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Osmo-priming with a Novel Actives Carrabiitol® Alleviates Abiotic Stresses in Sorghum and Fenugreek: Effect on Seed Germination and Seedling Growth

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ABSTRACT

Background: Abiotic stress is a serious threat to crops as it inhibits the seed germination and seedling growth. Seed treatment with osmolytes and plant growth regulators found to be effective in mitigating the abiotic stress. In this regard, the study was conducted to evaluate the osmo-priming effect of Carrabiitol®, a novel patented (IN) commercial product, on seed germination and seedling growth of sorghum and fenugreek under induced stresses.

Methods: The concentrations of Carrabiitol® tested for seed priming were 1000, 2000 and 3000 mg/L. The abiotic stresses *viz*. drought, salinity and excess water stresses, were induced using PEG 6000, sodium chloride and water, respectively. Experiments were arranged in a randomized complete block design with three replications.

Result: The germination percentage, root length and shoot length were reduced in non-primed stressed sorghum and fenugreek seeds (T2) as compared to unstressed seeds. Osmo-priming with 3000 mg/L of Carrabiitol® (T5) was found to be most significant in improving the seed germination and seedling growth of sorghum and fenugreek under the induced stresses ($p \leq 0.05$). Overall, the results suggested that application of Carrabiitol® was effective in improving the seed germination and seedling growth of sorghum and fenugreek by alleviating the applied abiotic stresses.

Key words: Abiotic stress, Carrabiitol®, Germination, Osmo-priming, Seedling growth.

INTRODUCTION

Plants are frequently subjected to a variety of abiotic stresses in the natural field environment, which have detrimental effects on agricultural plant performance. Among these, drought, salinity and water cause a variety of physiological, biochemical and metabolic changes in most of the plants. Therefore, plants may experience oxidative stress which may affect plant metabolism, performance and finally the overall yield (Jisha *et al*., 2013). Different strategies are currently being used to ameliorate the negative consequences of these stresses. Recently, seed priming has become a successful and useful method for increasing plant tolerance to various stresses (Hussain *et al*., 2022a).

Seed priming is a process in which seeds are imbibed in various solutions that may contain natural or synthetic substance as priming agents (Anjos Neto *et al*., 2020). This method promotes pre-germination metabolic activities, improve seed performance, speeds up membrane repair under various abiotic stress condition and offers synchronized and faster germination (Masondo *et al*., 2018). Seeds are generally very sensitive and vulnerable to extreme environmental conditions (Arief *et al*., 2021). Thus, it appears that studying plant reactions to induced abiotic stresses during the germination stage and seedling growth is required for the development of abiotic stress tolerant plants and the production of appropriate crop yields under stress conditions.

Currently, sea weed extract (SWE) is widely accepted as an organic bio-stimulant due to its beneficial effects under abiotic stress in agriculture and horticulture (Hernández-Herrera *et al*., 2019). The formulations derived from SWE typically contain substantial amounts of organic and inorganic osmolytes, which may be partially absorbed by the plant and/or cause moderate and transient osmotic stress when delivered *via* irrigation. The use of SWE as seed priming agent may be an effective method for alleviating the abiotic stress experienced by plants during seed germination and seedling growth (El-Boukhari *et al*., 2020). Although the aforementioned studies are laudable, the varied components of SWE make it difficult to understand the critical effects associated with various genotypes and bio-stimulants. MM *et al.* (2016) reported that the higher concentration of SWE negatively affects the seed germination percentage and protein content due to higher minerals content and other growth regulators.

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Therefore, there is a need to develop a formulation which can trigger specific pathway for specific abiotic stress tolerance.

Considering the above facts, the present study evaluated the effects of an organic osmolyte, Carrabiitol®, an oligosaccharide polyol composition (Shah and Patel, 2020), on seed germination and seedling growth of sorghum and fenugreek under various abiotic stresses.

MATERIALS AND METHODS

The seeds of sorghum (Variety: KAVERI COLONEL 6363) and fenugreek (Variety: MAHER-1) were obtained from the local market of Ankleshwar, Gujarat, India. The experiment was carried out at Agri Biochem Research Lab, Panoli, Gujarat, India during October 2020. Seeds were surface sterilized with 10% hypochlorite solution for 10 min followed by washing with sterile distilled water for 10 min. Experiments were arranged in a randomized complete block design with three replications. Abiotic stress treatments included: 1) Drought stress was induced using 25% PEG 6000; 2) Salinity stress was induced by two levels of salinity, *i.e.* 100 and 200 mM NaCl and 3) Excess water stress was induced by soaking the seeds under water for 12 and 24 h. The tested Carrabiitol® doses were 1000, 2000 and 3000 mg/L. The formulation at the required concentration was sterilized at 121°C for 15 min before application. For seed priming, the surface sterilized seeds were soaked in a required concentration of Carrabiitol® solution for 16 h. The unstressed seeds without priming were considered as absolute control, whereas stressed seeds without priming were considered as control. The details of each treatment along with treatment code have been shown in Table 1. After priming, the seeds were shed dried for 4 h and then transferred to sterile petri plates containing wet germination papers under aseptic condition. The petri plates were incubated at 25°C for 15 days by maintaining light (16 h)/ dark (8 h) photoperiods with light intensity of 400 µ moles/ms. The stress conditions were maintained by injecting 5 ml of the respective stress solutions into the seeds at intervals of three days. The parameters like germination percentage, root length and shoot length were evaluated after the completion of experiment. The vigor index was calculated using the formula proposed by Abdul Baki and Anderson (1973). Data were analyzed by one-way analysis of variance (ANOVA) and significant effects were evaluated at $p \leq 0.05$. The difference between treatment means were compared by Fisher's Post hoc LSD (Least significant difference) test.

RESULTS AND DISCUSSION

Effect of priming under drought stress

PEG 6000 was found to mimic drought stress and result in lowering the water potential of plants due to osmotic stress (Muscolo *et al*., 2014). The germination was reduced to 38% and 25% in sorghum and fenugreek seeds, respectively, under induced drought stress (T2) as compared to unstressed seeds (T1) (Fig 1a and 2a). The seed priming with 3000 mg/L (T5) of Carrabiitol® showed highest seed germination under induced drought stress. The germination efficiencies of Carrabiitol® primed seeds were enhanced by 60% and 33% for sorghum and fenugreek seeds, respectively, as compared to non-primed seeds (T2) under induced drought stress. The results of seed priming with Carrabiitol® solution at a concentration of 3000 mg/L (T5) were equally comparable to those of absolute control seeds (T1) in both plants. The highest seed germination efficiencies of Carrabiitol® primed seeds under drought stress may be due to the increasing uptake of water and activation of the osmo-protectant releasing pathway in response to maintaining lower osmotic potential. The increased germination percentage can be ascribed to the faster rate of cell division of osmo-primed seeds and the completion of pre-germinative metabolic activities and repair processes (Ghiyasi *et al*., 2008).

The root lengths were reduced to 92% and 73% for sorghum and fenugreek, respectively, under induced drought stress (T2) as compared to unstressed seedlings (T1) (Fig 1b and 2b). The reduction of root length under drought stress could be due to the reduction in the absorption of water and the delayed translocation of carbohydrates (Seleiman *et al*., 2021). The seedling raised after priming with 3000 mg/L of Carrabiitol® showed highest root length under induced drought stress. The comparative study of the data indicated that the seedling under induced drought-stress (T2) had significantly lower root length as compared to the seedlings raised from the Carrabiitol® treated seeds ($p<0.05$). The root lengths for T5 treatments were 29% and 27% lower in sorghum and fenugreek seedlings, respectively, as compared to unstressed non-primed seedlings (T1). The improved root lengths of seedlings raised from Carrabiitol® primed seeds may be due to the enhanced hydraulic conductivity following cell growth (Hattori *et al*., 2005).

Table 1: Details of different treatments.

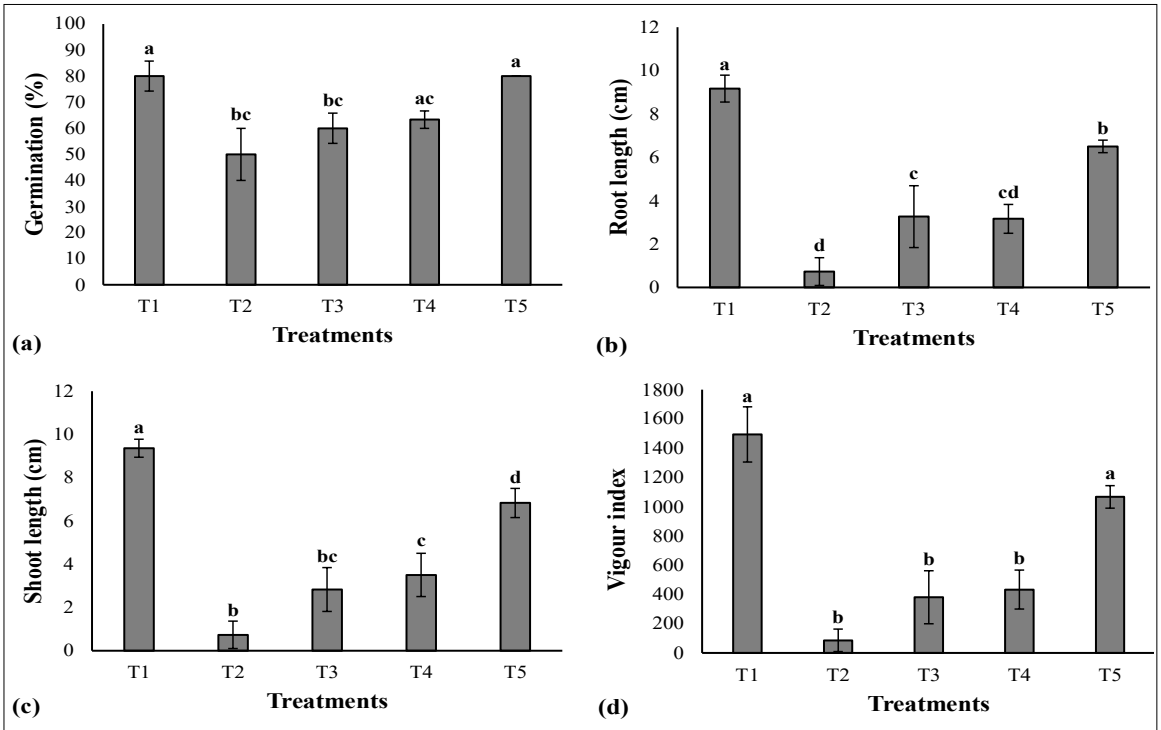
Treatment code	Details of treatment
T1	Absolute control, unstressed seeds without seed priming
T2	Control, seed treatment with either (a) 25% PEG for drought stress, (b)100 mM/ 200 mM NaCl for salinity stress or (c) 24 h/12 h water soaking for excess water stress
T3	Seed priming with Carrabiitol® at 1000 mg L^{-1} under respective stress
T4	Seed priming with Carrabiitol® at 2000 mg L^{-1} under respective stress
T5	Seed priming with Carrabiitol® at 3000 mg L^{-1} under respective stress

The shoot lengths were reduced to 93% and 53% for sorghum and fenugreek, respectively, under induced drought stress (T2) as compared to unstressed seedlings (T1) (Fig 1c and 2c). The reduction of shoot length under drought stressed condition may be due to the inhibition of processes like cell division, enlargement and differentiation associated with water deficit. The seedling raised after the priming with 3000 mg/L of Carrabiitol® showed highest shoot length under induced drought stress. The comparative study of the data indicated that the seedling under induced drought-stress (T2) had significantly lower shoot length as compared to the seedlings raised from the Carrabiitol® treated seeds (p<0.05). The shoot lengths for T5 treatments were 28% and 44% lower in sorghum and fenugreek seedlings, respectively, as compared to unstressed non-primed seedlings (T1). The improved shoot lengths of seedlings raised from Carrabiitol® primed seeds may be due to the increased metabolic activity and mobilization of reserves to the embryo axis, which promote primary shoot emission (Taiz and Zeiger, 2002).

The vigor index assesses the ability of seeds to produce normal seedlings under less than ideal or stress conditions, in addition to determining the percentage of viable seeds. It is an important parameter that needs to be evaluated for gaining insights into the performance of seeds under field conditions (Finch-Savage and Bassel, 2016). The vigor index was found to be maximum for T1 treatment in both sorghum and fenugreek (Fig 1d and 2d). The vigor index was 12.4-fold and 2.7-fold higher for Carrabiitol® primed seeds (T5) of sorghum and fenugreek, respectively, as compared to non-primed stressed seeds (T2).

Effect of priming under salinity stress

The cellular concentrations of Na^+ and Cl^- increase due to the higher concentration of NaCl. This causes osmotic stress and leads to toxic effects on plants. At lower concentrations (5-10 mM), NaCl was reported to positively influence the plant growth and can be used as a priming agent (Hongqiao *et al*., 2021). Therefore, the present study used higher concentrations of NaCl (*i.e*., 100 and 200 mM) to induce salinity stress. The reduction in germination was lesser at 100 mM salinity level as compared to 200 mM salinity level in T2 treatment subjected to sorghum and fenugreek seeds (Fig 3a and 4a). The germination was reduced to 28% and 7% in sorghum and fenugreek seeds, respectively, under salinity stress induced by 200 mM NaCl as compared to unstressed seeds (T1). This reduction could be attributed to the lower osmotic potential of the external medium, which reduces water absorption, metabolic disturbance, Na^+ toxicity to enzymatic activities, as well as hormonal imbalance (Chen *et al*., 2021). In case of the sorghum seed priming, all the tested concentrations of Carrabiitol® showed equally comparable and significant results on seed germination at 100 mM salinity level. On the other hand, the

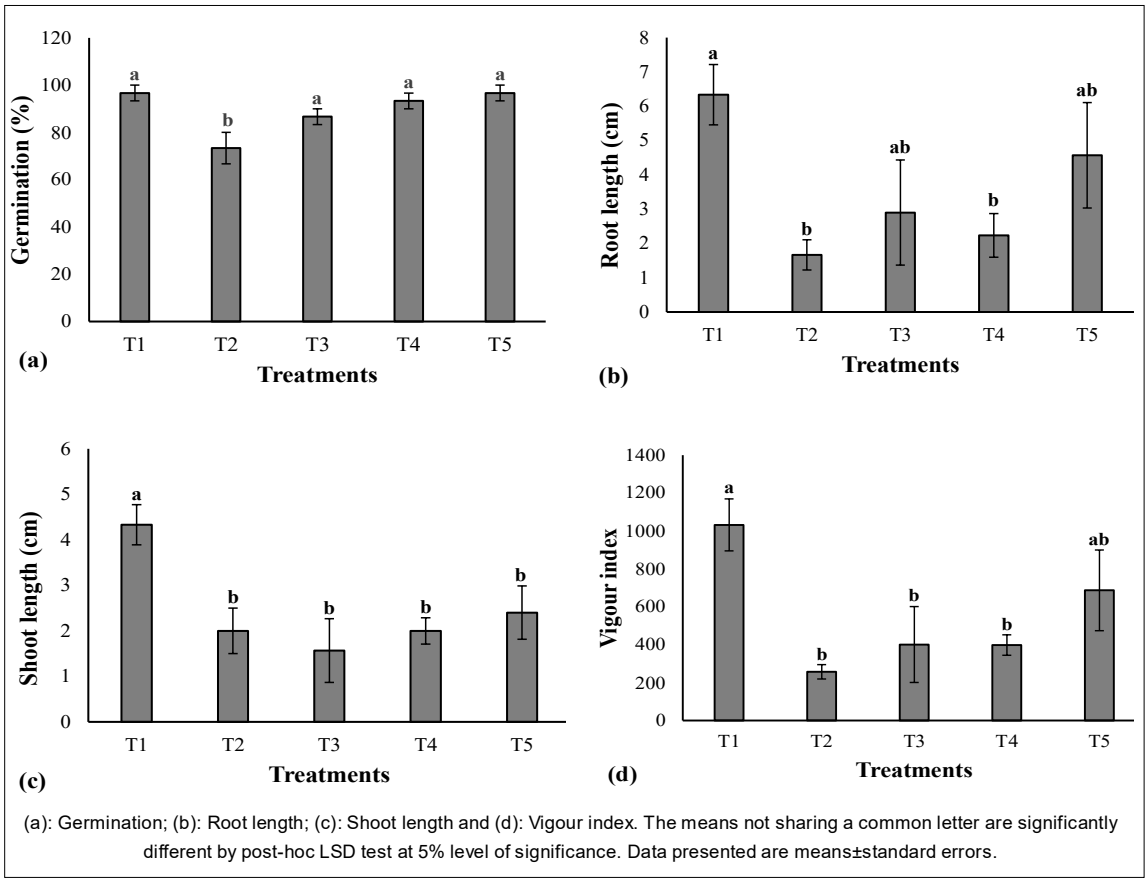


(a): Germination; (b): Root length; (c): Shoot length and (d): Vigour index. The means not sharing a common letter are significantly different by post-hoc LSD test at 5% level of significance. Data presented are means±standard errors.

Fig 1: Seed priming effect of Carrabiitol® on sorghum plant under drought stress.

increasing salinity level affected the germination efficiency at each tested concentration of Carrabiitol®. However, the germination efficiencies of T4 and T5 treatments were found to be equally comparable with absolute control (T1) and significantly higher than control (T2) at 200 mM salinity level. In case of the fenugreek seed priming, all the tested concentrations of Carrabiitol® showed equally comparable and significant results on seed germination. The germination efficiencies of T3 and T4 treatments were significantly higher as compared to absolute control (T1) and control (T2) treatments under salinity stress induced at 100 mM concentration (p<0.05). The germination efficiencies of T3 and T4 treatments were comparable with absolute control (T1) and control (T2) treatments under salinity stress induced at 200 mM concentration. The seeds primed with 3000 mg/L of Carrabiitol® (T5) showed statistically significant results on seed germination than absolute control (T1) and control (T2) treatments at both salinity levels (p<0.05). The significant seed germination efficiencies of primed seeds were due to the maintenance of osmotic balance under high salt concentrations by Carrabiitol®. Osmotic adjustments, ROS scavenging and subcellular structures stabilization are instance mechanisms by which Carrabiitol® may be involved in salt stress mitigation (Hossain *et al*., 2014). The findings clearly indicated that the sorghum and fenugreek seeds primed with a higher concentration of Carrabiitol® helped to alleviate the induced higher level of salinity stress and improved the germination efficiency.

The root lengths were reduced up to 65% and 53% for sorghum and fenugreek, respectively, under induced 200 mM salinity stress (T2) as compared to unstressed seedlings (T1) (Fig 3b and 4b). This reduction might be due to the toxic influence of NaCl and enhanced seed respiration, which diminishes the reserve substances accessible for seedling growth (Chen *et al*., 2021). The root lengths of primed sorghum seeds were longer as compared to non-primed seeds under salinity stress induced at 100 mM concentration and shorter as compared to unstressed absolute control treatment (T1). The root lengths of primed sorghum seeds were significantly higher as compared to non-primed seeds under salinity stress induced at 200 mM concentration (p<0.05) and comparable to T1 treatment. The seeds of fenugreek were able to withstand against the 100 mM salinity level and thus showed significant effects on root lengths of the seedlings raised after the priming with Carrabiitol® as compared to T1 treatment. Although the fenugreek root



(a): Germination; (b): Root length; (c): Shoot length and (d): Vigour index. The means not sharing a common letter are significantly different by post-hoc LSD test at 5% level of significance. Data presented are means±standard errors.

Fig 2: Seed priming effect of Carrabiitol® on fenugreek plant under drought stress.

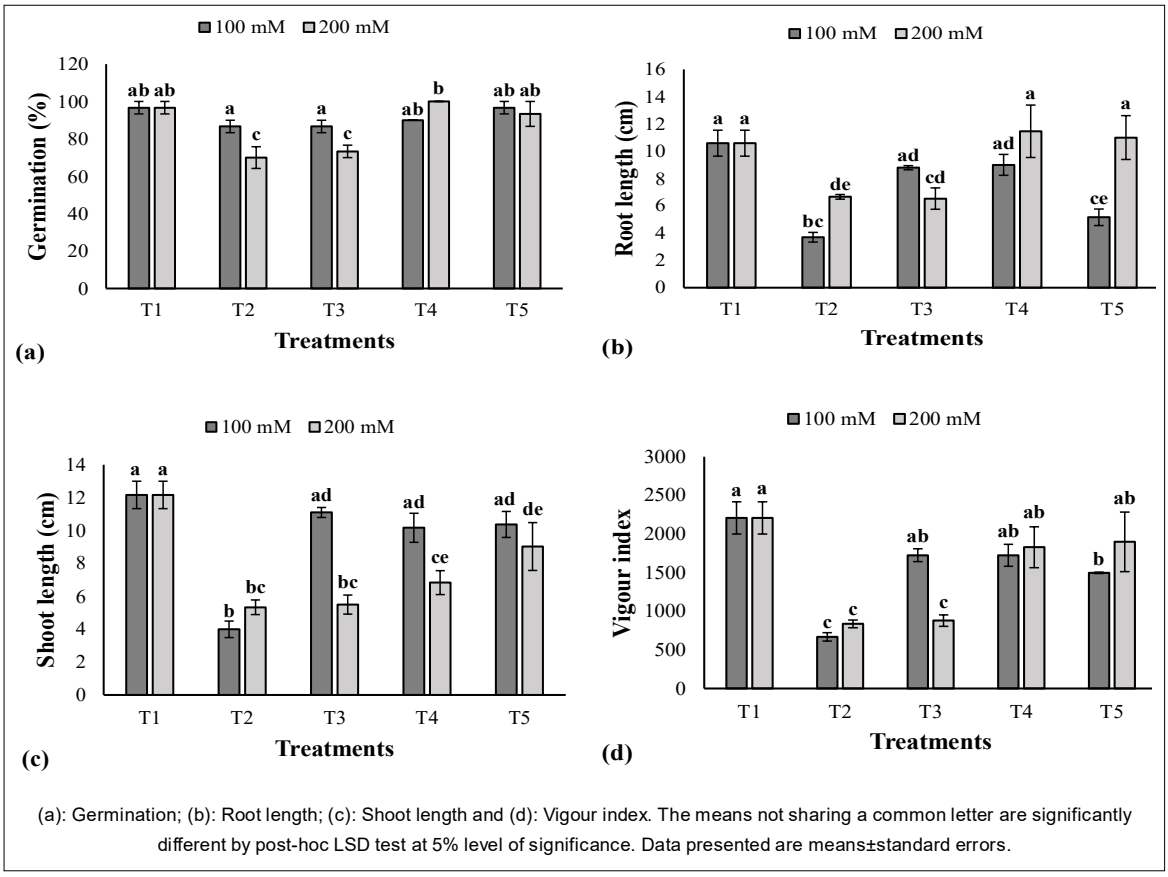
lengths of Carrabiitol® treated seeds were shorter as compared to T1 treatment under 200 mM salinity, it showed 61% better results as compared to T2 treatment. The flexibility of the embryo cell wall may explain the reason behind the longer roots of Carrabiitol® primed seeds as compared to non-primed seeds. Pre-treatment of seeds with priming solutions hydrates proteins and membranes and initiates a number of metabolic processes. This could be attributed to a balanced Na^+/K^+ ratio, which is crucial for maintaining turgor pressure, adjusting cell osmoregulation and absorbing water and vital minerals (Abraha and Yohannes, 2013).

The shoot lengths were reduced up to 67% and 33% for sorghum and fenugreek, respectively, under 100 mM salinity stress (T2) as compared to unstressed seedlings (T1) (Fig 3c and 4c). The decreases in shoot growth under salinity stress could be the result of both particular ionic and osmotic impacts on seedlings (Abraha and Yohannes, 2013). The shoot lengths were 2.8-fold and 1.7-fold higher in Carrabiitol® primed sorghum seeds (T5) as compared to non-primed seeds (T2) under 100 mM and 200 mM salinity, respectively. The growth patterns of shoots in fenugreek were found to be similar to those observed in root growth. The seeds of fenugreek were able to withstand against the 100 mM salinity level and thus showed 52% and 27% increasing results as compared to T2 and T1 treatments, respectively. Although the fenugreek shoot lengths of Carrabiitol® treated seeds were shorter as compared to T1 treatment under 200 mM salinity, they showed 48% better results as compared to T2 treatments. The increased root and shoot lengths of seedlings raised from primed seeds under salinity stress may be due to the involvement of Carrabiitol® in cell elongation or cell division and meristematic growth (Khan and Qaiser, 2006).

The vigor index was found to be higher in absolute control (T1) sorghum. It was found to be 2.2-fold higher in Carrabiitol® primed sorghum seeds (T5) as compared to non-primed sorghum seeds (T2) at both salinity levels (Fig 3d). The results of the vigor index were significantly higher in Carrabiitol® primed fenugreek seeds as compared to T1 and T2 at 100 mM salinity ($p<0.05$). The vigor index was found to be 1.8-fold higher in Carrabiitol® primed fenugreek seeds (T5) as compared to non-primed seeds (T2) at both salinity levels (Fig 4d).

Effect of priming under excess water stress

Water is generally used to transport the metals from the root to the upper plant parts. However, the excess water limits the growth of the plant through anaerobic respiration and causes a lower energy supply for the normal plant metabolism (Pan *et al*., 2021). Therefore, the present study

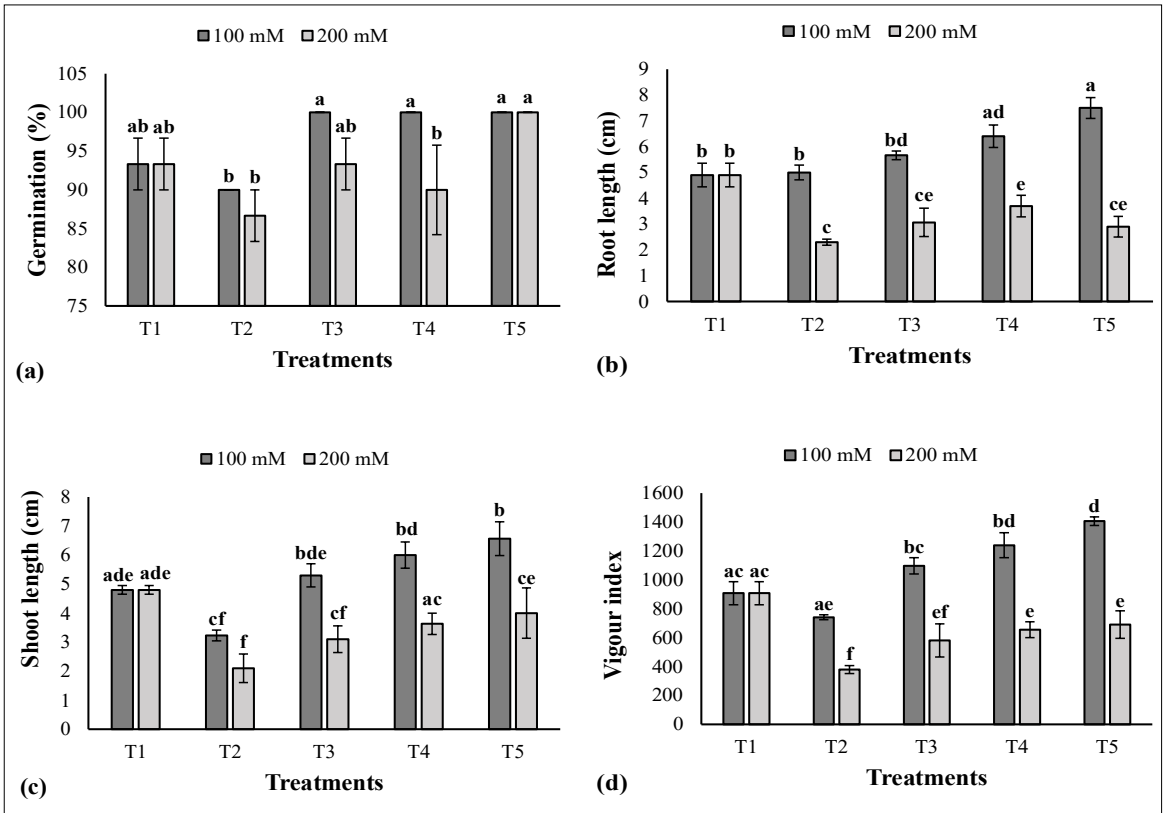


(a): Germination; (b): Root length; (c): Shoot length and (d): Vigour index. The means not sharing a common letter are significantly different by post-hoc LSD test at 5% level of significance. Data presented are means±standard errors.

Fig 3: Seed priming effect of Carrabiitol® on sorghum plant under salinity stress.

also evaluated the effect of Carrabiitol® priming under excess water stress by soaking the seeds at two different time periods *i.e.*, 12 h and 24 h. In contrast to drought and salinity stress, the responses of the tested concentrations of Carrabiitol® were different under excess water stress condition. The germination was reduced by 10-28% under excess water stress. The germination reduction of sorghum under 24 h of excess water stress was higher as compared to 12 h of excess water stress in non-primed seeds (T2). The comparative study of all treatments clearly indicated that the seed germination of primed seeds was higher than or comparable with absolute control (T1) and control (T2) treatments in both sorghum and fenugreek. The seed priming with 1000 mg/L of Carrabiitol® showed the highest seed germination under induced excess water stress at 12 h soaking time. The germination of Carrabiitol® primed sorghum seeds (T3) was increased by 39% and 56% as compared to absolute control (T1) and control (T2) treatment, respectively, under 12 h of excess water stress (Fig 5a). In case of fenugreek seeds, the germination of T3 treatment was increased by 16% as compared to control (T2) treatment under 12 h of excess water stress, while the germination was equally comparable with the absolute control (T1) under same condition (Fig 6a). The significant seed germination efficiencies of Carrabiitol® primed seeds may be due to the increased alpha amylase activity, which provides energy for seed germination under stress condition (Mondal *et al*., 2020). The findings clearly indicated that the sorghum and fenugreek seeds primed with 1000 mg/L concentration of Carrabiitol® were sufficient to alleviate the induced excess water stress, particularly for seed germination.

The root lengths were significantly reduced under excess water stress as compared to absolute control (T1). The reductions in root lengths were found to be 87% and 61% for sorghum seeds under 24 and 12 h of excess water stress, respectively (Fig 5b), while these reductions were found to be 64% in fenugreek seeds (Fig 6b**)**. Among all the tested concentrations of Carrabiitol®, 1000 mg/L and 3000 mg/L were effective in enhancing the root length in sorghum and fenugreek, respectively. The root lengths of primed sorghum seeds (T3) were increased by 6-fold and 2.8-fold under 24 h and 12 h of excess water stress, respectively. The root lengths of primed fenugreek seeds (T5) were enhanced by 5.7-fold and 3-fold under 24 h and 12 h of excess water stress, respectively. The results of root lengths under similar conditions (T3 for sorghum and T5 for fenugreek) were comparable or quite higher as compared to absolute control (T1). The improved root lengths of



(a): Germination; (b): Root length; (c): Shoot length and (d): Vigour index. The means not sharing a common letter are significantly different by post-hoc LSD test at 5% level of significance. Data presented are means±standard errors.

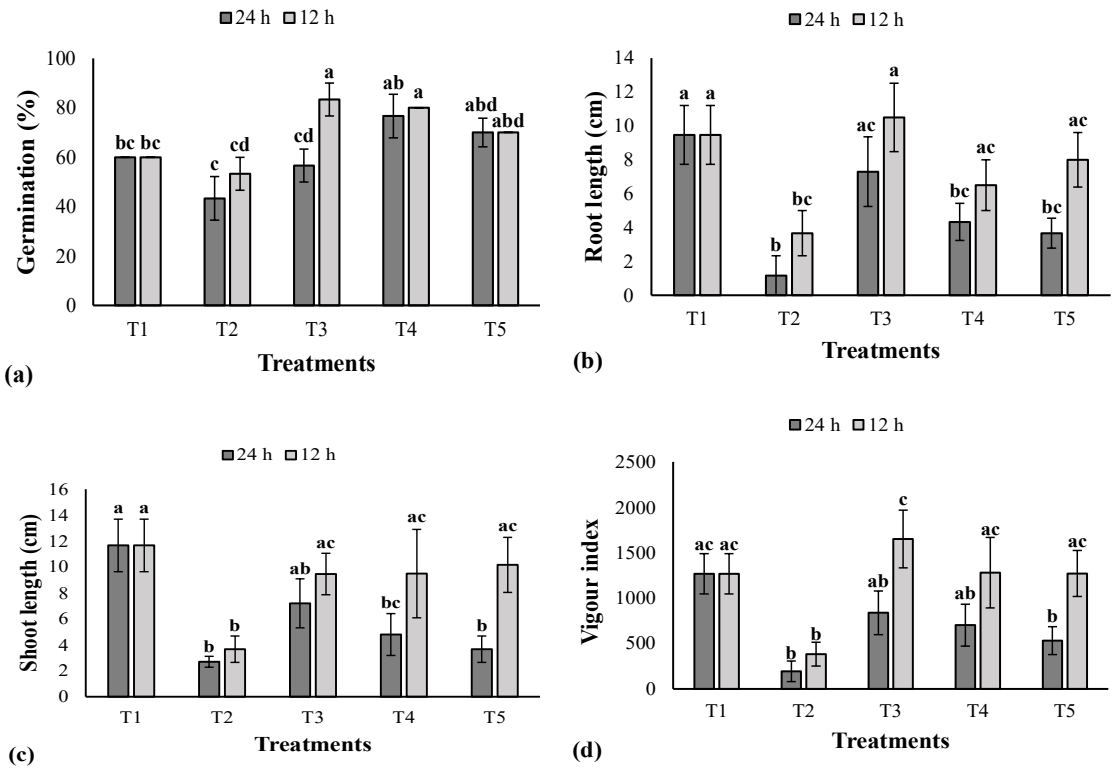
Fig 4: Seed priming effect of Carrabiitol® on fenugreek plant under salinity stress.

seedlings raised from Carrabiitol® primed seeds under excess water stress may be due to the increased soluble sugar content. This metabolic activity provides sufficient energy for the seedling establishment (Hussain *et al*., 2015).

The shoot lengths were significantly reduced under excess water stress as compared to absolute control (T1) (p<0.05). The reductions in shoot lengths were found to be 77% and 68% for sorghum seeds under 24 and 12 h of excess water stress, respectively (Fig 5c), while these reductions were lower in fenugreek seeds (Fig 6c). Among all the tested concentrations of Carrabiitol®, 1000 mg/L and 3000 mg/L were effective in enhancing the shoot lengths in sorghum and fenugreek, respectively. The shoot lengths of primed sorghum seeds (T3) were increased by 2.6-fold under both excess water stress conditions, while the shoot lengths of primed fenugreek seeds (T5) were enhanced by 2.4-fold and 2.9-fold under 24 h and 12 h of excess water stress, respectively. Overall, the results of shoot lengths for primed sorghum seeds were found to be lower as compared to absolute control (T1), while the results for primed fenugreek seeds were found to be higher than or equally comparable with absolute control (T1). Carrabiitol® may confers the turgidity of cells to regulate metabolic and hormonal activities under stress condition, which resulting in enhanced root and shoot growth.

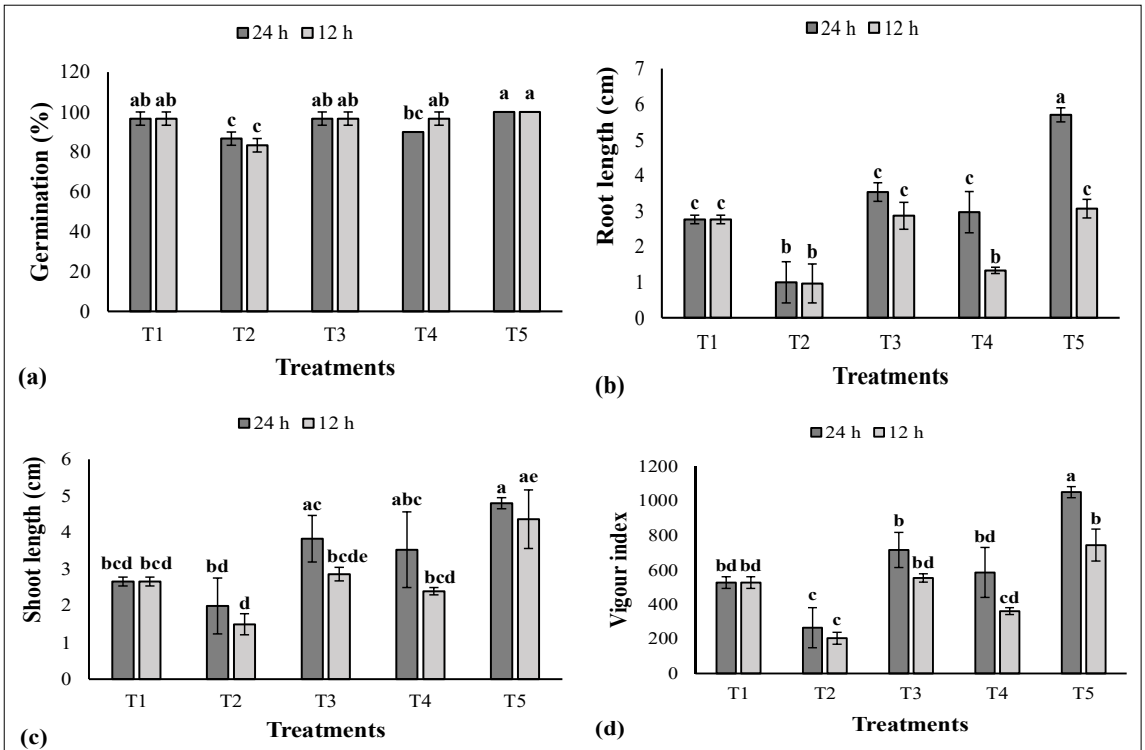
The vigor index was found to be higher or comparable in Carrabiitol® primed sorghum and fenugreek seeds as compared to absolute control (T1) (Fig 5d and 6d). The vigor index was found to be 4.3-fold higher in Carrabiitol® primed sorghum seeds (T3) as compared to non-primed seeds (T2) at both induced levels of excess water stress. In fenugreek seeds, the vigor index was found to be 4.0-fold and 3.6-fold higher at 24 h and 12 h induced excess water stress, respectively, in Carrabiitol® priming treatment (T5) as compared to non-primed seeds (T2). The vigor indices were found to be higher for Carrabiitol® primed seeds as compared to non-primed seeds under all induced stresses, which further confirms the improved viability of fenugreek and sorghum primed seeds under stress conditions.

Overall, results of the present study revealed that seed priming with Carrabiitol® could revitalize the sorghum and fenugreek seeds, resulting in greater germination performance and, thereby, seedling growth under various abiotic stress conditions (Fig 7). The germination efficiency and vigour index of PEG primed sorghum seeds were reported to be enhanced up to 43% and 2.5-fold, respectively, under drought stress conditions by Zhang *et al*. (2015). Hussain *et al*. (2022b) reported the enhanced germination index (37.52%) of two fenugreek cultivars under salinity stress by employing seed priming with gibberellic acid and PEG. Alike other organic osmolytes and plant growth regulators, Carrabiitol® maintains the osmotic potential and turgidity of the cells and activates a precise osmo-protectant



(a): Germination; (b): Root length; (c): Shoot length and (d): Vigour index. The means not sharing a common letter are significantly different by post-hoc LSD test at 5% level of significance. Data presented are means±standard errors.

Fig 5: Seed priming effect of Carrabiitol® on sorghum plant under excess water stress.



(a): Germination; (b): Root length; (c): Shoot length and (d): Vigour index. The means not sharing a common letter are significantly different by post-hoc LSD test at 5% level of significance. Data presented are means±standard errors.

Fig 6: Seed priming effect of Carrabiitol® on fenugreek plant under excess water stress.

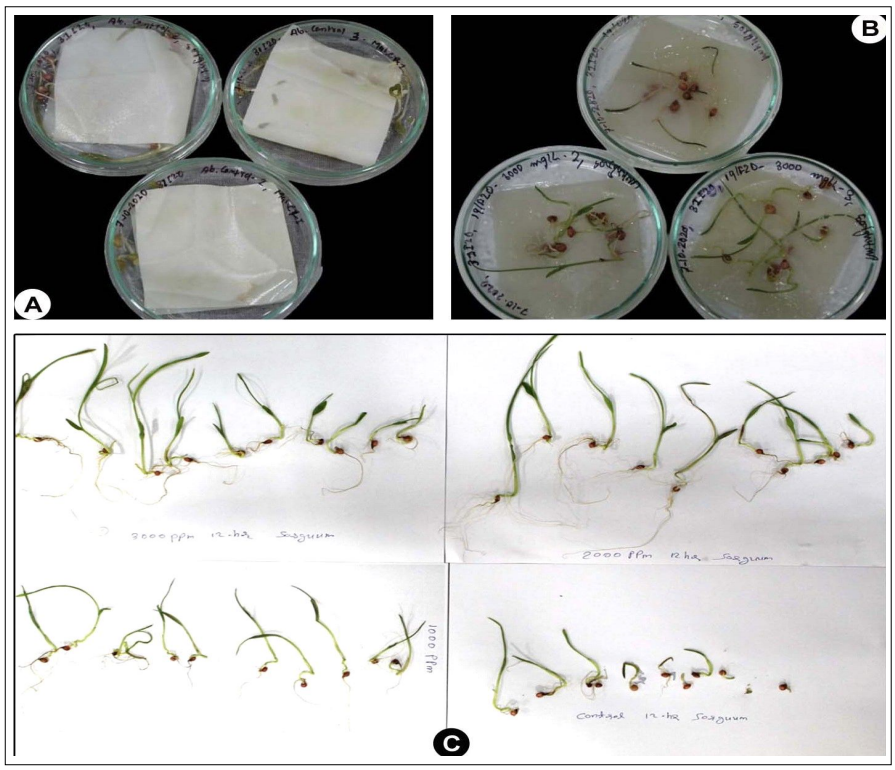


Fig 7: Seed priming effect of Carrabiitol®: Seed germination under (A): absolute control; (B): Carrabiitol® treatment and (C) Seedling growth under both control and Carrabiitol® treatment.

releasing signaling pathway under abiotic stress conditions. Therefore, the seeds of sorghum and fenugreek primed with Carrabiitol® resulted in improved seed germination and seedling growth.

CONCLUSION

Osmo-priming with Carrabiitol® is an effective approach to mitigate drought, salinity and excess water stress and to enhance the seed germination and seedling growth of sorghum and fenugreek under stress conditions. Nevertheless, further studies are required in order to understand the physiological and biochemical roles of Carrabiitol® during sorghum and fenugreek seed germination and seedling growth under various abiotic stresses.

Conflict of interest

All authors are employees of M/s. Pushpa J. Shah, with no stock options or incentives. Co-author Neil J. Shah is the Managing Partner of M/s. Pushpa J. Shah.

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