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Morpho-Physiological and Biochemical Responses to Salt Stress in Wheat (*Triticum aestivum* L.) at the Heading Stage

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1. Introduction

Abstract

The effect of soil salinity on the morpho-physiological and biochemical parameters in early heading stage was studied in two varieties of wheat, Ashtar and Salama, under three concentrations (50, 75 and 100 mM) of NaCl. Salinity stress has increased relative water content, chl 'a' and 'b' contents, membrane stability index, Na⁺ content and dry weight of aerial part. However, salt treatment has caused a decrease in plant height, number of leaves, leaf area, and specific leaf area, fresh and dry weight of roots, K+ content of roots and K+ /Na⁺ ratio. The results have showed a better salinity tolerance of the variety Achtar. This tolerance was manifested by the smallest decline in the shoot and root biomass.

The spatial coverage of arid ecosystems is approximately 33–40% of the land area and it is likely to increase as a result of climate change and desertification. The high illuminance and the scarce rainfall in the semi-arid and arid regions accentuate the salinization of irrigated perimeters and make them unsuitable to crops [1]. In these regions, the drought is a limiting factor for many organizations due to limited rainfalls and high evapo-transpiration. In addition, the expansion of irrigated agriculture and the intensive use of water resources, combined with a strong evaporation in the arid and semi-arid regions, inevitably lead to salinization of the soil and water tables [2].

The salinity plays an important role in the existence and distribution of plants. Unlike most of halophytes growing on salty soils [3], the glycophytes are exposed to changes in their behaviour physiological and biochemical effects depending on the degree of salinity in the environment [4, 5].

The reduction in the growth of the plant is due to decreases in osmotic potential in the soil, stomatal conductance, photosynthesis, and also by the increase in the concentration of ions Na^+ and Cl^- , which reach toxic levels for the plant [6]. Indeed, in the salty environments, plants osmotically adjust their cellular contents by synthesizing the amino acids such as the proline [7]. Plants' tolerance to salt also depends on their ability to maintain ions homeostasis, maintaining low concentrations in Na^+ and high in K^+ [8]. The Cells adapted as well to saline stress in now a low concentration of cytosolic Na^+ and controlling its movement across the plasma membrane and the tonoplast [9]. The H⁺-ATPase located in the plasma membrane forces the antiporter Na^+/H^+ to realize the efflux of Na^+ [10].

Under salt stress, the potassium is also crucial because it acts as a competitor of Na⁺ in the culture medium and it has an important role in stress signaling [11]. In addition, the opening of the stomata is done by the activation of K⁺ channels toward the inside, by the intermediary of the H⁺-ATPase of the plasma membrane which realizes the extrusion of protons H⁺ [12]. However, the response of plant species to the salt depends on the species themselves, their variety, salt concentration, culture conditions and the stage of plant development [13, 14].

Since the beginning of 1990's, wheat production (*Triticum aestivum* L.) has begun to stagnate in several major producing regions of the world [15, 16], and its performance is significantly influenced by the global climate change [17]. In Morocco, wheat contributes by an average for almost 47% of the gross value of cereals, followed by the durum wheat (27%), barley (23%), maize (2%) and other cereals (1%) [18].

This work aims to compare the behavior under salt stress of two varieties of common wheat (Achtar and Salama), by the study of the biomass and some settings morpho-physiological and biochemical analyzed in the early heading stage.

2. Materials and Methods

2.1. Plant material

The experiments have been conducted on two varieties of common wheat (*Triticum aestivum* L.): Achtar (early in the heading) and Salama (half-early to the heading) listed in the catalog of SONACOS (National Seeds Corporation Commercialization, Morocco). The Achtar variety has been obtained by the INRA 'Morocco' in 1988 and the variety Salama was obtained by Florimond Desprez 'France' in 2004.

2.2 Establishment of the experimental device

After disinfection with sodium hypochlorite (10%) for 10 min and rinsing with distilled water, the seeds of two varieties of common wheat were germinated in Petri dishes. The pre-germinated seed, the elderly a week, have been planted in pots (4 liters), the bottom of which has been drilled and then lined by gravel (1/10 of the volume) then completed by a mixture of sandy soil of Maamora forest (soil constituted by a cover of sand based on a level clay-sand to sandy clay-old) and black peat in respective proportions of 4V/1V. The pots, each containing 6 plants, have been placed under a plastic tunnel where they are arranged randomly with temperatures between 20°C at night and 34°C at day. Irrigation has been carried out twice per week up to the four-leaf stage, with a nutrient solution of Hoagland and Arnon [19].

A week after transplanting and from the 4 leaf stage (Tillering stage), 4 levels of NaCl (0, 50, 75 and 100 mM) have been applied, in 3 repetitions (or pots), each containing 6 plants. Irrigation was applied three times per week, with the first intake of 800 ml/pot of the nutrient solution with or without salt. After drainage, the mass of three jars was weighed for each stress level. The watering of compensation of losses of evapotranspiration has been carried out by the different solutions up to the capacity in the field. At the same time and in order to avoid the cumulative effect of the contribution of the salt in the substrate, a leaching to the water of drilling (electrical conductivity (EC): 0.46 mS/cm; pH: 7.5) has been practiced at the end of each week, followed by the restoration of the level of stress saline by adding 800 ml/pot of the nutrient solution with or without salt.

The treatment was applied until the beginning of the heading stage (index 50 according to the Zadoks scale), and the crop, the plants were aged 8 weeks, of which the last six weeks were under saline stress.

2.3 Parameters studied

- The plants height (length of the master-brin expressed in cm) and the leaves number were measured after 4th-5th-6th week of salt stress (corresponding to an age of plants, respectively 6, 7, and 8 weeks).

- The leaf area (LA), defined as the product of length by the average length of the leaves and by a correction factor calibrated for the leaves [20], is measured at the end of the test (after 6 weeks of stress).

- The specific leaf area (SLA), measured at the end of the test, by the ratio of the leaf area to the leaf mass of dry matter (SFS = leaf area / leaf mass of dry matter).

- The biomass (aerial and root) is determined at the end of the trial by the weighing of the mass of fresh material, and then dry after drying in an oven at ventilation at 75° C for 48 hours. It is measured for the aerial and root parties.

- In addition, analyses of the physiological and biochemical parameters have been carried out at the end of the test; from the front last leaf for the water content, the relative water content, chlorophyll, the membrane stability as well as for the determination of the organic solutes and inorganic.

* Water Content (WC) and relative water content (RWC) of leaves: the leaves have been cut at the base of the lamb, weighed immediately to obtain the fresh weight (FW), then put in test tubes filled with distilled water and placed in the dark in a cool place. After 24h, the leaves have been removed, past in a blotter paper in order to eliminate the water adsorbed to the surface and weighed again to obtain the weight at full turgor (FT) [21]. The samples were then put in a drying cabinet at 80°C during 48h and weighed for having the dry weight (DW). The relative water content was calculated by the formula RWC = [100. (FW-DW)/ (FT- DW)] [22] and the water content by the formula WC = 100. (FW-DW) / (FW).

* Chlorophyll content: the extraction was performed from 0.1g of matter fresh leaf (median third of younger leaves) finely ground by cold mortar in the presence of a few milligrams of sand, magnesium carbonate and anhydrous sodium sulfate. The obtained homogenate is supplemented with 5 ml of pure acetone and the supernatant is filtered into a volumetric flask of 25 ml. The acetone operation extraction is renewed on the residue remaining in the mortar until the plant material appears devoid of all traces of pigments and the filtrate is collected in the flask of filtration whose volume is adjusted with acetone up to the mark. From the extraction solution, it prepares a sample (8 ml of the acetone extract 2 ml of distilled water). In parallel, we prepare a a reference standard

to 80% of acetone. The optical density (OD) is then measured at 645 and 663 nm by using a spectrophotometer. The contents of chlorophylls were calculated using the equations [23]:

Chlorophyll a (mg/l) = 12.7 x OD663 - 2.63 X OD645

Chlorophyll b (mg/l) = $22.9 \times OD645 - 4.68 \times OD663$

Total chlorophyll (mg/l) = $(20.2 \times OD645) (8.02 \times OD663)$

* membrane stability: the membrane stability index (MSI) of leaves [24] is determined on a foliar disc whose surface is 1cm^2 , which have been cut before the last leave with a punch, then carefully washed with running water and then to the bi-distilled water to remove the surface contamination. The leaf disks were then placed in stoppered flasks filled with 10 ml of bi-distilled water and then incubated at ambient temperature (25°C) on a stirrer (100 rpm) during 24 hours. The electrical conductivity (EC) is then measured (C1), then the samples were autoclaved at 120°C for 20 min and a second reading (C2) was then taken after cooling of the solution to the ambient temperature. The membrane stability index was calculated by the formula [25]: MSI (%) = [(C1 / C2) × 100]. - Otherwise, for the determination of the organic solutes and inorganic, the extraction of solutes was conducted from 0.5 g of fresh material, levied from before last leaf. The samples were put in test tubes and kneaded in the presence of 5 ml of ethanol to 95%. After a night of maceration, the mixture is put to heat in a water bath (95°C) up to the total evaporation of the ethanol. The obtained residue is then solubilized in 25 ml of distilled water and the tubes were vigorously agitated using a vortex. The obtained supernatant is the plant extract which is used to meter the organic solutes and solutes minerals extractable by this method.

* The soluble sugars were dosed according to the technique described by Dubois *et al.* [26]. 1 ml of reagent to phenol (5% in distilled water) and 1ml of the plant extract or of the glucose range are homogenized in a test tube, and then 5 ml of concentrated sulfuric acid have been added in a single jet. The Assembly has been agitated quickly to the vortex then the tubes were placed in a water bath at 100°C for 5 min. After a rest of 30 min, the absorbance was measured at 492 nm.

* The proline was dosed according to the method of Dreier and Göring [27]. 1 ml of reagent to the ninhydrin (1g Ninhydrin in 24ml of acetic acid and 16ml of orthophosphoric acid) and 1 ml of the plant extract or of the proline range are homogenized in a test tube, and then 1 ml of glacial acetic acid has been added and the mixture was agitated to the vortex. The tubes have been closed and placed in the water bath at 100°C for 60 min until the color changes to pink. The tubes were then cooled by dipping in the melting ice for 5 min, then 5 ml of toluene were added and the mixture was waved to the vortex. After a rest of 30 min, the absorbance was measured at 528 nm.

* The cations Na^+ and K^+ , were quantified on the plant extract using a flame photometer and the content has been given in mg/g MS.

2.4. Statistical analysis

To highlight in order the effect of different factors as well as their possible interactions on the different morphophysiological and biochemical parameters, an analysis of the variance to two (variety and salinity) or three (variety, salinity and duration of treatment) criteria classification, has been carried out [28]. The device is in random blocks complete with a factorial trial (combination of 2 varieties to 4 concentrations of NaCl) with 6 repetitions. The data were subjected to analysis of variance and the effects revealed significant was then subjected to multiple comparisons of means by the Tukey test.

3. Results and Discussion

3.1. The effect of NaCl on the growth parameters

On all NaCl concentrations, the plants height (PH) was decreased in the two varieties of common wheat and the variation by report to the control has been accentuated by the concentration of NaCl (Fig. 1). The values recorded in the Salama variety were higher than those of the Achtar variety, but the reduction in relation to the control in the presence of the salt has always been more drastic in the Salama variety (55 leafs for the control and 48 leaves for the concentration 100 mM NaCl). The analysis of variance showed that the effects of salinity, the variety and the duration of the treatment have been very highly significant.

Concerning the leaves number, we have noted that the salinity has affected the leaves number that has decreased notably among the Salama variety (Fig. 2). In addition, from the 5th week, the recorded values among Salama have been relatively weaker than among Achtar (100 mM NaCl for example, the leaves number has been reduced by 13% and 4% respectively among Salama and Achtar). Indeed, the statistical analysis distinguishes two groups for the varieties (Achtar being less affected than Salama). For the leaf area (LA), the Salama variety is distinguished from Achtar by higher values (Fig. 3). On the other hand, even when the concentration of the salt is high (75 and 100 mM NaCl), the Salama variety has maintained the values close to the light (18 and 19 cm2 respectively in 75 and 100 mM NaCl) and always higher than Achtar (14 and 9 cm2 respectively in 75 and 100 mM NaCl). In effect, the analysis of variance showed an effect very highly significant of the factor variety (Salama being less affected than Achtar) but has not detected any significant difference between the salt concentrations.



Figure 1: Plants height of common wheat, Achtar and Salama varieties, watered for 4 to 6 weeks with different NaCl concentrations.



Figure 2: Leaves number of common wheat, Achtar and Salama varieties, watered for 4 to 6 weeks with different NaCl concentrations.

Concerning the specific leaf area (SLA), the increase in NaCl concentration caused a decrease in the SLA (Fig. 4) among the two varieties (100 mM NaCl specific leaf area was reduced by 12.4% and 22.5% respectively among Achtar and Salama). On the other hand, the values were comparable between the two varieties on the different concentrations of NaCl. In effect, no significant difference was detected for the effect of the variety, but the effect of salinity has been by against highly significant on the SLA variable.



Figure 3: Leaf area, at the early heading stage, in plants of common wheat, Achtar and Salama varieties, watered for 4 to 6 weeks with different NaCl concentrations.



Figure 4: Specific leaf area, at the early heading stage, in plants of common wheat, Achtar and Salama varieties, watered for 4 to 6 weeks with different NaCl concentrations.

For what is the fresh biomass, the increase in salinity appears to affect differently the fresh material (FM) of the aerial part (AP) of the two varieties (Fig. 5). In effect, the recorded values have shown a tendency to increase among Achtar (increase of 3.4% to 100 mM NaCl) contrary to a downward trend among Salama (decrease of 8.3% to 100 mM NaCl). However, no significant effect was detected for the salinity. The factor variety has showed an effect highly significant on this parameter and the comparison of the averages has distinguished the two groups (Achtar being less sensitive than Salama).

The fresh material mass of the root party (RP) was, by contrast, markedly affected by salinity in both varieties of common wheat (fig. 5). In addition, variety Salama has been more affected by the salt that the Achtar variety. Indeed, on 75 mM NaCl for example, reductions of 23% and 36%, compared to the control have been noted respectively among Achtar and Salama. The statistical analysis showed a significant effect for the salinity and very highly significant for the variety (Achtar was less affected that Salama).

Concerning the dry biomass, the effect of NaCl is translated by a slight stimulation of the production of the dry matter (DM) air (AP) of two varieties including 50 and 75 mM NaCl (75 mM NaCl for example, the air DM has been increased by 7.6% and 12.7% respectively among Achtar and Salama) (Fig. 6). However, this effect was not significant. Concerning the variety, a significant difference was observed in favor of Achtar.

For the mass of dry matter of the root party (RP), a reduction in the biomass was observed in the two wheat varieties, in parallel to the increase of the salt concentration (75 mM NaCl, the DM of root has been reduced by 27% and 33% and to 100 mM NaCl, the reduction was 33% and 69% respectively among Achtar and Salama) (Fig. 6). In effect, the salinity has showed an effect highly significant on this parameter. On the other hand, an effect very highly significant has been detected for the Factor variety in favor of Achtar. As well, in our experimentation, the increase in NaCl concentration in the irrigation water has caused a significant reduction of the specific leaf area (SLA) of two varieties and has also induces a decrease of fresh and dry biomasses, in particular in the root part of the plants. Similar studies also show that NaCl inhibits the growth of some Maghrebi varieties of wheat [29]. This

response to saline stress is more frequent among plants as glycophytes pepper [30], the Roselle [31], the chili pepper [32] and Yellow foxtail [33].



Figure 5: Fresh matter mass (g) aerial part (AP) and root part (RP), at the early heading stage, in plants of common wheat, Achtar and Salama varieties, watered for 4 to 6 weeks with different NaCl concentrations.



Figure 6: Dry matter mass (g) aerial part (AP) and root part (RP), at the early heading stage, in plants of common wheat, Achtar and Salama varieties, watered for 4 to 6 weeks with different NaCl concentrations.

The reduction in the plants growth can be attributed to a combination of effects osmotic and specific to the ions Na⁺ and Cl⁻ [34, 35]. This, therefore, leads to a reduction of the growth which is the result of a decline in the number of cell divisions [36]. The reduction of the growth may be the result of the increase in abscisic acid concentration in the aerial part or a reduction in cytokinin concentrations [37]. The decrease of the leaf area presents itself as being the main strategy developed by the durum wheat and the common wheat to mitigate the effects of the limitation of the availability of water in conditions of salt stress [38].

3.2. The effect of NaCl on the physiological parameters

The water status of the leaves of plants submitted to salt stress seems to be affected differently depending on the variety and NaCl concentrations (Fig. 7). However, no significant effect was detected for the two factors as well for the water content (WC) that for the relative water content (RWC). In effect, the values of these two parameters have always remained relatively high (higher respectively to 72% and 74%).

The water status of the plant, particularly expressed by the relative water content (RWC), has shown that the availability of water has been higher for Achtar in the presence of NaCl. In other work, it is also noted that the decrease of the RWC is more rapid in the susceptible varieties that among the resistant varieties [39] and that the salt stress resulted in a significant reduction in water content in some genotypes of common wheat [40].

Concerning the membrane stability index (MSI), the results showed an improvement in the stability in relation to the controls (Fig. 8) at the two wheat varieties (100 mM NaCl, the MSI has been increased by 21% and 155% respectively among Achtar and Salama). The analysis of variance has indeed shown a very highly significant salinity on this parameter. However, no significant difference was detected for the effect of the variety. Better membrane stability is also reported in genotypes of wheat resistant to drought [41]. However, in tomato, the sensitive species *Lycopersicon esculentum* had curiously revealed better membrane stability, unlike salt tolerant species (*cheesmanii* L. and *peruvianum* L.) [42].



Figure 7: Water content (WC) and relative water content (RWC), at the early heading stage, among of the plants leaves of common wheat, Achtar and Salama varieties, watered for 4 to 6 weeks with different NaCl concentrations.

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Figure 8: Membrane stability index (MSI), at the early heading stage, among of the plants leaves of common wheat, Achtar and Salama varieties, watered for 4 to 6 weeks with different NaCl concentrations.

The results concerning the chlorophylls content showed values of chlorophylls "a" and "t" higher in Achtar than among Salama (Fig. 9). For chlorophyll "a", the difference was highly significant for the varietal effect but has not depended on the salinity factor. By contrast, the total chlorophyll "t" showed significant variation under the salinity effect but not significant for the variety effect. Concerning the chlorophyll "b", no significant effect was detected for the variety effect but a significant effect was noted for the salinity.

In general, the chlorophyll decreases under salt stress [43]. However, several researchers have reported that the chlorophyll content increased in the conditions of salt stress for example among the Amaranth [44] and the Rice [45].



Figure 9: Chlorophyll content (Chl a, Chl b and Chl t), at the early heading stage, among of the plants leaves of common wheat, Achtar and Salama varieties, watered for 4 to 6 weeks with different NaCl concentrations.

3.3. The effect of NaCl on of the organic solutes

Concentrations of total soluble sugars appear to be little affected by salinity as well in leaves than in roots. Thus, for leafs for example, the values recorded were 16.1 and 20 μ mol/g FM for the controls and 16.2 and 20.5 μ mol/g FM for 100 mM NaCl respectively among Achtar and Salama (Fig. 10). Furthermore, concentrations appear to leaf slightly higher for the Salama variety. However, these differences were not significant. In other work, the tolerant varieties had accumulated levels more important in sugars that the susceptible varieties [46]. The accumulation of sugars seems to induce the gelling of cell contents by saturating the intracellular environment. This phenomenon, allowing avoiding the crystallization of the molecules contained in the cell, therefore limiting the damage at the level of cellular structures [47].





In addition, the salinity of the spray solution seems to be without effect on proline concentrations in leaves and roots (Fig. 11). However, the levels of foliage were significantly more important than those of the roots. On the other hand, proline concentrations in leaves and roots were generally higher in the Achtar variety that among the Salama variety (Fig. 11). However, these differences were significant only for the roots. According to some studies, it should be 100 to 150 mM NaCl to induce an increase of the accumulation of the proline [48]. This accumulation would be attributed to the inhibitory effect of stress on its oxidation in the mitochondria [49], as well as on its incorporation into protein [50]. The neosynthesis of the proline is, by contrast, triggered by the loss of turgor due to

salinity. The latter enables a series of complex events correlated with the level of stress, the tolerance of the plant and its stage of development [51]. Soluble compounds can represent a role of osmolytes or cryoprotection membranes and proteins. The osmolytes the most well-known are the proline, glycine-betaine and simple sugars [52, 53].



Figure 11: Proline content, at the early heading stage, of leaves (L) and roots (R) of common wheat, Achtar and Salama varieties, watered for 4 to 6 weeks with different NaCl concentrations.

3.4. The effect of NaCl on the inorganic solutes

The salinity effect on the content tissue in Na⁺ has deferred depending on the variety, the type of organ and the concentration in salt in the irrigation water (Fig.12). The salt stress caused a marked increase in the sodium content in the two varieties. Na⁺ concentrations were higher in the roots than in the leaves in the two wheat varieties (11.1 and 3.5 mg/ g DM for Achtar and 10.1 and 3.2 mg/g DM for Salama among controls). In addition, the highest values were recorded among the variety Achtar for the two organs types (leaves and roots). As well, the levels of leaves in Na⁺ in the presence of NaCl were increased compared with the control, 38, 162 and 200% among the Achtar variety and 52, 73 and 142% in the Salama variety, respectively on NaCl 50, 75 and 100 mM. For the root levels, the respective increases were 42, 47 and 65% among the Achtar variety and 28, 34 and 60% among the Salama variety. The statistical analysis showed an effect very highly significant NaCl on the sodium content in the two parts types. The variety effect has been highly significant for the leaves and very significant to the roots.

Concerning, potassium, K^+ levels were higher in leaves than in roots (Fig. 13). Foliar K^+ appears to vary slightly among the two varieties, but without any effect significant varietal. The root levels in K^+ declined under the salt stress effect among the two varieties and this reduction was highly significant. In effect, these levels spend 5.9 and 6.2 mg/GMS among the control to 3.7 and 3.04 mg/GMS on 100 mM NaCl, which represents a reduction of 36.7 and 51.6% compared to the control.







Figure 13: K^+ content, at the early heading stage, of leaves (L) and roots (R) of common wheat, Achtar and Salama varieties, watered for 4 to 6 weeks with different NaCl concentrations.

The K^+/Na^+ ratio has been significantly affected by the salinity of the irrigation solution (Fig.14). In addition, the values of this report in the roots have been lower than the unity among the two varieties. However, although the K^+/Na^+ ratio in the leaves decreases, it remains in favor of K^+ in the presence of 50 mM NaCl. The statistical

analysis showed an effect very highly significant of NaCl, but this effect was not significant for the variety. In addition, the values of the K^+/Na^+ ratio have remained higher in leaves than in roots.



Figure 14: K^+ /Na⁺ ratio, at the early heading stage, of leaves (L) and roots (R) of common wheat, Achtar and Salama varieties, watered for 4 to 6 weeks with different NaCl concentrations.

Some studies highlight the importance of mesocotyl in sodium retention and limiting its export to aerial parts in maize plants grown on NaCl [54]. In effect, the salt tolerance is all the better that Na^+ is less exported to the aerial parts [55]. The exclusion of Na^+ in aerial parts is characteristic of sensitive species to NaCl [56, 57].

The NaCl absorption inhibits the absorption of other nutrients, essentially the K^+ cation, which leads to a potassium deficiency [45]. Some researchers [58] have noted that the tolerant plants to salinity maintain a K^+/Na^+ ratio high in the aerial parts a result of discrimination of the absorption of K^+ and Na^+ at the level of the roots as well as of their transport in the aerial parts.

The effects of soil salinity have been studied also on other common wheat cultivars such as Kharchia 65 (tolerant) and KRL 19 (moderately tolerant) and researchers have found that the relative water content, the chlorophyll levels, the carotenoids, the proline, the glycine-betaine, the soluble sugars, the catalase and glutathione reductase, the index of membrane stability, biomass and grain yield, the reactive substances of the oxygen and superoxide dismutase (SOD) have been reduced in both cultivars at all growth and development stages [59].

Conclusions

At early heading stage (index 50 according to the scale of Zadoks), the study results have helped to make account of differences varieties with respect to the salinity. In effect, the Salama variety was more sensitive to salt that the Achtar variety. This last has shown itself to be more tolerant to salinity, having a decrease relatively more low in root biomass, concomitantly with the accumulation of Na⁺ levels higher in the two parts (leaves and roots) and in particular in the aerial part. As well, in 100 mM NaCl for example, Na⁺ concentrations in Achtar have increased by 200% in the aerial part and 16% in the root part.

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