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Influence of biostimulants-seed-priming on *Ceratotheca triloba* germination and seedling growth under low temperatures, low osmotic potential and salinity stress

Nqobile A. Masondo, Manoj G. Kulkarni, Jeffrey F. Finnie, Johannes Van Staden*

Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg Campus, Private Bag X01, Scottsville 3209, South Africa

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ABSTRACT

Extreme temperatures, drought and salinity stress adversely affect seed germination and seedling growth in crop species. Seed priming has been recognized as an indispensable technique in the production of stress-tolerant plants. Seed priming increases seed water content, improves protein synthesis using mRNA and DNA and repair mitochondria in seeds prior to germination. The current study aimed to determine the role of biostimulants-seed-priming during germination and seedling growth of *Ceratotheca triloba* (Bernh.) Hook.f. (an indigenous African leafy vegetable) under low temperature, low osmotic potential and salinity stress conditions. *Ceratotheca triloba* seeds were primed with biostimulants [smoke-water (SW), synthesized smoke-compound karrikinolide (KAR_1), Kelpak® (commercial seaweed extract), phloroglucinol (PG) and distilled water (control)] for 48 h at 25 °C. Thereafter, primed seeds were germinated at low temperatures, low osmotic potential and high NaCl concentrations. Low temperature (10 °C) completely inhibited seed germination. However, temperature shift to 15 °C improved germination. Smoke-water and KAR_1 enhanced seed germination with SW improving seedling growth under different stress conditions. Furthermore, priming seeds with Kelpak® stimulated percentage germination, while PG and the control treatment improved seedling growth at different PEG and NaCl concentrations. Generally, high concentrations of PEG and NaCl brought about detrimental effects on seed germination and seedling growth. Findings from this study show the potential role of seed priming with biostimulants in the alleviation of abiotic stress conditions during seed germination and seedling growth in *C. triloba* plants.

1. Introduction

Seeds are constantly exposed to harsh environmental conditions throughout their germination and seedling growth stages. These environmental factors include extreme temperatures, drought and salinity stress. Temperature is one of the most crucial climatic factors influencing seed germination. Changes in temperature significantly affect seed germination through the inhibition of radicle emergence and post-germination growth in seedlings (Probert, 2000). Successful seed germination and seedling establishment are dependent on surrounding temperatures with each species having a particular set of requirements. Outside these, seed germination declines gradually. Furthermore, drought and salinity stress severely affect seed germination by preventing water uptake and through the toxic effect of sodium and chloride ions. These factors result in inhibited or delayed seed germination and seedling growth (Ashraf and Foolad, 2005). In order to enhance seed germination under extreme temperatures, drought and salinity stress conditions, seed priming could be an indispensable technique in the production of stress tolerant plants (Jisha et al., 2012; Paparella et al., 2015). Seed priming agents including natural and synthetic compounds improve physiological processes in seeds prior to germination (Jisha et al., 2012).

Priming is a process by which seeds are hydrated in different solutions for the initiation of certain metabolic processes (e.g. protein synthesis using mRNA and DNA as well as repairing or synthesizing new mitochondria), which permits preliminary germination but not the final stage (Jisha et al., 2012; Paparella et al., 2015). The technique also improves seedling shoot and rooting frequency, vigour index and ultimately crop yields. There are several priming approaches currently applied in seeds of various species including hydropriming, osmopriming, chemical priming, hormonal priming, biological priming, redox priming and solid matrix priming. Seed germination after priming is dependent on the priming agent, severity of stress and crop species (Jisha et al., 2012). The use of biostimulants in order to

* Corresponding author.
E-mail address: rcpgd@ukzn.ac.za (J. Van Staden).

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counteract the effect of abiotic stress is well-recognized (Bulgari et al., 2014; Sharma et al., 2014). In addition, the promotory effects of biostimulants such as smoke-water (SW), karrikinolide (KAR_1) (Light et al., 2009; Kulkarni et al., 2011), Kelpak® (seaweed extract) (Stirk and Van Staden, 2006) and phloroglucinol [benzene-1,3,5-triol (PG); identified from *Ecklonia maxima*] have been documented (Kannan et al., 2013).

Ceratotheca triloba (Bernh.) Hook.f. (Pedaliaceae) is an indigenous leafy vegetable found in southern Africa. The nutritional, medicinal and pharmacological properties of the vegetable are well-documented (Masondo et al., 2016). *Ceratotheca triloba* is propagated through seed germination in spring or early summer in rich and well-drained soils. However, there is still limited information on seed germination of *C. triloba* particularly under abiotic stress conditions. Therefore, the current study aimed to determine the role of biostimulants-seed-priming during seed germination and seedling growth in *C. triloba* under low temperature, low osmotic potential and salinity stress conditions.

2. Materials and methods

2.1. Biostimulants and chemicals

Smoke-water and KAR_1 solutions were prepared according to previously described methods (Baxter et al., 1994; Flematti et al., 2004, 2005; Van Staden et al., 2004). Kelpak® [Kelp Products (Pty) Ltd, Simon's Town, South Africa] solution was prepared as indicated on the product label (0.4%). Phloroglucinol [benzene-1,3,5-triol (PG)], polyethylene glycol 6000 (PEG) (Merck, Darmstadt, Germany) and sodium chloride (NaCl) used were of analytical grade.

2.2. Seed imbibition, priming and germination

Seeds of *C. triloba* were purchased from Silverhill Seed Nursery, Cape Town, South Africa. In order to determine imbibing levels of *C. triloba* seeds, imbibition tests were carried out. In these tests, 90 seeds (30 seeds per Petri dish) were placed in 90 mm Petri dishes with two layers of filter paper (Whatman No. 1) moistened with 3 ml distilled water and allowed to imbibe for 6, 12, 24, 48 and 72 h at 25 °C. Thereafter, seed percentage imbibition by seeds was calculated as: Imbibition (%) = (weight of seeds after imbibition – initial weight of seeds)/initial weight of seeds (Govender et al., 2008).

Based on the imbibition results (Fig. 1), seeds were primed with different biostimulants (SW 1:500 v/v, KAR_1 10^{-6} M, Kelpak® 0.4%, PG 10^{-6} M) as well as with distilled water (control) and incubated for 48 h at 25 °C. During seed germination and seedling growth experiments, biostimulant treatments were separated as either smoke (SW, KAR_1) or seaweed (Kelpak®, PG) with their respective control treatment (water). For seed germination, 25 seeds were placed in 90 mm Petri dishes (5 seeds per Petri dish) lined with two layers of Whatman No. 1 filter paper. Thereafter, seeds were incubated at 10, 10/15 and 15 °C in a 16/8 h light and dark regime with a photosynthetic photon flux (PPF) of 45 μmol m^{-2} s^{-1} for 25 days. During temperature shift experiments, seeds were initially incubated at 10 °C for 15 days followed by another 10 days at 15 °C which is depicted as 10/15 °C above. In order to determine the effect of drought and salinity stress, different osmotic solutions were used to create low water potential during seed germination (e.g. PEG (enforces water stress) and NaCl (enforces salinity stress)) due to their lack of toxicity. For low osmotic potential and salinity stress, biostimulants-primed seeds were germinated with varying concentrations of PEG 6000 (0; − 0.05; − 0.15; − 0.30; − 0.49 MPa) as developed by Michel and Kaufmann (1973) and NaCl (0; 5; 15; 25; 50 mM). Respective biostimulants were used to prepare the different concentrations of PEG or NaCl. Furthermore, control treatments contained different PEG 6000 or NaCl concentrations prepared with distilled water. Solutions of PEG and NaCl were replaced on a 3 day interval for the duration of the experiment. Seeds were incubated at 25 °C in a 16/8 h light and dark regime with a photosynthetic photon flux (PPF) of 45 μmol m^{-2} s^{-1} for 25 days. Seed germination was recorded daily. Germination was considered successful when the radicle had protruded 2 mm. After 25 days of seed germination and seedling growth, survival rate, shoot length and root length were measured. Seedling vigour index was calculated as VI = seedling length (mm) × percentage germination (Dhindwal et al., 1991).

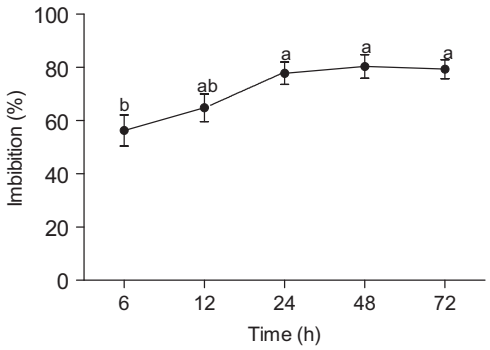

Fig. 1. Imbibition curve for *Ceratotheca triloba* seeds incubated at 25 °C. Bars (± SE; n = 30) with different letter(s) are significantly different ($P \leq 0.05$) based on Duncan's Multiple Range Test (DMRT).

3. Data analysis

Data were subjected to analysis of variance (ANOVA) using SPSS for Windows (SPSS®, Version 22.0. Armonk, New York, USA). The mean values were further separated using the Duncan's Multiple Range Test (DMRT) for statistical significance levels ($P \leq 0.05$).

4. Results

4.1. Effect of biostimulants-priming on seed germination and seedling growth under low temperature

Seed germination in *C. triloba* was completely inhibited at 10 °C (Fig. 2). Nevertheless, temperature shifts from 10 to 15 °C (10/15 °C)

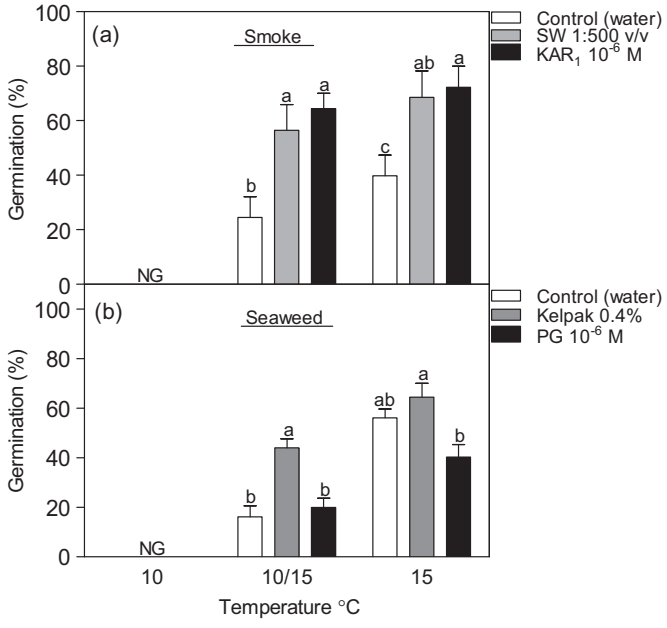

Fig. 2. Germination (%) of *Ceratotheca triloba* seeds primed with different biostimulants at low temperatures for 25 days. Bars (± SE; n = 25) with different letter(s) are significantly different ($P \leq 0.05$) based on Duncan's Multiple Range Test (DMRT). No germination (NG).