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Assessment of Zinc Transport in Different Organs and Various Growth Stages of Soybean under the Influence of Zinc, Iron and Manganese Fertilization

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ABSTRACT

In order to study of zinc transport at reproductive growth stages in different organs of soybean (*Glycine max* L.) under the influence of zinc, iron and manganese application, two field experiments were conducted at the research field of the Islamic Azad University of Kermanshah, Iran in 2010 and 2011 growing seasons. Three rates of zinc (0, 20 and 40 kg ha⁻¹ from ZnSO₄ source), iron (0, 25 and 50 kg ha⁻¹ from FeSO₄ source) and manganese (0, 20 and 40 kg ha⁻¹ from MnSO₄ source) were used. At sampling times, five plants selected randomly, zinc concentration was determined by atomic absorption spectrometry and then ratios of this element in soybean organs were calculated. Based on results obtained, with increases soybean old from onset flowering to final maturity, Zn concentration declined in stem and leaves. Also, Zn content in leaves was more than the stem in all the growth stages. [Zn]leaf/[Zn]stem ratio was increased with zinc, iron and manganese applications, So that, the ratio of [Zn]leaf to [Zn]stem was more than 6.5 times. In our experiment, [Zn]seed/[Zn]stem, [Zn]seed/[Zn]leaf and [Zn]seed/[Zn]pod ratios were recorded more than 9, 1.4 and 1.2 times, respectively.

Key words: Growth stages, micronutrient, seed, soybean, zinc transport

INTRODUCTION

Zinc is essential element for plant growth and development. Zinc deficiency in the soil is a major problem for agricultural production. It is estimated that about half of the cultivated soils in the world contains reduced amounts of bioavailable zinc. The Zn uptake is reduced as a result of zinc deficiency in the soil, resulting in reduces in crops yield and quality (Henriques *et al.*, 2012). The uptake of zinc happens actively through root membrane carriers. This element plays an important role in metabolism of nitrogen, stabilizes cytoplasmic ribosomes, catalyzes oxidation processes, synthesis of amino acid tryptophan, involve in the protein and carbohydrate metabolism, plants flowering and fruit set, increasing plant resistance to fungal disease and expanding plants roots (Fageria, 2009; Sadeghzadeh, 2013). Excess amount of this metal can exert toxicity to plant cells, so that the range between deficient and toxic levels of zinc is narrow. In comparison with total zinc in the soil, zinc concentration in the soil solution is usually small. Zinc in the soil is simply complexed with clay particles and organic matter, so in this form will not be absorbed. Soluble forms of zinc are very available for plant roots. Zinc has a high solubility and mobility in slightly

acid soils (Heitholt *et al.*, 2002; Kabata-Pendias, 2011). Cakmak (2008, 2011) reported that solubility of zinc in the soil is determined by amount of soil organic matter, pH, water content in the soil and microorganisms. In addition to that, other macro and micronutrients can affect on zinc absorption by roots and transport to shoots. Despite all the research done, there is little knowledge about the zinc homeostasis in plant organs. In this study, the authors aim to evaluate of zinc transport in different organs and various growth stages of soybean plant under the influence of zinc, iron and manganese fertilization.

MATERIALS AND METHODS

Two field experiments were conducted at the research field of the Islamic Azad University of Kermanshah province, Iran (34023' N, 4708' E; 1351 m elevation) in 2010 and 2011. Williams cultivar [*Glycine max* (maturity group III), supplied by the oilseed company of the Kermanshah Agricultural Administration, Iran], was selected as the experimental material. The experimental design was a 3×3×3 factorial experiment based on Randomized Complete Block with three replicates. Before planting, soil samples were collected from experimental area at 0-30 cm depth for soil analyses. The texture of the soil based on silty clay with pH 7.6, total organic matter 2.3%, Electrical Conductivity (ECe) 0.61 dSm⁻¹, total nitrogen 0.18%, available phosphorus 9.9 mg kg⁻¹, available potassium 563 mg kg⁻¹, zinc 0.71, iron 6.2 and manganese 4.3 mg kg⁻¹ soil for year of 2010 and pH 7.4, total organic matter 2.1%, Electrical Conductivity (ECe) 0.52 dSm⁻¹, total nitrogen 0.14%, available phosphorus 10.1 mg kg⁻¹, available potassium 389 mg kg⁻¹, zinc 0.83, iron 3.6 and manganese 4.0 mg kg⁻¹ soil for year of 2011. Fertilizers were used as follows: 200 kg P₂O₅/ha and 50 kg N/ha and mixed with soil and land was ploughed once and harrowed twice. Seeds were soaked in 10% sugar solution; then each kg of seeds inoculated with 2 g of *Bradyrhizobium japonicum*. This experiment included 27 treatments that were placed in 81 plots. The plots consisted of six rows, 5 m in length spacing 60 cm apart. The distance between plants within a row was 5 cm and plant density was 333000 plants/ha. The plant density was achieved by over planting and thinning at V3 stage. Usage amounts of fertilizers zinc (0, 20 and 40 kg ha⁻¹ from ZnSO₄ source), iron (0, 25 and 50 kg ha⁻¹ from FeSO₄ source) and manganese (0, 20 and 40 kg ha⁻¹ from MnSO₄ source) were calculated based on plots area surface; next, fertilizers were mixed with soft soil at the ratio of 1:5 and placed on furrows made manually next to the stacks. At the different reproductive growth stages of soybean (flowering stage, pod set stage, seed filling period stage and maturity stage) based on Fehr and Caviness (1977), five plants were selected from each plot randomly. To measure concentration of micronutrients in leaves, leaves on the most top trifoliolate of the plants were used and leaves were separated from stem. At the end of growing season, five plants were selected from each plot and seeds were separated from pods. Samples (leaves, stems, pods and seeds, separately) washed with distilled water and were dried in the oven at 70°C for 48 h, weighed and incinerated at 550°C. Dry ash samples were soluble in concentrated HNO₃ and HClO₄. Micronutrients were determined by Atomic Absorption Spectrometry (AAS) according to Kacar (1984) and then ratios of micronutrients in soybean organs were calculated. Excel software was used to draw figures.

RESULTS AND DISCUSSION

The effects of zinc fertilization on Zn concentrations in stem and leaf at reproductive growth stages of soybean are shown in Fig. 1. Based on results obtained, with increases soybean old from

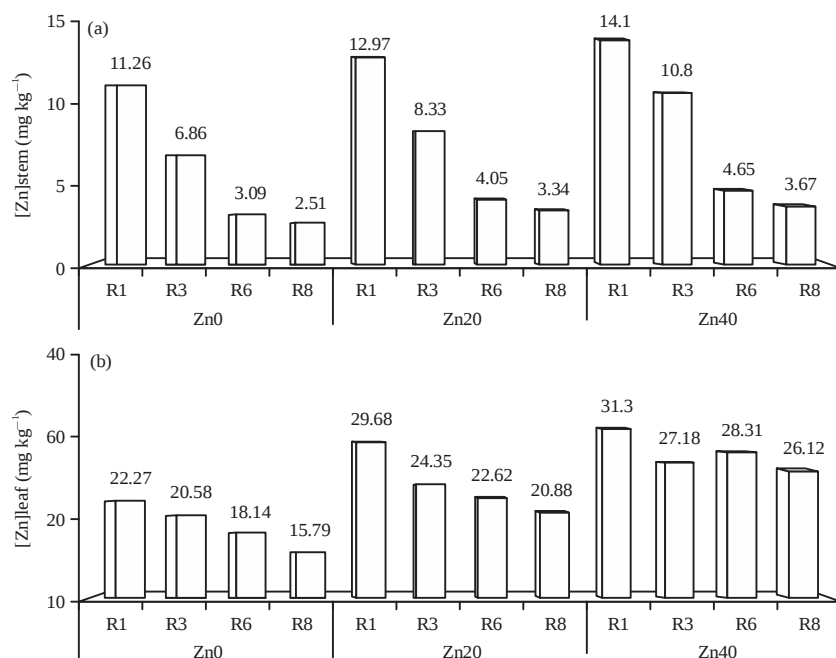


Fig. 1(a-b): Effects of zinc fertilization on Zn concentrations in (a) Stem and (b) Leaf of soybean (mg kg⁻¹) at R1: Early of flowering, R3: Early of pod set, R6: Seed filling period and R8: Full maturity

onset flowering to final maturity, Zn concentration declined in stem and leaves. These results were observed at all levels of zinc fertilizer (Zn0-Zn40). Hence, the zinc concentrations in stem and leaves at R1 stage were higher than the other stages. However, this decline was more in the stem compared the leaves. Zinc is transferred from the stem to leaves and then to pods and seeds coincidence to increase soybean old. In soybean stem and in Zn0, Zn20 and Zn40 treatments, Zn concentration at onset flowering compared final maturity was decreased and from 11.26, 12.97 and 14.10 mg kg⁻¹ reached to 2.51, 3.34 and 3.67 mg kg⁻¹ (-77.71, -74.25 and -73.97%, respectively), while, in soybean leaves these reductions were recorded -29.11, -29.65 and -16.55% (in Zn0, Zn20 and Zn40 treatments, 22.27, 29.68 and 31.30 mg kg⁻¹ in onset flowering reached to 15.79, 20.88 and 26.12 mg kg⁻¹ in final maturity, respectively). The Zn content in leaves was more than the stem in all the growth stages. These results agreed with the findings of Zhang and Brown (1999) and Phiv *et al.* (2003). The effects of zinc, iron and manganese fertilizations on [Zn]leaf/[Zn]stem, [Zn]pod/[Zn]stem and [Zn]pod/[Zn]leaf ratios at reproductive growth stages of soybean were shown in Fig. 2a-i. [Zn]leaf/[Zn]stem ratio was increased with zinc, iron and manganese applications. So, that, with application of the elements, the ratio of [Zn]leaf to [Zn]stem was more than 6.5 times (Fig. 2a-c). Indeed, applications of these micronutrients causes the more zinc transferred through the stem to the leaves. Transfer of zinc from the older parts of plant to growing organs was emphasized in wheat (Herren and Feller, 1994, 1997; Erenoglu *et al.*, 2001; Page and Feller, 2005) and dwarf bean (Ferrandon and Chamel, 1988). Minimum and maximum impact on this ratio was observed at stages of R1-R3 and R3-R6, respectively. R3 stage onward, transfer rate of zinc through the stem to the leaf was enhanced and this caused that [Zn]leaf/[Zn]stem ratio increased rapidly. The upward trend of [Zn]leaf/[Zn]stem up to R8 stage was maintained with zinc and manganese

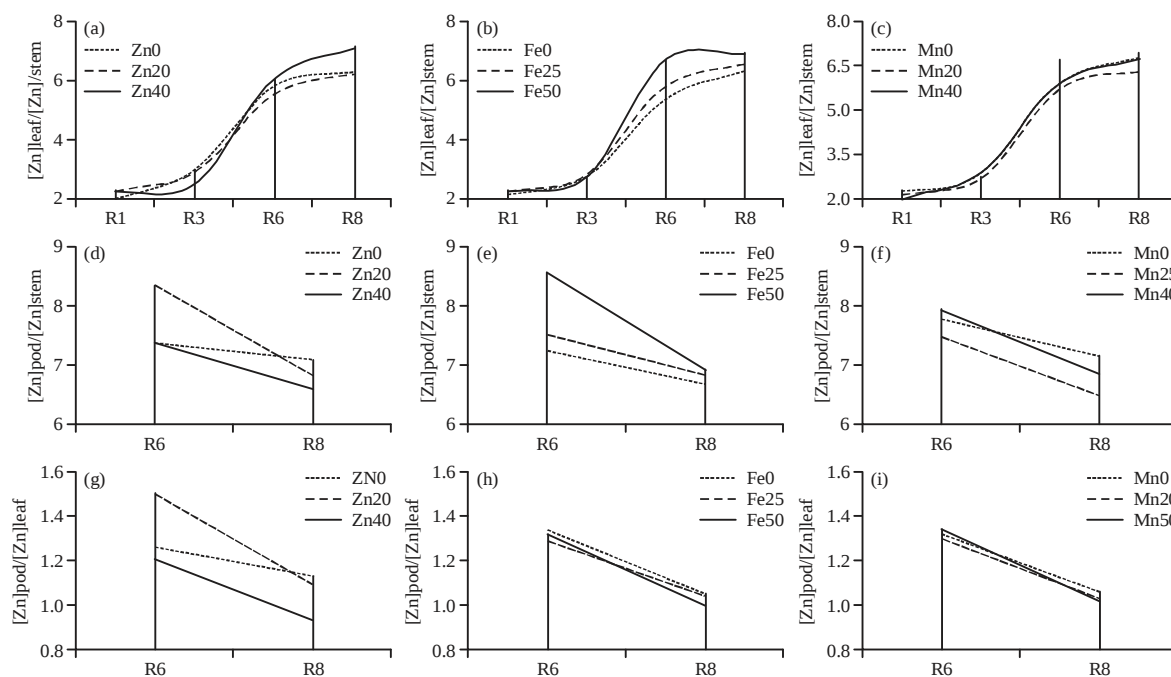


Fig. 2(a-i): Effects of zinc, iron and manganese fertilizations on soybean $[Zn]_{leaf}/[Zn]_{stem}$, $[Zn]_{pod}/[Zn]_{stem}$ and $[Zn]_{pod}/[Zn]_{leaf}$ ratios

application, whereas, iron application had no effect on this ratio at R6-R8 stages. Based on results, the range of pod set and seed filling period are critical stages for transfer of iron from stem to leave of soybean. The highest value of $[Zn]_{leaf}/[Zn]_{stem}$ was recorded when that Zn40, Fe50 and Mn40 were used. This ratio was varied from about 2-7.5 times in R1-R8 stages. It seems that, the leaves are the plant organs for Temporary storage of zinc. These results showed that in soybean, leaves role at the end of growing season (R8) as a reservoir for the accumulation of zinc is more important. Zhang *et al.* (2007) reported that leaves are temporary sink for the accumulation of zinc in plants. Therefore, soybean genotypes that hold more leave at the end of growing season are effective in transferring of zinc to the grain growing. The similar results were reported by Grusak *et al.* (1999) and Waters and Sankaran (2011). Zinc, iron and manganese fertilizations coinciding with increases soybean old (during R6-R8 stages) the ratio of $[Zn]_{pod}/[Zn]_{stem}$ was reduced (Fig. 2d-f). With application of 20 kg zinc, $[Zn]_{pod}/[Zn]_{stem}$ was more than eight times at R6 stage (Fig. 2d) while, at this stage, the maximum values of $[Zn]_{pod}/[Zn]_{stem}$ ratio were recorded in Fe50 and Mn40 treatments (Fig. 2e-f). At the end of the growing season of soybean, this ratio was dropped to seven times. Similar trends were observed in the ratio of $[Zn]_{pod}/[Zn]_{leaf}$. This result indicated that, zinc concentration in soybean pods was higher than the leaves. Finally, destination of zinc are seeds (Marschner, 1995) and at R6 and R8 growth stages, zinc transfer to the seeds, therefore, this is normally that $[Zn]_{pod}$ and seed more than the $[Zn]_{stem}$ and leaves. The ratio of $[Zn]_{pod}/[Zn]_{leaf}$ was equal to 1.5 times when that 20 kg zinc per hectare was applied (Fig. 2g) while, this ratio was less than 1.4 times when iron and manganese fertilizers were used (Fig. 2h-i). It seems that, zinc transfer from pods to grains at the R6 stage is a reason to reduction of $[Zn]_{pod}/[Zn]_{stem}$ and $[Zn]_{pod}/[Zn]_{leaf}$ ratios. The effects of zinc, iron and manganese fertilizations on soybean $[Zn]_{seed}/[Zn]_{stem}$, $[Zn]_{seed}/[Zn]_{leaf}$ and $[Zn]_{seed}/[Zn]_{pod}$ ratios were shown in Fig. 3. In this

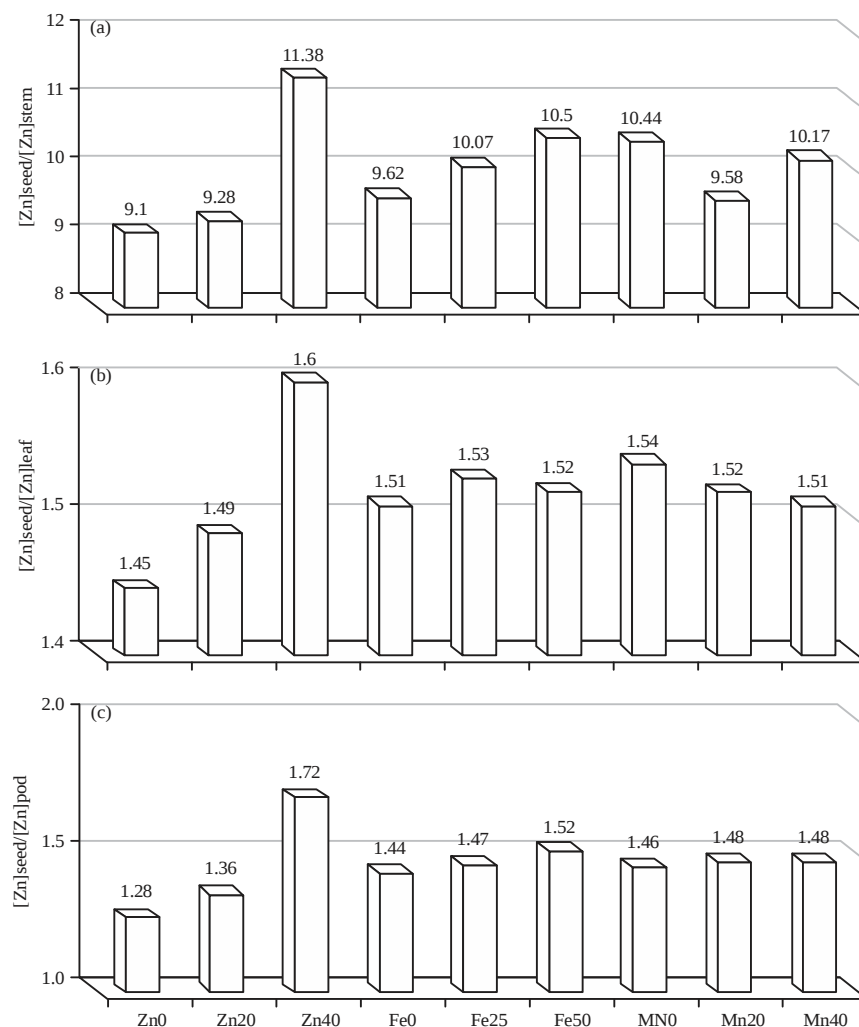


Fig. 3(a-c): Effects of zinc, iron and manganese fertilizations on soybean (a) $[Zn]_{seed}/[Zn]_{stem}$, (b) $[Zn]_{seed}/[Zn]_{leaf}$ and (c) $[Zn]_{seed}/[Zn]_{pod}$ ratios

experiment, $[Zn]_{seed}/[Zn]_{stem}$, $[Zn]_{seed}/[Zn]_{leaf}$ and $[Zn]_{seed}/[Zn]_{pod}$ ratios were recorded more than 9, 1.4 and 1.2 times, respectively. The highest $[Zn]_{seed}/[Zn]_{stem}$, $[Zn]_{seed}/[Zn]_{leaf}$ and $[Zn]_{seed}/[Zn]_{pod}$ ratios were obtained in Zn40 treatment (11.38, 1.60 and 1.72, respectively). Lack of zinc (Zn0 treatment) were reduced these ratios. This is consistent with previous reports on rice (Jiang *et al.*, 2008). Meng *et al.* (2005) stated that micronutrients fertilization was one of the most important ways for the enhancement of absorption, transport and accumulation of micronutrients in plants. The effects of zinc, iron and manganese on soybean seed $[Zn]/[Fe]$ and Seed $[Zn]/[Mn]$ were shown in Fig. 4. According to these results, iron application reduced $[Zn]_{seed}/[Fe]_{seed}$ ratio, while, the highest ratio of $[Zn]_{seed}/[Fe]_{seed}$ was recorded in Zn40 and Fe0 treatments. Manganese fertilization had no significantly effect on $[Zn]_{seed}/[Fe]_{seed}$ ratio. Concerning $[Zn]_{seed}/[Mn]_{seed}$ ratio, the lack of manganese had greater effect compared zinc application, therefore, the highest value of this ratio was obtained in Mn0 treatment (1.38).

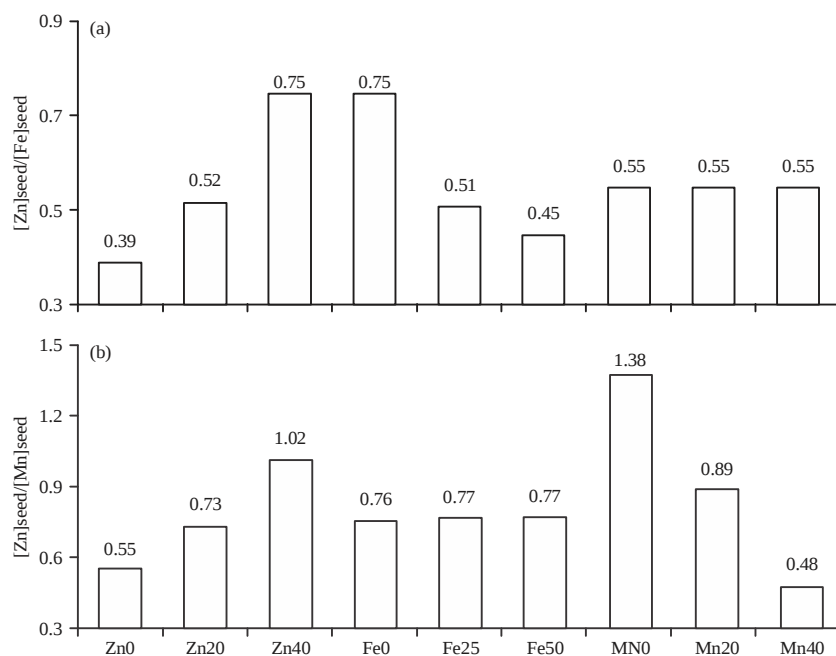


Fig. 4(a-b): Effects of zinc, iron and manganese on soybean (a) Seed [Zn]/[Fe] and (b) Seed [Zn]/[Mn]

CONCLUSION

Results of our experiment showed that with increases in soybean old and reach to full maturity stage, the micronutrient concentrations in tissue plant were decreased. [Zn]Leaf/[Zn]stem ratio in R1-R8 stages of about 2 fold to 7.5 times were altered. Also, in soybean with approaching maturity, leaves role as a reservoir for the accumulation of zinc is more important. After pod setting, transfer rate of Zinc from the stem to leaves increases, this causes [Zn] Leaf/ [Zn] stem ratio increase, severely.

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