

Seed germination of beans (*Phaseolus vulgaris*) with nano-particles of iron

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Abstract

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Nanoparticles (NPs) are used in agriculture for seed germination and their effect depends on factors such as chemical nature and size, concentration and time of exposure and/or aging of NPs). The objective of this study was to evaluate the potentiality of establishing whether Fe NPs could be used as a fertilizer for the improvement in germination in bean crops (*Phaseolus vulgaris*), and then to study the possible mechanisms of interaction between the NPs, the soil and the seeds. In this study, the effect of iron nanoparticles, obtained by green synthesis (NPs-FeG), on the germination of Creole black bean seeds was evaluated. The treatments were with 5 and 10 mg.L⁻¹ of NPs-FeG and a control. A completely randomized experimental design was performed with three treatments and five repetitions and an ANOVA. Germination was observed 5 days, then the length of the primary root was measured. The treatment of 5 mg.L⁻¹ of NPs-FeG showed higher germination than with 10 mg.L⁻¹ and the control. Longer and thicker roots were also observed. The NPs-FeG favor germination of the seeds, decrease the germination time and enhance the development of the root system.

Keywords: zero iron nanoparticles; green synthesis; seed; germination; beans

Introduction

The cultivation of beans (*Phaseolus vulgaris*) constitutes one of the staple foods in Mexico and in many parts of the world. This grain is an excellent source of proteins, essential amino acids and iron; beans contribute approximately 40% of iron to the human diet, however, it has been estimated that, of the total iron present in beans, only up to 20% is assimilated, so their contribution is low (Escamilla, 2013).

Iron (Fe) is a micronutrient, considered as one of the most important for plant life. An important portion of iron is located mainly in the chloroplasts of the leaves, where photosynthetic activity occurs, catalyzes the formation of chlorophyll

and acts as an oxygen transporter. It helps to form some enzymatic systems that act in the breathing processes. In their absence, all the green organs become discolored, because the Fe does not translocate inside the plant, the symptoms of deficiency appear first in the young leaves in the upper part of the plant (Rabotti et al., 1995). This element is also important to improve nutrition and achieve food security, there are rural populations, where women and children do not receive the necessary micronutrients to lead a healthy productive life, which leads to serious consequences on the physical and mental development of people and increases the risk of maternal death. Anemia is the most common and widespread nutritional disorder in the world and it is estimated that half

of all cases of anemia are due to iron deficiency (Holt et al., 2011). Currently, the effect of NPs of micronutrients such as Fe and Zn in plants is studied. It has been reported that NPs can be beneficial for the supply of biological molecules in plant cells, germination of seeds and plant growth, as well as the improvement of the application of herbicides (Lin and Xing, 2007).

Within the nanotechnology the green synthesis of NPs has emerged, a method that uses plant extracts (Wang et al., 2014) that replace chemical reducing agents such as sodium borohydride (NaBH_4) to obtain so-called green nanoparticles (green nanoparticle). One of the most studied extracts is the eucalyptus tea (*Eucalyptus globulus*), this species of the *Myrtaceae* family grows in different parts of the world and among its different applications its medicinal and therapeutic activity stands out (Du et al., 2017), the leaves of this plant contain polyphenolic organic compounds characterized by having in their structure one or more aromatic rings with one or more hydroxyl groups that are part of the plant defense system (Abadia et al., 2002). This method of synthesis is low cost, manages to stabilize the size of the nanoparticles and avoid the conglomeration of them.

In the agricultural sector, nanotechnology has a fundamental potential to facilitate and frame the following stages of the application of technology in the field, the adoption of nanotechnology plays a crucial role in the demand of a growing population with limited natural resources (Barrena et al., 2009). The objective of the present study was to evaluate the potentiality of establishing if Fe NPs could be used as a fertilizer for the improvement in germination and later in the extensive bean crops (*Phaseolus vulgaris*).

Materials and Methods

Synthesis of zero iron green nanoparticles

Eucalyptus tree leaves were washed with distilled water to remove dust from the surface, then dried at room temperature (RT). The eucalyptus extract was prepared with 15 g of dried leaves in 250 mL of deionized water at 80°C for 1 hour, the filtrate was kept at -4°C until its use. The nanoparticles were synthesized by adding 0.10 M FeSO_4 and green tea in a 2:1 volume/volume ratio at TA and stirring for 30 min. The disappearance of color indicates the reduction of Fe^{2+} , the solid obtained was filtered and rinsed with ethanol, dried under vacuum at 50°C (Herrera et al., 2010; Wang et al., 2014; Majumdar et al., 2016; Ramírez, 2017; Pariona et al., 2017) and were used immediately.

Obtaining and disinfection of bean seeds

Creole black bean seeds were donated by a producer from Cuauhtepac de Hinojosa, Hidalgo State, Mexico. A selection of seeds that did not present physical damage was disinfected and submerged in a 1% sodium hypochlorite solution for 10 minutes, then rinsed with distilled water.

Feasibility analysis of seeds

To avoid interference in the analysis of germination test results, a second seed selection was made. Bean seeds were placed in water for hydration, after 15 minutes those that floated were eliminated (Barrera, 2015).

Evaluation of germination with the use of NPs-FeG

Three treatments were performed with 5 repetitions and 25 seeds each, which were placed on filter paper in petri dishes with 20 mL of deionized water without NPs-FeG (T), with 5 (T1) and with 10 $\text{mg}\cdot\text{L}^{-1}$ (T2) of NPs-FeG. The Petri dishes with the treated seeds were kept in a germination chamber at a temperature of 25°C, with a relative humidity of 50% for 5 days (Fig. 1).

The variables evaluated in the germinated seeds were the following:

- Seeds with root development (SRD) every 24 hours a count of seeds was carried out. SRD is understood as all those that presented developed radicle.
- Seeds with break of testa (SBT) all those seeds that showed breaking of the seed coat.
- Average length of radicle (LR), the primary root of 50 seeds chosen at random for each treatment was measured by means of a vernier caller digital brand Truper, model CALDI-6MP. The results are expressed in mm.
- Secondary roots and absorbent hairs (SR and AH) by treatment were randomly selected 10 seeds, which were placed in Petri dishes in suspension system with NPs-FeG for 7 days, with the intention that the root system was developed

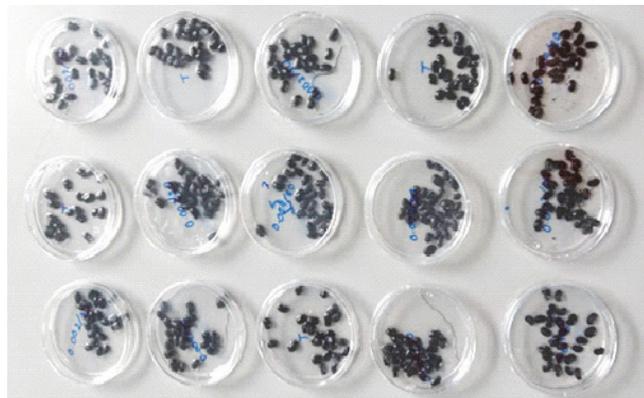


Fig. 1. Petri dishes with black bean seeds

to be able to observe and compare the root system of seeds using an Iroscope microscope model WB3T.

Statistical analysis

For the evaluation of the treatments, a linear model was used in a completely randomized design with three treatments (T, T1 and T2) and five repetitions. The normality of the data was tested by ANOVA and tests were made to compare means (Tukey $P < 0.05$), using the SAS statistical program.

Results and Discussion

Seeds with break of testa (SBT)

Fig. 2 shows the counting of seeds that showed breakage of the seed coat (the greater the break of the seed coat, the higher the incidence of germination and the development of the radicle), at 24 hours there are no significant differences between treatments because the process of imbibition lasts from 14 to 24 hours (Suárez and Melgarejo, 2010). A difference of 36% greater than the control was obtained.

This higher percentage of the testa break in T1 treatment that becomes constant after 48 hours, the T2 treatment at 48 hours has a decrease in the percentage of SBT, while the treatment T compared to T2 at 24 and 48 hours greater than SBT.

The results obtained coincide with that reported by Arredondo (2016), who indicated that the application of NPs at 0.5 mg.L^{-1} to broad chili seeds treated with NPs begin to show a greater break of the test, as well as the germination is favored with the application of 5 and 10 mg.L^{-1} of NPs, observed that the plumule and radicle reached longer lengths than the control. After 72 hours, the T2 and T treatments show no significant difference between them. For the 96 hours all the treatments are different, this was due to the germination process per day, at the end of 120 hours the T1 treatment was found to have the highest percentage of SBT (Fig. 2).

Seed with radicle development (SRD)

Fig. 3 shows the variations in the development of germinated seed radicles (SDR). At 24 hours, all treatments showed RDS. T1 and T2 have presented more SDR than the control T, same tendency that was shown until 120 hours. However, the T2 treatment is statistically equal to T at 120 hours (Fig. 2), some authors in similar experiments with iron and zinc NPs in pumpkin seeds (Ren et al., 2011), did not have significant differences in germination after 24 hours. In other studies (Hernández, 2015), evaluating roots and stems of sunflower and beans in greenhouses, presented similar results. It is pointed out that in many occasions, the seeds after their maturation and dispersion are not able to germinate, ei-

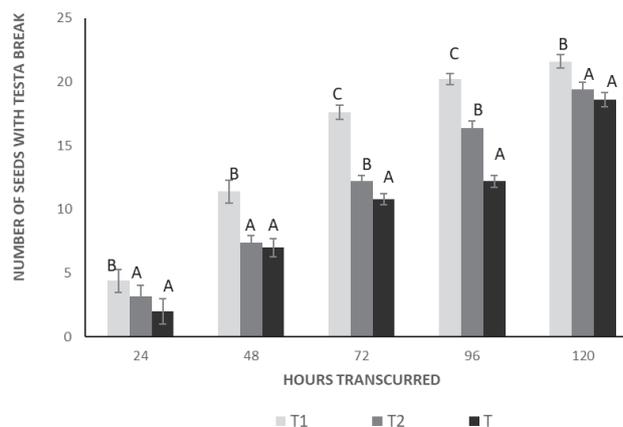


Fig. 2. Seeds with head breaking (SBT)

ther because they are dormant or because the environmental conditions are not favorable. In this situation the seeds begin to deteriorate which is manifested by the progressive loss of their ability to germinate (viability) and give rise to healthy and vigorous seedlings (vigor). The time it takes the seeds to lose their viability (longevity) is variable according to the species and dependent on both external factors (environmental temperature), and internal (moisture content, genotype, etc.) to the seeds themselves (Pérez and Pita, 2001).

The T1 treatment shows the best result of SDR every 24 hours, showing an increase with significant differences with the rest of the treatments (T2 and T) (Fig. 3).

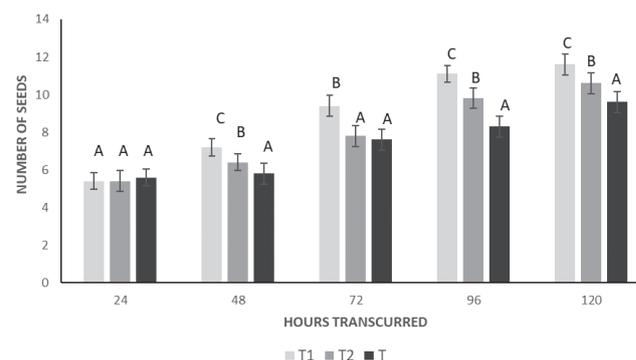


Fig. 3. Seeds with developed radicle (DR)

Average length of radicle (LR)

At the end of 120 hours, the length of the primary roots of 50 seeds per treatment, chosen at random, was measured. The mean root length of the seeds was 15.1, 23.6 and 17.3 mm for T, T1 and T2 respectively. Seeds treated with 5 mg.L^{-1} of NPs-FeG (T1) have longer roots, representing in treatment T1 26% higher than in T2 and 36% higher than the control T (Fig. 4).

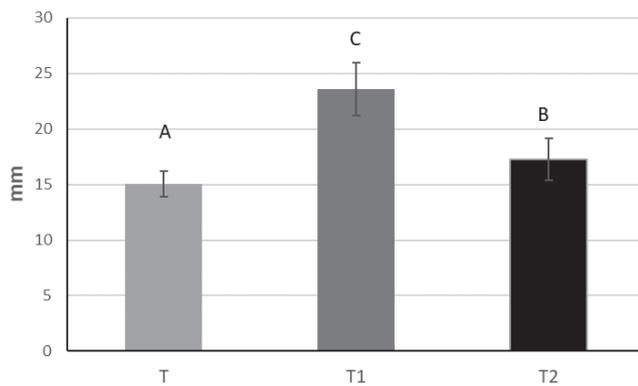


Fig. 4. Root length by treatment

Secondary roots (SR) and absorbent hairs (AH)

The application of NPs-FeG showed physiological changes in the root system. To observe these changes, 10 seeds were chosen per treatment after 120 hours, they were placed in petri dishes with a suspension of NPs-FeG for 48 hours. Fig. 5 shows the changes in the roots, treatment T (Fig. 5a)

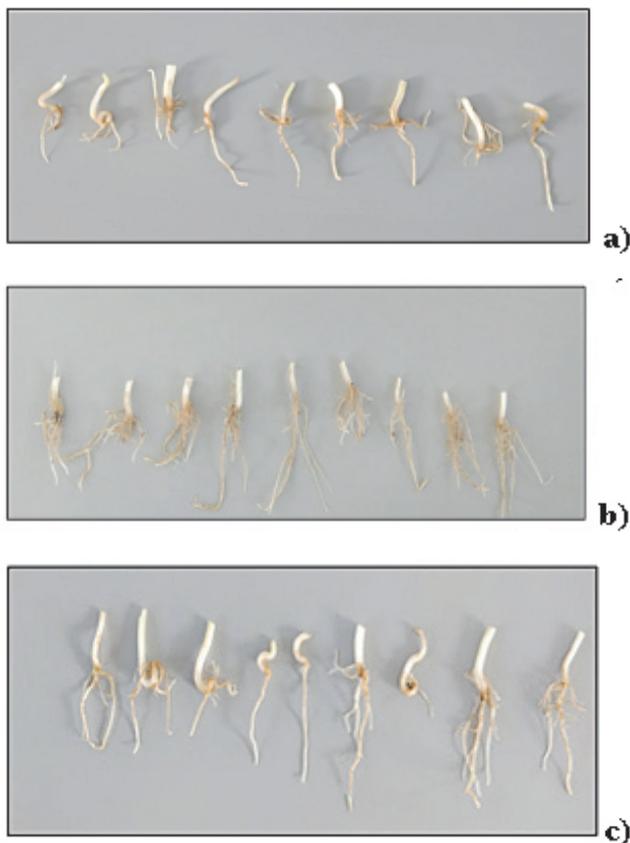


Fig. 5. Physiological changes of the root by treatments:
a) T; b) T1; c) T2

shows less amount of secondary roots (SR) and with smaller size in comparison with the two remaining treatments, for the treatment T1 (Fig. 3b) the secondary roots are more developed and larger root size compared to T (Fig. 5c) and T2.

The absorbent hairs (AH) come from the cells of the epidermis and their function is to increase the absorption capacity of the elements that the plant needs to take from the soil, dissolved in water, structurally they are evaginations of the epidermal cell wall in the form of tube, thin, just 1 mm long and covered with a mucilaginous substance (Ma, 2010). In the T2 treatment, there is a root system with a lower amount of AH compared to T1, but it shows a higher incidence in the amount of AH, in addition to a longer root length; being T the one that shows minor. As reported by Ramírez (2017) the application of NPsFe and MPsFe (iron microparticles), promoted the longitudinal development of the root system at concentrations of 25 and 50 mg.L⁻¹ up to 5.58% and 4.58% respectively.

Fig. 6 shows the differences by treatment in the absorbent hairs, the treatment T1 (Fig. 6b) showing a greater amount of absorbent hairs in comparison with T (Fig. 6a) and T2 (Fig. 6c).

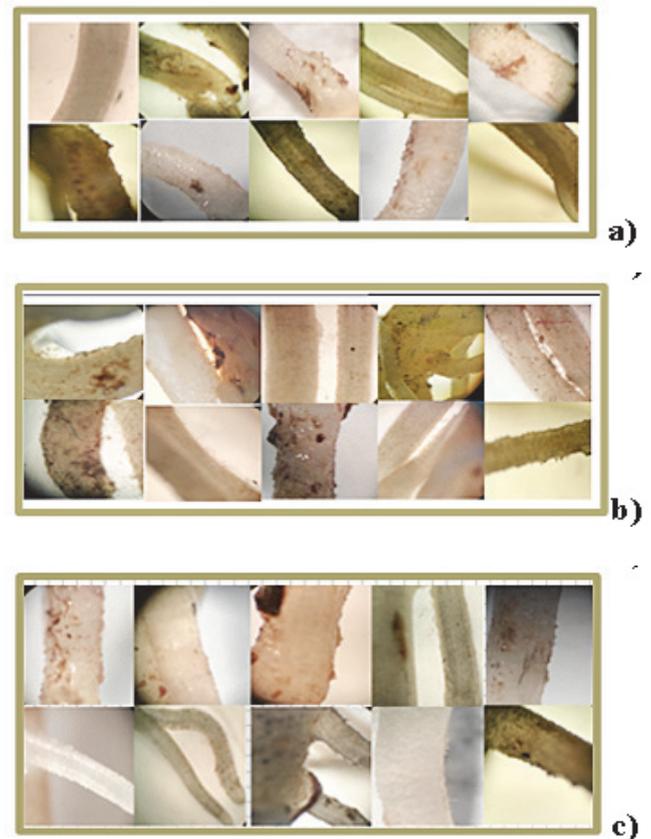


Fig. 6. Absorbent hairs per treatment:
a) T; b) T1; c) T2

Conclusion

The application of NPs-FeG improves the germination and length of the root system. The highest values of germination were obtained in the T1 treatment with a concentration of 5 mg.L⁻¹ of NPs-FeG, in comparison with the control. The T1 and T2 treatments show significant differences in the germination, development and size of the roots.

The application of NPs-FeG can function as a promoter of bean seed germination, since as the results show, the root system increased its length and quantity of absorbent hairs. Within the root system, the absorbent hairs are of great importance since the more quantity of them is on the surface of the epidermis the greater the absorption of nutrients, this is the case of T1 that shows more amount of absorbent hairs.

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