Research Article

Effect of sodium chloride, potassium chloride on germination and growth of Foxtail millet (*Setaria italica* L.)

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Abstract

Foxtail millet (*Setaria italica* L.) is the second most important species of Millet belonging to family Poaceae. It is a cereal crop mainly planted in poor sites of East Asia. In the present study the impact of different concentrations (100 mM, 150 mM, 200 mM, and 250 mM) of NaCl, KCl separate and in combination of both these salts in equal amounts was studied on germination rate and some growth parameters of foxtail millet (*Setaria italica* L.) using filter paper bioassay and pot experiment. Results showed that 100 mM of NaCl and KCl had the least effect on germination and growth of foxtail millet. However, under all the higher concentrations (150, 200, and 250 mM) of NaCl, KCl marked decrease in radicle length, plumule length, germination rate, stem length, stem thickness, leaves length, leaves number, number of tillers, whole plant weight, spike length, number of grains per spike and weight of 50 grains was recorded as compared to control plants. In combined salts stress, a marked reduction in all growth parameters of the test plant in all salt levels was recorded. It is concluded that with an increase in salt concentration, the effects of salt stress became more pronounced in all salt treatments. The results revealed that the effects of combined salts on the test plants were more pronounced as compared to the effects when the plants were treated with NaCl and KCl salts alone.

Keywords: Foxtail millet (*Setaria italica* L.); KCl; NaCl; Poaceae; Salt stress

Introduction

Millets are small-seeded annual C₄ grasses having a short life cycle. Millets belong to family Poaceae. It includes five genera: *Setaria*, *Pennisetum*, *Panicum*, *Echinochloa*, and *Paspalum*. Millets are rich in minerals, vitamins, phytochemicals, and protein and can be consumed as a good source of energy [1]. Foxtail millet (*Setaria italica* L.) and barnyard millet (*Echinochloa frumentacea*)

are the cultivated species. Foxtail millet (*Setaria italica* L.) is the second most important species of millet. It is a cereal crop for poor people, planted mainly in East Asia including Bangladesh, India, and China. Plant growth and yield are affected by a number of biotic and abiotic factors. Abiotic factors are drought [2], cold [3], high temperature [4], salinity [5], heavy metals [6] and ultraviolet radiations [7]. Increased soil

salinity is amongst the major factors affecting plant productivity as compared to other abiotic stresses [8, 9]. Salinity affects morphological development as well as a number of physiological processes such as gaseous exchange, protein synthesis, the activities of antioxidants, disruption of membrane functions [10-12] and oxidative stress [13]. Salinity also reduces CO₂ assimilation due to which metabolic activities of microorganisms get disturbed [14]. It also decreases the water potential and ionic imbalance due to the accumulation of certain ions like Na⁺ and Cl⁻ and the high concentration of these ions become toxic to root cell or may cause nutritional imbalances [15-17]. More than 800 million hectares of land around the world has been recorded unproductive due to salinity [18]. The decline in production of crops has been reached to 50 percent [19]. In Pakistan, almost 12.9% of the land is adversely affected by salinity [20]. Salts of sodium, calcium, magnesium, are found in soil in large amount while those of potassium, carbonate, and bicarbonate are found in small amounts [21]. Salt stress is divided into two main types of stresses on the basis of the origin of salts from different compounds i.e. natural salt stress and alkaline salt stress [22]. The salt tolerance of the plant can be checked and improved with the exogenous application of certain stress alleviating chemicals [23]. The main purpose of this study was to examine the effect of sodium chloride, potassium chloride and combination of both salts on various parameters of foxtail millet (*Setaria italica* L.) including germination rate, plumule length, radicle length in filter paper bioassay and stem length and thickness, number of tillers, number and length of leaves, spike length, weight of whole plant, number of seeds per spike and weight of 50 seeds under pot experiment.

Materials and methods

Filter paper bioassay

Sterilization of petri dishes

To avoid microbial activities petri dishes selected for lab experiments were first washed, and then sterilized for 72 hours at 100 degrees centigrade. Seeds of Foxtail millet (*Setaria italica*) were germinated in these sterilized petri dishes.

Seeds germination and salt treatments

Stock solutions of 100 mM, 150 mM and 200 mM each of NaCl and KCl. For combined effect, solutions of the respective concentrations of both the salts were mixed in equal proportion. All the solution was stored at 4 °C till use. Five seeds of foxtail millet were placed on two-fold filter paper in three Petri dishes and were provided with 100 mM of the respective salts solution or distilled water in case of control. After 10 days, different parameters like seed germination rate, and length of radicle and plumule of the seedlings were recorded.

Pot culture experiment

Three sets with 15 pots each were prepared by filling each pot with 5 kg mixture of soil and sand (3:1). The pots were lined with plastic bags to prevent leaching. After that, 10 seeds of foxtail millet were sown in each pot. - The first set was used for NaCl treatment in which 3 pots were treated with 100 mM salt concentrations, 3 pots with 150 mM, 3 pots with 200 mM, 3 pots with 250 mM and 3 pots were run as a control. Similarly, set 2 and 3 consisted of the same number of pots and treated with the same concentrations but utilized KCl solutions or combined salts treatment (NaCl +KCl). Thinning of plants was done by hand picking, leaving 5 plants in each pot. A total of three salt doses of the described concentrations were applied after every seven days. The first salt stress was applied at the two-leaf stage. Data of different parameters were recorded after every 7 days. The studied parameters included stem length and thickness, number

of leaves and tillers, leaves length, spike length, the weight of the whole plant, number of seeds per spike, the weight of 50 seeds in each concentration and control. The recorded data were then arranged into figures and tables.

Results

Laboratory work

The percent germination, plumule and radicle length of foxtail millet plants under different concentrations of NaCl, KCl, and combination of both salts were recorded after 10 days of the seedlings.

Effect of NaCl, KCl and combined salt stress on percent germination

All the treatments of different salts (NaCl, KCl, and NaCl+KCl) reduced percent germination of foxtail millet (Figure 1). Germination was reduced to 80% and 70% by 150 mM of NaCl and KCl solution. Treatments of 200 mM of NaCl and KCl also reduced 60% and 50% germination respectively. The maximum reduction in percent germination was observed in 200 mM concentration of NaCl+KCl solution. From the results, it is revealed that NaCl+KCl act as a stronger inhibitor for the germination of the plant as compared to individual plants stress.

Effect of NaCl, KCl and combined salt stress on plumule length

The influence on plumule growth increased with increasing salt levels (Figure 2). Least effect on plumule length of foxtail millet was

noted in 100 mM NaCl concentration, which is 2.9 cm compared to 5.9 cm in control. Results revealed that KCl inhibited plumule growth of foxtail millet in all the three concentrations than NaCl. The effect of combined salts on plumule length was more prominent as compared to the effect of individual salt. In high concentration of 200 mM, the effect on radicle length increased many folds reducing its length to 0.9 cm, 0.4 cm in NaCl and KCl respectively. The combined salts in high concentrations totally inhibited the plumule length of foxtail millet as compared to 5.9 cm recorded in control.

Effect of NaCl, KCl and combined salt stress on radicle length

Foxtail millet showed a significant reduction in radicle length in all concentrations of combined salts. The maximum reduction in radicle length has been recorded in 200 mM concentration of combined salts which is 0.2 cm compared to 3.7 cm in control. Lowest effect on radicle length is noted at 100mM of all the three types of salt treatments compared to control. With increasing salt concentrations their effects became more profound in all treatments. Radicle length in 100 mM, 150 mM, and 200 mM NaCl concentration is 1.9 cm, 1.8 cm, and 1.5 cm respectively. Similarly, radicle length in 100 mM, 150 mM, and 200 mM of KCl concentrations is 1.1 cm, 0.7 cm and 0.5 cm respectively (Figure 3).

Figure 1. Effect of NaCl, KCl and Combined salt stress on Percent germination

Figure 2. Effect of NaCl, KCl and Combined salt stress on plumule length

Figure 3. Effect of NaCl, KCl and Combined salt stress on radicle length

Field Work

Effect of NaCl, KCl and combined salts on stem length and stem breadth

Stem length

Maximum stem length was observed at control in all three doses while dramatic effects of NaCl, KCl and combined salts on stem length were observed at higher salt concentrations. At 100 mM concentration, a slight reduction in stem length has been observed. At 150 mM of NaCl, stem length was 5.8 cm, 9.7cm and 52 cm for dose 1, dose 2, and dose 3 respectively. Similarly, a gradual reduction in stem length was

observed at 200 mM NaCl. At 250 mM of NaCl stem length reduced to 4.2 cm, 7.4 cm, and 38 cm in dose 1, dose 2, and dose 3 respectively (Table 1). Lowest effects on stem length of foxtail millet were noted in 100 mM KCl concentration i.e. 5.8 cm, 9.6 cm, 48 cm in all three doses respectively. After the application of dose 3, the length of the stem was 45 cm, 40 cm and 36.8 cm in 150 mM, 200 mM, and 250 mM respectively. By comparing results of stem length in case of combined salt treatment with individual treatments, it is clear that stem length is badly

affected in all the concentration of combined salts of NaCl and KCl.

Stem breadth

In (Table 1) indicated that stem breadth recorded to be 0.2 in control which is similar to the effect of NaCl stress at 100 mM concentration. In 150 mM concentration of NaCl stem breadth was recorded to be 0.2 cm, 0.2 cm and 0.3 cm for dose 1 dose 2 and dose 3 respectively. While 200 mM and 250 mM have the same effect at all the three doses. Maximum stem breadth was recorded at dose 3 (0.4 cm) in control plants. The minimum stem breadth was recorded (0.2 cm) in 250 mM concentration of NaCl. In case of KCl (Table 1), the effect of 100 mM concentrations on stem breadth in dose 1 and dose 2 was niggling while maximum in dose 3 (0.3 cm) as compared to control (0.4 cm). Maximum reduction (0.2 cm) in stem breadth has been observed at 200 mM and 250 mM in dose 3 (0.2 cm) as compared to control (0.4 cm). In comparison to NaCl, the effect of KCl stress on stem breadth was slightly adverse. The treatments of 150 mM, 200 mM, and 250 mM concentrations of combined salts negatively affected stem breadth and it was minimum in 250mM (0.2 cm) compared to control (0.4 cm) in dose 3. Results showed that combined salts adversely affected stem breadth compared to NaCl and KCl.

Effect of NaCl, KCl and combined salts on leaf length and number

Leaf length

In (Table 2) revealed that in 100 mM and 150 mM treatment of NaCl, no marked reduction in leaf length was observed. In 250 mM of NaCl, leaf length was reduced to 6.9 cm, 7 cm and 14 cm in dose 1, dose 2, and dose 3 respectively. An inverse relationship was observed among leaf length and increasing salt concentrations. Under high concentration of KCl, leaf length was adversely affected as compared to control (24 cm) and the leaf length recorded in dose 3 was 16 cm, 15 cm and 13 cm in 150 mM, 200 mM and 250 mM

respectively. KCl is posing stronger stress on leaf length in comparison to NaCl. At 150 mM combined salt stress, the leaf length was recorded to be 6.8 cm, 7.5 cm and 15 cm for dose 1, dose 2 and dose 3 respectively. In 250 mM leaf length has been reduced to 5.8 cm, 6.3 cm and 12 cm for dose 1, dose 2 and dose 3 respectively. It is clear that leaf length is severely affected in all the concentration of combined salts of NaCl and KCl.

Number of leaves

The higher number of leaves were counted in all doses of control plants (Table 2). The lower concentration (100 mM) of NaCl had the least effect on the number of leaves. In 150 mM NaCl treatment 5, 8.4 and 11.5 mean number of leaves was counted in dose 1, dose 2 and dose 3 respectively. The KCl treatment decreased the mean number of leaves more effectively as compared to NaCl (Table 2). The maximum number of leaves were counted in control (16) in dose 3 and a minimum number of leaves (7) were counted in 250 mM KCl solution in dose 3. In 200 mM KCl treatment mean a number of leaves recorded to be 5, 7.5 and 9 in the three doses respectively. In 150 mM and 200 mM, leaves mean number reduced to 7 and 6.4 after application of dose 2 as compared to 9.7 in control plants. Minimum values of leaf number i.e. 6.5 and 7 were observed in dose 2 and dose 3 respectively at 250 mM concentration.

Effect of NaCl, KCl and combined salts on number of tillers

In 100 mM of KCl 0, 2 and 4 tillers were observed in dose 1, dose 2 and dose 3 respectively, whereas the number of tillers was counted to be 0, 2 and 3 at same doses in 150 mM of KCl. The number of tillers was approximately the same in both 100 mM and 150 mM concentrations of KCl. However, in 200 mM and 250 mM of KCl, only one tiller appeared in dose 2, while in 200 mM of one more tiller appeared at dose 3. KCl treatment highly reduced the number of tillers as

compared to NaCl. The impact of combined salt on a number of tillers is more pronounced (Table 3). In 200 mM and 250 mM concentration of NaCl+KCl highly reduced tiller number in dose 2 and dose 3 i.e. only one tiller was observed in both salt doses. Combination of both salts (NaCl+KCl) profoundly affected the appearance of tillers in all salt concentration as compared to KCl and NaCl stress alone.

Effect of NaCl, KCl and combined salts on Spike length, plant weight and weight of 50 grains

Spike length

In (Table 4) showed that the spike length was maximum (10.2 cm) at control and minimum (2.1 cm) at 250 mM of combined salts. Lowest effect on spike length of foxtail millet was noted in 100 mM concentration of NaCl with the spike length of 6.1 cm compared to 10.2 cm in control. Combined salts profoundly affected the spike length in all treatment as compared to NaCl and KCl alone. In higher salt concentration of 250 mM, the effect on spike length increased many folds reducing its length to 2.7 cm in NaCl and in KCl respectively. Spike length of the plant in 250 mM of combined salts was 2.1 cm, which is very low as compared to control plants i.e. 10.2 cm.

Plant weight

Results (Table 4) showed that in case of NaCl treatment, plant weight was 3.4 g, 2.4 g, 1.6 g and 1.1 g in 100 mM, 150 mM, 200 mM, and 250 mM concentrations respectively. The effect of salt stress was more pronounced under higher salt levels of NaCl. The maximum reduction in plant weight has been recorded at 200 mM of combined salts (0.6 g) and KCl salt. Similar plant weight (1.3 g) has been recorded at 150 mM of both NaCl and KCl.

The weight of 50 grains

Weight of 50 grains was recorded to be 0.8 g, 0.6 g, 0.4 g and 0.3 g at 100 mM, 150 mM, 200 mM and 250 mM concentrations respectively. This showed that the effect of salt stress became more pronounced at higher salt levels of NaCl (Table 4). Maximum weight has been recorded at control (1.2 g) and minimum at 250 mM in KCl (0.2 g) and combined salts (0.1 g). In both KCl and combined salts treatments, the weight of grains was highly reduced as compared to NaCl stress. The effect of KCl and combined salts on the weight of grains were much profound as compared to NaCl stress (Table 4).

Table 1. Effect of NaCl, KCl and combined salt stress on stem length and stem breadth (cm)

Parameter	Treatments	NaCl			KCL		Combined salts			
		*D1	**D2	***D3	D 1	D2	D 3	D 1	D2	D 3
Stem length (cm)	100 mM	6.1	10.3	56.6	5.8	9.6	48	5.1	8.6	46.1
	150 mM	5.8	9.7	52	5.2	8.9	45	4.7	7.5	40.4
	200 mM	5.2	8.2	43.5	4.3	7.8	40	4.1	6.9	36
	250 mM	4.2	7.4	38	4	7.2	36.8	3.6	6.6	32.2
	Control	6.8	12.2	62.7	6.8	12	63.3	6.8	12.2	62.9
stem breadth (cm)	100 mM	0.2	0.3	0.3	0.2	0.3	0.3	0.1	0.2	0.2
	150 mM	0.2	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.2
	200 mM	0.2	0.2	0.3	0.1	0.2	0.2	0.1	0.2	0.2
	250 mM	0.1	0.2	0.2	0.1	0.2	0.2	0.1	0.2	0.2
	Control	0.2	0.3	0.4	0.2	0.3	0.4	0.2	0.3	0.4

*D1= Dose 1 (After 7 days), **D2= Dose 2 (After 14 days), ***D3= Dose 3(After 45 days)

Table 2. Effect of NaCl, KCl and combined salt stress on leaf length (cm) and a number of leaves (mean)

Parameter	Treatments	NaCl			KCL			Combined salts		
		*D 1	**D2	***D 3	D 1	D2	D 3	D 1	D2	D 3
leaf length (cm)	100 mM	7.9	10.9	21.6	10	19	7.3	7.2	8.8	18
	150 mM	7.6	9.2	18.9	7.9	16	7	6.8	7.5	15
	200 mM	7.3	8.2	17	6.8	15	6.2	6	7	13
	250 mM	6.9	7	14	6.4	13	6	5.8	6.5	12
	Control	8.2	12.9	24.9	12.9	24	8.3	8.3	12.9	24
number of leaves (mean)	100 mM	5.1	9.7	16	5	8.6	12	5	7.4	9.9
	150 mM	5	9.6	13	5	8	10	5	7	7.6
	200 mM	5	8.4	11.5	5	7.5	9	5	6.4	6.9
	250 mM	5	7.4	9	5	6.5	7	5	5.6	6
	Control	0.2	0.3	0.4	5.1	9.7	16	5.1	9.7	15

*D1= Dose 1 (After 7 days), **D2= Dose 2 (After 14 days), ***D3= Dose 3(After 45 days)

Table 3. Effect of NaCl, KCl and combined salt stress on a number of tillers (mean)

Parameter	Treatments	NaCl			KCL			Combined salt		
		*D1	**D2	***D3	D1	D2	D3	D1	D2	D3
number of tillers (mean)	100 mM	0	3	5	0	2	4	0	1	3
	150 mM	0	2.2	5	0	2	3	0	1	2
	200 mM	0	2	4	0	1	2	0	1	1
	250 mM	0	2	3	0	1	1	0	1	1
	Control	0	3	6	0	3	6	0	3	6

*D1= Dose 1 (After 7 days), **D2= Dose 2 (After 14 days), ***D3= Dose 3(After 45 days)

Table 4. Effect of NaCl, KCl and combined (NaCl, KCl) salt stress on spike length (cm), plant weight (g) and weight (g) of 50 grains of foxtail millet (*Setaria italica*)

Treatments	spike length (cm)			plant weight (g)			weight (g) of 50 grains		
	NaCl	KCl	Combined salt	NaCl	KCl	Combined salt	NaCl	KCl	Combined salt
100 mM	6.1	4.6	4.2	3.4	2.5	2.1	0.8	0.6	0.5
150 mM	4.6	4.1	3.7	2.4	1.3	1.3	0.6	0.5	0.4
200 mM	4	3.6	3.1	1.6	1.2	1.1	0.4	0.3	0.2
250 mM	2.7	2.7	2.1	1.1	0.7	0.6	0.3	0.2	0.1
Control	10.2	10.3	10.2	5.3	5.2	5.2	1.2	1.2	1.2

Discussion

Salinity is the most important abiotic factor and badly affects seeds germination and radicle elongations of seedlings [24]. The present findings showed that salinity stress (NaCl, KCl and combined) adversely affected seed germination and other growth parameters in foxtail millet including, length

and breadth of stem, number of leaves and tillers, leaves length, spike length, weight of the whole plant, number of seeds per spike, weight of 50 seeds in all concentration. These adverse effects are concentration dependent as the plant is severely affected at higher concentration. Higher concentration of NaCl adversely affected all growth parameters i.e.

relative shoot growth weight (RSGR), shoot and root dry weight, biochemical characters like total chlorophyll content and mineral elements i.e. K^+ and K^+/Na^+ ratio in maize (*Zea mays* L.) [25]. Increasing salt concentrations dramatically affect both seedling emergence and growth of sunflower (*Helianthus annuus* L.) [26]. In comparison to NaCl, the effect of KCl stress on stem breadth was slightly adverse. Salt stress has significantly reduced stem diameter in several other different plant species [27-29]. Similarly, the effect of combined salts is more severe as compared to the effect of NaCl and KCl alone. The effect of KCl and combined salts on plant weight were much profound as compared to NaCl. Maximum plant weight was recorded at control (5.3 g) and minimum at 250 mM in KCl (0.7 g) and combined salts (0.6 g). Similar results in different plant species have also been found [30, 31]. The combination of NaCl and KCl is a strong stressor for stem breadth in foxtail millet in higher salt concentrations. The combined stress of NaCl and KCl also reduced stem diameter of wheat [32]. The leaf length is severely affected in all the concentration of combined salts of NaCl and KCl. Similarly reduction in different parameters including leaf length and a number of leaves in tomato plant has been observed [32]. By comparing results of stem length in case of combined salt treatment with individual treatments NaCl and KCl, it is clear that stem length is badly affected in all the concentration of combined salts of NaCl and KCl. Similar results have been obtained by many researchers in different plant species when grown under salt stress [33, 34].

Conclusion

It was concluded that salinity affects various growth parameters of the foxtail millet and the effects are more devastating at high concentrations in all salt treatments. Similarly, the effects of combined salts on the

test plants were more pronounced as compared to the effects when the plants were treated with NaCl and KCl salts alone.

Authors' contributions

Conceived and designed the experiments: K Natasha & SIU Haq, Performed the experiments: K Natasha, SIU Haq & S Ahmad, Analyzed the data: K Natasha, Z Ullah & Z Rahim, Contributed materials/analysis/ tools: K Natasha, SIU Haq & S Ahmad, Wrote the paper: SIU Haq.

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