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Reducing The Adverse Effect of Irrigation Water Salinity Stress on Yield of Barley (*Hordeum vulgare* L.) Cultivars by Foliar Application of Zinc Sulfate

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RESEARCH ARTICLE

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ABSTRACT

BACKGROUND: Zinc is an essential low-use element involved in the activity of various enzymes. Carbonic anhydrase, which catalyzes the reversible conversion of carbon dioxide and water into carbonic acid, contains zinc and requires zinc for its activity. Zinc plays a role in protein metabolism, gene expression, structural and functional integrity of biological membranes and photosynthetic carbon metabolism.

OBJECTIVES: The present study was conducted with the aim of investigating the foliar application of zinc sulfate fertilizer on the amount of proline and zinc element in leaves and the yield of barley cultivars under saline water irrigation conditions.

METHODS: This research was done according split plots experiment based on randomized complete block design (RCBD) with three replications was carried out in the Station of Agricultural Research in Kabutrabad, Isfahan. Zinc sulfate application three levels (0, 0.5 and 1%) as the main factor and three cultivars (Armaghan (sensitive to stress), Goharan (tolerant to drought) and Mehr (tolerant to salinity)) were considered as secondary factor.

RESULT: The highest content of zinc in leaves (45.9 $mg.kg^{-1}$) and number of seeds per spike (37.2) was observed with 1% zinc sulfate solution in Mehr cultivar. With 1% zinc sulfate foliar application, Mehr and Goharan cultivars had more leaf proline, 21.3% and 15.3%, respectively, compared to Armaghan cultivar. Foliar appkication of zinc sulfate with a concentration of 1% in Mehr, Goharan and Armaghan cultivars significantly increased seed yield by 22.2, 25.7 and 0.29%, respectively, compared to no foliar application of sulfate fertilizer.

CONCLUSION: In general, the results of the research showed that under irrigation water salinity conditions, Mehr varieity was superior to Gohran and Armaghan cultivars in terms of seed yield in response to zinc sulfate application.

KEYWORDS: *Chlorophyll, Fertilizer, Low-use elements, Nutrition, Proline.*

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1. BACKGROUND

Barley, with the scientific name *Hordeum vulgare L*., is an annual plant (of the Gramineae or Poaceae family), with a height of 50 to 130 cm and usually has many tiller. Barley is the fourth most important seed after wheat, rice and corn. Compared to wheat, barley requires a soil with less fertility and its adaptability to all kinds of soils is wider than wheat (Tigre *et al*., 2014). One of the methods of fast supply of nutrients for plants is spraying them. In this method, food elements are directly available to the aerial organ. Due to the fact that the factors affecting soil absorption of nutrients are variable, spraying is considered an effective method in correcting nutritional disorders of plants (Khan *et al*., 2003). Foliar feeding is a method to reduce the fixation of chemical fertilizers in the soil and as a result reduce environmental risks, including reducing soil and water pollution, and it makes nutrients available to plants in a controlled manner (Kannan, 2010). Today, in addition to high-consumption food elements, the use of micronutrient elements as an important tool to achieve maximum yield per unit area is considered (Mosavi *et al*., 2007). Zinc is an essential low-use element involved in the activity of various enzymes. Carbonic anhydrase, which catalyzes the reversible conversion of carbon dioxide and water into carbonic acid, contains zinc and requires zinc for its activity (Weisany *et al*., 2012). Zinc plays a role in protein metabolism, gene expression, structural and functional integrity of biological membranes and photosynthetic carbon metabolism. Zinc interferes in regulating the opening of the stomata due to its role in maintaining the integrity of the membrane. In zinc deficiency, the K+ content of stomatal guard cells decreases (Tester and Davenport, 2003). Continuous cultivation, year-round and excessive use of phosphorus fertilizers, leaching and other conditions governing calcareous soils, including the presence of large amounts of calcium carbonate, alkaline pH, and the lack of use of fertilizers containing micronutrients and organic fertilizers reduce the storage of this element in the soil and as a result reduced yield (Seyed Sharifi *et al*., 2015). Zinc deficiency in cases caused by the limitations of the subsoil, dryness of the surface soil and diseases cannot be completely and definitively resolved through the consumption of fertilizers containing zinc. Therefore, the use of effective genotypes for the absorption of zinc can be an effective and sustainable solution for the production of more crops in conditions of zinc deficiency (Sadeghzadeh, 2013). The use of resistant and efficient genotypes in absorbing and using zinc can be considered as a complement to soil application and foliar application of zinc, especially when farmers are not aware of the lack of zinc in their farm soil or access to zinc fertilizer. The use of cultivars with high efficiency of zinc element can increase the quantity and quality of the product with the minimum amount of available zinc in addition to reducing the consumption of chemical fertilizers (Sadeghzadeh and Rengel, 2011). Salinity of water and soil resources is one of the most important agricultural problems in Iran. In salinity conditions, the availability of nutrients in the soil solution is reduced due to the high concentration of chlorine and sodium ions, and sometimes calcium, and it leads to a disturbance in nutrition and disrupting the balance of plant nutrients. Therefore, the role of proper nutrition in these conditions is very important to help maintain the balance of nutritional elements and provide the proper growth and plant yield (Ahmadi *et al.,* 2006). One of the most effective micronutrients for plants grown in calcareous, saline and sodium soils with high pH is zinc. The use of treatments with the help of which plants can be grown in saline soils is interest to many researchers today. Zinc is one of the important elements in reducing salinity stress and the application of zinc reduces the toxic effects of sodium and chlorine in plants (Vojodi Mehrabani *et al*., 2018).

2. OBJECTIVES

The present study was conducted with the aim of investigating the foliar application of zinc sulfate fertilizer on the amount of proline and zinc element in leaves and the yield of barley cultivars under saline water irrigation conditions.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

This research is carried out in the agricultural research station of Kabutar Abad, Isfahan, with a geographical longitude of 51 degrees and 49 minutes east, a geographical latitude of 32 degrees and 30 minutes north, and an altitude of 1541 meters above sea level, and according to the coupon classification, it has a very hot dry climate with hot summers and dry and semi-cold winters, the average annual rainfall and temperature are 122 mm and 16.1 degrees Celsius respectively. Some physicochemical characteristics of the soil of the test site are presented in table 1.

Table 1. Some of physical and chemical characteristics of field soil

Clay (%)	**Silt** (%)	**Sand** (%)	**O.C** (%)	**pH**
38	46	16	0.95	7.1
P (mg.kg^{-1})	**K** (mg.kg^{-1})	**Cu (mg.kg^{-1})**	**Zn (mg.kg^{-1})**	**Mn (mg.kg^{-1})**
11.5	304	0.76	0.30	7.64
pH	**Mn (mg.kg^{-1})**	**EC** (dS.m^{-1})	**Fe (mg.kg^{-1})**	**B (mg.kg^{-1})**
7.1	7.64	13.2	1.22	1.68

A split-plot experiment was conducted according randomized complete block design (RCBD) with three replications in an environment with saline irrigation (with a water salinity of more than 6 $ds.m^{-1}$). The test treatments include spraying zinc sulfate at zero, 0.5% (2 kg of zinc sulfate per hectare or equivalent to 659 grams of zinc element per hectare and 1% (4 $kg.ha^{-1}$ zinc sulfate or equivalent to 1319 grams of zinc element) per hectare) as the main factor and three cultivars (Armaghan (sensitive to stress), Goharan (tolerant to drought) and Mehr (tolerant to salinity)) were considered as secondary factors.

3.2. Farm Management

Zinc sulfate solution application was done at the tillering stage with an interval of 7 days and in 3 times. The area of each plot was 4.8 square meters and the density of 400 seeds per square meter was considered.

3.3. Measured Traits

In order to measure the proline content, three flag leaves from each experimental unit were randomly sampled at the spike stage and the proline content was measured by the Bates (1973) method. In this method, 400 milligrams of the fresh leaves of the plant, which were previously kept at -20 degrees Celsius, were transferred into a Chinese mortar and pounded inside the mortar by adding 10 milliliters of 3% sulfosalicylic acid. Then the resulting solution was passed through filter paper and 2 milliliters of filtered solution was poured into a test tube along with 2 milliliters of ninehydrin solution and two milliliters of acetic acid solution. The resulting solution was placed in a bain-marie bath at a temperature of 90 degrees Celsius for one hour. The tubes containing the solution were immediately placed in ice to stop the reaction, and then four milliliters of toluene was added to the solution and shaken for 15 to 20 seconds. After shaking, a two-phase solution was formed, the upper phase containing proline. Toluene solution was used to calibrate the spectrophotometer, after calibrating the device, the samples were read at a wavelength of 520 nm. The amount of proline obtained during this method was obtained based on micromoles of proline per gram of fresh leaves. In order to measure the element of zinc in the flag leaf, the samples were prepared at the seed filling stage and after washing and drying, they were ground with a mortar. Then put 0.5 grams of this sample in the oven at 550 degrees Celsius for 3 hours until the samples turn into ash. Then, 2 normal hydrochloric acid (5 ml) was added to each ash sample and its final volume was brought to 5 ml by adding distilled water. This prepared extract was used to measure the concentration of zinc element with the help of atomic absorption device (BC-Avanta-PM, Australia) (Costa *et al.*, 2020). Also, in order to determine the number of seeds per spike, the 1000 seed weight and seed yield, 10 plants were randomly picked from the plants in each experimental plot and weighed with an accuracy of 0.01 grams.

3.4. Statistical Analysis

After collecting the data, they were analyzed using MSTATC software and comparing the averages with the LSD test at the 5% of probability level. The correlation coefficients of traits were also estimated using SPSS software.

4. RESULT AND DISCUSSION

4.1. *Leaf proline*

The results of analysis of variance (Table 2) indicated that the main effects of fertilizer, cultivar and also the interaction of these two factors on leaf proline content were significant at the 1% of probability level. The results of the interaction of investigated factors showed that the highest level of leaf proline (260.8 $\mu g.g^{-1}$) belonged to the Mehr cultivar and under the condition of 1% zinc sulfate solution application (Table 3). In the conditions of 1% zinc sulfate foliar application, Mehr and Goharan cultivars had more leaf proline, 21.3% and 15.3%, respectively, compared to Armaghan varieity (Table 3). Research results showed that the difference of barley cultivars was significant in terms of proline content of flag leaves, and proline content of leaves was higher in salinity-tolerant and semi-tolerant cultivars compared to semi-saline-sensitive cultivars (Mahlooji, 2017). Researchers stated that barley cultivars with high Fv/Fm ratio and the highest proline production capacity under stress conditions had less yield reduction (Mamnoei and Seyed Sharifi, 2010). The lowest amount of leaf proline was observed in the condition of not using zinc sulfate fertilizer, and with foliar application of zinc fertilizer, especially at a higher concentration (1%), the content of leaf proline in all three cultivars increased significantly. Foliar application of 1% zinc sulfate in Mehr, Goharan and Armaghan cultivars caused a significant increase in leaf proline by 52.2, 49.8 and 30.6%, respectively, compared to no application of zinc fertilizer (Table 3). Increasing the amount of proline in plants under salt stress is actually a reaction of the plant to the reduction of water in the root environment. Proline helps to regulate osmosis during stress and maintain the primary structure of macromolecules and membranes during increased dehydration. In addition to osmotic regulation, proline has other functions such as protecting the plasma membrane and removing hydroxyl radicals and active oxygen (Sun *et al*., 2013). On the 1 hand, the use of zinc element by affecting the production of osmolytes such as proline reduces the effects of stress and on the other hand by indirectly preventing the destruction of chlorophyll, it causes continued growth and optimal distribution of assimilates in the plant (Abbasi and Shekari, 2016). In a research, the highest amount of proline was obtained in severe stress treatment with 1% zinc sulfate solution application (Fallah, 2019). The results of a research on mung bean plant under drought stress conditions showed that foliar application with 10 grams of zinc nanooxide had the greatest effect on leaf proline with a 32% increase compared to the control (Makarian *et al*., 2017).

Table 2. Analysis of variance of studied traits in barley cultivars effected by foliar application of zinc sulfate

S.O.V	df	Proline content	Zinc content	1000-seed weight	Seeds per spike	Spike per m^2	Seed yield
Replication	2	8361.48**	29.07^{ns}	17.22^{ns}	5.55^{ns}	2446.17^{ns}	197428.9^{ns}
Zinc sulfate	2	12371.7**	397.66**	66.65^{ns}	167.77**	29412.8**	3378907.2**
Error (a)	4	62.75	4.47	14.97	5.15	597.14	41655.4
Cultivar	2	2243.51**	30.97**	21.73^{ns}	26.64**	3484.24**	586173.1**
Zinc sulfate × cultivar	4	416.46**	9.62**	18.06^{ns}	3.09*	788.74*	51030.2*
Error (b)	12	65.37	1.32	6.99	0.94	234.33	15325.6
C.V (%)	-	3.97	3.23	7.43	3.05	3.05	2.29

^{ns}, * and ** are not significant, significant at the 5% and 1% probability levels, respectively.

Table 3. The results of interaction effects of zinc sulfate and barley cultivars on studied traits

Zinc sulfate (%)	Cultivars	Proline content (μg.g^{-1} FW)	Zinc content (mg.kg^{-1})	Seeds per spike	Spike per m^2	Seed yield (kg.ha^{-1})
0	**Armaghan**	164.5e	27.5e	24.2f	415.7f	4530.5f
	Goharan	165.4e	28.5e	27.5e	422.4f	4764.0e
	Mehr	171.3de	29.1e	29.8d	482.0e	5003.3d
0.5	**Armaghan**	183.5d	36.0d	31.8c	501.7de	5078.6d
	Goharan	199.0c	36.8cd	32.2c	516.8cd	5508.1c
	Mehr	225.5b	37.9cd	34.0b	524.3cd	5865.2b
1	**Armaghan**	214.9b	38.5bc	34.7b	535.4bc	5847.4b
	Goharan	247.9a	40.0b	35.2b	558.3ab	5989.0ab
	Mehr	260.8a	45.9a	37.2a	563.5a	6118.5a

Means in each column followed by similar letter(s) are not significantly different at the 5% probability level (LSD test).

4.2. *The amount of zinc in the leaves*

Based on the results of analysis of variance, the main effects of the studied factors (fertilizer and cultivar), their interaction was significant at the 1% of probability level on the amount of zinc in the leaves (Table 2). In the condition of no zinc consumption, there was no significant difference between cultivars in terms of zinc content in leaves, and all three cultivars were included in the same statistical group. But with foliar application of zinc sulfate, especially at a higher concentration (1%), the amount of zinc in the leaves increased significantly. Application 1% zinc sulfate in Mehr, Goharan and Armaghan cultivars increased the amount of zinc in leaves by 57.7, 40.3 and 40.0%, respectively, compared to the control treatment (Table 3). The increase in the concentration of zinc in the leaves is the result of the direct effect of spraying this element, which is easily absorbed by the leaves (Vatankhah *et al*., 2016). The results of a research showed that increasing the concentration of zinc sulfate and nano-chelate fertilizers increased the concentration of zinc element in the leaves of Abu Jahl watermelon medicinal plant (Nikbakht *et al*., 2021). In another study, the highest concentration of zinc in grape leaves (49.1 mg.kg^{-1}) was obtained from the 0.2% zinc sulfate treatment, which was significantly different from the control (Vatankhah *et al.* ., 2016). In a research that was conducted to investigate the effect of zinc fertilizer on seed corn under salinity stress conditions, the highest amount of zinc application increased zinc and potassium in the aerial parts of the plant by 27.7 and 5%, respectively, compared to the condition of no application of zinc (Karmollachaab and Gharineh, 2013). According to the comparison of the average data, the highest amount of Zn in the leaf was observed with 1% zinc sulfate solution in Mehr cultivar, which had a significant difference with all treatments. In the conditions of foliar application of 1% zinc sulfate, Mehr cultivar had more zinc content in leaves compared to Goharan and Armaghan cultivars, 14.7% and 19.2%, respectively (Table 3). In the study of the effect of irrigation water salinity and foliar application of zinc fertilizer on barley genotypes, the results indicated that the reaction of barley cultivars in terms of zinc element in aerial parts was significant at the probability level of 1%, so that the highest concentration of zinc element in four salinity genotypes (tolerant to salinity) were observed (Mahlooji, 2017). Correlation results showed that the amount of zinc element in leaves had a positive and significant correlation at the probability level of 1% with seed yield (0.80**) (Table 4). In this regard, the researchers stated that the addition of zinc fertilizer in saline conditions may lead to more growth and to some extent increase the yield of wheat by improving the condition of nutrients in the plant and reducing the effects of salinity (Ahmadi *et al.*, 2006).

4.3. *Number of seeds per spike*

The results showed that in addition to the main effects of the investigated factors (at the 1% of probability level), their interaction also had a significant effect on the number of seeds in the spike at the probability level of 5% (Table 2). The highest and lowest number of seeds per spike belonged to 1% zinc sulfate solution application in Mehr cultivar and Armaghane cultivar treatment and no zinc fertilizer application (Table 3). Parallel to the increase in the consumption of zinc element, the number of seeds per spike also increased, so that the highest average number of seeds per spike in all three cultivars was related to the concentration of 1% zinc fertilizer. Foliar application of zinc sulfate with a concentration of 1% in Mehr, Goharan and Armaghan cultivars caused a significant increase in the number of seeds per spike by 24.8, 28.0 and 43.3%, respectively, compared to no foliar application (Table 3). In this regard, the research results showed that the difference between the studied cultivars of barley was statistically significant on the number of seeds per spike, so that the highest number of seeds in the spike belonged to the stress-tolerant genotype(Mahlooji, 2017). Feeding the plant with zinc increases the life of the pollen seed due to the increase in the carbohydrates stored in the pollen seed, and as a result, increases pollination and the formation of more seeds in the plant (Hafeez *et al.*, 2013). The researchers stated that the sterility of spike flowers of plants is more severe in zinc deficient soils and adding zinc reduces the problem of spike sterility (Bagci *et al.*, 2007). In a research on barley, the highest and lowest number of seeds per spike belonged to the treatment of using 60 $kg.ha^{-1}$ of zinc sulfate fertilizer and the treatment of no fertilizer application, respectively, which was consistent with the results of this research (Seyed Hayat Gheyb *et al.*, 2019). The results of the trait correlation estimation showed that the number of seeds per spike had a negative and significant correlation (%1 level) with the 1000- seed weight(Table 4). In this regard, the research results showed that the correlation between the 1000-seed weight and the number of seeds in barley spike was negative and significant (Nikkhah *et al.*, 2015). In another study, the researchers found that the number of seeds per spike in barley, showed a significant negative correlation with the 1000-seed weight and the total number of tillers, and there was a positive and significant correlation between the 1000-seed weight and the number of tillers (Soleimani *et al.* ., 2017). The correlation study of traits showed that the number of seeds per spike had a significant and positive correlation (0.88**) with seed yield (Table 4). The number of seeds pr spike actually determines the capacity of plant reservoirs. If the number of seeds in the plant is higher, there are usually more reservoirs for the grown materials produced, and any factor that can increase this component will also increase the yield.

4.4. *Number of spikes per square meter*

The results of analysis of variance (Table 2) showed that the main effects of fertilizer and cultivar at the 1% of probability level and their interaction at the 5% of probability level affected the number of spikes per square meter. The lowest number of spikes per square meter was related to Armaghane cultivar in the condition of not using zinc fertilizer (Table 3). In the lower concentration of zinc sulfate fertilizer (0.5%), no significant difference was observed between the cultivars in terms of the number of spikes per square meter, but in the higher concentration (1%), Mehr cultivar had more spikes compared to Armaghane. In the condition of foliar application of 1% zinc sulfate, Mehr cultivar produced 5.2% more spikes compared to Armaghan cultivar(Table 3). Different mechanisms influence the difference in the response of different plant cultivars to the application of nutrients (Baligar *et al*., 2001; Erenoglu *et al*., 2002; Karimian & Moafpouryan, 1999). These mechanisms are: interactions related to the root in order to increase the ability to use the zinc element for absorption by the root, increasing the absorption and transfer of zinc from the root to the aerial organs, changing the intracellular composition of zinc in the aerial organs of the plant (so that more amount of zinc is placed in the cytoplasm) and improvement or increase in the biochemical utilization efficiency of zinc in plant cells (Malian *et al*., 2014). It seems that by increasing photosynthesis, photosynthetic substances and auxin hormone, zinc fertilizer increases vegetative growth and improves fertile spikes in the plant. Also, this issue is related to improving plant nutrition (Seyed Hayat Gheyb *et al*., 2019). The results of a research showed that the highest and the lowest number of spikes per square meter of barley was assigned to the treatment of 60 $kg.ha^{-1}$ and no application of zinc fertilizer, respectively (Seyed Hayat Gheyb *et al*., 2019).

4.5. *Seed yield*

Based on the results of analysis of variance, the effect of zinc fertilizer and cultivar ($p \leq 0.01$) and their interaction was significant at the 5% of probability level on seed yield (Table 2). The highest seed yield (with an average of 6118.5 kg per hectare) belonged to the Mehr cultivar under the conditions of 1% zinc sulfate application (Table 3). The higher seed yield in Mehr cultivar was due to the number of seeds per spike and the number of spikes and the significant and positive correlation of the aforementioned traits with seed yield. The results of yield comparison tests in the lands under salinity stress at stations in the temperate regions of the country showed the superiority of the Mehr cultivar over the Khatam cultivar, so that this cultivar with an average seed yield of 4751 kg.ha^{-1}, 655 kg.ha^{-1} (16 percent) compared to the average of the witness cultivar had increased yield (Nikkhah *et al*., 2019). In the study of the effect of irrigation water salinity and zinc fertilizer foliar application on barley genotypes, the results showed that the highest seed yield was related to line four, which was tolerant to salinity, which showed a 100.5% increase compared to Morocco genotype (sensitive to stress) (Mahlooji, 2017). The results of a research showed that different wheat cultivars had different responses to zinc fertilization. Some cultivars showed a greater response to the application of fertilizer in comparison with other cultivars and the seed yield in fertilizer treatments had a significant increase in comparison with the control. In contrast, some cultivars showed a lower response to fertilizer application, or fertilizer application did not have a significant effect on their seed yield compared to control (Malian *et al*., 2014). The lowest seed yield of 4530.5 kg per hectare was observed in Armaghan cultivar and in the condition of not using zinc sulfate fertilizer. Foliar application of zinc sulfate with a concentration of 1% in Mehr, Goharan and Armaghan cultivars caused a significant increase in seed yield by 22.2, 25.7 and 0.29%, respectively, compared to no foliar application of zinc sulfate. (Table 3). The increase in yield with the use of zinc sulfate fertilizer can be attributed to increasing auxin biosynthesis in the presence of zinc element, increasing chlorophyll concentration, increasing phosphoenol pyruvate carboxylase and ribulose biphosphate carboxylase, reducing sodium accumulation in plant tissues and increasing nitrogen and phosphorus absorption efficiency (Khan *et al*., 2003). The correlation results of the traits showed that among the components of yield, the 1000-seed weight had a negative and significant correlation at the level of 1% with seed yield (Table 4). It should be noted that there is a negative correlation between the constituents of yield, so that with the increase in the number of seeds, due to the increase in the sink capacity against a fixed amount of storage material, it is natural that a smaller amount of material is stored in each sink capacity. It is noteworthy that the nature of the relationships between components is not only genetic and changes from one environment to another, and therefore different results are seen in the experiments (Ahakpaz *et al*., 2020). The researchers stated that the 1000-seed weight had a negative and significant relationship with barley yield. With the increase in the 1000-seed weight, the number of seeds per spike, which is one of the components of the yield, decreases, which will subsequently be effective in reducing the yield (Baraty *et al*., 2014).

5. CONCLUSION

The results of the present study showed that the interaction of zinc sulfate fertilizer and barley cultivars on traits such as number of seeds per spike, number of spikes per square meter and seed yield was significant. The highest yield and yield components were observed with 1% zinc sulfate solution in Mehr cultivar. The reason for this can be attributed to the superiority of Mehr cultivar in salinity conditions and so to the positive role of zinc element in improving plant growth. Therefore, use of this amount of zinc sulfate fertilizer in this cultivar is suitable and recommended for the production of barley under similar conditions to this research. Considering the positive effect of zinc fertilizer in improving barley yield and so, considering the lack of this element in most of lands where barley is cultivated, special attention should be paid to this micronutrient in nutrition programs of plant.

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FOOTNOTES

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