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Effects of temperature, moisture and salinity on seed germination of *Artemisia annua* L. grown under Tarai conditions of Uttarakhand

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Abstract

Each plant species has its own set of germination requirements consisting of both intrinsic and extrinsic factors. The present investigation was aimed to study the effect of various extrinsic factors *viz*., temperature, moisture and salt concentrations affecting *ex situ* seed germination of different populations of *Artemisia annua* growing in Tarai region of Uttarakhand. All the populations were susceptible to changes in abiotic conditions *viz*., moisture and salinity levels in dose dependent manner. All, invariably, showed maximum germination at alternate day/night temperature (25/20 ºC) than under constant temperatures. Among the different populations, V-IV, a non-pigmented, early flowering population was the most tolerant one as it showed broader range of germination percentage ranging from 66±6.1 at -5 bar to 40.0±7.6 at -15 bars and 62.7±7.0 at 0.2% NaCl to 9.3±1.3 at 0.8% NaCl, respectively.

Keywords: *Artemisia annua*, artemisinin, seed germination, moisture stress, salinity

Introduction

Seed germination is the most critical factor determining success or failure of plant establishment (Kader and Jutzi, 2004). Each plant species has characteristic set of requirements for seed germination as a result of adaptive radiation and changing environments (Thompson, 1974a). In nature, seeds and seedlings are subjected to different environmental factors like moisture, temperature and salinity. Failure to cope with the adversity caused by the extremes of these factors results in poor germination, poor seedling development, and eventually, reduced crop yields. Thus, for any plant, understanding the basic seed physiology is essential for predicting how species may respond to changes in abiotic conditions. The effect of moisture, temperature and salinity levels on germination of various medicinal plants has been studied by many workers (Helmerick and Pfeifer, 1954; Onen, 2006; Zheng *et al*., 2005).

Artemisia annua L. is a very valuable medicinal plant used for many centuries in traditional Chinese medicine for the treatment of fever and malaria. The importance of the plant is due to the presence of artemisinin, a sesquiterpene lactone endoperoxide, that has been found to have strong anti-malarial properties. Unfortunately, the artemisinin content in *A. annua* is very low (0.01-1% dry weight), yet the demand for artemisinin is increasing worldwide (Maguire, 1973). The chemical synthesis of artemisinin is achieved, however the yield is low and is quite uneconomical. The isolation of artemisinin from the plant still holds the best alternative and hence it needs to be cultivated on mass scale. Population studies showed that the Indian populations of *A. annua* is a racial mixture of multiple introductions from diverse secondary populations (Sangwan *et al.*, 1999). The present study was undertaken to evaluate the effect of different abiotic factors *viz*., different temperature regimes, moisture and salt concentrations on seed germination behaviour of eight populations of *A. annua* growing in Pantnagar and find out optimum conditions for seed germination and to identify resistant and susceptible genotypes against abiotic stresses.

Materials and methods

Seed collection: The seeds were collected from 8 different populations of *A. annua*, *viz*., V-I, V-II, V-III, V-IV, V-V, V-VI, V-VII and V-VIII grown at different sites in Pantnagar (Bisht *et al*., 2010).

Germination Experiments: The seeds of individual populations of *A. annua* were sterilized with 0.01% $HgCl_2$ and thoroughly washed with sterilized distilled water. Fifty seeds of each population were kept at equal distances in sterilized Petri plates (9 cm dia) containing two Whatman No.1 filter papers, in triplicates. The germination of seeds was examined under different regimes of temperature, water and salt. Seeds were moistened with distilled water and kept at three constant temperature regimes (15, 25, and 35 ºC) obtained with the help of incubators. Effect of alternating day/night temperatures (25 ºC/20 ºC) obtained in the laboratory was treated as control. Mannitol was used to maintain desired levels of osmotic stress (Jami al Ahmadi and Kafi, 2006). The seeds were moistened either with distilled water (0 bar /control) or with different concentrations of mannitol to obtain different levels of osmotic potential (-5, -10 and -15 bars) to create different levels of osmotic stress. NaCl was used to maintain desired levels of salinity. The seeds were moistened with distilled water (control) and with different concentrations of salt solution (0.2, 0.4, 0.6 and 0.8% NaCl) prepared in sterilized dist. water for use in germination experiments. Seeds were considered germinated with the emergence of radicle and observations were made at an interval of 24 h (1 d) up to 15 days and expressed as percent seed germination.

Results and discussion

The seed germination (%) of *A. annua* under control conditions ranged from 72.0±9.9 to 84.0±3.1 in V-II and V-VI, respectively (Table 1, 2 & 3). All the populations, invariably, showed optimum seed germination in alternate day/night temperature (25 ºC/20 ºC) compared to constant temperatures (Table 1). Similar trend has been reported for few forest species (Schutz and Milberg, 1997; Bargali and Singh, 2007). Germination responses of *A. abyssinica* indicated 30/20 ºC as the most favourable temperature (Basahy, 1996). Other studies also reported stimulated seed germination in response to alternating high and low temperature (Thompson, 1974b; Zheng *et al.*, 2005). Alternating temperature creates a shift in the inhibitor-promoter balance where the inhibitor is decreased during low temperature cycle and promoter increases during high temperature leading to germination (Copeland and McDonald, 2001). In nature also, species experience alternate temperature during day and night. Temperature regulates seed germination by affecting enzymatic activities and several other metabolic activities. In the study, under constant temperature regimes, the percentage seed germination, in general, decreased with increasing temperature and the decrease was significantly different. The least per cent germination was observed at high temperature *i.e.*, 35 ºC (Table 1). The reduction percentage was maximum (99.3) at high temperature in V-I, V-III and V-VII and minimum (10.1) at low temperature in V-I (Fig. 1a). The possible reason can be that low temperature inhibited the catabolic activity and high temperature denatured proteins and inactivated certain enzymes (Maguire, 1973). Onen (2006) observed highest percent germination in *A. vulgaris* at temperature ranging from 15 ºC to 30ºC and found variation in optimum temperature among different seed lots. However, the present study reports uniformity in optimum temperature among seed lots belonging to different populations.

In all the populations, the per cent seed germination was maximum in dist. water and decreased significantly with increasing levels of osmotic stress (Table 2). Under osmotic stress condition per cent seed germination ranged from 66.0±6.1 at -5 bar for V-IV to 14.7 ± 3.5 at -15 bars for V-III respectively. Boydak *et al.* (2003) also found a marked reduction in germination percentage, germination speed and germination value with decrease in water potential in six provenances of *Pinus brutia* from different bioclimatic zones. Duan *et al.* (2004) also reported decreased germination percentage with decrease in osmotic potential. Reduced germination under water stress conditions could be attributed to the fact that seeds develop an osmotically enforced "dormancy" under water stress conditions, which may be an adaptive strategy of seeds to prevent germination under stressful environment thus ensuring proper establishment of the seedlings (Singh *et al.*, 1996; Prado *et al.*, 2000). Per cent reduction over control in different populations was maximum in V-II (79.7) at high osmotic stress and minimum in V-IV (19.5) at low stress (Fig.1b). At all the levels of osmotic stress, V-IV showed maximum germination percentage (66.0±6.1 at -5 bar to 40.0 at -15 bar) thus, emerging as the most tolerant population for osmotic stress during germination phase. This may be due to high relative water content in the seeds of this population (Siddique *et al.*, 2000) which helped the plant to sustain the effect of osmotic stress.

Table 1. Effect of different temperature regimes on seed germination (%) in different populations of *A. annua* (Mean ±SE)

Population	Control (20/25 ºC)	Temperatures (ºC) 15	25	35
V-I	79.3±9.3	71.3±10.7	47.3±8.4	0.6±0.0*
V-II	72.0±9.9	29.3±5.5*	47.3±8.4	0.0±0.0*
V-III	81.3±4.1	55.3±3.7*	56.7±7.1**	0.6±0.0*
V-IV	82.0±4.0	57.3±4.7*	64.0±1.2*	4.7±0.6*
V-V	73.3±12.7	42.7±1.8	38.7±4.0	6.7±0.6*
V-VI	84.0±3.1	56.0±5.0*	54.66±8.7*	0.0±0.0*
V-VII	78.0±4.2	36.0±8.1*	42.0±8.1*	0.6±0.0*
V-VIII	78.0±2.0	37.3±0.7*	39.3±5.5*	0.0±0.0*

Table 2. Effect of osmotic stress on seed germination (%) in different populations of *A. annua*(Mean± SE)

Population	Control (0 bar)	Osmotic stresss (bar) 5	10	10
V-I	79.3±9.3	62.7±10.4	35.3±4.7*	28.7±7.7*
V-II	72.0±9.9	47.3±3.7	21.3±4.4*	14.7±3.5*
V-III	81.3±4.1	58.0±5.0*	37.3±3.5*	14.0±2.0*
V-IV	82.0±4.0	66.0±6.1	60.7 ±1.3*	40.0±7.6*
V-V	73.3±12.7	54.7±2.4	39.3±4.1	33.3±4.4*
V-VI	84.0±3.1	58.0±4.2*	45.3±5.2*	38.0±2.0*
V-VII	78.0±4.2	48.0±4.0*	36.0±6.1*	24.0±4.6*
V-VIII	78.0±2.0	49.3±5.7*	30.7±5.8*	16.0±3.5*

Table 3. Effect of salinity (NaCl) stress on seed germination (%)in different populations of *A. annua* (mean±SE)

Populations	Control	Salt concentrations (NaCl %) 0.2	0.4	0.6	0.8
V-I	79.3±9.3	26.0±2.3*	12.7±1.8*	6.0±1.2*	1.3±0.0*
V-II	72.0±9.9	12.7±1.8*	0.7±0.0*	1.3±0.0*	0.0±0.0*
V-III	81.3±4.1	5.3±2.0*	4.0±2.0*	1.3±0.0*	2.7±0.6*
V-IV	82.0±4.0	62.7±7.0	30.0±8.1*	12.7±3.5*	9.3±1.3*
V-V	73.3±12.7	36.0±1.2*	20.0±6.1*	14.7±3.5	7.3±1.3*
V-VI	84.0±3.1	42.0±11.7*	27.3±9.0*	16.0±5.0*	1.3±0.0*
V-VII	78.0±4.2	8.0±4.0*	2.0±1.0*	1.3±0.0*	0.0±0.0*
V-VIII	78.0±2.0	15.3±1.8*	18.0±21.0*	3.3±1.3*	0.6±0.0*

t- test was applied.* and ** indicate significant at $P<0.05$ and $P<0.01$,, respectively

Germination of seeds was also adversely affected by salinity. The germination percentage in all the populations was significantly decreased by increasing levels of salinity (NaCl) stress (Table 3). At all the levels of salinity, V-IV showed maximum germination percentage ranging from 62.7± 7.0 at low(0.2%) salinity level to 9.3±1.3 at high salinity (0.8%) level. In all the populations, per cent reduction over control was maximum in V-III at 0.2% salinity, V-II at 0.4%, V-II, V-III and V-VII at 0.6% and V-II, V-VII at 0.8% salinity level (high), respectively; minimum per cent reduction was in V-IV (25.2) at low salinity (0.2%) level (Fig. 1c). Overall, seeds of all the populations were found to be highly susceptible to changes in salinity levels. Other investigations on seed germination under salinity stress also indicated that seeds of most species are very sensitive to elevated salinity at germination and seedling phases of development (Duan *et al.*, 2004; Mensah *et al.*, 2006; Qu *et al.*, 2008). Of the different stress conditions, salinity stress proved most detrimental during germination phase for all the populations. In *Mimosa tenuiflora*, seed germination was found to have lower tolerance to salinity stress than to water stress (Bakke *et al.*, 2006). Germination inhibition due to salinity appears to be osmotic. The detrimental effect of salinity occurred because of osmotic stress and specific ion toxicity. The interaction of specific ion and osmotic effects induces a reduction in the number of seeds germinated and retardation in the rate of germination (Jami al Ahmadi and Kafi, 2006).

The perusal of results of the present study indicated that alternate day/night temperature was the best for germination of *A. annua* irrespective of the populations. Further, as the seeds of *A. annua* could germinate over different levels of osmotic stress, it indicated that all the populations were more tolerant to osmotic stress than salinity or temperature stress. Our results also identifies V-IV, an early flowering population (Bisht *et al.*, 2010) grown in tarai, as a tolerant population at germination phase. Due to its broader range of tolerance, it could be recommended for cultivation in environments where salinity and moisture stress of soil is a constraint and could also be utilised in future breeding programmes for improving osmotic and salinity stress tolerance of the species. Overall, the results of the study are important to understand germination requirements of *A. annua*, and hence would definitely help in improvement of management strategies specific to the crop in field conditions.

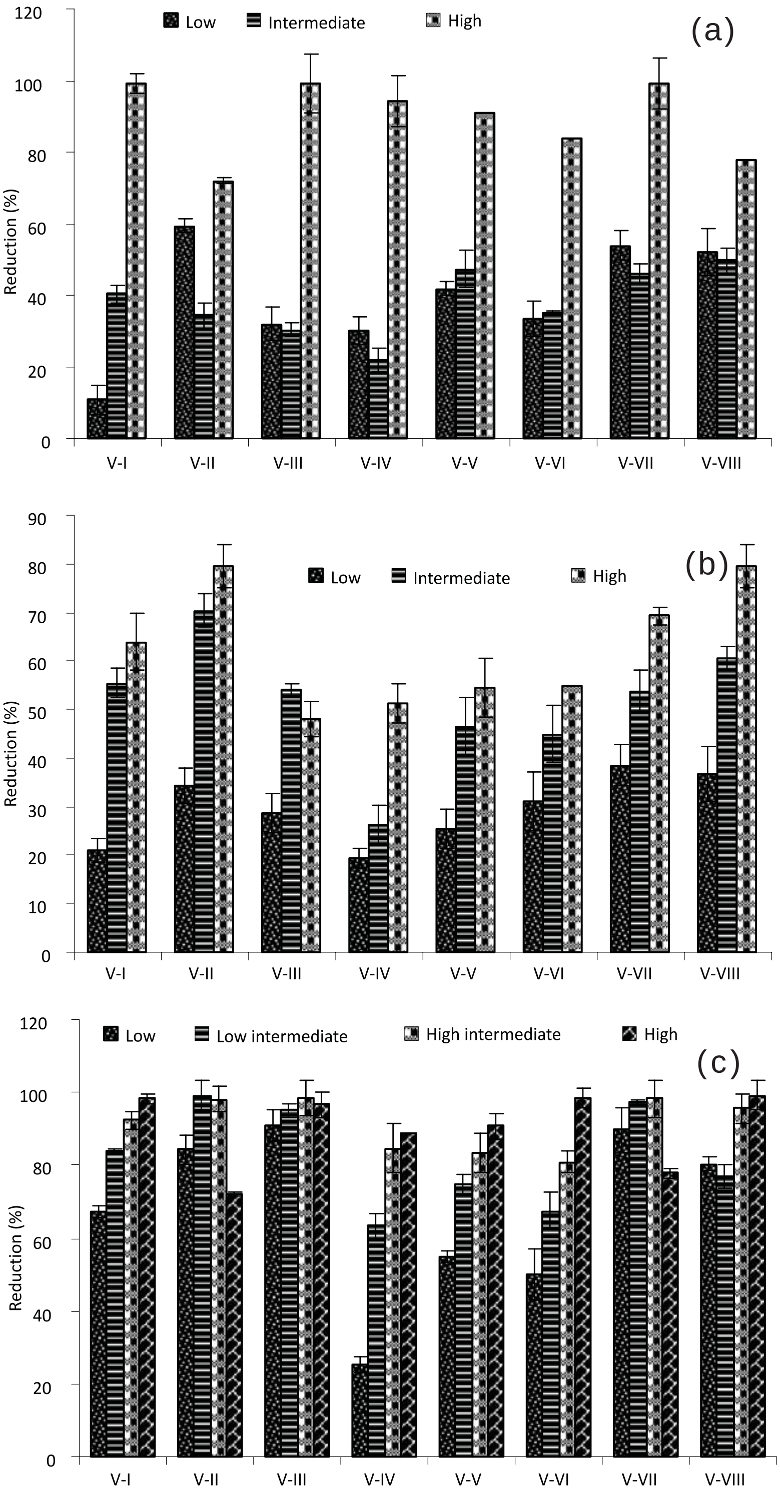

Fig.1. Effect of different temperature regimes (a),different levels of osmotic stress (b) and salinity stress (c) on germination reduction (%) in different variabilities of *A. annua*.

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