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## **Research Article**

## Effect of Physiological Acid Salts on Swelling, Germination and Growth Processes of Seedlings

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## Abstract

The effect of physiological acid salts (KCl and NH<sub>4</sub>Cl) on wheat (C3), corn (C4) seeds hydration, swelling, germination processes and growth rate of sprouts was studied. It is determined that swelling is a very complex and phased process, and it follows the same way during effects of salts on kinetic regard. As in control, in experience the absorption of water, regardless of the object, was also specified with 3-phase curves, and the only difference was quantitative. It was revealed that germination of seeds and growth of sprouts gets weakened under the physiological effects of acid salts. Adverse effect of salts was higher on corn seeds. No germination in higher concentrations of salts or the low growth may be due to specific effects of ions. During the short-term effects of salt, i.e. the first stages of germination (within 3 days), the seeds were more sensitive to the adverse effects of salt. At the later stages of ontogenesis (within 7 days), higher germination and growth indicators of seeds can be explained with transition from heterotrophic feeding into autotrophic feeding, because of the defense mechanism, and adaptation to the adverse effects of salt during this period. NH4Cl has more negative impact on germination and growth processes compared to KCI. Because compared to K<sup>+</sup>, NH<sub>4</sub><sup>+</sup> cations are more rapidly absorbed and immediately join to metabolism, thus, result in more rapid acidation.

#### Keywords

Physiological acid salts; Swelling; Germination energy; Germination percentage; Germination; Growth rate

## Introduction

Most of the soils on earth are characterized by an excess amount of salt. Improper irrigation, high amounts of mineral fertilizers and other anthropogenic factors impact the increase in the amount of salts in soils. According to Pitman and Lauchli [1] 20% and according to Stroganov [2] 25% of irrigated soils on earth are salinized. According to Mahajan and Tuteja [3] because of the intense salinization until the mid-21<sup>st</sup> century 50% of the cultivated soils will get useless. Extreme salinity causes negative impact on the growth of cultivated plants, reduces productivity, at times completely destroying them [4,5]. Due to soil salinization million tons of agricultural products are destroyed every year. In this regard, study of plants' salt sustainability is an important area of study. Salt sustainability of plants depends on

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the stage of their growth. More effect of high salinity on plants occurs at the beginning of their first developmental stages. Seedlings are

more affected by salinization compared to mature plants [6-8].

Some saline soils show a high degree of acidation. It manifests excessive use of fertilizers such as KCl and NH<sub>4</sub>Cl. On such soils the pH can reach up to 3.5-4.0 [9,10]. Considering that the optimum reaction condition for cultivated plants is 5.0-7.0, the theoretical and practical study of the effect of acid salts on the growth of plants especially at the first stages of growth gains importance.

Taking into account all the above factors growth speed, intensity, hydration, swelling and germination processes under the physiological effects of acid salts at the first developmental stages were investigated.

## The Object and Methodology of the Study

Wheat (C3 plant) and corn (C4 plant) seeds were selected as the subjects of study. Grain seeds are rich in starch and corn seeds contain about 72% [11]. Physiological acid salts of KCl and NH<sub>4</sub>Cl isoanions as stress factors at different concentrations of 0.05-1.0M were used. The ions in both salts are negatively hydrated ions. Swelling process of seeds was studied by determining their weights as dry, absolute dry and wet weights within 24 hours. Each of them was weighed on analytical, torsion and electronic scales with gravity method. Determination of wet weight was done for every 30 minutes during the first 3 hours and later for it was carried out for every 3 hours. The seeds were kept on thermostat under  $105^{\circ}$ C for the determination of absolute dry weight and periodically their weights were determined on analytical KERN ABJ scales.

Seed germination energy and germination percentage were also determined during the experiment. Germination energy is defined as the total number of germinated seeds within 3 days and the germination percentage as the total number of 10-days sprouts. Daily growth size and relative growth of sprouts were recorded in order to determine the speed and intensity of growth. Lengths of root and stem of sprouts were measured for 7 days to determine the overall growth and relative growth is determined by dividing obtained size of each day with the results of the sizes of rootlet of the previous day and stem after germination.

To germinate the seeds, they were soaked in Petri cups, each cup containing 10 seeds, in distilled water (control) and 0.05; 0.1; 0.2; 0.4; 0.6; 0.8 and 1.0M (experience options) concentrated solutions of physiological salts KCl and NH<sub>4</sub>Cl and were placed inside a thermostat at a temperature of 25°C. After 24 hours, swollen seeds were put inside tubs filled with distilled water and salt solutions, and after required growth of root and stem (2-3 cm) they were put inside a thrice and average displacements were calculated on the average sizes obtained from the results [12]. Failure was not more than 5 per cent.

## **Results and Discussion**

To define the effect of different concentrations of KCl and  $\rm NH_4Cl$  physiological acid salts on swelling and germination processes of wheat and corn seeds dry, absolute dry and wet weights of the seeds were determined. As the humidity and hydration level on absolute

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dry seeds was zero, they did not show any germination in spite of soaking. Thus, seed germs are completely destroyed under the influence of a high temperature and metabolic processes and the breathing intensity stops completely. Germination of dry seeds with 10-12 per cent of moisture occurs with their water absorption and consists of swelling, cracking and heterotrophic nutrition phases of sprouts.

The determination of wet weights of seeds for every 3 hours up to 24 hours the swelling process is a very complex and phased process. Like in water, in salt version also the absorption of water, regardless of the object, was specified with 3-phase curves, i.e. swelling process observes the same rule of kinetic regard Ismayilova et al. [13] (Figures 1 and 2).

Water absorption was very rapid during the first 3 hours in all versions. During the absorption of water by dry seeds hydrostatic pressure develops up to 100 atm. due to the hydration and swelling. This water absorption speed in the first phase is linked to the physical process-diffusion. Water absorption is slowed down in the second phase. Sprouting can be visually seen in this phase which lasts for 3-12 hours. This phase is related to biochemical processes - activation of hydrolytic enzymes with water absorption and strengthening of the decomposition process. Water absorption was accelerated in the third phase and stabilized after 18 hours [14]. In general, roots and stems of sprouts begin to grow rapidly in the third phase, lasting for 60 hours. The growth of sprouts, transition to autotrophic nutrition and other accelerated physiological processes under the photosynthetic activity are the main reasons for the water absorption in this phase.

Though swelling process was the same in kinetic regard in all versions and characterized by 3-phase curves, angle coefficients (tg  $\alpha$ ) shows that there is a quantitative difference in results obtained from control and experience options. The tg  $\alpha$  calculated for wheat seeds was found to be 0.509 in control; 0.608 in KCl concentrate of 0.05M; 0.75 in 0.1 M; 1.4 in 0.2 M; 1.0 in 0.4 M and for corn seeds it was 1.31; 2.4 in KCl concentrate of 0.05M; 2.98 in 0.1M; 1.53 in 0.2M and 0.67 in 0.4 M. In the experiments conducted with NH<sub>4</sub>Cl the results for wheat seeds were found to be: 1.67 (control); 2.93 (0.05M); 2.5 (0.1M); 1.88 (0.2M); 1.0 (0.4M) and for corn seeds the results were: 1.47 (control); 1.50 (0.05M); 1.59 (0.1M); 1.85 (0.2M); 1.85 (0.4M). These quantitative differences are more apparent in figures from the control and experimental options (Figures 1 and 2).

The effect of physiological acid salts on seed germination energy and germination percentage was also determined. Germination energy is the index of energy stock owned by the seed and indicates the intensity of germination and the germination percentage indicates the quality of seed showing the effectiveness of germination.

The figures show the obtained results (Figures 3 and 4).

As shown in the figures, in experience options seed germination energy and germination percentage were lower compared to control. Adverse effects of salts were higher on corn seed germination energy compared to wheat seeds. When the effect of KCl and  $NH_4Cl$  salt solutions on the germination energy of seeds is compared, the latter had more negative effects because  $NH_4^+$  cations are more rapidly absorbed by plants as a source of nitrogen and it causes a more rapid acidation.

The adverse effect of salt solutions on seed germination percentage was not severe when compared to their effect on the germination energy. In the first stages of germination (within 3 days), seeds were more sensitive to the effects of salt. In the later stages of ontogenesis (within 7 days), higher germination and growth indicators of seeds can be explained with adaptation to the adverse effects of salts, because of the defense mechanism, and transition from heterotrophic nutrition into autotrophic during this period.

The results obtained from the experiments shows that wheat seeds are more salt-resistant compared to corn seeds. The seeds of salt-resistant plants (varieties) are characterized with higher germination energy and swelling speed, and more intensive root growth in salt conditions. The more number of sprouts in salt conditions evidence their more salt-resistant nature. Wheat seeds have germinated in KCl solutions of 0.4 M (10%) and in NH<sub>4</sub>Cl solutions of 0.4 M (35%), whereas corn seeds have not germinated. Compared to wheat seeds, corn seeds were more sensitive to the negative effects of NaCl salts [14].

Low growth rates and no germination under the influence of salts may be due to the specific effect of ions.

The effect of physiological acid salts on the growth of sprouts was also studied. Growth is the most integral indicator of life in plants; the nature of growth depends on unity of the exchange processes running in plant organisms. The intensity of growth depends on the peculiarities of species and their life conditions. Increase in the size of plants or their organs, their number, wet and dry weights, increase in cells, proteins or DNA molecule etc. are criteria for growth. Although none of these parameters separately reflect the growth, the increase in the linear size and volume of cells are considered to be important indicators of growth.

General and relative growth of sprouts have been defined in the experiments as indicators of growth, and figures were set defining the nature and intensity of growth for 3-, 5-, and 7-days seedlings (Figures 5-8).

As seen in the figures, different concentrations of KCl had negative effect on the growth of 3-, 5- and 7-days wheat seedlings (Figure 5), and the stem is more sensitive to the effects of salt. The increase in concentration resulted in a gradual decrease in the root size of seedlings compared to control, whereas the stem size of 3-days seedlings was decreased much in low concentrations (0.05 M), gradually decreased in higher concentrations (0.1 and 0.2 M), and no germination took place in 0.4M concentrations at all (Figure 5a).

Salts had more negative impact on the stem growth rather than on root in all 5-days seedlings and in high concentrations in 7-days seedlings. Unlike 3-days wheat a seed, in 5- and 7-days seedlings in the concentrations of 0.4M solution germination has occurred, the length of the root in both cases was 6 mm, and the length of the stem was 2 mm (Figure 5b, c).

Based on measurements of corn sprouts, it can be noted that stem and root sizes of 3-days seedlings soaked in KCl salt solution in low salt concentrations (0.05 M) have changed very little compared to the control, no germination occurred in other concentrations (Figure 6a).

Stem (0.05 M and 0.1 M) and root (0.05 M) sizes of 5-days seedlings in low concentrations increased compared to the control, in higher concentrations (0.2M in roots; 0.1M and 0.2M in stem) it decreased slightly, but in 0.4M the germination has not occurred (Figure 6b). In other words, salts had stimulating effect on the growth indicators in 0.05 and 0.1M concentrations. Its stimulating effects to seedlings' growth in low concentrations are confirmed by the data in the literature [15-18].



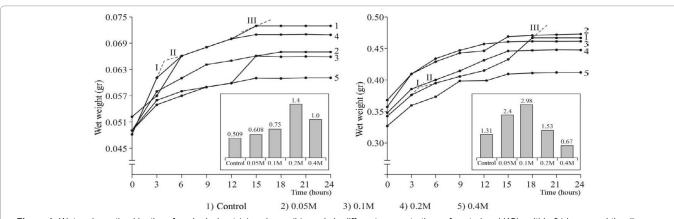


Figure 1: Water absorption kinetics of soaked wheat (a) and corn (b) seeds in different concentrations of control and KCI within 24 hours and the diagrams drawn on the basis of angle ratios.

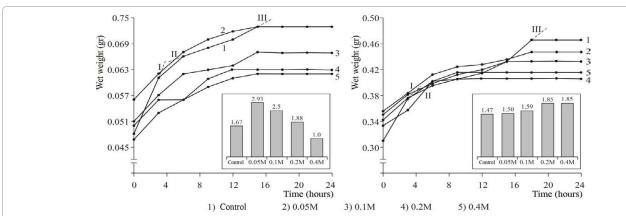
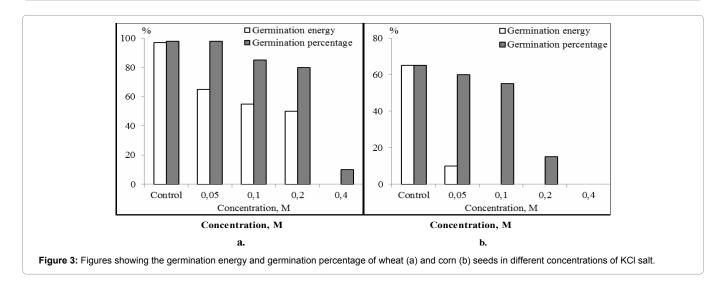


Figure 2: Water absorption kinetics of soaked wheat (a) and corn (b) seeds in different concentrations of control and KCl within 24 hours and the diagrams drawn on the basis of angle ratios.



The size of the root and stem of the 7-days seedlings were bigger compared to the 5-days seedlings. For example, the root was 20 mm and the stem was 4 mm in 5-days seedlings in 0.2 M concentration of KCl, whereas these indicators in 7-days seedlings were 32 mm and 16 mm accordingly. Under the long-term effect of salt, germination capacity of seeds increased. No germination occurred in 7-days seeds in 0.4 M concentration of KCl (Figure 6c). The effect of different concentrations of KCl salt on 3-(a), 5-(b), and 7-days (c) corn seedlings' root and stem growth. Similar results were obtained from the experiments with  $NH_4Cl$ . Wheat seedlings' root and stem growth decreased

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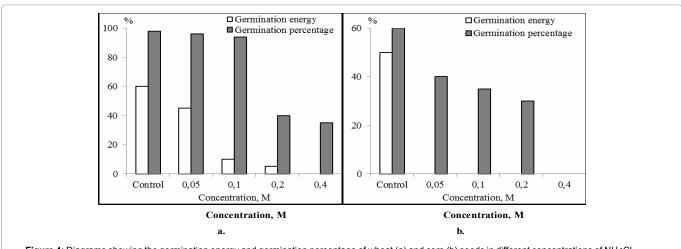
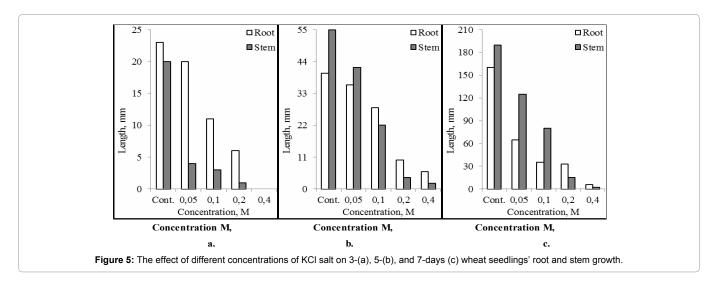


Figure 4: Diagrams showing the germination energy and germination percentage of wheat (a) and corn (b) seeds in different concentrations of NH4CI.



with the increase of salt concentration, the negative effect of the salt on the stem was higher, and no germination occurred in 0.4 M concentration (Figure 7).

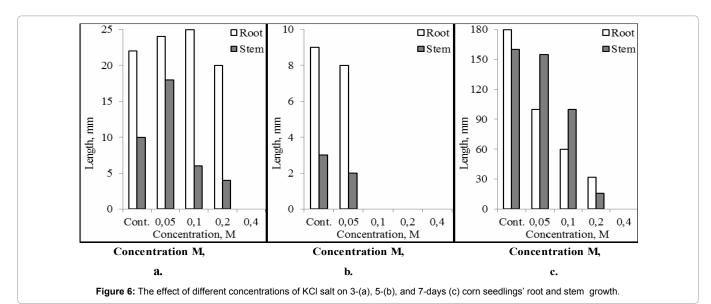
The studies conducted on the effect of different  $NH_4Cl$  concentrations on the growth indicators of corn seedlings, show that during short-term effect (within 3 days) there was no germination in any experience options, and within 5 and 7 days in all cases germination occurred, excluding 0.4 M concentration, and as concentration increased, the root and stem sizes reduced compared to control. The root and stem sizes were about 2-3 times higher in 7-days seedlings compared to 5-days seedlings (Figure 8).

So, by comparing the ability of germination and growth indicators between 3-, 5-, and 7-days seedlings of wheat and corn soaked in different concentrations (0.05 M, 0.1 M, 0.2 M and 0.4 M) of KCl and  $NH_4Cl$  physiological acid salts, it can be concluded that the harmful effects of salts in plants can be seen starting from seed germination and as we increase concentrations of salt a salt delay germination of seeds occurs and weakens the growth of seedlings. This conclusion is confirmed in the studies by other authors Khan et al. [19] Khan et al. [20] Trophimov [21] Qin et al. [22] Khasan [23] Jalal Abdu Khaid Khasan Almiklafi [24] Belozerova [25] Belozerova et al. [26]. Despite of the increased water absorption by seeds under high pressure (100 atm.) during the swelling process, no germination under the influence of salts can be explained by specific effect of ions during the short-term effects of salts, i.e. at the first stages (within 3 days) of germination the seeds were more sensitive to the adverse effects of salts. At the later stages of ontogenesis (within 7 days), the higher germination capacity is related to the increased salt sustainability of seeds and transition from heterotrophic nutrition into autotrophic.

It became clear that though each of KCl and NH<sub>4</sub> is negative hydrated ions, there are some peculiarities in their absorption by plants. NH4Cl has more negative impact on germination and growth processes compared to KCl because compared to K<sup>+</sup>, NH<sub>4</sub><sup>+</sup> cations are more rapidly absorbed and immediately join to metabolism, thus result in a more rapid acidation.

Based on the relative growth determination, it can be said that in the early days of germination the growth of the root system was higher compared to stem. Root formation results in a better absorption of water and mineral substances, formation of absorbing wires, the diversity of the root system, and therefore acceleration of growth. In most cases, the maximum growth of roots was noted in the 6<sup>th</sup> day,

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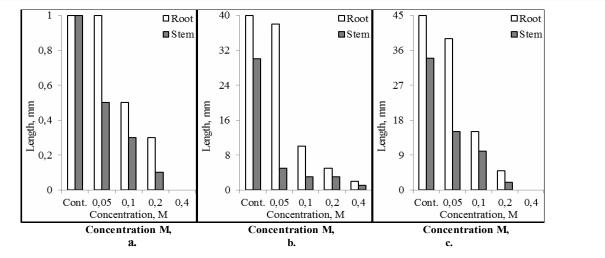
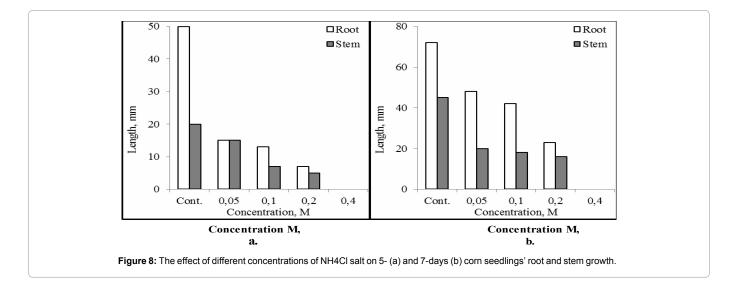


Figure 7: The effect of different concentrations of NH4Cl salt on 3-(a), 5-(b), and 7-days (c) wheat seedlings' root and stem growth.



coincided with the relative growth of the stem. The salts had negative effects on root system and the root growth decreased, stem growth increased on the contrary. The negative effect of salts on the growth of the root system has been defined in other studies as well Wanqs et al. [27]. Because the roots are in direct contact with the salts, salt directly goes to roots and accumulate for a long time, and thus, gradually affects the very organ and simultaneously rapid growth occurs in the stem. In the following days with the high concentrations of salts relative growth decreased in roots and stem. The decreased relative growth and the stabilization can be explained by specific effect of ions.

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