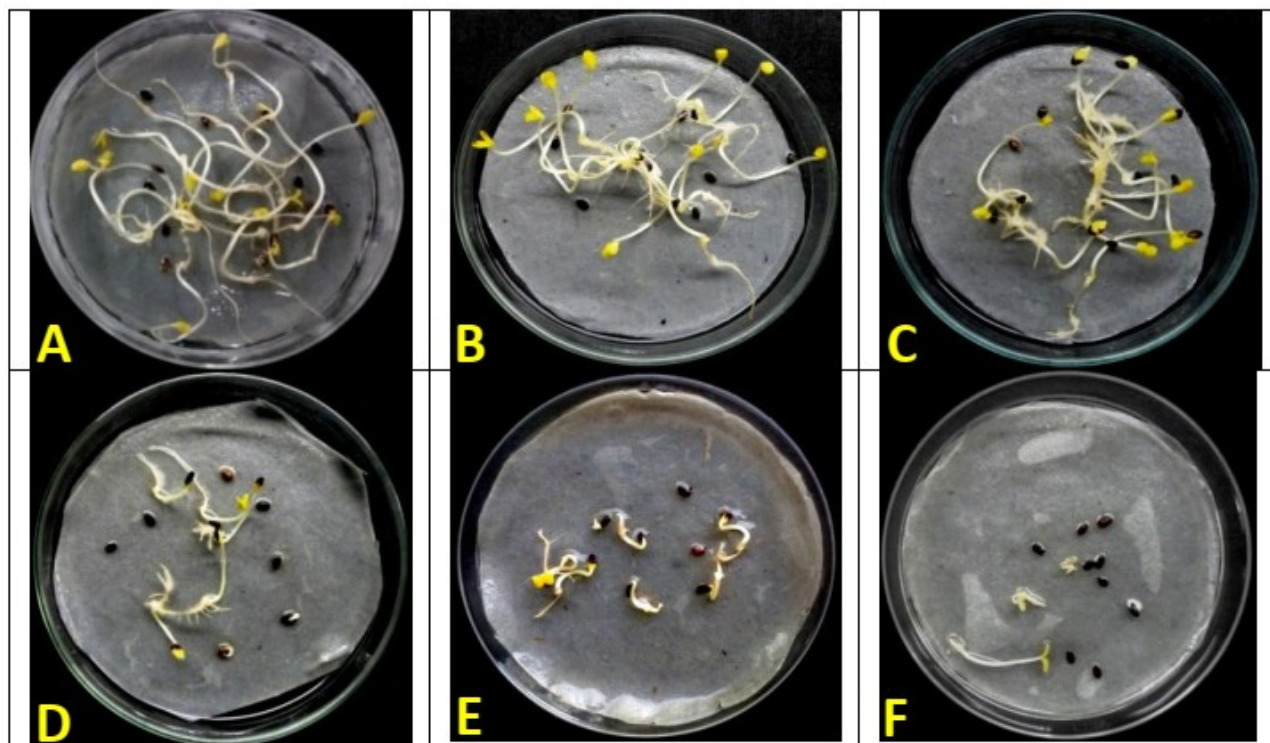


Figure 5. (A-F) *Sesamum indicum* L., Sorbitol (Water Stress) Treatments – (A) Control (B) 10mM (C) 25mM (D) 50mM (E) 100mM (F) 250mM of sorbitol solutions – after 7 days of treatments.

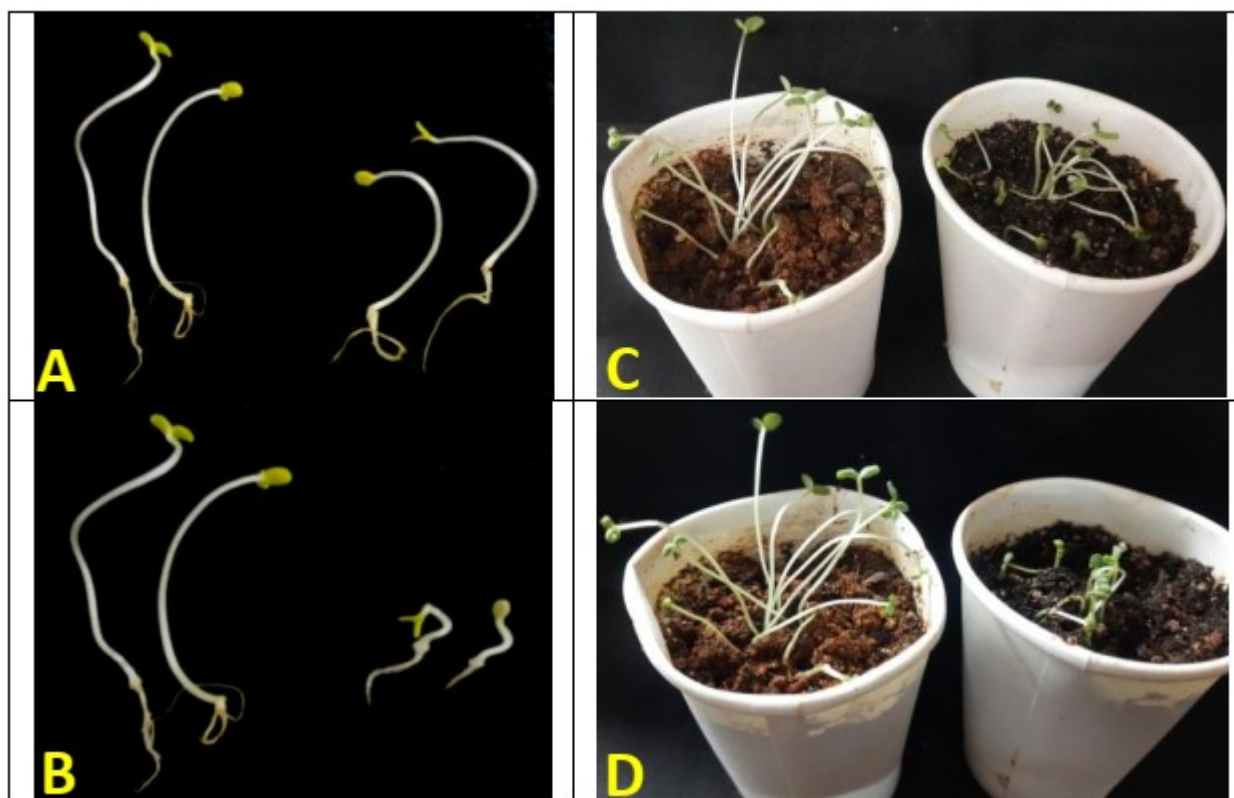


Figure 6. (A-D) *Sesamum indicum* L., Sorbitol – Water Stress Treatments – (A) Control + 10mM (B) Control + 250mM - seedlings after 7-days of treatments and (C) Control + 10mM (D) Control+250mM of sorbitol solutions (Seedlings were transferred on paper cups after 10 days of treatment).

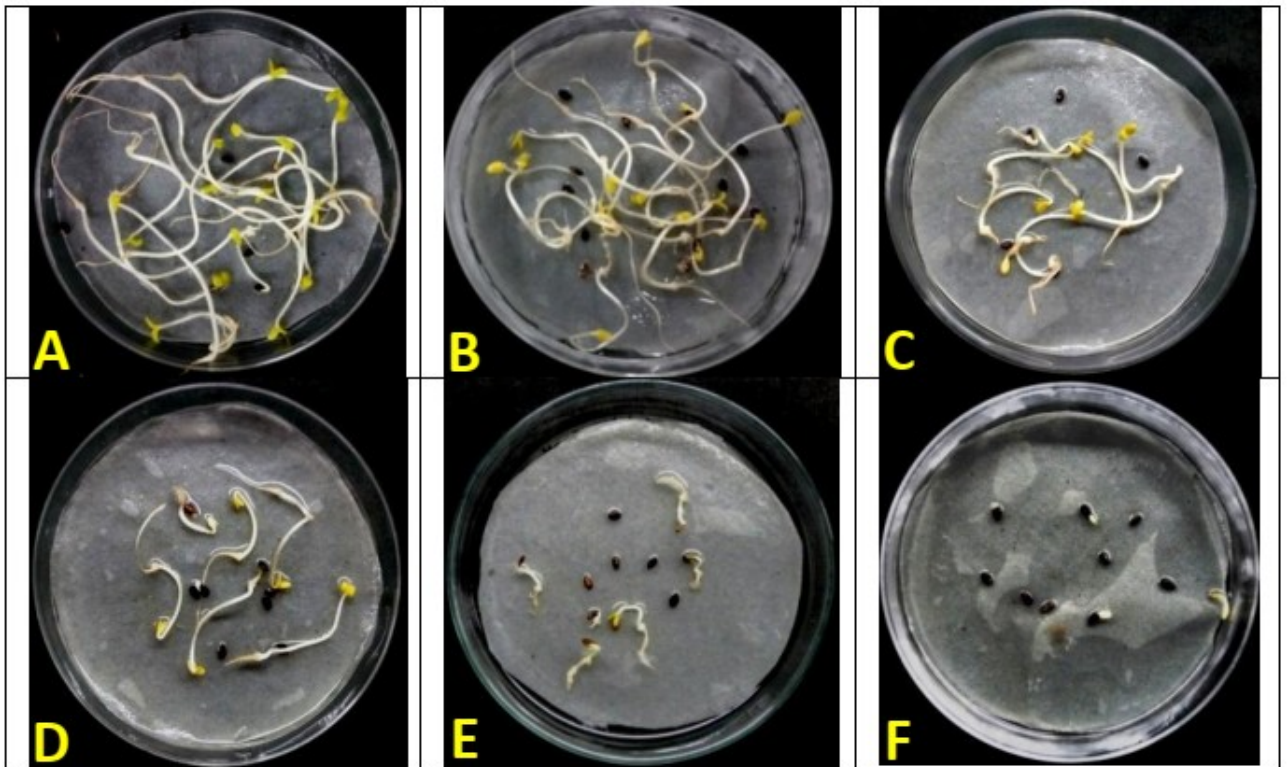


Figure 7. (A-F) *Sesamum indicum* L., Polyethylene glycol (PEG) (Water Stress) Treatments – (A) Control (B) 5% (C) 10% (D) 15% (E) 25% (F) 50% of PEG solutions after 7 days of treatments.

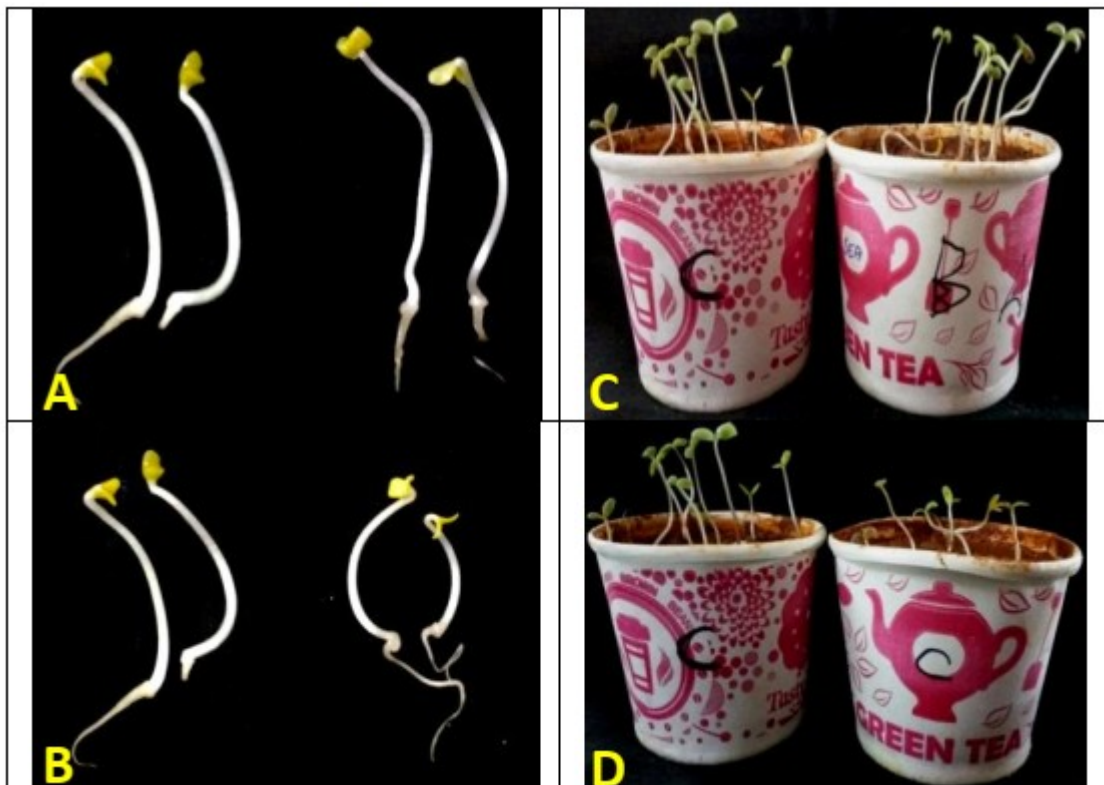


Figure 8. (A-D) *Sesamum indicum* L., Polyethylene glycol (PEG) (Water Stress) Treatments – (A) Control + 5% (B) Control + 15% of PEG solutions (Seedlings after 7 days of treatments) (C) Control + 5% (D) Control + 15% of PEG solutions (Seedlings were transferred on paper cups after 10 days of treatment).

Table 1. Effects of various concentrations of NaCl solutions on seed germination and early seedling growth in black sesame (*Sesamum indicum* L.).

S. No.	NaCl Concentration (mM)	Germination (%) Mean \pm S.E		Root length (cm) Mean \pm S.E	Shoot length (cm) Mean \pm S.E	Total Proline Content ($10^{-3}g^{-1}$ of fresh extract)
		3 rd Day	7 th Day			
1	Control	100 \pm 1	100 \pm 1	2.07 \pm 0.13	3.31 \pm 0.17	7.22
2	10	100 \pm 1	100 \pm 1	1.47 \pm 0.20	3.29 \pm 0.15	10.94
3	25	100 \pm 1	100 \pm 1	1.03 \pm 0.12	2.39 \pm 1.18	28.34
4	50	100 \pm 0.5	100 \pm 0.5	0.5 \pm 0.21	2.1 \pm 0.78	75.23
5	100	80 \pm 0.0	80 \pm 0.0	0	0.12 \pm 0.08	128.3
6	250	0	0	0	0	0

Table 2. Effects of various concentrations of mannitol solutions on seed germination and early seedling growth in black sesame (*Sesamum indicum* L.).

S. No.	Mannitol Concentration (mM)	Germination (%) Mean \pm S.E		Root length (cm) Mean \pm S.E	Shoot length (cm) Mean \pm S.E
		3 rd Day	7 th Day		
1	Control	100 \pm 1	100 \pm 0.5	3.11 \pm 1.04	3.77 \pm 0.19
2	10	100 \pm 1	100 \pm 1	1.56 \pm 0.25	2.96 \pm 0.11
3	25	80 \pm 1	100 \pm 0.5	1.19 \pm 0.21	1.72 \pm 0.19
4	50	70 \pm 0.5	100 \pm 1	0.31 \pm 0.21	0.87 \pm 0.15
5	100	50 \pm 0.5	50 \pm 0.5	0	0.25 \pm 0.41
6	250	0	0	0	0
7	500	0	0	0	0

Table 3. Effects of various concentrations of sorbitol solutions on seed germination and early seedling growth in black sesame (*Sesamum indicum* L.).

S. No.	Sorbitol Concentration (mM)	Germination (%) Mean \pm S.E		Root length (cm) Mean \pm S.E	Shoot length (cm) Mean \pm S.E
		3 rd Day	7 th Day		
1	Control	100 \pm 1	100 \pm 0.5	3.5 \pm 1.63	4.04 \pm 1.06
2	10	100 \pm 0.5	100 \pm 0.5	2.81 \pm 0.12	3.2 \pm 0.11
3	25	100 \pm 1	100 \pm 0.5	1.79 \pm 0.14	2.11 \pm 0.31
4	50	80 \pm 0.5	90 \pm 1.5	1.55 \pm 0.28	1.5 \pm 0.64
5	100	50 \pm 1.5	70 \pm 0.5	0.71 \pm 0.27	0.93 \pm 0.32
6	250	40 \pm 1	60 \pm 1	0.38 \pm 0.14	0.44 \pm 0.16
7	500	10 \pm 0.5	10 \pm 0.5	0	0.21 \pm 0.03

Table 4. Effects of various concentrations of Polyethylene glycol (PEG) solutions on seed germination and early seedling growth in black sesame (*Sesamum indicum* L.).

S. No.	PEG Concentration (%)	Germination (%) Mean \pm S.E		Root length (cm) Mean \pm S.E	Shoot length (cm) Mean \pm S.E
		3 rd Day	7 th Day		
1	Control	100 \pm 1	100 \pm 0.5	3.2 \pm 0.57	3.33 \pm 1.69
2	5	80 \pm 2	100 \pm 2	2.32 \pm 0.76	2.82 \pm 0.44
3	10	80 \pm 1	90 \pm 2	1.47 \pm 0.35	1.75 \pm 0.33
4	15	60 \pm 1.5	70 \pm 0.5	0.79 \pm 0.31	1.43 \pm 0.32
5	25	40 \pm 1	40 \pm 0	0	0.78 \pm 0.34
6	50	0	0	0	0

DISCUSSION

In order to increase crop productivity, development of stress tolerant plants and genotypes could be of always a meaningful approach. Under the current prevailing climatic conditions, the selection of abiotic stress tolerant crop cultivars is considered as a feasible alternative to sustain the productivity of the crops globally.

Effects of Salinity Stress

Germination and seedling growth have been reported to decline with many abiotic factors such as salt and drought stress that are perhaps two of the most important and common abiotic stresses that restrict the number of seedlings and later during seedling growth (Ashraf *et al.*, 2008). Moreover, the presence of high amount of various salts in the germinating medium may prevent or retard the absorption of water and cause toxicity to the embryo and endosperm. This osmotic absorption might be the limiting factor due to the high salt concentration and cause delay in the germination (Ramya Sree and Sivaprasad, 2019).

Moreover, it is known that increased salinity can influence seed germination either by toxic effects of specific ions, such as Na⁺, Cl⁻, and SO₄²⁻, or by forming osmotic pressure which impedes seed water uptake (Kaya *et al.*, 2006; Shaikh *et al.*, 2007). Soil salinity and water deficit decrease the soil water potential that leads to late and inadequate germination associated with failure of crop growth. As a result, the crop productivity is adversely affected (Willenborg *et al.*, 2005).

It is argued that salinity stress during germination

and early seedling growth stage may result in serious damage in seedling establishment. However, various approaches like conventional breeding and selection, transgenics production (Zhao *et al.*, 2006), exogenous application of osmolytes, osmo-protectants or plant hormones (Ashraf *et al.*, 2008) and pre-sowing seed treatments with priming agents (Afzal *et al.*, 2006) have been employed for alleviation of salinity stress in crops (Pal *et al.*, 2017).

Thus, it could be suggested that, in sesame, early selection for salt tolerance should be based on germination percentage and on root and shoot length (El Harfi *et al.*, 2016). In fact, it was reported that root and shoot lengths were the most important parameters for salt stress sensitivity evaluation, providing a relevant index to the response of plants to this stress (Jamil and Rha, 2004; Jamil *et al.*, 2006).

Although studies have indicated the sesame crops are sensitive to salinity (Rhoades *et al.*, 2000; Suassuna, 2013); however, additional studies indicate that sesame seeds could be moderately tolerant to salt stress (Abbasdokht *et al.*, 2012; Bahrami and Razmjoo, 2012). Therefore, it is evident that the use of saline water for irrigation is an alternative approach to increase the tolerance of crops to salinity (Lima *et al.*, 2015). However, the tolerance level of plants to salinity may vary depending on the species and cultivars of the same species in addition to other factors, such as both type and concentration of salt as well as exposure time (Garcia *et al.*, 2010; Dias *et al.*, 2017).

During present study based on NaCl-salinity stress treatments, sesame seed was found to be very sensitive

and growth of complete seedlings with roots was lacking in seeds that were growing with the NaCl (100mM) solution and moreover, further high concentration of NaCl (250mM) solution was turned out to be toxic and lethal for seed germination. The reduction in the germination percentage at higher concentrations may be due to the presence of excess amount of ions that cause depletion of acids from tri-carboxylic acid cycle which reduces the respiration rate (Munns, 2008).

In previous study, the emergence, growth and production was evaluated in sesame (cv. CNPA-G3), after irrigation with saline water (Dias *et al.*, 2017). Moreover, the effect of salinity stress for all traits was found to be significant in sesame. With the increase in salinity stress, germination percentage, germination, normal seedling percentage, seedling length and dry weight were found to reduce by the osmotic potential. In sesame, increasing salinity retards all germination associated parameters (Tabatabaei and Naghibalghora, 2014).

Moreover, in recent study, the stress treatments of NaCl (10, 25, 50, 100, and 200 mM) were given and resulted a significant reductions in parameters like germination percentage mean, germination time, speed of germination, seedling length, and fresh weight along with increasing in salinity. However, *Sesamum indicum* L. cv *Thilak* was found to show the maximum tolerance level up to 50mM of sodium chloride solution (Ramya Sree and Sivaprasad, 2019).

Significantly, during early seedling growth with NaCl-solution treatments in present study, seedlings heights were seen to decrease with the increase in NaCl-salt concentrations in the treatment solutions and therefore 100mM was found to be the very strong inhibitor for the complete seedling growth. Moreover, germinated seeds were failed completely to show root formation indicating that NaCl induced salinity stress causes more inhibitory effects during root development in comparison to shoot development and root-shoot length ratio (0.0cm/0.12±0.08cm). In contrast, seedlings that were growing under control conditions were found to exhibit the maximum height (2.07±0.13cm/3.31±0.17cm).

Moreover, literatures reveal that salt stress is known to increase the intake of toxic ions that may have altered

certain enzymatic or hormonal activity in seeds during germination (Smith and Comb, 1991; Begum *et al.*, 2013). In the higher concentrations, the osmotic relationship between the seed and water is found to get inhibited due to excess amount of salts resulting in the reduced water uptake. Besides, the germinating seeds get low amount of oxygen in the dissolved form and this reduces the energy supply to carry out the effective mobilization (Gaballah *et al.*, 2007). In sesame cultivars, the effect of salt stress caused by the salinity resulted induction of high anti-oxidative enzyme activities (Koca *et al.*, 2007).

In another study, similar salt stress decreases the percentage (Almodares *et al.*, 2007b) and increases the duration of germination (Gill *et al.*, 2003) in sweet sorghum. However, significant differences could be detected in the sensitivity of germination to high salinity among cultivars (Samadani *et al.*, 1994). Moreover, 824 genes have been found that differentially express when plants are subjected to early salt stress conditions, including 84 transcription factors (Gruber *et al.*, 2009).

Effects of Water Stress

Sesame is a resilient crop with a strong adaptation to drought prone environments. In fact, sesame is known to grow in arid and semi-arid areas where high temperature, high solar intensity, high rates of water evaporation are common leading to drought stress which greatly impairs the productivity (Dossa *et al.*, 2019). Compared to other crops, sesame has better drought tolerance; however, it is particularly sensitive to drought occurring during germination and seedling stages (Orruno and Morgan, 2007; Boureima *et al.*, 2011).

In general, drought stress has been known to affect adversely the seed germination, plant growth and development (Ashraf *et al.*, 2002; Almaghrabi and Abdelomoneim, 2012; Vibhuti Shahi *et al.*, 2015). Previous studies on sesame reveal the negative effect of drought stress (Boureima *et al.*, 2011; Bahrami *et al.*, 2012; Keshavarzi, 2012) have been seen on seed germination and seedling growth.

Moreover, it is suggested that germination inhibition may be caused due to lower infusibility of water through

the seed coat and initial water uptake of the seed under stress condition (Khayatnezhad and Gholamin, 2011; Bahrami *et al.*, 2012). Moreover, drought stress promotes stomatal closure and this increases the oxidative stress on the plant tissues resulting in harm to others important bio-molecules (Sairam and Tyagi, 2004).

It is suggested that plants are known to protect themselves from drought stress due to some morphological adaptations towards drought stress that leads to multiplicity of biochemical processes and physiological processes which act as mechanism of resistance against stress. A group of anti-oxidative enzymes like SOD, CAT, GPX, APX etc are activated during stress to bind to the reactive oxygen species (Sayfzadeh and Rashidi, 2011).

During present study, water stress causing agent mannitol concentrations (10mM, 25mM, and 50mM) have been proved ineffective to cause stress inhibitions during seed germination and moreover, germination response ($100\pm 1\%$) was recorded similar to control treatments ($100\pm 0.5\%$). However, with further increase in mannitol concentration (100mM) solution, partially germinated seeds ($50\pm 0.5\%$) could show complete inhibitions for root developments. However, further increase in mannitol concentrations (250mM and 500mM) was proved to be lethal and toxic.

Additionally, during present study, seedling growth and heights were found to gradually reduce with the increase in mannitol concentrations in the treatment solutions and moreover, even low concentration of mannitol (50mM) was turned out to be inhibitory for seedling growth and hence seedling height as root and shoot lengths ratio was recorded the minimum as ($0.31\pm 0.21\text{cm}/0.87\pm 0.15\text{cm}$) in comparison to control seedling ($3.11\pm 1.04\text{cm}/3.77\pm 0.19\text{cm}$) while with further high concentration of mannitol (100mM) solution, seedlings lengths ($0.0\text{cm}/0.25\pm 0.41\text{cm}$) was recorded indicating that 100mM of mannitol proves to be toxic level for root developments during sesame seed germination.

In contrast to mannitol, sorbitol proves to be relatively weak inhibitor during sesame seed germination and early seedling growth. Even high

concentration of sorbitol solution (250mM) was found to be slight inhibitory and germination response ($60\pm 1.0\%$) was obtained while further high concentration of sorbitol solution (500mM) was turned out to be strongly inhibitory ($10\pm 0.5\%$), however, root development was lacking completely in germinated seeds.

Moreover, the minimum seedling heights in terms of root-shoot lengths ratio ($0.38\pm 0.14\text{cm}/0.44\pm 0.16\text{cm}$) in comparison to control ($3.5\pm 1.63\text{cm}/4.04\pm 1.06\text{cm}$) was recorded in seedlings that were growing high concentration (250mM) of sorbitol solution. Significantly, seedlings lengths ($0.0\text{cm}/0.21\pm 0.03\text{cm}$) were found with 500mM of sorbitol solution treatment.

Moreover, polyethylene glycol (PEG), a non-penetrating osmotic agent that lowers the water potential of the medium, has been used extensively to stimulate drought stress in plants (Smith *et al.*, 1986). PEG molecules do not enter in the seed and once water potential of the seed and its surrounding environment are in equilibrium, the seed will not continue to imbibe (Michel, 1983; Mehra *et al.*, 2003). Similar findings were reported by in barley (Zhang *et al.* 2010), in soybean (Khajeh-Hosseini *et al.*, 2003) and in safflower (Zraibi *et al.*, 2011).

During present study, another water stress causing agent polyethylene glycol (PEG) proves to be strong inhibitor at the 7th day of treatments and frequency of seed germination ($40\pm 0.0\%$) was recorded in seeds that were treated with PEG (25%) solution. However, PEG (25%) solution was proved to be strongly inhibitory and root development was significantly inhibited. Moreover, seedling heights ($0.0\text{cm}/0.78\pm 0.34\text{cm}$) were also found to be the minimum in seeds that were grown with 25% of PEG-solution. However, 50% of PEG solution was proved to be completely lethal and toxic.

Previous studies indicate that NaCl and PEG adversely affect the germination and early seedling growth of sesame. This is may be due to alteration of enzymes and hormones found in the seed (Botia *et al.*, 1998) or to the metabolic disorders induced by stress and generation of Reactive Oxygen Species (Almas *et al.*, 2013). It could also be a deficit of hydration of the seeds due to high osmotic potential causing inhibition of the mechanisms leading to the output of the radicle out

of the coat and therefore a seed germination delay (Gill *et al.*, 2001, 2003; El Harfi *et al.*, 2016).

On the other hand, by comparing the effect of PEG and NaCl on germination percentage and root and shoot length, water and salt stresses, regardless of their level, had lesser inhibitory effect on seed germination than seedling growth. Present result supports the results obtained during earlier studies with different safflower varieties under drought and salt stress (Zraibi *et al.*, 2011). A similar finding was also obtained in sunflower (Kaya *et al.*, 2006).

However, higher germination percentage under NaCl stress has been observed in comparison to PEG in sesame, at the same water potential, indicates that adverse effect of PEG on the germination is due to osmotic effect rather than specific ion accumulation. Moreover, such findings have been also recorded in various species, such as barley (Zhang *et al.*, 2010), safflower (Zraibi *et al.*, 2011), sunflower (Kaya *et al.*, 2006), soybean (Khajeh-Hosseini *et al.*, 2003), durum wheat (Sayar *et al.*, 2010), pea (Okçu *et al.*, 2005) and cowpea (Murillo-Amador *et al.*, 2002).

Moreover, since, drought cannot be easily controlled in the field because of rainfall that can impede water deficit (Muscoloa *et al.*, 2014), therefore, evaluating plant response to drought at early seedling stage could be achieved by using chemical desiccators such as mannitol, sorbitol and polyethylene glycol (PEG). Significantly, during recent study, seed germination and seedling establishments have been found to be inhibited under drought stress due to the drop of water potential, which results in the decline of water uptake (Farooq *et al.*, 2009; Kokkanti and Rayalacheruvu, 2019).

Endogenous Proline Status during Salinity Stress

The determination of accumulated proline content induced under stress is considered as one of the fast and efficient techniques for evaluating the salt tolerance in plants. It is suggested that the accumulation of proline results from the decrease of protein synthesis, conversion of glutamate to proline, and induced pH regulation (Steward, 1981; Venekamp, 1989). Moreover, proline also acts as a compatible osmolyte and accumulates during abiotic stresses.

In this study, proline content was found to gradually increase in tissues that were growing with increased concentrations of NaCl-solutions. Significantly, proline accumulation was observed to be the maximum ($128.3 \times 10^{-3} \text{g}^{-1}$) in tissues that were growing with high concentration (100mM) of NaCl-solution as compared to control ($7.22 \times 10^{-3} \text{g}^{-1}$) sample. Moreover, similar results also have been reported in rice seed germination under *in vitro* salinity stress treatments where proline accumulation was found to increase with the increase in NaCl concentration from low concentration to high concentration (Rajakumar, 2013).

CONCLUSION

Abiotic stresses such as salinity and drought in soil impose serious constraints to plant growth and development leading to yield reduction. In order to increase crop productivity against these abiotic stresses, production of stress tolerant plants and genotypes are of great importance. During NaCl salinity stress treatments, this cultivar of sesame (TMV3) was proved to be salt tolerant and 100mM of NaCl solution was proved to be ineffective to suppress seed germination but was strongly inhibitory during early seedling growth. Moreover, proline accumulation was found to gradually increase with the increase in NaCl concentrations in the treatment solutions.

However, in case of water stress treatments, mannitol (100mM) and sorbitol (500mM) solutions were proved to be toxic for seed germination and seedling growth while (25%) of PEG solution was found to be strongly inhibitory.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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