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Salinity Effects on Germination Properties of *Kochia scoparia*

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Abstract: In order to examine seed germination responses of kochia to different levels of salinity, an experiment was performed in the Institute of Sustainable Agriculture (CSIC), Cordoba, Spain, in a Completely Randomized Design with two replications. Treatments were different levels of salinities equal to 0, 5, 10, 15 and 20 dS m^{-1}, obtained by mixing NaCl and $CaCl_2$ in a 2:1 molar ratio. Evaluated properties were germination percentage, rate and final number of germinated seeds. The results showed that the germinated seed number and the germination percentage had an inverse relation with salinity of substrate. About 91% of seeds germinated in distilled water. This value reduced to about 36% in 20 dS m^{-1}. Increasing salinity up to 10 dS m^{-1} did not have any significant effect on germination, but after that, the germination rate and percentage began to reduce significantly. Salinity also increased the time required for 50%germination from 28 h in distilled water to 78 h in 20 dS m^{-1}, showing a delay in germination as salinity increased. Regarding linear reduction in germination rate in response to increase in salinity, it seems that as salinity exceeds 30 dS m^{-1}, germination rate of kochia approaches to zero. This perhaps can be accepted as the threshold of kochia germination tolerance to salinity. According to the results, the germination stage of kochia has a good tolerance to elevated levels of salinities and it seems that a good stand establishment in saline soils and water conditions can be insured, if proper management is exerted in farms.

Key words: Salinity, germination percentage, germination rate, tolerance, *Kochia scoparia*

INTRODUCTION

Arid and semi arid regions are areas with unpredictable precipitation in which potential evapotranspiration exceeds precipitation[1]. Furthermore, agriculture has faced with soil salinity as a major problem in many of these regions. Alarming extent of arid and semiarid regions in Iran (90% of total area) and extended surface of lands affected by salinity and alkalinity (15% of total area)[2] and also in other arid countries, demonstrate increasingly the need of attention to alternative systems and species which have a more coincidence with these harsh conditions and can not only use resource optimally, but also have a high production. Occurrence of these harsh conditions has led to the evolution of distinct biological forms in desert species in order to maximize their adaptation to environment[1], as to be used to provide human and livestock needs with a proper management.

The cultivation of some palatable xero- or halophytes under arid or saline stress, using available water for irrigation is a promising solution for forage shortage problem in these areas[3]. Kochia is one of these plants that with its rapid establishment on saline soils can not only produce protective short-lived vegetation coverage, but also is being used as an alternative forage crop, especially in regions faced with forage shortage[4]. Kochia also has a high yield potential. In arid climate in Texas, Sherrod[3] has reported an annual forage yield up to 11 t ha^{-1}. Also, primary studies in New Mexico showed a good irrigated and fertilized green cover of kochia with four harvests during season had a total biomass production about 26 t ha^{-1}[5].

Seed germination is usually the most critical factor determining success or failure of plant establishment[5]. Initial species establishment in saline habitats depends on their seed germination response to thermal and saline regimes and it is the level of this response that usually determines whether a population can persists until reproductive maturity or not[6]. Though increase in salinity causes decrease or delay in seed germination of both halo-and glycophytes[5-10], halophytes are differentiated

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from glycolphytes with their ability to tolerate saline conditions[11]. Most of halophytes germinate much better under non saline situations[8], but their restriction to saline environment demonstrates their requirement to relatively high salt concentration, tolerance to excessive salt levels, or competition reduction of other plants, compared to less stressful environments[11].

Kochia has a rapid germination under optimum conditions and its seed coat would penetrate usually during 24 h after imbibition[12], it also has a good tolerance to non-saline situations and germinates well under moderate and even high salinity level[4,6,7,12]. Everitt *et al.*[12] found that seed germination of kochia in distilled water was equal or more than 88%, but when seeds were subjected to distinct solutions of different salts with concentrations up to 20 dS m^{-1}, Germination Percentage (GP) started to decline significantly. Steppuhn and Wall[4] used equal NaCI and $CaCI_2$ volume to generate salinity concentrations from zero to 30 dS m^{-1} and observed salinity reduced GP generally. In their study, although GP was 95% in distilled water, it declined to 14% in 30 dS m^{-1}. Reduction of germinations percentage and rate had been reported in another species of Kochia (*Kochia americana*) and also other halpohytes[7-10].

Although there are several studies were done about the effects of salinity on seed germination of Kochia and other halophtes, most of them were on the effects of only one salt and there is not any report about unequal combination of salts on Kochia seed germination. Thus the purpose of this experiment was studying seed germination characteristics of *Kochia scoparia* under unequal combination of NaCI and $CaCI_2$ salts and threshold of its germination tolerance to salinity.

MATERIALS AND METHODS

The mature seeds used in germination tests were collected during fall 2003, from a field experimental conducted in research farm of Birjand University, Brijind, South Khorasan, Iran. Seeds were stored in paper bags at room temperature (20°C). The germination experiment was conducted when the seeds were less than 1 year old. All tests of Kochia seed germination response to different salinities were conducted in growth chambers with automatic temperature control at the Institute of sustainable agriculture (Institute De Agricultural Sostenible, CSIC), Cordoba, Spain. During the germination, temperature was maintained at 20±1°C. As light is not required for kochia germination[12], all tests were conducted in darkness. Since kochia seed coat has no effect on its germination[13], intact seeds were used.

All germination tests were done using plastic petridishes (90 mm diameter in 16 mm depth) containing 2 ashless filter papers (Watman, No. 1). One of papers placed at bottom of petridishes; seeds distributed on it randomly and covered with another one and then 10 mL saline solution were added to each petridishes. The saline solutions were prepared by adding proper amounts of NaCl and $CaCl_2$ salts in a 2:1 molar ratio to distilled water, obtaining an electrical conductivity range from 0 to 20, with 5 dS m^{-1} intervals. After adding saline solutions, petridishes were put in the second plastic container and to avoid evaporation, this second container was encased with a plastic film.

Each treatment consisted of 2 replications of 30 seeds and experiment replicated twice (2 series). Finally, results of each treatment calculated based on 4 replications of 2 series of experiment (a total of 120 seeds). The experiments were conducted in completely randomized design. Seeds were considered germinated when the tip of radicle with a 5 mm length was uncoiled[14]. Germination counting was done at 12 h intervals and germinated seeds were removed due to evasion of interference between results. Germinated seeds in each count were calculated cumulatively, until cumulative germinated seeds became stable in 2 consecutive records. Germination was considered as complete when 100% germination was done or no more germination occurred. At the end of experiments, total Germination Percentage (GP) was calculated for each replication and then, after pooling data, for each treatment. Germination Rate (GR) was determined with dividing cumulative germinated seed number in each counting per total germinated seeds and also the inverse of time taken to 50% germination (T_{50}) was calculated as an index of germination rate[1,15,16]. As the least germinated seed number was equal to 8 seeds in one of 20 dS m^{-1} replications, the time taken to germination of 8 seeds was calculated as an arbitrary index of germination rate. Before statistical analysis, normality test was performed for all data and data were transformed (Arc Sin $\sqrt{\%}$) when required. Data were analyzed by the analysis of variance and the means were compared using Duncan's Multiple Range test.

RESULTS AND DISCUSSION

Germination Percentage (GP): There was a reverse relation between the peak of cumulative germination with salinity of medium (Fig. 1). GP reduction with increase in salinity had been reported in Kochia and other halophytes[4,6-10,12]. In addition to the reduction of total germinated seeds, enhancement of salinity caused the time taken to germination stability to be increased.

Table 1: Effects of salinity on germination properties of *Kochia scoparia*

Salinity (ds m^{-1})	Average germinated seed in each petri dishes	Germination Percentage	Time taken to germination of 8 seeds (h)	Time taken to 50% germination (h)
0	27.25**a	90.84**a	20.0*b	27.8**b
5	27.5a	91.67a	25.3b	34.25b
10	26.0ab	86.67a	30.25b	37.8b
15	21.75b	72.5ab	54.5b	60.30a
20	10.75c	35.84b	143.3a	77.8a

In each column, non similar letters indicate significant difference at 0.05 (*), or 0.01(**) probability level

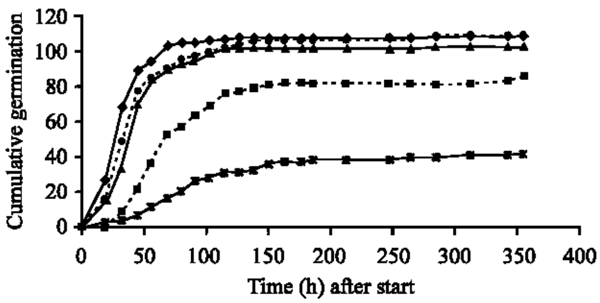

Fig. 1: The trend of cumulative kochia seed germination at 0(◇), 5(●), 10(Δ), 15(■) and 20(✳) dS m^{-1} salinity levels

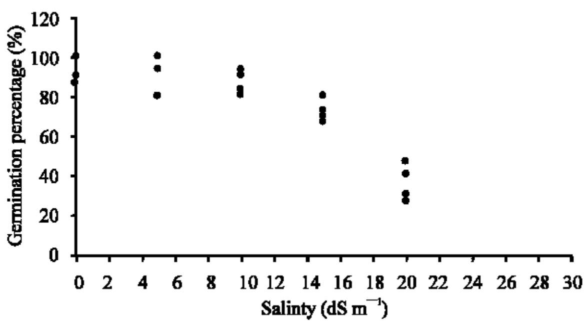

Fig. 2: Changing trend of germination percentage in different salinity levels

Though peak of germination occurred at first 100 h of experiment, but a few seeds germinated even after passing 14 days from the start of tests (Fig. 1) that indicates high genetical diversity of Kochia seed lots which maybe a reason of its success to establish in harsh environments and also shifting to a successful weed in agrosystems.

Salinity enhancement up to 10 dS m^{-1} did not have considerable effect on seed germination, but passing this threshold, germination percentage and rate decreased significantly ($p<0.01$, Fig. 1, 2 and Table 1). The extreme reduction occurred at 20 dS m^{-1}, but still more than 35% of seeds germinated at this level of salinity. In the study of Khan *et al.*[6], salinity enhancement had no effect on germination up to 200 mmol NaCl and at salt concentration of 1000 mmol, still 24% of seeds germinated. *Haloxylon ammodendron,* a halophyte, had a 50% germination at high salt concentration (1 mol salt per liter; 8) and any seeds of *Triglochin maritima* germinated at 400 mmol[10], but as Steppuhn and Wall[4] reported, there was 14% germination for kochia at 30 dS m^{-1}. Regardless of inequality of units used by researchers to explain salinity concentration, this reports clearly showed that each species has very particular germination requirements and response to salinity and also each species displays a distinct germination, based on the location and dominated climate conditions during growth and seed production.

Figure 2 clearly shows the decline of germination percentage in salinities above 10 dS m^{-1}. Of course, with this data it is very difficult to obtain the strict threshold of kochia seed tolerance to salinity, which above that, seed germination severely affected by salinity and would be reduced and would need further experiments with more salinity levels. Also with germination percentage it is very difficult to attain ultimate limit of germination tolerance to salinity, in which germination of kochia seeds will cease, maybe using of germination rate will give us better result.

Decrease of germination percentage with increase in salinity may be due to osmotic effects or exclusive ion toxicity. In a comparison between the effects of NaCl and PEG, Katembe *et al.*[9] stated NaCl decreased germination index of two Atriplex species more than PEG, because of the effect of sodium toxicity on membranes of seeds. As salinity prevents seed germination with reduction of water availability or interference with some aspects of metabolism like changing equilibrium of growth regulators[10], possibly in this experiment mixing of NaCl and $CaCl_2$ caused more disruption in critical process of seed than applying NaCl alone.

Germination rate: Increase in salinity also caused reduction of seed germination rate (Fig. 3) and seeds required a longer period to complete their germination in high salinity levels (Table 1). Figure 4 indicates as salinity increased, seed germination rate (the inverse of time taken to 50% germination) decreased linearly, as time required to this stage reached from 28 h in distilled water to 78 h at 20 dS m^{-1}. On the other hand, when about half of seeds completed their germination at the first day of test in control, it prolonged to 3 days in 20 dS m^{-1}, that clearly shows a delay in germination due to salinity

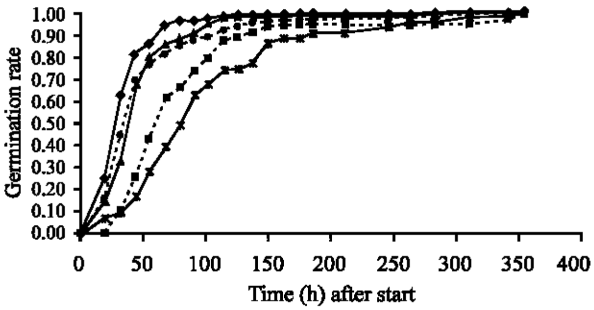

Fig. 3: The trend of germination rate of kochia seeds at 0(◇), 5(●), 10(Δ), 15(■) and 20(✳) dS m^{-1} salinity levels

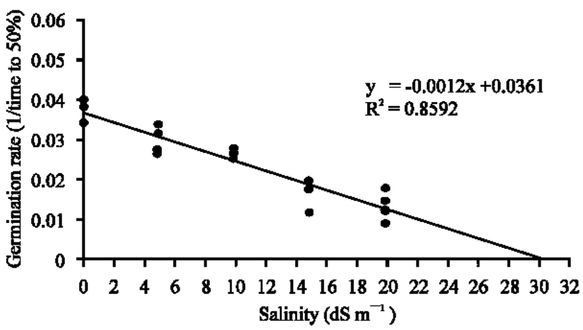

Fig. 4: Germination rate (inverse of time taken to 50% germination) of *K. scoparia* under different salinity levels. Each series of point belong to 4 replications of each treatment

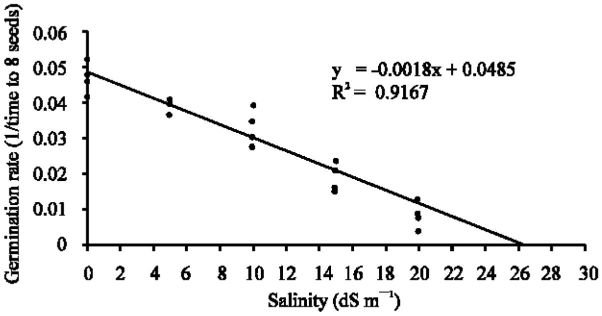

Fig. 5: Germination rate (inverse of time taken to germination of 8 seeds) of *K. scoparia* under different salinity levels. Each series of point belong to 4 replications of each treatment

enhancement; However there was not any significant difference between salinity levels for germination rate, up to 10 ds m^{-1}. in the study of Khan *et al.*[6] also as salinity increased, average required time to reach germination stability (the end of germination) increased, from 4 days in distilled water reached to 9 days in 24 and 30 dS m^{-1}.

Fitting a line between data points of GR and extrapolating it till crossing horizontal axis, the threshold of germination tolerance appears to be slightly more than 30 dS m^{-1} (Fig. 4). It seems after this salinity level, GR approaches to zero; however according to Steppuhn and wall[4], there was still 14% germination at 30 dS m^{-1}. Of course, they used equal volume of NaCl and $CaCl_2$ in preparing saline solution and this may be the reason for difference between results. It should be noted in present study that the effects of concentrations higher than 20 dS m^{-1} were not evaluated. Also, our used temperature (20°C) was different from temperature used in their study (15°C) and concerning interaction between salinity and temperature[6], this temperature difference may contribute in difference between results.

Germination rate based on 8 seeds (the inverse of time taken to 8 seeds germination) also showed a linear decrease as salinity increased (Fig. 5), however the declining slope of GR was slightly more than that of GR calculated based on 50% germination (Fig. 4). It maybe because at the start of germination, GR was very high and with elapsing time, it decreased gradually (Fig. 1 and 3). Accordingly, it is reasonable that GR of 8 seeds is higher than 50% of seeds.

Seeds of many halophytic species show optimum germination at distilled water[5,6,8,9], that indicates improving of germination with a reduction in soil salinity that usually occurs under field conditions and after spring rainfall[9]. Considering the point that there was not any significant reduction in GR and GP up to 10 dS m^{-1} (Table 1, Fig. 2 and 3), it seems it is possible to cultivate *K. scoparia* at farm using saline water with same concentration, provided soil is wetted until establishment of seedlings so that soil water potential remains at a level that does not interfere with seeds. Furthermore, it should be considered that several factors (including water. temperature, light and salinity) which interfere at soil surface regulate seed germination and may besides seasonal variation in temperature, effects temporal pattern of germination. Osmotic and matric potentials restrict effective thermal range for germination of temperate halophytes[6]. Thus at planting time in the early of spring, two issues should be consulted: if air temperature (prior, soil temperature) is high, it is evident that GP is more important than GR. In this situation, one can sow more seeds to insure a good crop stand. If temperature is low or water is salty, GR would be reduced and other factors such as microorganisms may affect the seed; thus in this situation irrigation should be done with a water that its salinity is not in such a level that reduces GR severely. Considering these points, it could be expected a desirable plant establishment with presence of saline water or soil is achievable.

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