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Research Article
Salt and PEG Induced Osmotic Stress Tolerance at Germination and Seedling Stage in *Camelina sativa*: A Potential Biofuel Crop

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Abstract

Background: *Camalina sativa* L. (Crantz), a non-edible oilseed crop has been introduced in India as a potential feedstock for biofuel production. In order to avoid resource sharing with food crops, Indian National Biofuel Policy states that biofuel crops are to be cultivated only on marginal degraded lands. Therefore, the present investigation was designed to study salt and osmotic stress tolerance in Camelina. **Materials and Methods:** In the present investigation, salt (NaCl 50, 100, 150, 200, 250 or 300 mM) or polyethylene glycol 6000 (PEG 6000; 5, 10, 15 or 20% w/v) induced osmotic stress tolerance at germination and on seedlings of *C. sativa* cv. Calena was studied. **Results:** Seed germination or opening of cotyledonary leaves was not affected by treatment with up to 150 mM NaCl. When treated with 20% PEG, seed germination was not inhibited, however, percent and rate of opening of cotyledonary leaves was reduced. Treatment of seedlings with 150 mM NaCl for 11 days resulted in no differences in growth and relative water content compared to the untreated control seedlings. Treatment with PEG affected growth and relative water content compared to the control. Relative water content was reduced and membrane damage significantly increased in PEG treated seedlings over the control or NaCl treated seedlings. **Conclusion:** Thus, the results suggest that *Camelina* cv. Calena is tolerant to salt (NaCl, 150 mM) and may be suitable for cultivation under salt affected areas for biofuel production.

Key words: *Camelina sativa*, salt, PEG, germination, RWC, membrane damage

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Biofuels are renewable alternatives to petroleum-based fuels which may reduce greenhouse gas emissions. Non-food crops which have the lowest resource sharing are grown worldwide to produce fuels or fuel-like precursors, at lower costs with higher productivity. To make biofuel cost-competitive with petroleum-based fuels, production costs must be reduced. *Camelina sativa* L. (Crantz) typically contains 35-38% oil in its seed and is a potential biofuel feedstock. *Camelina* is fast-growing and early maturing crop with harvest within 90 days. *Camelina* has relatively high resistance to disease and pests and competes well against weeds once established. It has wider adaptation to varied climate conditions and can be grown on nutritionally poor soils[1]. It is being grown to determine its biofuel potential[2,3].

Due to its potential as a biofuel crop work continues in the area of crop improvement[4-6]. The higher seed yield could enhance the value of the crop for biofuel production and for various industrial and agricultural applications[5]. However, it needs to be evaluated for economic returns. *Camelina* cv. Calena (EC 643910) has been introduced in India as a potential biodiesel feedstock[3]. Experiments are being carried out at DIBER, Haldwani, on standardization of *Camelina* intercropping in Jatropha (*Jatropha curcas* L.) plantations, especially during winter months when Jatropha sheds its leaves, to have an uninterrupted supply of raw materials for biodiesel production[7]. As per National Biofuel Policy of Government of India, biofuel crops have to be grown on degraded or fallow land. Since fertile land can not be brought under cultivation of biofuel crops with less, or no resource sharing, with food crops, it is necessary to determine the tolerance of these crops to environmental stresses including salt and drought. Though *Camelina* is considered to be adapted to diverse environmental conditions, scientific studies on *Camelina* in general and the cultivar Calena, in particular, are sparse[8]. The investigation was designed to determine effects of NaCl or polyethylene glycol induced osmotic stress tolerance of *Camelina* cv. Calena at germination and seedling stage.

MATERIALS AND METHODS

Seed material: *Camelina sativa* cv. Calena was obtained from Dr. Vollmann, BOKU-University of Natural Resources and Applied Life Sciences, Vienna, Austria and introduced in India through NBPGR, New Delhi. The nucleus seed was multiplied at DIBER, Field Station, Pithoragarh, Uttarakhand, India.

Effect of salt concentrations on seed germination in *Camelina*: Seed (50 per petri plate) were sown on filter papers placed in glass petri plates. The filter paper disc was moistened with NaCl solutions (50, 100, 150, 200, 250 or 300 mM) or water. Seed germination and opening of cotyledonary leaves were recorded. The rate of germination as Timson's index was calculated using formula as described earlier[9].

The speed of opening of cotyledonary leaves was calculated as Timson's index with slight modification, using the data on percent opening of cotyledonary leaves calculated for total period of observation in days.

Effect of polyethylene glycol-PEG induced osmotic stress on seed germination in *Camelina*: Seed (50 per petri plate) were sown as above and the filter paper moistened with PEG 6000 solution (5, 10, 15 or 20%) or water as the control. Seed germination as radical protrusion and opening of cotyledonary leaves were recorded. Percent and rate of germination as well as the opening of cotyledonary leaves was calculated as explained in case of salt tolerance.

Effect of salt or PEG stress exposure in *Camelina* at seedling stage: *Camelina* seedlings were exposed to 150 mM NaCl or 20% w/v PEG 6000 for 11 days along with untreated controls. Treatment of salt and PEG solutions prepared in Hoagland liquid medium was initiated 17 days after sowing (DAS). Control seedlings received equal volumes of Hoagland liquid medium. For each treatment, solutions were added to plastic bowls in which pots were placed. The evapo-transpirational losses were replenished with distilled water twice a day. Solutions were changed every 3 days. Entire seedlings from were harvested at the end of the treatment period and all analyses performed in quadruplet.

Leaf senescence were recorded at the end of the treatment period. Plants were removed from pots and roots cleaned of the sand and washed with deionized water. The internal water balance was determined as Relative Water Content (RWC) of three seedlings per replicate[8]. Fresh Weight (FW) was recorded immediately after uprooting seedlings and Turgid Weight (TW) measured after 24 h saturation with deionized water in the dark. Dry Weight (DW) was determined after drying the leaves for 48 h in a hot-air oven at 80°C.

Membrane Damage (MD) was estimated from three seedlings per replicate as electrolytic leakage measured with an Electrical Conductivity (EC) meter[10]. Seedlings were washed with distilled water and incubated in glass tubes containing distilled water for 24 h with intermittent shaking.

At the end of incubation, initial EC reading (EC1) of the bathing solution was recorded. Tubes were capped and autoclaved at 121°C for 20 min and EC reading (EC2) recorded after cooling the solution to room temperature. Leaf membrane damage (MD) was calculated using formula:

$$MD\ (\%) = \frac{EC\ 1}{EC\ 2} \times 100$$

Statistical analysis: Treatments and controls were replicated four times. CropStat for Windows (7.2.2007.2 module), developed by the Biometrics Unit, IRRI, Philippines was used for analysis of variance (ANOVA) of experiments laid out in a completely randomized design. Percent data were arcsine (angular) transformed before statistical analyses. Treatment means were compared using the Duncan's New Multiple Range Test (DNMRT) at $p \leq 0.05$.

RESULTS AND DISCUSSION

Seed germination as radical protrusion was synchronous and 80% or higher within 4 days after sowing in control or NaCl concentration up to 150 mM. However, the NaCl concentration of 200 mM or higher reduced radical protrusion (Fig. 1). The rate of germination calculated as Timson's index was reduced at NaCl concentration of 150 mM or higher. Opening of cotyledonary leaves was reduced in NaCl concentration of 200 mM or higher (Fig. 2). The rate of opening of cotyledonary leaves was reduced at NaCl concentrations of 100 mM or higher. The NaCl concentration of 300 mM completely inhibited opening of cotyledonary leaves.

Seed germination was not affected by PEG concentration up to 20% (Fig. 3). However, rate of seed germination was higher by the PEG concentration of 10 or 15%. In the other

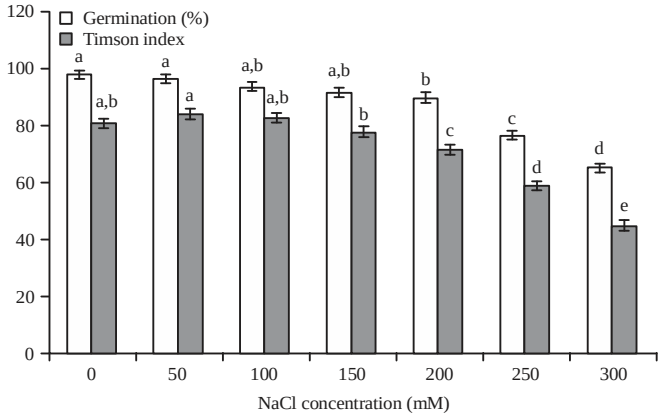

Fig. 1: Effect of NaCl on seed germination in *Camelina sativa* cv. Calena. Seeds were treated with 50-300 mM NaCl along with a water only control. Values are means of three replicates. Error bars indicate SEM. Mean values in a series indicated by different letters are significantly different at $p \leq 0.05$, LSD

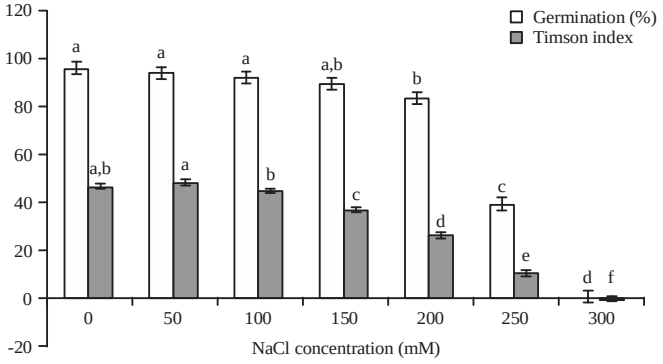

Fig. 2: Effect of NaCl on opening of cotyledonary leaves in *Camelina sativa* cv. Calena. Seeds were treated with 50-300 mM NaCl along with a water only control. Values are means of three replicates. Error bars indicate SEM. Mean values in a series indicated by different letters are significantly different at $p \leq 0.05$, LSD

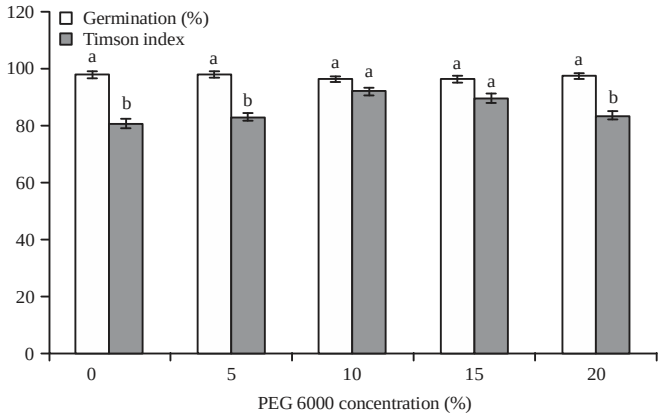

Fig. 3: Effect of polyethylene glycol on seed germination in *Camelina sativa* cv. Calena. Seeds were treated with 5-20% PEG 6000 concentration along with a water only control. Values are means of three replicates. Error bars indicate SEM. Mean values in a series indicated by different letters are significantly different at $p \leq 0.05$, LSD

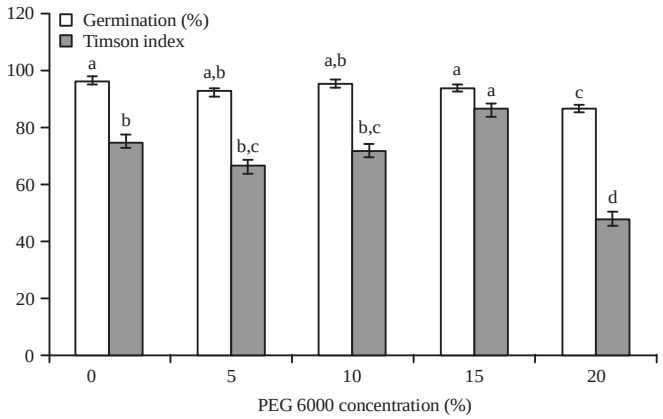

Fig. 4: Effect of polyethylene glycol on opening of cotyledonary leaves in *Camelina sativa* cv. Calena. Seeds were treated with 5-20% PEG 6000 along with a water only control. Values are means of three replicates. Error bars indicate SEM. Mean values in a series indicated by different letters are significantly different at $p \leq 0.05$, LSD

PEG concentrations, rate of seed germination was lower. Opening of cotyledonary leaves was reduced in PEG concentration of 20% (Fig. 4). At lower PEG concentrations opening of cotyledonary leaves was not affected. Similarly, the rate of opening of cotyledonary leaves was lowest in response to PEG concentration of 20%.

Seed germination is one of the important stages in seedling establishment and required for successful completion of the plant life cycle. Salt concentration upto 150 mM NaCl or 20% PEG 6000 did not affect seed germination in *Camelina* cv. Calena. Within 4 days after sowing synchronous and maximum (80% or more) germination occurred in control or these salt stress treatments. Neither total seed germination nor its rate as Timson's index was reduced in these treatments. Also, the seedling establishment was not affected in these stress treatments. Russo *et al.*[8] reported that seed germination in *Camelina* is affected at 32°C with no effect at 27°C or lower. Environmental effects on seed germination and seed yield attributing characters have also been reported earlier[11].

The seedlings at 17 DAS were exposed to the salt or PEG stresses for 11 days. There was no leaf senescence in NaCl treated or control seedlings. However, all seedlings exposed to the PEG exhibited leaf senescence. Seedling growth, as fresh weight was lowest in PEG treated seedlings than in NaCl treated or control seedlings. Mean fresh weight of salt stressed seedlings was similar to controls (Fig. 5). At least part of the membrane damage is caused by uncontrolled production of Reactive Oxygen Species (ROS) in response to plant exposure to salt, osmotic or other abiotic stress[12]. Lower oxidative

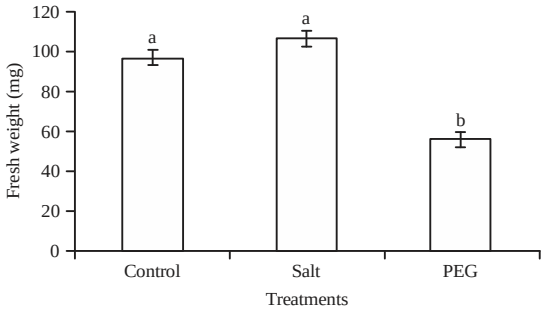

Fig. 5: Effect of 150 mM NaCl and 20% PEG (w/v) on growth of *Camelina sativa* cv. Calena seedlings exposed at 17 DA 11 days. Plant growth was estimated as fresh weight (mg) of three seedlings at the end of treatment. Values are means of three replicates. Error bars indicate SEM. Mean values in a series indicated by different letters are significantly different at $p \leq 0.05$, LSD test

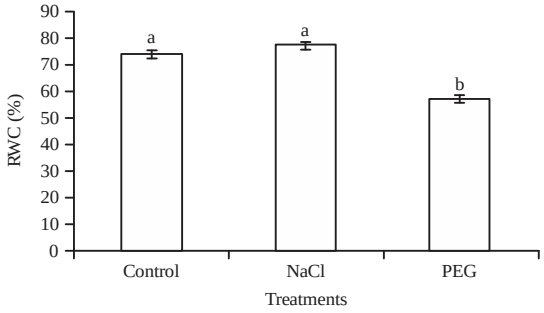

Fig. 6: Effect of NaCl and PEG exposure on *Camelina sativa* cv. Calena relative water content. Seedlings at 17 DA were exposed to NaCl (150 mM) or PEG 6000 (20%) for 11 days. Plant water balance was estimated as RWC (%) of three seedlings at the end of treatment. Values are means of three replicates. Error bars indicate SEM. Mean values in a series indicated by different letters are significantly different at $p \leq 0.05$, LSD

damage to biological macromolecules is necessary for survival of plants exposed to stress[10]. In addition to membrane damage, RWC is an important index for screening salt, drought and other abiotic stresses in plants, higher RWC and lower membrane damage are indicators of tolerance[10,13,14]. Relative water content was not affected in NaCl treated seedlings compared to controls (Fig. 6). However, the relative water content was reduced in response to PEG exposure. Membrane damage was lowest in NaCl treated seedlings (Fig. 7). Membrane damage was higher in controls than in NaCl treated seedlings, but was lower than the PEG treated seedlings. The highest membrane damage was in the PEG stressed seedlings. Lower membrane damage in NaCl treated

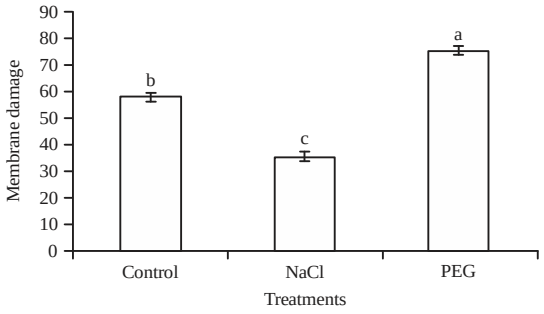

Fig. 7: Effect of NaCl and PEG in *Camelina sativa* cv. Calena on membrane damage. Seedlings at 17 DAs were exposed to NaCl (150 mM) or PEG 6000 (20%) for 11 days. Oxidative damage to membranes balance was estimated as MD (%) of three seedlings at the end of treatment. Values are means of three replicates. Error bars indicate SEM. Mean values in a series indicated by different letters are significantly different at $p \leq 0.05$, LSD

plants could be due to better antioxidant metabolism activated in *Camelina* seedlings for efficient scavenging of salt induced oxidative stress. Lower oxidative damages to the biological macromolecules in NaCl treated plants could have resulted in better survival than in PEG treated seedlings. The salt tolerance in stressed seedlings was associated with better internal water balance and lower membrane damage. PEG treated seedlings could not maintain the water balance as RWC.

CONCLUSION

Seed germination remained unaffected up to 150 mM NaCl or 20% PEG stress under laboratory conditions. However, at seedling stage, tolerance to these stresses varied. Seedling growth was unaffected in salt (NaCl 150 mM) stressed seedlings compared to the control. Salt tolerance was associated with better growth, higher RWC and lower membrane damage. However, on exposure to PEG (20% w/v) seedling growth was reduced and the sensitive response associated with lower RWC and higher membrane damage. Effects of these stresses on seed yield, oil content or quality need to be explored at field level. The data can be used as a starting point to determine whether the introduced *Camelina* variety can be grown for biofuel production on marginal lands with lesser agricultural inputs.

SIGNIFICANCE STATEMENTS

- The results confirmed salt (NaCl 150 mM) stress tolerance in *Camalina* cv. Calena at germination and seedling stage

under laboratory conditions. Thus, the results suggest suitability of the *Camelina* cultivar for cultivation under salt affected areas for biofuel production

- Further, the results need to be confirmed based on trials under field conditions

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