Journal of Stress Physiology & Biochemistry, Vol. 19, No. 2, 2023, pp. 114-123 ISSN 1997-0838

ORIGINAL ARTICLE

Seed priming: An efficient method for enhancing growth and yield of *Sesamum indicum* L. under drought stress

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Received January 30, 2023

Seed priming is a pre-germination physiological treatment of seeds with natural or synthetic compounds which provides faster and synchronized germination. In the present research work, the seed priming effects of NaCl and KNO_3 in *Sesamum indicum* L.var. Thilothama under drought stress were studied. Sesame is a well known oil yielding plant and the seeds of these plants are used for oil extraction. Morphological, physiological and biochemical analysis were conducted in the seedlings raised from primed and non-primed seeds under field conditions. The results of analyses showed significant reduction in the growth of seedlings under drought stress conditions. But both the seed priming treatments resulted in an increase in the germination percentage, growth and yield. The germination percentage and growth attributes were found to be high in KNO_3 primed plants, but as far as the yield parameters were concerned, the fruit weight and seed weight were found to be maximum in NaCl primed plants. Thus from the present research work, it was concluded that both the seed priming methods improved the drought stress tolerance potential of sesame and among the two seed priming treatments, we recommend seed priming with 15 mM NaCl as the most effective method for enhancing yield under drought stress in sesame.

Key words: *Carbohydrate, chlorophyll, drought, proline, protein, seed priming*

Plant growth is frequently come across with different environmental stresses in their growth period. Among the growth stages, germination and early vegetative growth are more prone to these stresses. Abiotic and biotic stresses severely affect the crop's growth and development at every stage of growth cycle. Abiotic stresses adversely affect the growth and productivity and trigger a series of morphological, biochemical and molecular changes in plants (Wang *et al*., 2001). During the course of evolution, plants have developed precise mechanisms to sense the subtle changes of growth conditions, and trigger signal transduction cascades, which in turn activate stress responsive genes and ultimately direct to changes at the physiological and biochemical levels (Gao *et al*., 2007). Drought stress is one of the major abiotic stress for crop plants particularly in the tropics. According to Kim *et al.* (2012), drought is one of the major constraints for reduction of crop yield in the tropical world. Water availability is a major limiting factor for the germination and early establishment of arid and semi-arid plants (Zeng *et al.,* 2010). Plant response to water deficient are depend on the amount of water loss, the rate of water loss, the duration of drought stress, the plant variety/species under consideration, developmental stages of the plant and other environmental variables, such as temperature, relative humidity, etc. (Soren *et al*., 2010).

Several methods are regularly employing in the plant breeding research for imparting abiotic stress tolerance in different plants. Among these methods, seed priming is a promising one. It has proved to be an effective method to impart abiotic stress tolerance to plants. It is a pre-germination physiological treatment of seeds with natural or synthetic compounds which provides faster and synchronized germination. It is a controlled procedure followed by re-drying which will allow the seed to imbibe water, stimulate the internal biological process essential for germination but this process will not permit the seed to be truly germinates (Mustafa *et al.,* 2017*).* Seed priming is an efficient method for improving the seed germination and reducing the maturation time in any plants under field conditions. However, the effectiveness of any seed prming methods relay on different conditions like type of priming agent employed, priming duration, concentration of priming agent, temperature, etc. Thus, in the present research work, effects of two different chemical priming (KNO_3 priming and NaCl priming) on *Sesamum indicum* L. under drought stress in the field conditions were compared by analyzing the different growth attributes and stress indicators of seedlings and also the yield of plants raised from primed and non-primed seeds.

MATERIALS AND METHODS

Plant material

Sesame (*Sesamum indicum* L.) is a member of Pedaliaceae family and is an important oil crop. Seeds of sesame variety Thilothama were procured from Kerala Agricultural University, Thrissur, Kerala, India.

Seed priming techniques

Identical and healthy seeds of sesame were washed with 0.1% $HgCl_2$ and the process was followed by washing with detergent solution and distilled water respectively. The surface sterilized seeds were then kept in various concentrations of NaCl and KNO_3 solutions in glass beakers. The seed priming concentrations of NaCl and KNO_3 were identified based on standardization procedures. After seed priming treatments, the seeds were surface dried on absorbent paper and kept until original weight was re-gained.

Raising of seedlings under field conditions and application of drought stress in the field

The primed and non-primed seeds were sowed in grow bags separately. For each treatments, ten bags were used and one seed was sowed in each bag. All the polythene bags were filled uniformly with soil, cow dung, sand and bone meal in 1:1:1:1 ratio. The bags were watered twice a day regularly. After attaining a specific growth, ie two week old seedlings were collected from each bag and shoot length, root length, fresh weight and dry weight of seedlings were recorded. Estimation of photosynthetic pigments, Malondialdehyde (MDA), proline and total protein were done. The plants were then subjected to drought stress for two weeks. Drought was applied by withdrawing the water supply to plants.

The physiological and biochemical attributes of the plants under drought stress were also analyzed.

Growth parameters

The major growth parameters studied were shoot length, fresh weight, dry weight and moisture content. For shoot length measurements, graduated scale was used and expressed in centimeters. Fresh weight and dry weight measurements were carried out with the help of an electronic balance. For the measurements, the plants were brought from the field and the fresh weight of plants was measured immediately in the laboratory. For dry weight measurements, the plants were kept in hot air oven at 100˚C. After 48 hours the samples were allowed to cool and then weighed.

Physiological and biochemical studies

Quantification of photosynthetic pigments was carried out as per the method suggested by Arnon (1949). Proline content was analysed as per the method of Bates *et al* (1973) and the MDA content was estimated according to the method of Heath and Packer (1968). Protein content of plants was quantified using Folin – Ciocatteau reagent as per the method of Lowry *et al*. (1951).

Yield parameters

The fruits were collected from the mature plants and the fresh weight was determined using the electronic balance. After that the fruits were dried under shade. Then the seeds were collected from the fruits and the seed weight was determined with the help of electronic balance.

Statistical analysis

The results were analysed by using Microsoft excel. Standard deviation and standard error were determined for each category of data.

RESULTS

Determination of halopriming concentration of NaCl and duration of seed priming

Leaching of solutes from the seeds was observed when the seed priming duration prolonged beyond 12 hr. The priming duration which caused minimum leaching out of solutes from the seeds and which resulted in maximum enhancement of seedling growth attributes was fixed as the priming duration. From the results, the best priming duration was selected as 12 hr. For the determination of halopriming concentration, the seeds of *Sesamum indicum* were kept in various concentrations of NaCl solutions (0, 5, 10, 15, 20 and 25 mM) and were germinated as per the methods explained in materials and methods. The seedlings raised from primed seeds were examined and the seedlings which showed maximum shoot lengths when compared to control seedlings where noted and from the observations, the most effective halopriming concentration of NaCl solution was selected as 15 mM that showed highest shoot length (Fig.1A).

Selection of optimal concentration of KNO_3 for osmopriming

The seeds of *Sesamum indicum* were immersed in different concentrations of KNO_3 solutions (10, 20, 30, 40, 50 mM) and were further germinated as described in materials and methods. The shoot length, fresh weight and dry weight of seedlings raised from differently osmo-primed seeds were recorded. From the results, the most effective priming concentration of KNO_3 solution was selected as 30mM (Fig. 2A, B, C).

Germination under field conditions

The KNO_3 primed seeds germinated faster than the other two treatments. The KNO_3 primed seeds germinated in the polythene bags on the 12^{th} day after sowing which was slow when compared to the time taken in Petri plates. The NaCl primed seeds germinated much more slowly than KNO_3 primed seeds which showed synchronized germination. The germination of control seeds was very slow and many of the seeds failed to germinate in the field. From the results, it was clear that KNO_3 priming of seeds was successful for producing synchronized germination in sesame seeds when compared to other treatments. KNO_3 primed seeds showed 100% germination but the NaCl primed seeds showed only 50% germination and the un-treated control seeds showed only 25 % germination (Fig. 3A).

Seedling growth parameters- shoot length, root length, fresh weight and dry weight

When the shoot length, root length, fresh weight and dry weight were recorded, it was found that the shoot length (12.75cm), root length (4.3cm), fresh weight (0.781g) and dry weight (0.06g) were higher for the seedlings raised from KNO_3 primed seeds. The least value for each parameters were found in the seedlings raised from un-primed seeds. That is, shoot length (6.1cm), root length (3.2cm), fresh weight (0.466g) and dry weight (0.03g) respectively (Table 1).

After the application of drought stress, the fresh weight and dry weight of seedlings were recorded. The fresh weight was highest for the seedlings raised from unprimed seeds (37.8g) and the least fresh weight was recorded for the seedlings raised from unprimed stress applied seeds (0.74g). The dry weight was observed highest for the seedlings raised from unprimed seeds (15.31g) and the least dry weight was recorded for the seedlings raised from unprimed stress applied seeds (0.33g) (Fig. 3B).

Photosynthetic Pigment composition

The chlorophyll *a* and *b* content raised from KNO_3 primed seeds showed increase under stressed and unstressed conditions when compared to the seedlings from non-primed seeds. The highest total chlorophyll content was recorded in the primed seedlings under stressed conditions (738.96 µg/g fw). The lowest total chlorophyll content was found in the seedlings primed with NaCl under unstressed conditions (236.04 µg/g fw). The carotenoid content of seedlings were highest in the seedlings raised from primed seeds under stressed condition (304.35 µg/g fw) and the minimum carotenoid content was observed in seedlings raised from NaCl primed seeds under unstressed conditions (122.40 µg/g fw) (Fig. 4).

MDA Content

MDA content of the seedlings were found highest in the non-primed seeds under stressed conditions (10.13 µg/g fw). On seed priming, the MDA content was found decrease in the seedlings under both stressed and unstressed conditions. The maximum reduction in MDA content was found in seedlings raised from KNO_3 primed seeds under unstressed conditions (2.06 µg/g fw) (Fig. 5A).

Proline Content

Maximum accumulation of proline was recorded in the seedlings raised from NaCl primed seeds under stressed condition (274.63 µg/g fw). It is followed by the seedlings raised from un-primed seeds under stressed conditions (178.38 µg/g fw). The least proline content was recorded from the seedlings raised from KNO_3 primed seeds under stressed conditions (5.46 µg/g fw) and the proline content was very low in the seedlings raised from NaCl primed seeds under un-stressed condition(6.83 µg/g fw) (Fig. 5B).

Total protein content

The total protein content was found to be highest in the seedlings raised from un-primed seeds under drought stress (769.93 µg/g fw). Then it was found higher in the seedlings raised from KNO_3 primed seeds under un-stressed conditions (500 µg/g fw). The lowest protein content was found in the seedlings raised from un-stressed seeds under unstressed conditions (86.96 µg/g fw) (Fig. 5C).

Yield parameters

The fruit weight was higher in the NaCl primed plants (88.15 g/plant). It was followed by fruits of un-primed plants (77.98 g/plant) and in the KNO_3 primed plants, the fruit weight was found to be 65.74 g/plant. When the seed weight was recorded, it was found that the maximum seed weight was for NaCl primed plants (15.82g/plant) and it was followed by control (11.18g/plant) and KNO_3 primed (8.85g/plant) (Table 2).

DISCUSSION

Efficient seed germination is important for agriculture. Successful establishment of early seedling indeed requires a rapid and uniform emergence and root growth. Seed priming is such a practice in agriculture. The time interval of seed priming is an important aspect of seed priming. Previous reports emphasis the importance of several factors including priming duration (Parera and Cantliffe, 1994). In the present research work, as per the results, 12h was taken as the halo priming duration which caused maximum enhancement of seedling growth attributes.

The most appropriate concentration of NaCl which caused the priming effects in the seedlings was identified and was found to be 15mM NaCl. Concentration of NaCl higher than 15 mM was failed to induce any increase in the growth characters of seedlings. This indicates that a specific concentration of NaCl itself is capable of inducing saturating priming effects in the growth of seedlings. According to Yunpeng *et al.* (2014), in chemical priming, high concentration of beta amino butyric acid (BABA) above 50 mM caused growth inhibitory effects like shoot length, fresh weight, dry weight and root vitality in soyabean seedlings.

From the results obtained from the field experiment we observed that in specific concentration of KNO_3 (30 mM) the sesame seeds showed synchronized germination and better germination percentage which was absent in unprimed seeds. It may be due to the imbibitions and increased metabolite mobilization during the priming treatments. Basra *et al*. (2005) reported that seed priming treatment can lead to better germination and establishment in many crops such as maize, wheat, rice and canola. Razaji *et al*. (2012) also found that seed priming of safflower increased the germination percentage and seedling dry weight under drought stress conditions.

Seed priming with KNO_3 caused enhancement of seedling growth attributes when compared to seedlings raised from non-primed seeds. The increase in growth parameters of the seedlings raised from primed seeds is may be because of the enhanced cell elongation or cell division rate because of the high water intake during the priming treatments. The enhanced seedling growth attributes like shoot length, fresh weight and dry weight of seedlings raised from primed seeds could be the result of fast germination procedures that occurred as a part of priming treatments in the seeds which reduces the lag time between imbibition and radical emergence during seed germination (Bradford *et al*., 1990). In sesame, priming treatments significantly enhanced the seedling emergence as well as seedling growth parameters (Root length, shoot length, seedling length, fresh weight, dry weight and vigour index) (Shabbir *et al*., 2014).

In the present research work, it was found that the highest total chlorophyll content was recorded in the primed seedlings under stressed conditions. The increased chlorophyll content of seedlings raised from primed seeds may be due to the increased production of photosynthetic pigments or may be due to the increased growth attributes of seedlings achieved through primng treatments. The enhanced leaf chlorophyll content as a result of priming treatments was already reported in maize (Tabrizi *et al.* 2011) and moringa (Nouman *et al*. 2012). From the present study, it was found that seed priming caused a reduction of the MDA content in the seedlings under stressed and unstressed conditions. The highest reduction in MDA content was observed in the seedlings raised from KNO_3 primed seeds under unstressed conditions. The lipid peroxidation in biological membranes is the most obvious symptoms of oxidative stress in plants. Malondialdehyde (MDA) is one of the final products of oxidative modification of lipids. When the ROS (Reactive Oxygen Species) levels inside the plants increase, lipid peroxidation occurs and as result many physiological processes of the cell will be altered. Seed priming treatments may repair the damaged membranes and thereby guarantee the constant operation of the physiologic process in the seedlings raised from primed seeds. According to some authors, the functional mechanism of seed priming is to initiate the repairing system for damaged membranes and also to help the metabolic preparation for germination by controlling the imbibition rate of seeds (Mittal and Dubey, 1995). The reduction in MDA content in seedlings raised from primed seeds was already reported in maize by Randhir and Shetty (2005).

Proline is an important organic osmolyte which accumulates in the cells of plants that are exposed to different types of stresses. In the present research work proline accumulation was fund to be high n the seedlings raised from NaCl primed seeds under stressed conditions. Thus form the observation it was clear that the seed priming treatments interfere with the stress mitigation pathway by accelerating the production of proline which is considered as an important metabolite in stress tolerance. According to Kavi Kishore *et al*. (2005), the proline accumulation in plants under abiotic stress conditions depends on the species and

also on the extent of stress experienced by the plants.

In the present research work, enhancement of total protein content was observed in the seedlings raised from non-primed seeds under drought stress. The increase in the total protein content of seedlings under stressed conditions may be due to the enhanced synthesis of some specific proteins that are concerned with the stress mitigating mechanisms. According to Wang and co-workers (2003; 2004), under osmotic stresses, different proteins like heat-shock proteins (HSPs), late embryogenesis abundant (LEA) proteins and molecular chaperons which are found to accumulate in plants thereby providing abiotic stress tolerance in plants.

As far as the yield parameters were concerned, it was observed that the maximum yield was obtained for the plants which were raised from NaCl primed seeds and minimum yield was obtained for plants raised from KNO_3 primed seeds. This result implies that even though the KNO_3 priming caused early and synchronized germination in sesame, it failed to continue the priming effects till the yield when compared to NaCl priming.

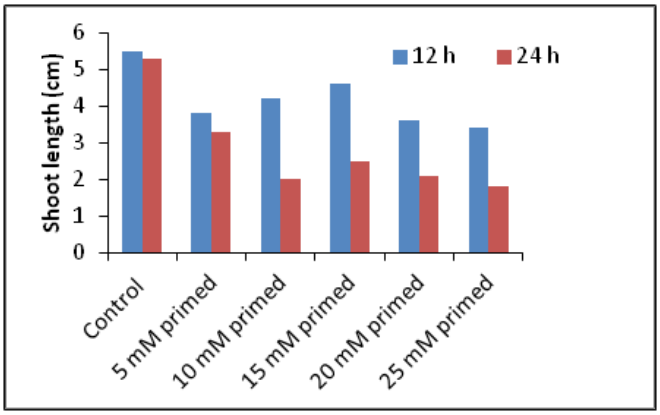

Figure 1. *D*etermination of priming concentration and priming duration of *Sesamum indicum* L. var Thilothama.

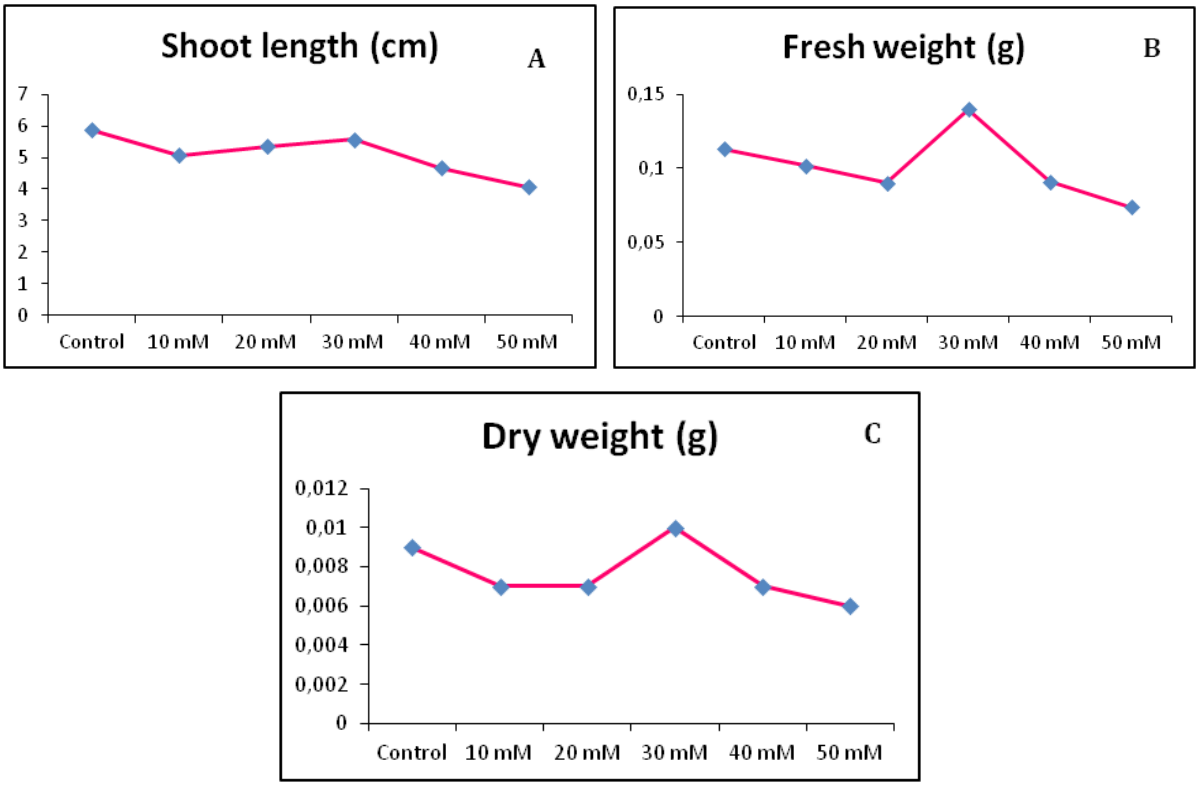

Figure 2. Growth parameters (A-shoot length, B-fresh weight and C-dry weight) of sesame seedlings raised from different concentrations of KNO3 primed seeds

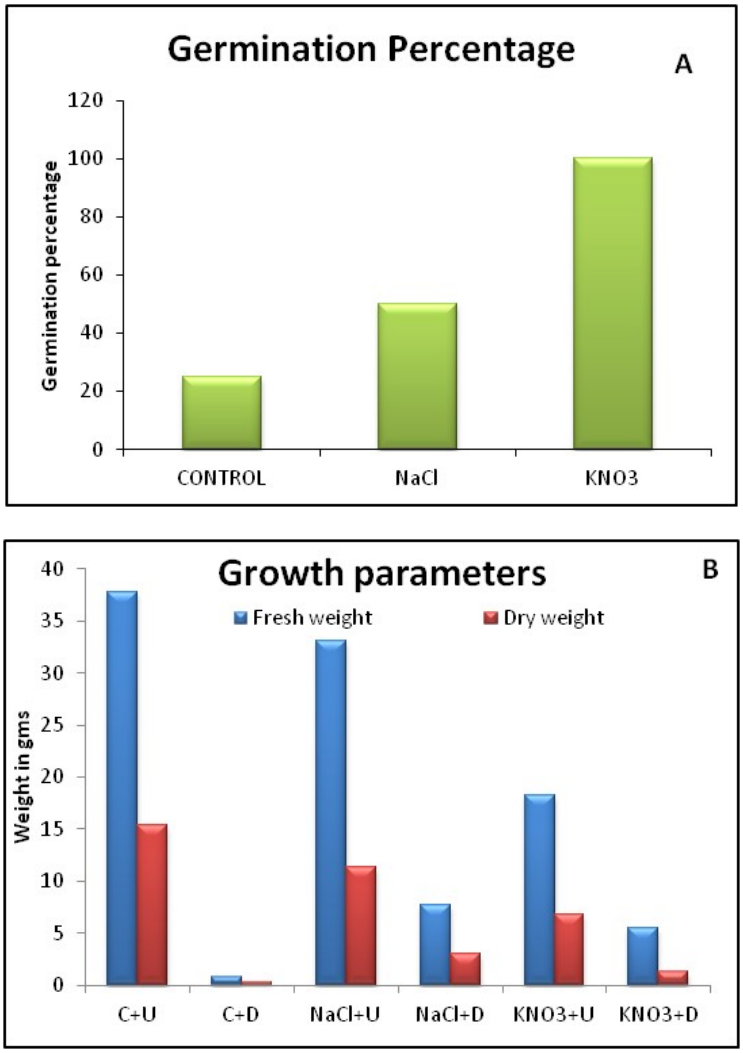

Figure 3. Germination percentage (A) and growth parameters (B) of primed (NaCl and KNO3) and non-primed sesame seeds under field conditions

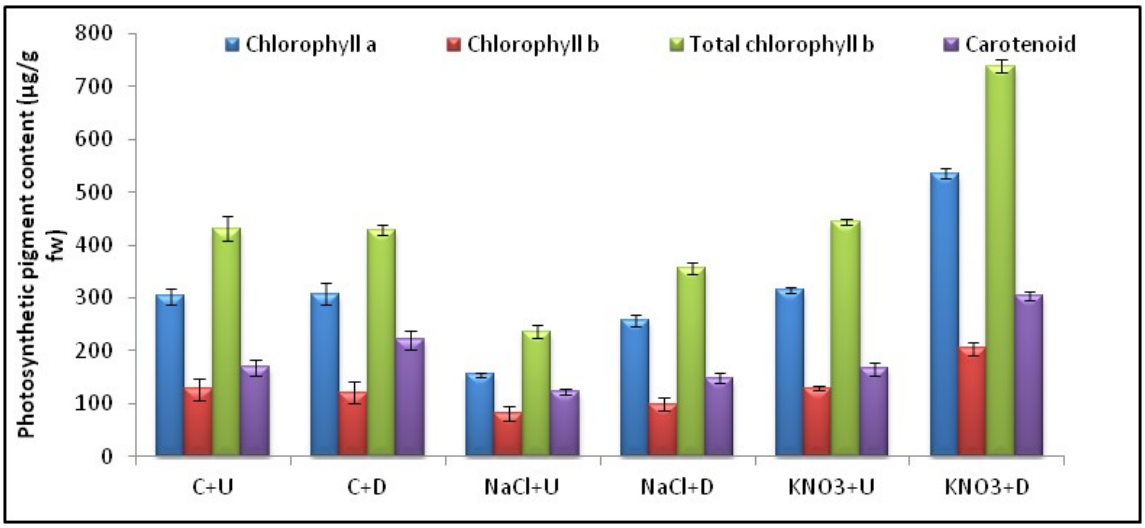

Figure 4. Photosynthetic pigment composition of primed (NaCl and KNO_3) and non-primed sesame seeds under stress and unstressed conditions. The vertical bars represent SE of the mean value of recordings.

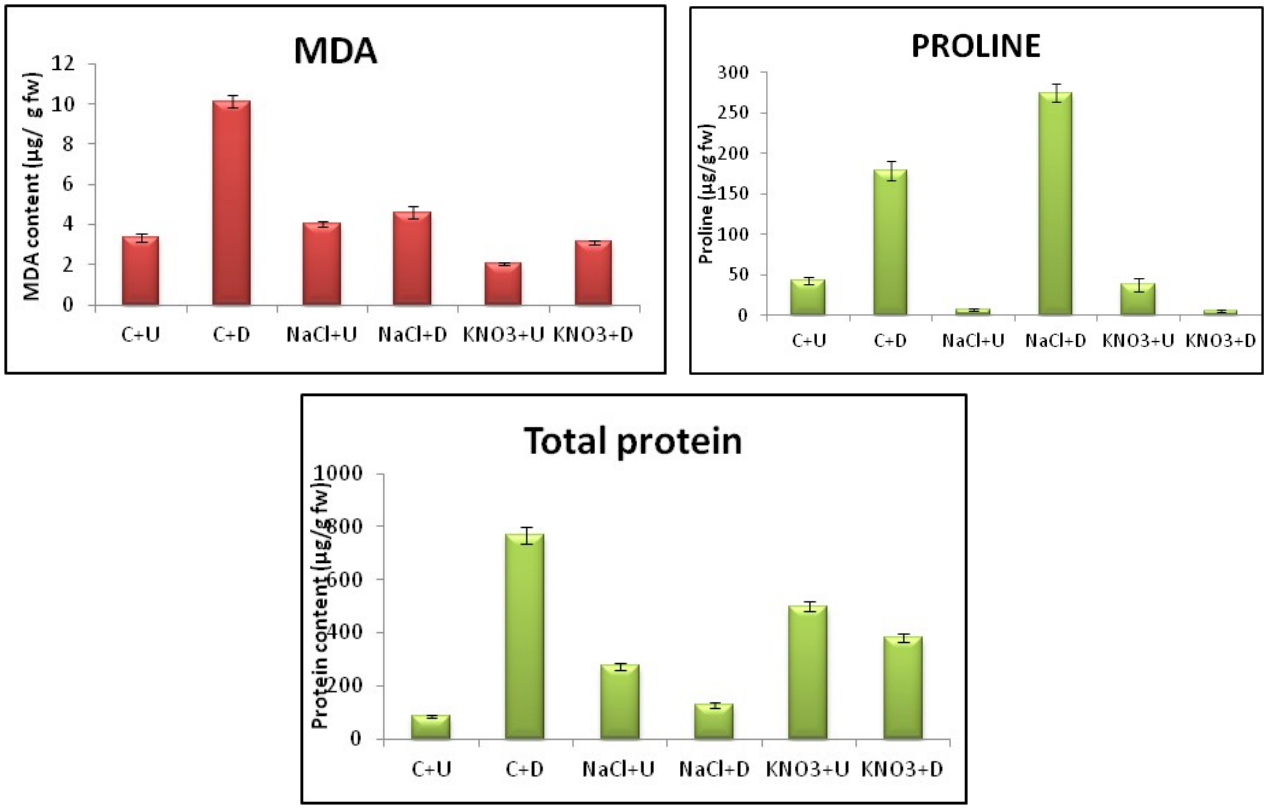

Figure 5. MDA (A), proline (B) and total protein (C) content of primed (NaCl and KNO3) and non-primed sesame seeds under stress and unstressed conditions. The vertical bars represent SE of the mean value of recordings.

Table 1: Growth parameters of sesame seedlings raised from primed (NaCl and KNO3) and non-primed seeds under field conditions.

	SHOOT LENGTH(cm)	ROOT LENGTH(cm)	FRESH WEIGHT(g)	DRY WEIGHT (g)
KNO_3 PRIMING	12.75	4.3	0.781	0.03
NaCl PRIMING	6.5	3.3	0.762	0.05
CONTROL	6.1	3.2	0.466	0.06

Table 2: Yield parameters of sesame plants raised from primed (NaCl and KNO3) and non-primed seeds.

	Fruit weight(g)/plant	Seed weight(g)/plant
Un-primed	77.88	11.18
NaCl primed	88.15	15.82
KNO_3 primed	65.74	8.85

CONCLUSION

Abiotic and biotic stresses severely affect the crop's growth and development at every stage of growth cycle. Drought stress is one of the major abiotic stress for crop plants particularly in the tropics. Seed priming is an important method for imparting stress tolerance in various crop plants. In the present study, the halo and osmopriming effects on *Sesamum indicum* L. var. Thilothama under drought stress were analyzed. The results obtained from the present study suggest that seed priming with NaCl sigmificantly increased the yield parameters while seed priming with KNO_3 increased the germination percentage along with synchronised germination. From the research work, the most

appropriate concentration of KNO_3 for priming was found to be 30 mM. The seedlings raised from primed seeds imparted good resistance against drought stress also. Among the two seed priming methods, KNO_3 priming imparted good seed germination and early seedling growth but the yield was found to be higher in NaCl priming. Thus we suggest both these seed priming methods for increasing drought tolerance in sesame under field conditions. Moreover, in terms of cost effectiveness, we recommend NaCl priming for imparting drought stress tolerance in sesame rather than KNO_3 because NaCl is cheaper than KNO_3 and is easily available too.

CONFLICTS OF INTEREST

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

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