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Effect of potassium chloride-induced stress on germination potential of *Artemisia annua* L. varieties

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ABSTRACT

Artemisia annua L. is a well-known antimalarial plant cultivated across the globe. In this study we are reporting the effect of potassium chloride-induced stress on germination potential of two *A. annua* varieties ('CIM- Arogya' and 'Jeevan Raksha') under *in vitro* conditions. The percentage of germination, seedling vigour index I & II, enzymatic and non-enzymatic, biochemical changes were observed by varying the salt concentration in the range of 0 mM (control) to 200 mM at 15 °C coupled with 16 h light and 8 h dark photoperiod. The results revealed a non-significant decrease in the germination percentage and significant decrease in seedling vigor index while proline and lipid peroxidation increased with a rise in the potassium chloride concentration irrespective to varieties. Comparatively, 'CIM-Arogya' variety showed higher germination percentage, seedling vigor index, carbohydrate, protein, catalase, proline and lipid peroxidation except total phenolic content which was superior in 'Jeevan Raksha' at 150 mM KCl. Furthermore, 'CIM-Arogya' showed a better adaptation and tolerance potential (up to 150 mM) to potassium chloride-induced stress than 'Jeevan Raksha' (up to 100 mM).

1. Introduction

Artemisia annua L. is an annual Chinese herb/shrub grown in many countries of Asia, Africa, Australia, America and Europe (Gupta et al., 2002). It is a well known source of anti-malaria drug 'artemisinin', which was approved by World Health Organisation for cerebral malaria (World Health Organization, 2006). The plant derived artemisinin production still has a limitation to fulfil the demand of the drug by pharmaceutical industries worldwide. There are many studies available on the fertilization such as application rate of NPK (Rahman et al., 2014) and NaCl stress on seed germination of *A. annua* (Keshavarzi, 2012; Aftab et al., 2010; Kumar et al., 2014). The effect of physical treatment (cold and warm water), chemical treatment (H_2SO_4 and CH_3OH), hormone treatment (gibberellic acid) at different concentration and time interval on seed germination of *A. annua* revealed a positive response (Muhammad et al., 2014; Sada et al., 2015).

Potassium is the seventh most abundant element in the lithosphere which is essential to all forms of life including plants. The status of potassium in the vertisol of India are 65×10^4 ppm, 21×10^4 ppm, 15×10^4 ppm, 34×10^3 ppm, 26×10^3 ppm, 17×10^3 ppm, and 17×10^3 ppm for Rajasthan, Haryana, Punjab, Gujarat, Uttar Pradesh, Bihar and Tamil Nadu respectively (Murthy, 2012; Prasad, 2012). Although, there is no known harmful effect of potassium chloride to the human health, but the consequences of its inadequate level cause severe degradation of soil characterized by high acidity, low nutrients and an unbalanced ecosystem. It has direct effect on plant growth particularly chloride ion sensitive crops and interrupt with efficient utilization of other nutrients such as N, Mg and P. Priming effect of potassium chloride on seed germination showed a significantly higher percentage of germination than unprimed maize seeds at different concentration of sodium chloride (Badar-uz-Zaman Ali et al., 2012). Misra and Dwibedi (1980) reported that seeds soaked in 2.5% KCl for 12 h before sowing increased wheat yield by 15%. Paul and Choudhury (1991) observed that seeds soaked with 0.5–1% of KCl or K_2SO_4 significantly increased plant height, yield attributes and grain yield in wheat. However, contradictory results were also observed which suggest that potassium ions often play a role in saline toxicity (Lauchli, 1990; Niu et al., 1995). Romero-Aranda (1998) revealed that seedling of citrus varieties treated

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Fig. 1. *Artemisia annua* plant.

with chloride salt (15 mol m^{-3} $CaCl_2$, 30 mol m^{-3} CaCl and 30 mol m^{-3} KCl) showed physiological and anatomical disturbance in seedlings. Adiloglu et al. (2007) reported that seedling of wheat treated with different level of NaCl and KCl salt (0–60 mM) resulted in decrease in growth and biological index of the seedling compared to control. However, no such study is available on *A. annua.*

The present study aim is to assess the effect of a direct exposure of different concentrations of potassium chloride (0 mM–200 mM, present in different vertisol of India) on percent germination, seedling vigour index I & II, enzymatic and non-enzymatic biochemical changes in two varieties viz. ('CIM-Arogya' and 'Jeevan Raksha') of *A. annua.*

2. Material and methods

2.1. Seeds

The seeds of two varieties ('CIM–Arogya' and 'Jeevan Raksha') of *A. annua* (Figs. 1 and 2) were collected in January 2015 from the Experimental Farm of CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, Uttar Pradesh, India and stored in the National Seed Gene Bank of CSIR-CIMAP until experiments were conducted.

2.2. Seed germination and seedling vigor test

The experiment was conducted on two varieties using five different concentrations (0 mM (control), 50 mM, 100 mM, 150 mM and 200 mM) of potassium chloride on top of the filter paper in Petri-dishes (16 cm diameter × 3 cm deep) placed at 15 °C coupled with 16 h light/8 h dark photoperiod. The treatments (salt concentration and varieties) were replicated three times and each Petri dish comprising of 100 seeds. The 7th day was established as final count day for the germination study (Kumar et al., 2013).The germination percentage, seedling vigor index I (SVI-I) and vigor index II (SVI-II) were calculated with the formula shown below:

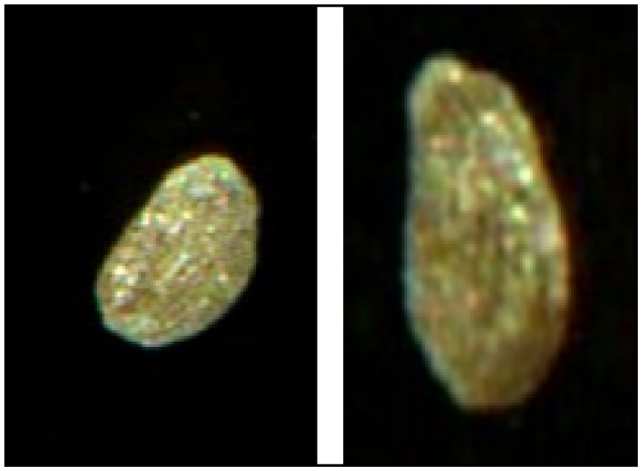

Fig. 2. 'CIM- Arogya' (L) and 'Jeevan Raksha' (R) seeds.

$$\text{Germination Percentage} = \frac{\text{Number of seeds germinated} \times 100}{\text{Total nunber of seeds}}$$

Seedling vigor index I = Germination (%) × Average seedling length

Seedling vigor index I = Germination (%) × Average seedling dry weight

2.3. Determination of biochemical (non-enzymatic and enzymatic) assays

One gram of seedlings from each treatment were immediately crushed with liquid nitrogen in ice cold mortar and pestle and further 3 ml of extraction buffer containing 50 mM $NaPO_4$ (pH 7) were added for preparation of extract. The extract was centrifuged at 10,000 × g for 10 min. The supernatant was kept at −20 °C until used for the measurement of carbohydrate, protein and catalase. The extraction for total phenolic content, proline and lipid peroxidation were done separately.

2.4. Protein

To an aliquot (50 µl) of the supernatant,1 ml of extraction buffer and 5 ml of Coomassie brilliant blue (CBB) G-250 was added and mixed thoroughly. The absorbance was read at 595 nm in a spectrophotometer against a reagent blank. The amount of protein was calculated using standard prepared with different concentrations of bovine serum albumin (BSA) ranging from 0.1 to 1 mg/ml (Bradford, 1976).

2.5. Carbohydrate

An aliquot (100 µl) of the supernatant was diluted with 1 ml extraction buffer and 1 ml of 5% phenol (aqueous w/v) then 5 ml of concentrated sulphuric acid were added rapidly and mixed. The samples were incubated for 10 min at 37 °C. The colour development was read at 490 nm using a UV–vis spectrophotometer. The reagent without the sample served as a blank. The amount of carbohydrate was estimated using standard graph prepared with different concentration of D-glucose ranging from 0.1 to 1 mg/ml (Dubois et al., 1956).

2.6. Catalase

The catalase activity was measured by Chandlee and Scandalios (1984) method with slight modification. The reaction mixture contains 0.2 ml of enzyme extract, 2.5 ml of $NaPO_4$ and 0.1 ml of 10 mM. H_2O_2 was added to observe the catalase activity. The absorbance was recorded at 230 nm upto 75 s at every 15 s interval. The enzyme activity was expressed in U/mg protein (U = 1 mM of H_2O_2 reduction per min per mg of protein).

2.7. Lipid peroxidation

The lipid peroxidation level was estimated by measuring the malondialdehyde (MDA) production using thiobarbituric acid (TBA) method as reported by Heath and Packer (1968). One gram of seedling was homogenized in 1 ml of 0.5% trichloracetic acid (TCA). The homogenate was centrifuged at 19,000 × g for 20 min. The 0.5 ml of