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The Effect of Different Levels of Salinity and Potassium Nitrate on the Germination of Cocks Comb (*Celosia cristata*)

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Abstract

Cocks Comb (*Celosia cristata*) is a hot-season annual species which is grown from seeds. A study was carried out in the horticulture laboratory of Gorgan University of Agriculture Science and Natural Resources of February 2013 to evaluate the impact of salinity and potassium nitrate on the germination of cockscomb with five salinity levels (0, −2, −4, −6, and −8 bars) and three potassium nitrate levels (0%, 0.2%, and 0.4%) at 25˚C on the basis of a Randomized Complete Block Design. Analysis of variance showed significant differences among salinity levels in germination percentage, radicle length, plumule length, and seed vigor at the 1% probability level. Mean comparison for germination percentage revealed that higher salinity reduced seed germination percentage so that it was decreased from 80% in no salinity to 15% in −8 $dS \cdot m^{-1}$. The highest percentage of germination was related to zero percent salt and potassium nitrate 0.2%. Also, the highest radicle length of 2.48 cm was related to no salinity and the lowest one (0.61 cm) to −6 dS salinity. The highest radicle length and seed vigor were also observed in no salinity. Potassium nitrate by itself had no impact on the measured traits. Among interactions between salinity and potassium nitrate, the highest germination percentage was observed under 0 salinity × 0.2% potassium nitrate.

Keywords

Amarantaceae, Cocks Comb, Germination Percentage, Potassium Nitrate, Salinity

1. Introduction

Cocks Comb (*Celosia cristata*) is an annual growing up to 30 - 90 cm in height.

Its inflorescence looks like a spike. The flowers are yellow, pink, red, and purple in color. They are very durable and can be dried and used as dry flower bunch for winter [1]. Cockscombs are propagated by the seeds [2]. It is highly heliotrope and despite it is drought resistant, keeping soil moisture in hot sunny days can help the production of good flowers [1]. Cockscombs are affected by soil salinity in arid and semi-arid regions due to high temperatures and evaporation and low precipitation. Salinity reduces water potential in root zone by reducing available water potential on the one hand and some ions leave toxic impacts on plants' physiological and biochemical processes on the other hand. Both phenomena disrupt the uptake of the nutrients by the roots, and finally, reduce plants' growth [3] [4]. Salinity susceptibility of plants (both agronomical and ornamental) varies at different growth stages [5].

Grime and Campbell (1991) stated that most plants show the highest susceptibility to salinity stress at germination and flowering stages, whereas suggesting seed germination stage as the most susceptible stage [6]. Seed germination percentage and rate are among the most effective factors in salinity stress conditions [5] [7].

One adverse impact of salinity on plants is the reduction of photosynthetic activity that results in the loss of chlorophyll, CO_2 uptake, and photosynthetic capacity [8].

Germination percentage and rate are the most important factors among seed germination parameters that are affected by salinity stress [5].

Grime and Campbell (1991) stated that plants are the most susceptible to salinity stress at seed germination and early seedling growth [6]. Edward and Bison (1996) reported that the presence of certain ions and their effect on membrane permeability and germination-related enzymatic activity may accelerate germination under salinity stress [9].

There are various strategies to cope with the negative impacts of stresses. Seed priming is a method to reduce the adverse effects of stresses like salinity [10] [11], and to induce initial resistance to environmental stresses. As well, seed priming is a pre-germination physiological method to improve the seed yield and help quicker and more uniform germination [11]. The examination of the effect of different priming treatments on the germination of watermelon seeds under osmotic and thermal stresses revealed the loss of mean germination time and the increase in final germination of the seeds hydro-primed and primed with potassium nitrate as compared to control [12]. In another study, priming with potassium nitrate improved the germination of the sunflower seeds in salinity stress and hydropriming had the most favorable influence on radicle and plumule growth [13]. As salinity stress was intensified, the sunflower seeds primed with potassium nitrate and sodium chloride exhibited higher emergence rate than the unprimed seeds [14].

Today, the saline lands are growing by improper management and other related activities. As previous studies have shown, seed priming can be a good managerial approach to improve plant growth under environmental stresses, especially soil and irrigation water salinity.

Potassium nitrate is a widely used chemical for germination enhancement. The 0.1% and 0.2% solutions are common in conventional germination trials and have been recommended by AOSA and ISTA for the germination trial on most species [15].

The present study aimed at examining the influence of different treatments of salinity and potassium nitrate on germination parameters of Cocks Comb (*Celosia cristata*) seeds and determining their salinity resistance.

2. Materials and Methods

The experiment was carried out in horticulture laboratory of Gorgan University of Agriculture Science and Natural Resources, as a factorial experiment based on Randomized Complete Block Design. The experimental treatments included five levels of salinity stress (2, 0, −4, −6 and −8 bars) and three levels of potassium nitrate (0%, 0.2% and 0.4% KNO_3) with three replications and 30 seeds in each replication [16]. The prepared F_1 seeds were cultured in sterilized Petri dishes and then, they were applied with the different levels of salinity stress. To apply potassium nitrate treatments, the seeds were soaked in pre-prepared potassium nitrate solution for two hours. Then, they were placed in the Petri dishes and distilled water was added to them. Afterwards, the seed-containing Petri dishes were placed in an incubator at 25˚C, and the germinated seeds were counted twice a day (in the morning and evening) to estimate germination percentage. The trait measurements were terminated after germination and cotyledon growth stopped. The measured traits included germination percentage, germination speed, seed vigor, plumule length, radicle length, plumule fresh weight, radicle fresh weight, and normal seedling.

Germination speed (GR) was estimated by the following equation [16].

Formula 1:

$$\mathrm{GR} = \frac{\text{Germinated seed number at the first day}}{\text{Days to the first counting}} + \cdots + \frac{\text{Germinated seed number at the last day}}{\text{Days to the last counting}}$$

The following equation was used to estimate seed vigor [17].

Formula 2:

$$\text{Seed vigor index} = \frac{\text{Mean plumule length }(\text{in mm}) \times \text{germination percentage}}{100}$$

Germination percentage was obtained from the following equation [18].

Formula 3:

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}}$$

Plumule and radicle lengths were measured by a ruler, and their fresh weights were read on a digital scale. Normal seedlings were also counted in each replication when they were green and the cotyledons were fully grown.

The collected data were statistically analyzed by SAS Software Package, and the means were compared by the LSD test at the 1% and 5% probability levels. Graphs were, also, drawn by MS-Excel Software Package.

3. Results

Analysis of variance showed that germination percentage and speed, seed vigor, plumule and radicle length, plumule and radicle fresh weight, and normal seedlings were significantly affected by different levels of salinity and their interactions with potassium nitrate at the 1% and 5% probability level (Table 1).

3.1. Germination Percentage

According to means comparison, the highest germination percentage (80.05%) was obtained from no salinity, and potassium nitrate did not change it significantly. Among interactions, the highest germination percentage of 88.33% was obtained from no salinity and no nitrate potassium application, which did not have significant differences with the application of 0.2% KNO_3 under −2 bars salinity and 0.2% KNO_3 under −4 bars salinity (Tables 2-4).

3.2. Germination Speed

Means comparison revealed that the maximum germination rate (5.57) was obtained from no salinity level, and KNO_3 could not by itself affect it significantly. Among interactions, the highest germination rate (5.94) was observed in 0.4%

Table 1. Analysis of variance of measured characteristics of Cocks Comb under salinity stress.

S.O.V	df	Germination percentage	Germination speed	Plumule length	Radicle length	Plumule fresh weight	Radicle fresh weight	Seed vigor	Normal seedling percentage
Nitrate	2	170.556^{ns}	0.056^{ns}	0.001^{ns}	0.023^{ns}	1503.438**	0.0000072^{ns}	196.519**	166.280^{ns}
Salinity	4	6524.444**	34.308**	7.831**	8.777**	315.598**	0.00028**	1205.900**	10464.821**
Salinity × Nitrate	8	124.028^{ns}	0.574^{ns}	0.078^{ns}	0.454*	311.870**	0.0000013^{ns}	319.183**	85.628^{ns}
Error	30	125.000	0.878	0.052	0.160	8.244	0.00001344	27.781	113.938

**, * and ^{ns}: Respectively significant difference and at 5% and 1% and non-significant.

Table 2. The effect of salinity stress on measured characteristics of Cocks Comb.

Salinity	Germination percentage (%)	Germination speed (Number of germinated seeds in day)	Plumule length (cm)	Radicle length (cm)	Plumule fresh weight (g)	Radicle fresh weight (g)	Seed vigor (%)	Normal seedling percentage
0	80.56^{a}	5.57^{a}	2.26^{a}	2.48^{a}	13.09^{a}	0.015^{a}	31.21^{a}	85.19^{a}
−2 bars	76.11^{ab}	5.041^{ab}	2.22^{a}	1.87^{b}	10.27^{b}	0.008^{b}	25.28^{b}	80.48^{a}
−4 bars	71.67^{ab}	4.45^{bc}	1.85^{b}	1.35^{c}	5.76^{c}	0.008^{b}	23.1^{b}	62.8^{b}
−6 bars	69.44^{b}	4.062^{c}	1.41^{c}	0.61^{d}	0.026^{d}	0.006^{b}	14.08^{c}	51.87^{c}
−8 bars	15^{c}	0.61^{d}	0^{d}	0^{d}	0^{d}	0^{c}	1.5^{d}	0^{d}

In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test.

Table 3. The effect of potassium nitrate on measured characteristics of Cocks Comb.

Potassium nitrate levels	Germination percentage	Germination speed (Number of germinated seeds in day)	Plumule length (cm)	Radicle length (cm)	Plumule fresh weight (g)	Radicle fresh weight(g)	Seed vigor (%)	Normal seedling percentage
zero	58.66^{a}	3.93^{a}	1.54^{a}	1.24^{a}	17.39^{a}	0.008^{a}	20.25^{a}	2.24^{a}
0.2 %	64.66^{a}	4.01^{a}	1.56^{a}	1.24^{a}	0.30^{b}	0.007^{a}	19.94^{a}	57.66^{a}
0.4 %	64.33^{a}	3.89^{a}	1.54^{a}	1.31^{a}	0.07^{b}	0.007^{a}	21.89^{a}	58.30^{a}

In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test.

Table 4. The interaction between salinity stress and potassium nitrate on measured characteristics of Cocks Comb.

Potassium Nitrate	salinity	Germination percentage (%)	Germination speed (Number of germinated seeds in day)	Plumule length (cm)	Radicle length (cm)	Plumule fresh weight (g)	Radicle fresh weight (g)	Seed vigor (%)	Normal seedling percentage (%)
zero	zero	88.33^{a}	5.33^{a}	2.26^{a}	2.50^{a}	39.21^{a}	0.01^{a}	39.21^{a}	84.74^{a}
	−2	70^{c}	5.03^{a}	2.23^{a}	2.10^{a}	30.50^{b}	0.009^{dc}	30.50^{ab}	81.66^{ab}
	−4	63.33^{cd}	4.94^{ab}	1.7^{b}	1^{bc}	17.23^{c}	0.009^{dc}	17.23^{d}	55.19^{d}
	−6	58.33^{d}	3.53^{c}	1.53^{b}	0.62^{cd}	0.02^{d}	0.007^{bc}	12.47^{d}	39.62^{e}
	−8	18.33^{e}	0.85^{d}	0^{c}	0^{d}	0^{d}	0^{d}	1.83^{e}	0^{f}
0.2 percentage	zero	81.66^{ab}	5.44^{a}	2.43^{a}	2.46^{a}	0.03^{d}	0.01^{a}	1.83^{e}	85.66^{a}
	−2	80 ^{ab}	5.22^{a}	2.30^{a}	2.16^{a}	0.04^{d}	0.008^{c}	36.01^{ab}	78^{ab}
	−4	76.66^{bc}	4.62^{b}	1.73^{b}	0.96 ^{bc}	0.03^{d}	0.007^{bc}	21.01^{cd}	64.55^{c}
	−6	76.66^{bc}	4.44^{b}	1.33^{b}	0.63^{cd}	0.03^{d}	0.006^{b}	15.13d	60.11^{cd}
	−8	8.33^{f}	0.32^{d}	0^{c}	0^{d}	0^{d}	0^{d}	0.83^{e}	0^{f}
0.4 percentage	zero	76.66^{bc}	5.94^{a}	2.10^{a}	2.50^{a}	0.03^{d}	0.01^{a}	34.81^{ab}	85.17^{a}
	−2	78.33^{c}	4.86^{b}	2.13^{a}	1.36^{b}	0.28^{d}	0.008^{c}	27.11^{bc}	81.78ab
	−4	75^{bc}	3.79^{c}	2.13^{a}	2.10^{a}	0.03^{d}	0.008^{c}	31.06^{e}	68.67^{c}
	−6	73^{bc}	4.21^{b}	1.36^{b}	0.6^{cd}	0.02^{d}	0.005^{b}	14.63^{d}	55.87^{d}
	−8	88.33^{a}	5.33^{a}	2.26^{a}	2.50^{a}	39.21^{a}	0.01^{a}	39.21^{a}	84.74^{a}

In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test.

KNO_3 and no salinity, but it showed no significant differences with the interaction of 0% KNO_3 × 0 salinity, 0% KNO_3 × −2 bars salinity, 0.2% KNO_3 × 0 salinity, and 0.2% KNO_3 × −2 bars salinity. The lowest germination rate (0.61) was observed at salinity level of −8 bars (Tables 2-4).

3.3. Plumule Length

According to means comparison, the maximum plumule length was observed at no salinity level and among interactions, the maximum one was observed in 0.2% KNO_3 × 0 bars salinity. The minimum plumule length was related to −6 bars salinity, and no plumule grew at −8 bars salinity. KNO_3 could not alone make any significant differences in plumule length (Tables 2-4).

3.4. Radicle Length

Means comparison showed that the maximum radicle length was related to 0 bars salinity and that among interactions, 0% KNO_3 × 0 bars salinity and 0.4% KNO_3 × 0 bars salinity were related to the maximum radicle length. The lowest radicle length was observed at salinity level of −6 bars and no radicles were produced at salinity level of −8 bars. Potassium nitrate could not alone change radicle length significantly (Tables 2-4).

3.5. Plumule Fresh Weight

Means comparison showed the maximum plumule fresh weight at salinity level of 0 bars and among interactions, under 0% KNO_3 × 0 bars salinity. The lowest plumule fresh weight was observed at salinity level of −6 bars and no radicles were produced at salinity level of −8 bars. Among different rates of potassium nitrate, the highest plumule fresh weight was observed at 0% KNO_3 rate (Tables 2-4).

3.6. Radicle Fresh Weight

As means comparison showed, the maximum radicle fresh weight was observed at salinity level of 0 bars and among interactions, under 0% KNO_3 × 0 bars salinity, 0.2% KNO_3 × 0 bars salinity, and 0.4% KNO_3 × 0 bars salinity. Salinity levels of −2, −4 and −6 bars exhibited no significant differences in radicle fresh weight, and no radicles were observed at −8 bars salinity. Potassium nitrate by itself could not affect radicle fresh weight significantly (Tables 2-4).

3.7. Seed Vigor

According to means comparison, the maximum seed vigor was associated with 0 bars salinity and no significant differences were found among different rates of potassium nitrate in seed vigor. Among interactions, the highest seed vigor was observed at 0% KNO_3 × 0 bars salinity which was not significantly different from that obtained from 0.2% KNO_3 × 0 bars salinity and 0.4% KNO_3 × 0 bars salinity (Tables 2-4).

3.8. Normal Seedling Percentage

As means comparison revealed, maximum normal seedling percentage was obtained from 0 and −2 bars salinity. Potassium nitrate did not influence normal seedling percentage significantly. Among interactions, the highest normal seedling percentage was obtained from the application of 0%, 0.2% or 0.4% KNO_3 under no salinity. It was not significantly different from that obtained from the interaction of 0%, 0.2%, or 0.4% KNO_3 with −2 bars salinity (Tables 2-4).

4. Discussion

As the salinity is intensified and the electrical conductivity of the solution is increased from control to 12 dS, germination percentage is lost [16].

According to Fenando *et al.* (2000), the increase in salinity resulted in the loss of germination of *Chenopodium quinona* [3]. In addition, Rajabi and Poustini (2005) reported that 0 and 3 $dS \cdot m^{-1}$ salinities were significant different in terms of germination percentage and that the stress caused ion toxicity and the loss of germination [7].

The loss of germination percentage and rate by higher salinity can be related to the excessive presence of cations and anions that, beside causing toxicity, reduce water potential because of their solubility in water, so that despite the existence of water in the environment, their reactionary capacity is occupied by the ions and so, plants cannot uptake them, facing a kind of water deficiency [19].

In Rajabi and Poustini (2005) and Khaleghi and Moallemi (2009), higher salinity and EC decreased root length [7] [16].

Overall, the loss of germination and seedling growth under higher concentrations of salts in the medium is related the physico-chemical effects or toxic-osmotic consequences of the minerals contained in saline solution. In fact, as osmotic pressure is increased by higher salinity of the medium, seed imbibitions are disrupted on the one hand, and higher concentration of anions and cations in the medium becomes toxic to seeds, hindering their germination [4] [7]. Furthermore, the negative impacts of salinity on membrane permeability, cell division, protein synthesis, and enzymatic activity extends mean germination time and reduces germination speed and radicle elongation [20].

In a study on *Aragania spinosa*, significant differences were found among salinity levels in germination rate, radicle length, and dry weight and as salinity concentration was increased, germination time was not affected; rather, germination rate, root length, and radicle dry weight were decreased [21]. Also, relatively numerous studies on different plants have shown that higher salinity results in the loss of plumule and radicle length and dry weight as compared to control [22].

The increase in salinity level under in vitro conditions was associated with the loss of germination percentage of all priming treatments, so that 4 $dS \cdot m^{-1}$ salinity resulted in lower germination percentage in control, hydroprimed seeds and seeds primed with 3 and 6 $g \cdot l^{-1}$ KNO_3 than no salinity by 34%, 14% and 10% [23].

Higher germination percentage and emergence of primed seeds under salinity conditions are related to the fulfillment of some germination processes during seed priming including imbibitions and the synthesis of nucleic acids [24] which shortens germination time and then, seeds would need shorter time for germination that unprimed seeds under salinity conditions. Furthermore, mean germination time and emergence are important factors in improving emergence percentage of primed seeds under stress conditions as compared to control (unprimed) seeds. It has been suggested that faster exit of radicle and plumule from primed seeds is caused by more efficient water uptake and metabolic activity during germination [25] and that higher water uptake capability of primed seeds vs. unprimed seeds influences germination percentage and rate positively [26]. Also, accelerated germination of primed seeds can be related to higher cell division rate in these seeds [27] and the stimulation of some metabolic activities engaged in the first phase of germination [28].

It has been reported that the priming of cotton seeds decreased mean germination and emergence time, thereby playing a key role in improving the measured parameters of the seedlings in laboratorial conditions and of the greenhouse-grown plants [23]. Indeed, priming shortened this phase of plant life, helping them to exploit the environmental resources for the growth and salinity adaptation more quickly. Also in unstressed conditions, seed hydropriming can be a good strategy to improve initial growth of the cotton plants due to its inexpensiveness and availability in different conditions. However, in case of the salinity stress, priming with the appropriate dosages of potassium nitrate (below toxicity level) can be a suitable method to make the cotton plants cv. "Sahel" and the resulting seedlings stress resistant.

The salinity induced loss of plant growth may be caused by the disruption of the uptake of the nutrients, disturbance of ion balance or the loss of water potential in soil and osmotic stress, or may be caused by the variation of the availability of the enzymes effective on photosynthesizing system. Stem and root length are the main parameters to monitor the impact of environmental stresses, especially salinity and drought because root is in a direct contact with soil absorbing water and minerals and stem mobilizes them towards other parts. Therefore, the longitudinal variations of these two parameters (stem and root) signal the plant responses to drought stress [29].

The desirable impact of seed priming on germination percentage and rate has been reported by many studies. For example, Demir Kaya *et al.* (2006) observed higher germination percentage and rate in sunflower seeds primed with potassium nitrate under salinity stress [12]. Farooqh *et al.* (2006) related germination improvement of two rice cultivars to priming with potassium chloride and calcium chloride [30]. Germination rate has been reported to be improved by hydropriming in plants like corn and cotton [31]. Some researchers suggest that higher water uptake in the primed seeds vs. unprimed seeds positively affected germination percentage and rate [26].

Some studies have revealed the improvement of radicle and plumule length in primed seeds. It has been shown that seed priming with potassium nitrate increased radicle and plumule length of seedlings grown from the seeds of different plants under salinity conditions [32].

Kattimani *et al.* (1999) reported higher seed vigor due to priming with nitrate solutions [33] and Artola *et al.* (2003) noted the favorable impact of hydropriming on the seed vigor of *Melilotus officinalis* [34].

Soleimani *et al.* (2012) concluded that salinity reduced germination percentage and rate, plumule and radicle length, seedling fresh weight, and seed vigor [23]. The species did not show similar responses to different pre-treatments, so that priming of *Nigella sativa* with potassium nitrate, distilled water, and potassium chloride improved germination and seedling features under salinity but priming with calcium chloride and sodium chloride did not have any positive effects.

5. Conclusion

Germination percentage was 80.05% in control, reached 69.44% at −6 bars salinity and was reduced to as low as 15% under −8 bars salinity. So, it can be said that Cocks Comb (*Celosia cristata*) is a semi-resistant species to salinity. Also, normal seedling percentage was 85.19% in control which was reduced to 51.87% in −6 bars salinity. Most traits were lost by higher salinity. Potassium nitrate by itself had no significant effect on the measured traits, but its interaction with salinity was significant for radicle length, plumule weight, and seed vigor.

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