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AGRONOMIC BIOFORTIFICATION OF BEET PLANTS WITH ZINC VIA SEED PRIMING[1]

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ABSTRACT - One-fifth of the world's population consumes too little zinc (Zn) causing deficiencies that can damage cells, stunt growth, and decrease immune response. This study evaluated the effect of time on the priming of beet seeds, in solutions enriched with Zn, on physiology, growth, production, and root biofortification. Two greenhouse experiments were conducted during spring 2015 and autumn 2016. In each experiment, 24 treatments were tested which comprised various combinations of three Zn concentrations (0, 10, and 30 mg mL^{-1}), two Zn sources (sulphate and chloride), and four time periods (12, 16, 20, and 24 h), arranged in a randomised block design with four replicates. The concentration of Zn, mainly as sulphate, affected all parameters evaluated in the beet plants, such as fresh and dry root mass, photosynthesis, and root Zn concentration (biofortification). Compared to the control, fresh root mass increased 70 and 100 g per plant with 10 mg mL^{-1} of Zn during the experiments in 2015 and 2016, respectively. The same concentration for 16 h produced the highest Zn concentration in the roots, achieving 121 and 42 mg kg^{-1} in 2015 and 2016, respectively. Priming seeds in solutions enriched with Zn, thus, benefited the physiological response of the beet plants by promoting increases in growth, production, and biofortification of beet roots. Therefore, this method can be used to biofortify beet plants agronomically, regardless of the Zn source.

Keywords: *Beta vulgaris* var. *vulgaris*. Nutritional value. Seed treatment. Micronutrient.

BIOFORTIFICAÇÃO AGRONÔMICA DE BETERRABA COM ZINCO VIA CONDICIONAMENTO OSMÓTICO DE SEMENTES

RESUMO - Uma quinta parte da população mundial consome pouco zinco (Zn), causando deficiências que podem causar danos celulares, retardo no crescimento e diminuir o sistema imunológico. Nesse trabalho avaliou-se o efeito do tempo de condicionamento osmótico de sementes de beterraba em soluções enriquecidas com Zn sobre a fisiologia, o crescimento, a produção e a biofortificação da raiz. Dois experimentos em estufa foram conduzidos durante primavera de 2015 e outono de 2016. Em cada experimento foram testados 24 tratamentos os quais compreenderam as combinações de três concentrações de Zn (0, 10 e 30 mg mL^{-1}), duas fontes de Zn (sulfato e cloreto) e quatro tempos (12, 16, 20 e 24 horas), sob arranjo de blocos casualizados com quatro repetições. A concentração de Zn, principalmente como sulfato, afetou todos os parâmetros avaliados nas plantas de beterraba, massa fresca e seca de raiz, fotossíntese e a concentração de Zn na raiz (biofortificação). Em comparação ao controle, a massa fresca da raíz incrementou 70 e 100 g por planta com 10 mg mL^{-1} Zn durante os experimentos de 2015 e 2016, respectivamente. A mesma concentração por 16 horas provocou a maior concentração de Zn nas raízes atingindo 121 e 42 mg kg^{-1} em 2015 e 2016, respectivamente. Assim, o condicionamento osmótico de sementes em soluções enriquecidas com Zn, melhora as respostas fisiológicas das plantas, promovendo incrementos no crescimento, na produção e na biofortificação das raízes de beterraba. Portanto, esse método pode ser utilizado para biofortificar agronomicamente plantas de beterraba, independente da fonte de Zn.

Palavras-chave: *Beta vulgaris* var. *vulgaris*. Valor nutricional. Tratamento de sementes. Micronutriente.

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INTRODUCTION

Soils worldwide contain low levels of available zinc (Zn), which reduces Zn uptake and crop yields (WHITE et al., 2018). The critical level of Zn deficiency in soils varies from 0.5 to 2.0 mg kg $^{-1}$, depending on the extractant used and the variety of crop (MOREIRA; MORAES; DOS REIS, 2018), whereas for sugar beet leaves, an adequate concentration can range from 20 to 100 mg kg^{-1} (TRANI; VAN RAIJ, 1997).

The quality of agricultural products is affected by Zn deficiencies in soils which in turn affects human nutrition and health (WHITE et al., 2018; HEFFERON, 2019). Problems associated with malnutrition, such as Zn deficiency, is already recurrent and of great concern to international authorities, and could increase dramatically as the world population is expected to exceed 9 billion by 2050 (GODFRAY et al., 2010). Zn deficiency in humans is associated with growth retardation, impaired foetal brain development, poor birth outcomes in pregnant women, and increased susceptibility to infectious diseases, therefore a daily Zn intake of 15 mg is recommended (INSTITUTE OF MEDICINE US, 2001).

Agronomic biofortification has been proposed as a strategy to help combat malnutrition and improve the health of the world's population by increasing both the concentration of nutrients in the edible parts of food and crop yields (BOUIS; SALTZMAN, 2017), thus benefiting both consumers and producers. Some factors, however, may decrease the impact of interventions, such as high degrees of Zn adsorption to soil constituents, low mobility of Zn in the phloem of plants (BROADLEY et al., 2012), or its tight genetic regulation (KUMAR et al., 2018).

Adequate Zn fertilisation, by soil (in solution) and/or foliar application, has positively affected various crops (GOBARAH et al., 2014; BARRAMEDA-MEDINA et al., 2017; WHITE et al., 2018), which is attributed to the participation of Zn in several metabolic processes. Barrameda-Medina et al. (2017) and Barlóg, Nowacka and Blaszyk (2016) found that different doses of Zn increased the foliar area of cabbage and sugar beet, respectively. Among vegetables, sugar beet is one of the most commonly produced vegetables in the world and its seeds represent an important commercial value in Brazil, with about US$ 61 million in 2010 (ABCSEM, 2011). It has a high capacity to uptake Zn (SAGARDOY et al., 2009), and therefore, has the potential to be biofortified.

The priming of seeds in solutions enriched with inorganic salts has been used in several agricultural crops to improve germination, emergence, and initial establishment of seedlings in regions with unfavourable environmental conditions (MUNAWAR et al., 2013; HASSAN et al., 2019). This technique may also contribute to the increase in the quality of agricultural products because it can increase nutrient concentrations in the edible parts of plants (FAROOQ; WAHID; SIDDIQUE, 2012; CARMONA et al., 2019).

We hypothesise that priming beet seeds with Zn might be an efficient way to agronomically biofortify this vegetable, therefore the duration of priming and the source and concentration of Zn in solution are important variables to study. Our objective was to evaluate the effect of time on the priming of beet seeds in solutions enriched with Zn on the physiology, growth, production, and root biofortification of *Beta vulgaris* var. *vulgaris*.

MATERIAL AND METHODS

Study location

Two experiments were conducted, one during each of two growing seasons: spring 2015 (September to November) and autumn 2016 (May to July), in an unheated greenhouse at Sao Paulo State University - UNESP, *Campus* Jaboticabal, SP, Brazil (21°15'22"S, 48°18'58"W; 575 m a.s.l.). Average temperature and relative air humidity in the greenhouse ranged from 21.4 to 30.4 °C and 20 to 70% in 2015 and from 14.7 to 26.1 °C and 53 to 91% in 2016.

Treatments and experimental design

Each experiment evaluated 24 treatments combining three factors: Zn concentration (0, 10, and 30 mg mL^{-1}), Zn source (sulphate and chloride), and duration of seed priming (12, 16, 20, and 24 h). Each experiment had a randomised block design, with treatments arranged in a 3 × 2 × 4 factorial scheme, with four replicates. Each experimental unit was adequate labelled.

Installation and conduction of experiments

Zn solutions used in the treatments were prepared with deionised water (pH 7.0) and calculated amounts of $ZnSO_4 \cdot 7H_2O$ (Synth® at 22.7% Zn, 11.1% S) and $ZnCl_2$ (Dynamic® at 48% Zn). Two grams of beet seeds (approximately 15 seeds of the cultivar Early Wonder Tall Top) were completely immersed in 10 mL of Zn solution (MUNAWAR et al., 2013) in polyethylene bags and kept in a dark at room temperature (~24 °C) for the duration specified for each treatment.

The treated seeds were sown in 200-cell polyethylene trays with a vermiculite substrate. The trays were kept in the greenhouse during initial growth of seedlings. The seedlings were irrigated periodically with the nutrient solution of Hoagland and Arnon (1950) prepared with deionised water,

without a supply of Zn, and at 50% ionic strength.
The seedlings were transplanted when the first pair of leaves developed (18 days after sowing (DAS)) into pots filled with 6 dm^3 of coconut-shell fibre (AMAFIBRA, Golden Mix 98) without fertiliser and pre-moistened with deionised water. Two seedlings were placed 10 cm apart in each pot. The pots were carefully labelled and separated by 5 cm.
The plants were fertigated from 24 and 30 DAS in 2015 and 2016, respectively, until the end of the experiment; the nutrient solution was prepared with deionised water, without a Zn supply, and at 100% ionic strength. The plants were fertigated until drainage of the solution was observed. Fertigation was alternated daily with irrigation with deionised water to avoid salinisation of the substrate. The experiments ended at 66 DAS.

Plant growth

The leaf area (LA, cm^2 $plant^{-1}$) of all plants in both experiments was evaluated at 66 DAS using an electronic leaf area meter Li-3100® (LI-COR Inc., Lincoln, NE, USA). The leaves were then washed, first in running water and then in a solution of 0.1M HCl and neutral detergent (3 mL L^{-1}); then rinsed in running water and finally deionised water. The samples were subsequently dried on a paper towel, packed, and identified for dehydration at 65 °C in a forced-air circulation oven to a constant weight, then the aboveground dry mass (AGDM, g $plant^{-1}$) was quantified. The plants were harvested and the fresh-root mass (FRM) was immediately obtained using a precision scale. The roots were sliced and dried in the forced-air circulation oven, and the dry root mass (DRM) was obtained.

Measurement of gas exchange

The photosynthetic rate (*A*), stomatal conductance (*gs*), and transpiration rate (*E*) of a recently fully developed leaf without visible blemishes from each plant was evaluated at 47 and 55 DAS in 2015 and 2016, respectively, using an infrared gas analyser (LCPro System, ADC) under ambient CO_2 concentration (370 ± 10 μmol m^{-2} s^{-1}) and a photosynthetically-active photon flux of 1000 μmol m^{-2} s^{-1}. The measurements were carried out during morning periods on cloudless days.

Leaf Zn concentration (ZnL)

A newly developed leaf was collected from each plant after the analysis of gas exchange, as described by Trani and Van Raij (1997). The leaves were washed as previously described, and the samples were dried on a paper towel, packed, and identified for dehydration at 65 °C in the forced-air circulation oven to a constant weight. The dried leaves were ground in a Wiley-type mill and passed through a 2-mm sieve to determine the Zn concentration (mg kg^{-1}), as described by Miyazawa et al. (2009).

Biofortification of roots with Zn (ZnR)

The Zn concentrations (ZnR, mg kg^{-1}) in the dried roots were evaluated after harvest. The procedures for the preparation of the root samples for Zn evaluation were the same as those described for ZnL.

Statistical analysis

The variance of the data was analysed for each experiment using the AgroEstat application (BARBOSA; MALDONADO JÚNIOR, 2015). When the mean squared ratio of the residue of a given parameter was < 7, a joint analysis of the experiments for this parameter was performed (PIMENTEL-GOMES, 2000). The effect of the treatments on the analysed parameter was determined separately for each experiment when the joint analysis identified a significant effect. The means for each parameter were compared by Tukey tests at $P<0.05$.

RESULTS AND DISCUSSION

Zn concentrations affected LA, AGDM, FRM, DRM, and ZnR in both experiments. AGDM and FRM were also affected by priming duration in 2015, whereas only FRM was affected in 2016. There were no effects of Zn source on these parameters and no interaction among factors was found.

Priming the seeds with Zn affected both FRM and DRM, increasing root growth in both experiments (Figure 1), which is supported by previous research that also found positive effects of Zn application on beet yield and quality (GARTLER et al., 2013; GOBARAH et al., 2014; BARLÓG; NOWACKA; BLASZYK, 2016). However, from our understanding, the effects on beet root growth promoted by seed priming in solutions containing Zn has not been reported before.

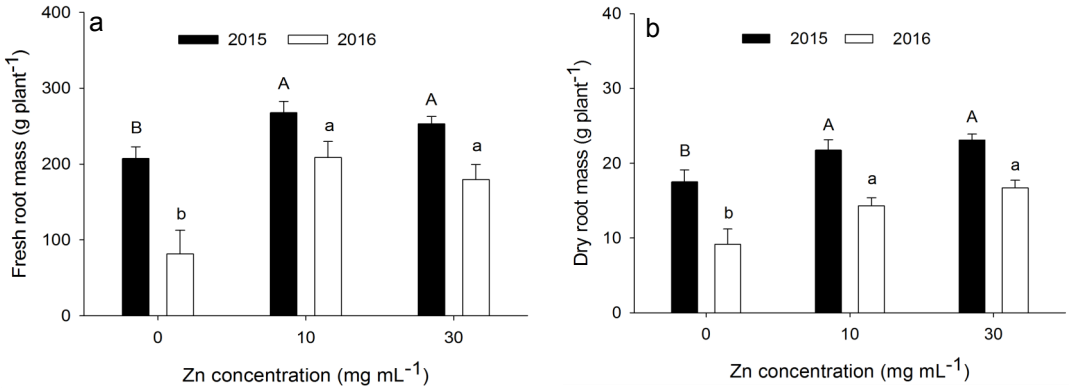

Figure 1. Fresh (a) and dry (b) root mass of beet plants grown in a soilless culture system after priming of seeds in solutions enriched with Zn. Different letters in the columns indicate statistically significant differences (P<0.05) for each growing season.

The response to treatment was more variable in plants cultivated in 2016 than 2015, with increases in FRM of 100 and 70 g, respectively, when plants were grown at 10 mg mL^{-1} as compared to the control plants (Figure 1a). In addition, under these treatment conditions, Zn priming also increased DRM approximately 82 and 32% for 2016 and 2015, respectively (Figure 1b), highlighting Zn as a constituent of cellular structure and its involvement in the activation of numerous enzymes involved in stress tolerance and synthesis of carbohydrates and proteins (BROADLEY et al., 2012), which directly affect biomass production (BARRAMEDA-MEDINA et al., 2017; HASSAN et al., 2019).

Duration of seed priming only affected the FRM of beet plants in both experiments. Periods of 16 and 20 h presented similar results for this parameter, reaching a high value of 277 g $plant^{-1}$ at 16 h and 175 g $plant^{-1}$ at 20 h in 2015 and 2016, respectively (Figure 2), indicating that 16 h presented the most efficient treatment for increasing FRM, even though it did not biofortify the roots. The efficiency of seed priming might be affected by different ambient factors (FAROOQ; WAHID; SIDDIQUE, 2012) such as duration of priming treatment and the crop species studied (MUNAWAR et al., 2013; HASSAN et al., 2019).

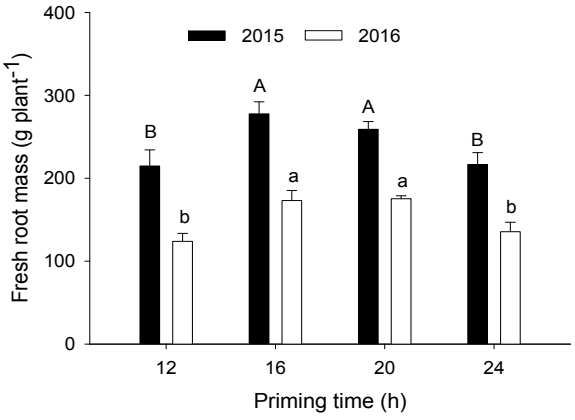

Figure 2. Fresh root mass of beet plants grown in a soilless culture system after priming of seeds in solutions enriched with Zn. Different letters in the columns indicate statistically significant differences (P<0.05) for each growing season.

In addition to its participation in many metabolic processes that promote growth in plants, Zn is an essential element involved in the biosynthesis of tryptophan, an amino acid precursor of indol-3-acetic acid, a hormone that regulates plant growth (TAIZ et al., 2017). Thus, it can be explained that LA and AGDM were higher in primed than unprimed plants in both experiments (Figure 3). LA was higher in 2016 than in 2015, mainly at 30 mg mL^{-1} (Figure 3a). AGDM, however, was nearly two-fold higher in 2015 than in 2016 for all Zn concentrations (Figure 3b). An increase in LA is considered a key factor for beet production (JAGGARD; QI, 2006). Zeinab, Soudi and El-Shenawy (2011) reported that an increase in LA in beet plants with foliar application of Zn increased yield. Engels, Kirkby and White (2012) found that increasing foliar photosynthetic activity and/or LA increased the productivity of agricultural crops, because a larger photosynthetic area implies the absorption of more light by the plants.

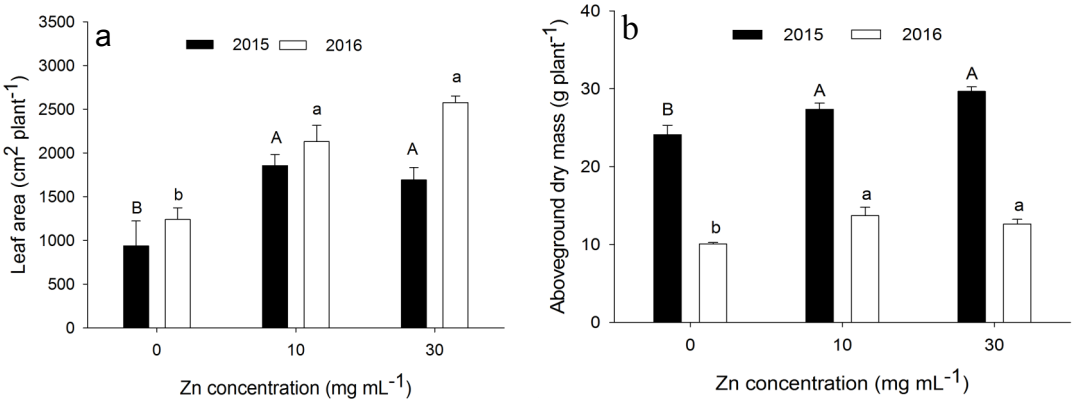

Figure 3. Leaf area (a) and aboveground dry mass (b) of beet plants grown in a soilless culture system after priming of seeds in solutions enriched with Zn. Different letters in the columns indicate statistically significant differences (P<0.05) for each growing season.

As shown in Figure 4, the photosynthetic parameters assessed in our work were affected by the treatments. In 2015, *E* was affected by Zn concentration and source and by their interaction. *E* was 31% higher in plants whose seeds were primed in chloride solutions at 10 mg mL^{-1} Zn compared to control plants (Figure 4a). On the other hand, there was no difference in *E* when sulphate was used.

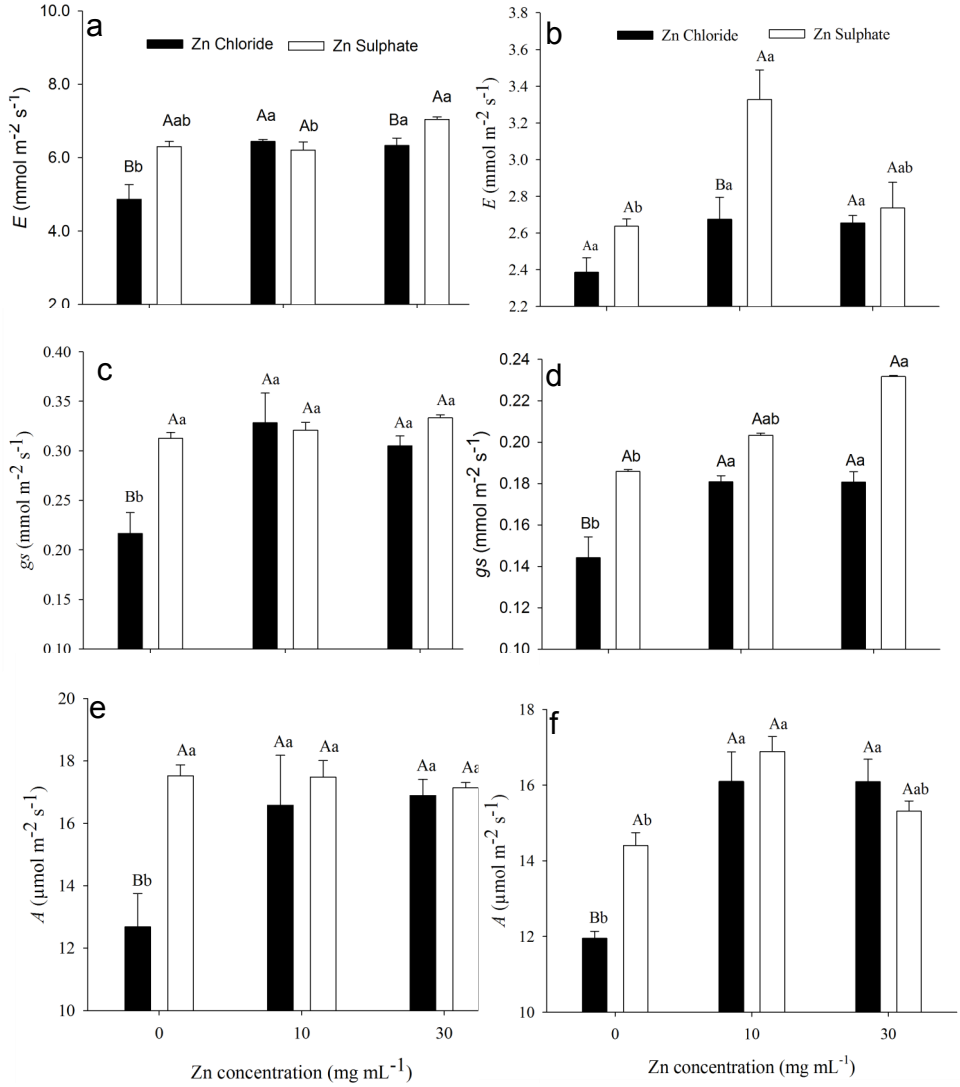

Figure 4. Transpiration rate (*E*), stomatal conductance (*gs*), and photosynthetic rate (*A*) of beets plants grown in 2015 (a, c, e) and 2016 (b, d, f) after seed treatments in solutions enriched with Zn from different sources. Different capital and lowercase letters in the columns indicate statistically significant differences (P<0.05) between Zn sources and concentrations, respectively.

In 2016, *E* was only affected by Zn concentration and source, not by their interaction. *E* was highest in plants treated with a sulphate solution containing 10 mg mL^{-1} Zn. Only at this concentration, was *E* higher in plants treated with the sulphate solution as compared to the chloride solution (Figure 4b).

Stomatal conductance (*gs*) was affected by the interaction between Zn concentration and source in 2015, but only by Zn concentration and source in 2016. The *gs* increased in 2015 and 2016 in beet plants treated with Zn chloride, but only in 2016 when treated with Zn sulphate (Figure 4c, d). In 2016, *gs* was higher in plants treated with 30 mg mL^{-1} Zn as sulphate than the control plants, whereas 10 mg mL^{-1} Zn produced no significant difference in plants without Zn and with 30 mg mL^{-1} Zn. On the other hand, when seeds were treated with Zn in chloride solution, the *gs* of plants at both concentrations (10 and 30 mg mL^{-1}) was higher than the control plants.

A was affected by the interaction between Zn concentration and source in both years and also by the interaction between Zn concentration and priming duration in 2016. In 2015, *A* was higher than the control only when Zn chloride was used as the Zn source, and did not differ significantly between the 10 and 30 mg mL^{-1} Zn concentrations. Priming using the Zn sulphate source did not affect *A* (Figure 4e). In 2016, *A* was affected by both Zn sources (Figure 4f). *A* was highest at 10 mg $mL^{-1}$for Zn sulphate source and at 10 and 30 mg mL^{-1} for the Zn chloride source, with increases of 20 and 46%, respectively, relative to the control. Priming duration did not affect gas exchange in either year. *A* is directly correlated with *E* and *gs* because the loss of water from transpiration is necessary for absorption of nutrients and assimilation of atmospheric carbon (TAIZ et al., 2017); it is also the main mechanism of thermal regulation. An increase in *E* and *gs* has thus been correlated with an increase in photosynthesis in plants grown under suitable conditions (SAGARDOY et al., 2009), consistent with our study. The increase in *A* improved beet production as a function of Zn application to the seeds (Figure 4) and accounted for the higher FRM in beets grown in spring 2015 (slightly higher temperatures) than in autumn 2016.

ZnL was only affected by Zn concentration in both growing seasons. In 2015, ZnL was 10% higher with Zn present in solution than without (Figure 5), but ZnL was > 80 mg kg^{-1} in all plants. In 2016, ZnL was lower, but the highest value in plants primed with 10 mg mL^{-1} Zn (at 47 mg kg^{-1}) was approximately 100% higher than control plants. Seed priming in our study increased ZnL (Figure 5) without exceeding the critical limit of 100 mg kg^{-1} in the mass of dry leaves (TRANI; VAN RAIJ, 1997) and consequently improved physiological activity and growth (Figures 1–4), as also reported by Munawar et al. (2013). Both *E* and *gs* are associated with the loss of water vapour through the stomata and are strongly influenced by temperature (TAIZ et al., 2017). Nutrients are mainly translocated via the xylem (transpiratory current); nutrients with low mobility in plants, such as Zn, tend to have higher concentrations in organs with higher *E* (WHITE et al., 2018), which may account for the higher ZnL in 2015 when ambient and foliar temperatures averaged 5.5 and 8.0 °C higher than in 2016, respectively.

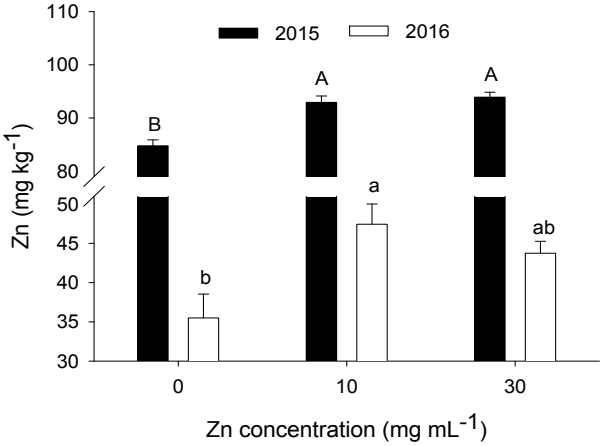

Figure 5. Leaf Zn concentration in beet plants grown in a soilless culture system after priming of seeds in solutions enriched with Zn. Different letters in the columns indicate statistically significant differences (P<0.05) for each growing season.

In 2015, ZnR was highest (121 mg kg^{-1} Zn) in plants primed with 30 mg mL^{-1} Zn, but did not differ significantly (114 mg kg^{-1} Zn) from the treatment with 10 mg mL^{-1} Zn (Figure 6). In 2016, ZnR was also high (42 mg kg^{-1} Zn) in plants primed with 10 mg mL^{-1} Zn; about 25% higher than the control. ZnR was nearly three-fold higher in 2015 than in 2016, similar to ZnL. Root enrichment with Zn was verified in both experiments, with the highest concentration in 2015 (Figure 6) attributed to the

increase in gas exchange parameters, ZnL, and growth characteristics of the plants (Figures 1, 4, 5). Although Zn mobility through the phloem is considered low, Zn is distributed between plant organs mainly directed to growth points represented by root reserves which become the main drain of the plant for photosynthates and nutrients after reserve accumulation (GONDIM et al., 2011). The results of this study show that roots were biofortified using Zn to prime seeds with a concomitant increase in FRM and DRM. More studies focusing on Zn enrichment in beets could aid development of this technique for biofortification.

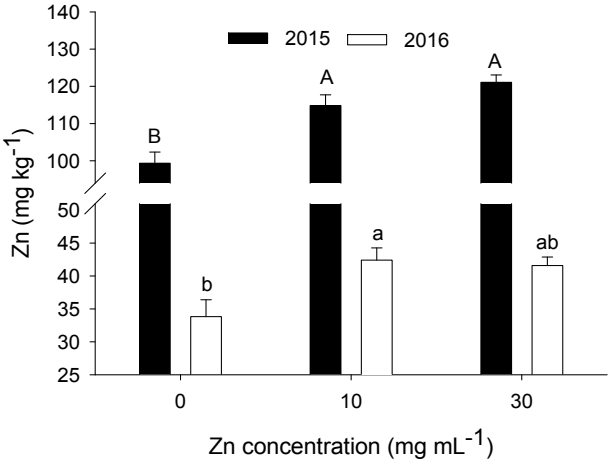

Figure 6. Zn concentration in roots of beet plants grown in a soilless culture system after priming of seeds in solutions enriched with Zn. Different letters in the columns indicate statistically significant differences (P<0.05) for each growing season.

The recommended adult intake of Zn is 15 mg d^{-1} (INSTITUTE OF MEDICINE US, 2001); considering that about 90% of beet biomass is water, an adult would meet 8% of their daily demand for Zn by consuming a portion of 100 g d^{-1} of beets biofortified in 2015 (121 mg kg^{-1} of Zn) and 3% from the same amount of beets in 2016 (42 mg kg^{-1} of Zn) (Figure 6). Biofortification of beets by priming seeds with Zn for 16 h is therefore a potential strategy for agronomic biofortification, regardless of the source of Zn, because this duration of priming was found to favour a high FRM.

CONCLUSION

Priming seeds with Zn benefited the physiological responses of the beet plant by promoting increases in ZnL, growth, production, and root biofortification. Priming seeds for 16 h with 10 mg mL^{-1} Zn is recommended for the agronomic biofortification of beets, regardless of the Zn source (sulphate or chloride).

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