Halopriming: An effective approach to enhance the NaCl tolerance potential of *Sesamum indicum* L.

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Abstract

Various strategies are routinely employed in plant breeding programmes to overcome the deleterious effects of these stresses. Seed priming is such a simple and cost effective strategy which improves the seed germination percentage and seedling vigour under stressed conditions. In the present project work, the seed priming effects of NaCl on the NaCl stress tolerance potential of *Sesamum indicum* L. were studied. Morphological, physiological and biochemical analysis were conducted in the seedlings raised from NaCl primed and non-primed seeds. The study showed significant reduction in the growth of seedlings under NaCl stress conditions. But the seed priming treatments resulted in the amelioration of the NaCl stress impacts in the seedlings. Thus from the studies it was concluded that 12h seed priming with 15 mM NaCl was the best seed priming method for sesame under NaCl stress.

Keywords: Seed priming, halopriming, stress tolerance, germination, metabolites

Introduction

Seed germination is a vital process in the life cycle of a plant. Seed germination and early seedling growth are invariably associated with many environmental factors. Among these environmental factors, many of them become stressful to plants at certain stages of their life period. Salt stress is one of the major environmental constraints for crop plants particularly in arid and semi arid regions (Kaymakanova and Stoeva, 2008; Turan *et al*., 2009).

Various technologies have been adapted from time to time to improve the stress tolerance performance of plants. Many research studies have shown that seed priming is an efficient method for increasing plant growth and yield in saline conditions. Seed priming is a control procedure where seeds are soaked in solutions of different concentrations to enhance seed germination and growth in stress environment. Various studies confirmed that seed priming has several advantages including early emergence, higher water use efficiency, deeper root germination in broad range of temperature and resistance against disease and environmental stresses (Mustafa *et al*., 2017).There are different types of seed priming treatments in practice like hydropriming, halopriming, priming with chemicals, etc.

Sesame (*Sesamum indicum* L.) is an important oil crop of semi arid regions, belongs to the family Pedaliaceae. A major constraint to seed germination and seedling establishment of sesame is soil salinity. Halopriming of sesame seeds with NaCl could be used as an adaptation method to improve salt tolerance of seeds. It could be an effective technique for enhancing seed germination and shortening maturation time in the field condition, but the efficiency of priming treatments to the seeds would be strongly dependent on individual or integrated conditions of priming agent, concentration, temperature, duration etc.

In the present research work, the effects of NaCl seed priming in *Sesamum indicum* L. were analyzed by studying the various morphological, physiological and biochemical changes in the seedlings raised from primed seeds in comparison with seedlings raised from non-primed seeds.

Materials and Methods

Plant material

Sesame (*Sesamum indicum* L. Var. Thilothama) belongs to the family Pedaliaceae. The seeds of sesame were collected from Kerala Agricultural University, Mannuthy, Thrissur.

Method

Determination of stress imparting concentration of NaCl

The concentration of stress imparting solution of NaCl was determined through a series of standardisation procedures. Healthy and uniform sesamum seeds were washed with 0.1% $HgCl_2$ followed by detergent solution. Then the seeds were thoroughly washed with distilled water. Sterilized seeds were germinated in Petri plates containing absorbant cotton soaked with different concentrations of NaCl (5, 10, 15, 20 and 25 mM) along with control (absorbant cotton soaked with distilled water). The Petri plates were kept under alternating light and darkness. The shoot length and fresh weight of seedlings were recorded on the 7th, 8th, 9th and 10th day of germination. From the results, the concentration of NaCl which caused 50% growth retardation in terms of shoot length was selected as the stress imparting concentration of NaCl. Moreover, the day on which maximum seedling shoot length recorded was selected as the analysis day for further physiological and biochemical analysis.

Seed priming techniques

Plump, healthy and uniform seeds of sesame were washed with 0.1% $HgCl_2$. Then the seeds were washed with detergent solution and wash thoroughly with distilled water. The seeds were then immersed in different concentrations of NaCl solution. During seed priming treatment in an open beaker, the seeds along with the solution were swirled regularly to ensure proper aeration. The seed priming period and concentration of priming solution were determined through a series of standardisation process. After priming treatment, the seeds were washed with distilled water and surface dried on absorbent paper.

Determination of seed priming concentration of NaCl and duration of priming treatments

The seeds were primed in various concentrations of NaCl (5mM, 10mM, 15mM, 20mM and 25 mM) in two open beakers. One beaker is kept for 12 hours and other for 24 hours. The seeds were washed and surface dried on an absorbent paper after the priming treatments. The non-primed seeds were also washed with $HgCl_2$, detergent solution and distilled water respectively and surface dried. Both the primed (12h and 24h) along with non-primed seeds were allowed to germinate in Petri plates containing absorbant cotton soaked with distilled water (control) and 20 mM NaCl (stress imparting concentration of NaCl) solution. After germination, the shoot length and fresh weight of seedlings were recorded on the fixed day using a graduated scale and determined the priming concentration of NaCl which caused maximum increase in seedling shoot length when compared to seedlings raised from non-primed seeds. Similarly, the seed priming duration was also selected by comparing the shoot length of seedlings raised from 12h and 24h primed seeds with the seedlings raised from non-primed seeds and the priming duration which caused maximum enhancement in seedling shoot length and fresh weight was selected as the seed priming duration for further studies.

In order to study the effect of NaCl priming on sesame seeds, primed as well as non-primed seeds were germinated in Petri plates containing absorbent cotton soaked with distilled water (control) and NaCl solution. The Petri plates were kept under alternating light and darkness. The growth and biochemical attributes of primed, non-primed as well as stressed and control seedlings were recorded on 8th day of germination.

Growth parameters

The shoot length of seedlings raised from both primed and non-primed seeds were measured using a graduated scale and was expressed in centimetres. Samples (seedlings) were weighed using electronic balance. For fresh weight and dry weight measurements, the seedlings were blotted and wrapped separately in paper boats. Fresh weight of the samples was determined by weighing them immediately after wrapping. For dry weight measurements, the samples were kept in hot air oven at 100˚C . After 48 hours the samples were allowed to cooled and then weighed. Then dry weight percentage was calculated by using the following formula:

$$Dry\ weight\ prcentage = \frac{Dry\ weight}{Fresh\ weight} * 100$$

Moisture content percentage was calculated by using the following formula

$$\text{Moisture content percentage} = \frac{\text{Fresh weight - Dry weight}}{\text{Fresh weight}} \text{ x } 100$$

Physiological studies

Various physiological parameters were measured by using standard protocols. Estimation of chlorophyll and carotenoids were done according to the method of Arnon (1949). The proline content was estimated according to the method of Bates *et al* (1973) and MDA content estimation was done according to Health and Packer (1968). Total protein content of the plant material was estimated using Folin – Ciocatteau reagent according to the method of Lowry *et al*. (1951).

Results

Determination of stress imparting concentration of NaCl and duration of seed priming

The concentration of NaCl which imparted 50% retardation in the seedling shoot length was selected as stress imparting concentrations of NaCl. Sesame seeds showed 50% growth reduction at 20mM NaCl (Fig. 1A). 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 (Fig. 1B).

Selection of optimal concentration of NaCl for halopriming

The seeds of *Sesamum indicum* L. were immersed in different concentrations of NaCl solutions (0, 5, 10, 15, 20 and 25 mM) and were further germinated as described in materials and methods. The shoot lengths of seedlings raised from differentially haloprimed seeds were recorded. From the results, the most effective halopriming concentration of NaCl solution was selected as 20Mm (Fig.1B).

Seedling growth parameters

From the results, it was clear that halopriming significantly decreased the extent of NaCl stress induced reduction in shoot length, fresh weight and dry weight of the seedlings as compared to the seedlings raised from non-primed seeds.

The seedlings grown under NaCl stress showed reduction in shoot length of seedlings when compared seedlings raised under control conditions. NaCl priming was found to be effective in bringing about prominent increase in shoot length of seedlings under unstressed (4 cm) and NaCl stressed (2.2cm) when compared to seedlings raised from non-primed seeds (Fig. 2A). As far as the fresh weight of seedlings was concerned, it was found that fresh weight of seedlings raised from primed seeds in unstressed conditions is higher when compared to the fresh weight of seedlings raised from primed seeds in stressed conditions. There was no major difference in fresh weight of seedlings raised from primed and non-primed seeds (Fig. 2B). Under stressed conditions, the dry weight of seedlings raised from both primed and non-primed seeds were higher when compared to seedlings raised in unstressed conditions (Fig. 2C).

Halopriming in unstressed condition showed lesser dry weight percentage while in stressed conditions the dry weight percentage was high. In the case of moisture content percentage, seedlings raised under unstressed conditions showed high value when compared to that of stressed conditions (Fig. 3A & 3B).

Photosynthetic pigment composition

The chlorophyll *a* & *b* content of seedlings raised from NaCl primed seeds showed a prominent increase under stressed and un-stressed conditions when compared to seedlings raised from non-primed seeds. The highest total chlorophyll content (chlorophyll *a+b*) was recorded in the primed seedlings under unstressed conditions (7.5mg/g dw) followed by primed seedlings under NaCl stressed conditions (7.3mg/g dw). The lowest total chlorophyll content was found in seedlings raised from non-primed seeds under unstressed conditions (5.5mg/g dw) (Table 1).

The carotenoid content of seedlings raised from non-primed seeds was more in both unstressed and stressed conditions. The seedlings raised from non-primed seeds under stressed conditions showed maximum carotenoid content (1.7mg/g dw) and the minimum carotenoid content was observed in seedlings raised from primed seeds under stressed conditions (1.5mg/g dw) (Table 1).

Proline and MDA content

Under stressed conditions, maximum accumulation of proline was recorded in the seedlings raised from primed seeds. The proline content of seedlings raised from primed seeds was higher especially under stressed conditions (614µg/g dw). The primed seedlings under unstressed condition showed least proline content (59µg/g dw). There was a prominent difference in the proline content of seedlings grown under stressed and unstressed conditions on halopriming (Fig. 4A).

From the results, it was clear that MDA content of the seedlings increased under stressed conditions when compared to the seedlings raised under unstressed condition. But on seed priming, the MDA content was found decrease in the seedlings under both stressed as well as unstressed conditions. The maximum reduction in MDA content was found in the seedlings raised from primed seeds under unstressed conditions (27µg/g dw) (Fig. 4B).

Total Protein Content

Total protein content was found to be higher in the seedlings raised under NaCl stress conditions. The NaCl seed priming further enhanced the amount of total protein in the seedlings growing under both stressed and unstressed conditions. The highest total protein content was recorded in the seedlings raised from NaCl primed seeds under NaCl stressed conditions (3.6mg/g dw). Under unstressed conditions, the protein content was comparatively lower than that in NaCl stressed conditions in both primed and non-primed seedlings. The lowest protein content was found in the seedlings raised from non primed seedlings under unstressed conditions (1.4mg/g dw) (Fig. 4C).

Discussion

Seed priming is a popular practice in agriculture. It has been proved to be a cost-effective, easy and a safe technique for the enhancement of seed germination and also for increasing the capacity of seedlings for tolerating stress along with enhanced growth and productivity.

The NaCl stress tolerance potential of any plants could be studied by determining the stress imparting concentration of NaCl. In the present study, it was found that 20mM NaCl caused 50% growth reduction in the shoot length of sesame seedlings when compared to control seedlings. The further priming analysis were carried out by using this concentration of NaCl as the stress imparting concentration for sesame seeds. Seedling growth difference under stressed conditions depends on the tolerance nature of varieties. In *Medicago truncatula*, among the two different genotypes studied, the NaCl tolerant one showed more seedling growth under NaCl stress when compared to that of the sensitive genotype subjected to NaCl stress (De Lorezo *et al*., 2007).

It was also essential to check the optimal concentration of NaCl for bringing about effective seed priming effects in sesame seedlings. It was found that sesame seeds were more effectively haloprimed (in terms of better shoot length) at 15mM NaCl. From the results, it was also clear that priming of seeds with a concentration higher than 15 mM of NaCl could not bring about any enhancement in the growth of sesame. This implies that a particular concentration of NaCl might bring about some saturating effects in the growth of seedlings. Recently it was reported that treatment with high concentration ($\geq$50 mM) of BABA in soyabean plants showed growth inhibitory effects, resulting in significant reduction of shoot length, fresh and dry weight and root vitality in the treated plants (Zhong *et al*., 2014).

The duration of priming is a key component of any seed priming technique for any crop. Earlier works showed that the success of seed priming is influenced by a combination of factors, among which the duration of seed priming is an important one (Parera and Cantliffe, 1994). In the present study, 12h was selected as the halo priming duration, because above this period of priming, leaching out of metabolites from the seeds was observed.

Growth retardation occurred in the sesame seedlings which were subjected to NaCl stress but interestingly, due to the influence of seed priming treatments, this reduction in growth attributes of seedlings were significantly reduced. Seed priming with NaCl 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 due to the increased cell division or cell elongation due to increased water uptake of seeds during the seed priming treatments, which finally resulted in an increase of the seedling vigour. The increased shoot and root length in plants raised from primed seeds can also be due to change in germination events such as reducing the lag time between imbibition and radical emergence (Bradford *et al.*, 1990).

In the present study, NaCl stress did not caused reduction in the chlorophyll content of seedlings, but caused slight increase in the amount of chlorophyll content of the seedlings. The priming treatments further enhanced the amount of chlorophyll pigments where as the carotenoid content reduced on priming when compared to seedlings raised from non-primed seeds. More than a direct role for seed priming in influencing the synthesis of chlorophylls and carotenoids, the enhanced seedling fitness would have facilitated the increased levels of pigment content in the leaves of seedlings. According to Tabrizi *et al.* (2011) and Nouman *et al*. (2012), seed priming caused an increase in leaf chlorophyll content of maize and moringa seedlings, respectively.

Proline is a major organic osmolyte that accumulates in a variety of plant species in response to environmental stresses such as drought, salinity, extreme temperatures, UV radiation and heavy metals. In the present study significant accumulation of proline was noted in the seedlings which were raised under NaCl stress. This accumulation of proline is may be the stress tolerance behaviour of the seedlings under NaCl stress. Seed priming with NaCl caused accumulation proline in the sesame seedlings even in the absence of a stress. The accumulation of proline in the seedlings raised from primed seeds was maximum under NaCl stress. Thus from the study, t was clear that the seed priming treatments significantly enhanced the NaCl stress tolerance potential of sesame seedlings. Proline accumulation in the *O. sativa* seedlings raised from hydroprimed seeds was already reported by Yuan-Yuan *et al.* (2010) and Mondal *et al.* (2011).

Lipid peroxidation of biomembranes is a common outcome of abiotic stresses and it impairs various metabolic functions by changing the physicochemical properties of cell membranes through disruption of lipid bilayers which further promote leakage of solutes, leading to cell death (Jisha and Puthur, 2014). In the present study, sesame seedlings on exposure to NaCl stress showed increased MDA content, which implied that these stresses caused lipid peroxidation of biomembranes in the seedlings. But the NaCl seed priming method decreased the MDA content in the sesame seedlings and thus implying that the stress to which the seedlings were exposed is reduced to some extent through seed priming treatment of the seeds. The results are in accordance with the findings of Amooaghaie (2011), wherein the MDA content reduced under hydropriming of alfalafa seeds.

In the present study, enhancement of total protein content was observed in the seedlings which were subjected to NaCl stress. The increase in the total protein content of seedlings under stressed conditions may be due to the over production of certain proteins involved in the stress combating mechanisms. Wang and co-workers (2004) have reported that under osmotic stresses, heat-shock proteins (HSPs), molecular chaperones and LEA protein families are known to get over expressed, which are involved in conferring abiotic stress tolerances in plants. Sesame seedlings raised from NaCl primed seeds showed even more accumulation of total protein content than the seedlings raised from non-primed seeds. The enhanced primary metabolism and the resulting increase of primary metabolites will certainly aid in increasing the seedling vigour of plants.

Summary and Conclusion

Abiotic stresses are the major limitations of crop establishment and development in the world. Among these stresses, salinity plays an important role that causes major crop lose. Seed priming is an important method for imparting stress tolerance in various crop plants. In the present study, the seed priming effects of NaCl on the NaCl stress tolerance potential of *Sesamum indicum* L. were analyzed. Sesame is an important oil crop plant which belongs to the family Pedaliaceae. Morphological, physiological and biochemical analysis were conducted in the seedlings raised from NaCl primed and non-primed seeds. In general, the application of NaCl stress resulted in inhibitory effects on growth, and also resulted in the enhanced accumulation of proline and MDA content, in sesame seedlings. The results obtained from the present study suggest that seed priming with NaCl significantly ameliorated the adverse effects of NaCl stress by altering the various metabolic pathways. In the present project work, the most appropriate concentration of NaCl (15 mM) and the most suitable priming interval for NaCl seed priming (12h) for bringing out the priming effects in sesame were determined. Thus from the studies it was concluded that 12h seed priming with 15 mM NaCl was the best seed priming method for sesame under NaCl stress.

Thus in the current scenario of increasing salt stress, our findings have great importance as the seed priming with NaCl increases the seedling vigour of sesame. Moreover the seed priming treatments imparts osmotic stress tolerance to sesame seedlings. Hence the seed priming technique, which is a very simple and cheap form of treatment, can be very well recommended to the farmers, so they can get better synchrony of seedling emergence and better crop stand in sesame.

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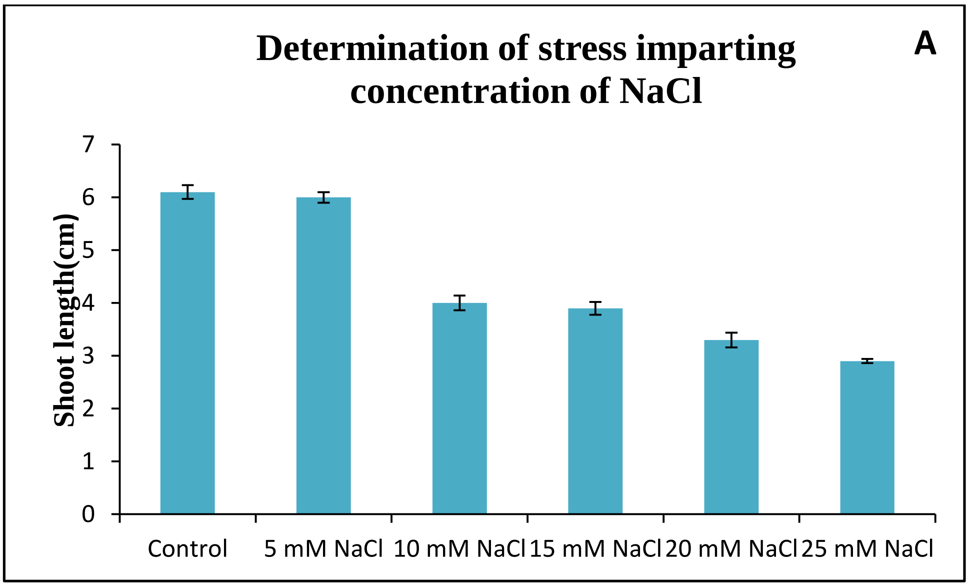


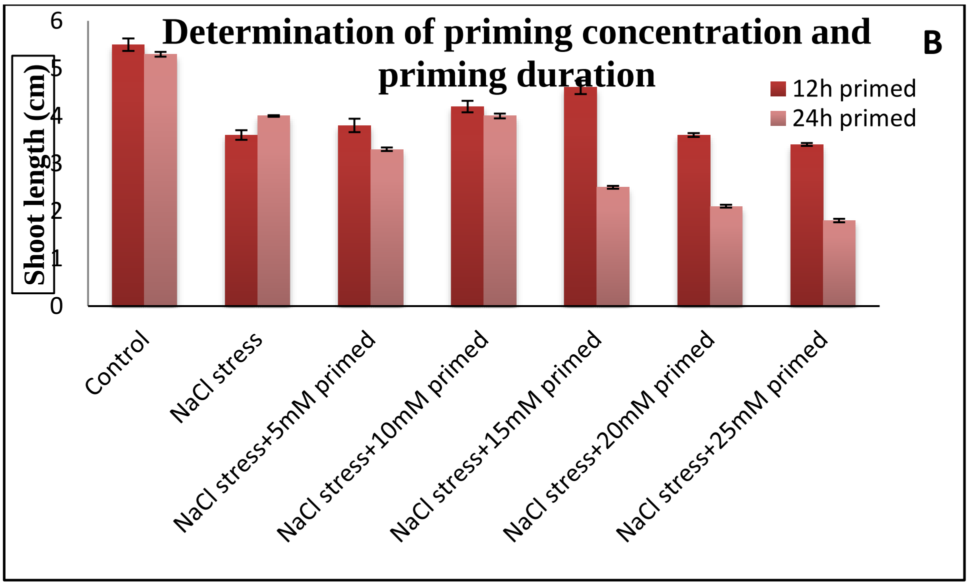
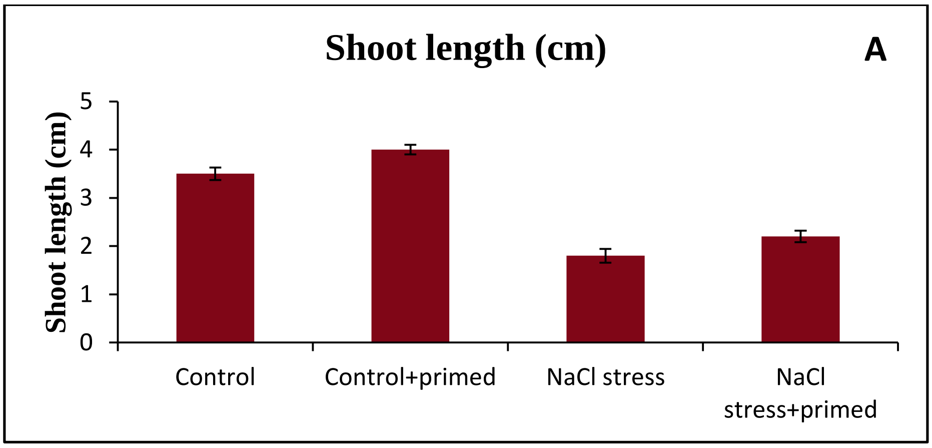
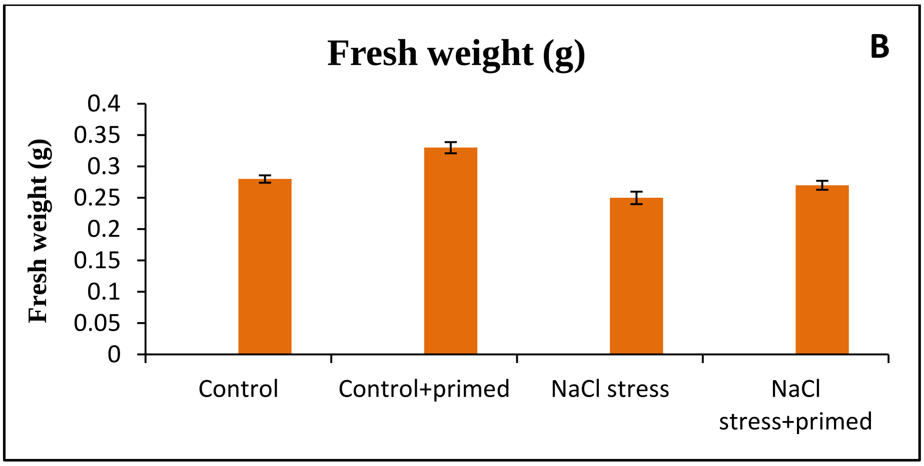


Figure 1: (**A**) Determination of stress imparting concentration of NaCl and (**B**) determination of priming concentration and priming duration of *Sesamum indicum* L. var.Thilothama grown under unstressed and stressed conditions. The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates.





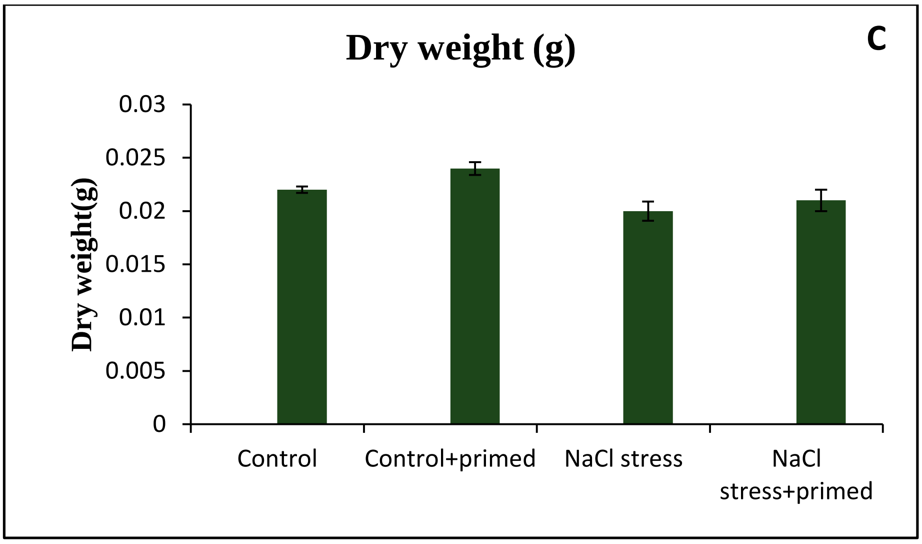
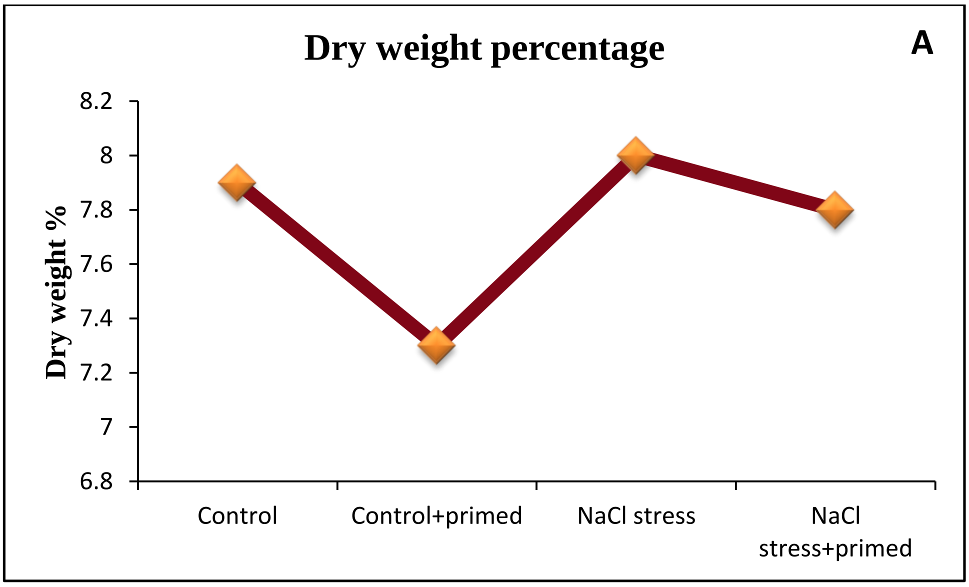


Figure 2: Shoot length (**A**) fresh weight (**B**) and dry weight (**C**) of *Sesamum indicum* L. var.Thilothama seedlings raised from primed and non-primed seeds and grown under unstressed and stressed conditions. The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates.



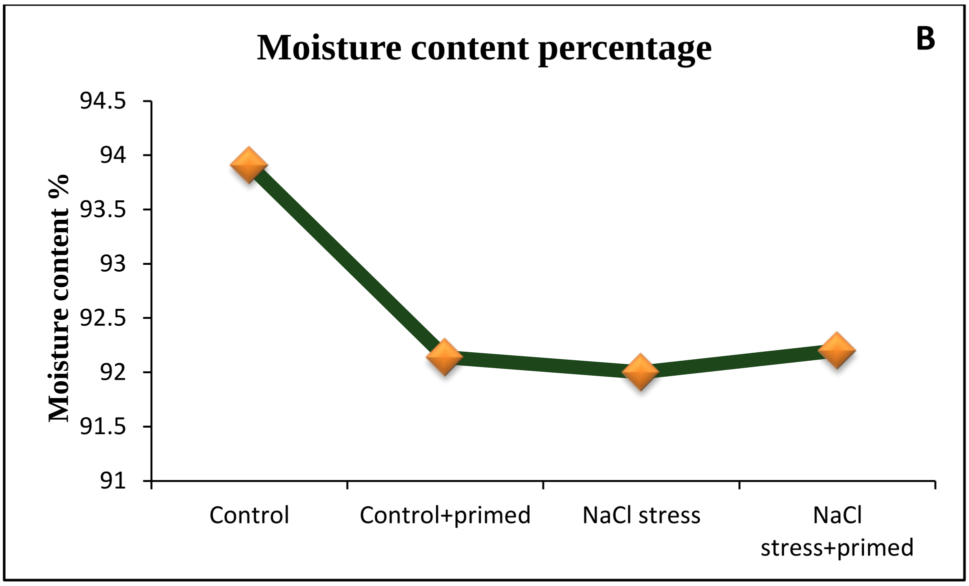
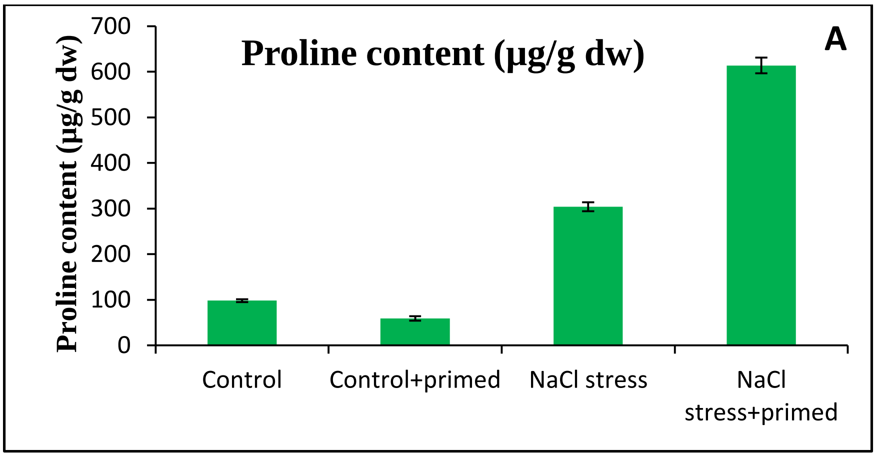


Figure 3: Dry weight percentage (**A**) and moisture content percentage (**B**) of *Sesamum indicum* L. var.Thilothama seedlings raised from primed and non-primed seeds and grown under unstressed and stressed conditions. The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates.



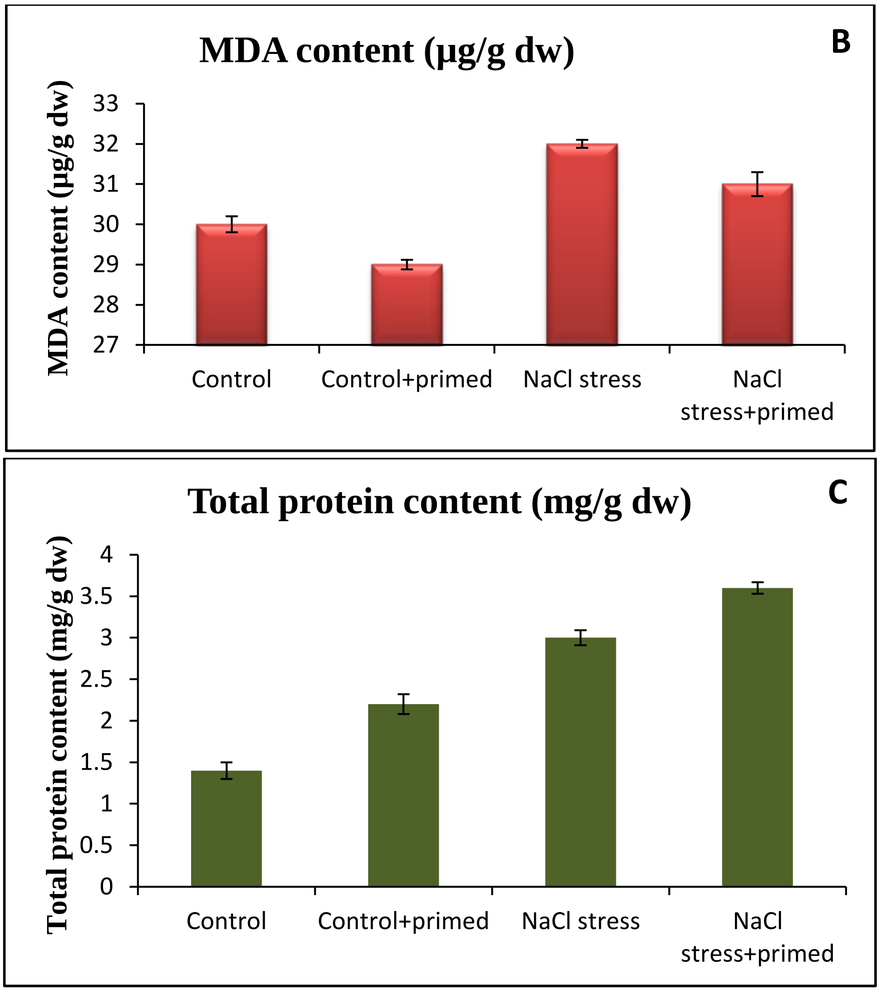


Figure 4: Proline content (**A**), MDA content (**B**) and total protein content (**C**) of *Sesamum indicum* L. var.Thilothama seedlings raised from primed and non-primed seeds and grown under unstressed and stressed conditions. The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates.

Table 1: Photosynthetic pigment content of sesame seedling leaves under unstressed and NaCl stressed conditions raised from primed as well as non-primed seeds. The data is an average of recordings from three independent experiments each with three replicates (i.e. n=9). The data represent mean±standard error.

Chlorophyll a (mg/g dw)				*Chlorophyll b (mg/g dw)*			
Control	**Control+primed**	**NaCl stress**	**NaCl stress+primed**	**Control**	**Control+primed**	**NaCl stress**	**NaCl stress+primed**
3.21±0.16	4.14±0.11	3.71±0.06	4.02±0.17	2.27±0.12	3.36±0.12	2.74±0.08	3.28±0.05
Total chlorophyll (mg/g dw)				***Carotenoids (mg/g dw)***			
Control	**Control+primed**	**NaCl stress**	**NaCl stress+primed**	**Control**	**Control+primed**	**NaCl stress**	**NaCl stress+primed**
5.47±0.11	7.48±0.06	6.43±0.03	7.28±0.09	1.63±0.11	1.56±0.14	1.70±0.07	1.51±0.06