

## DETERMINATION OF THE EFFECT OF SILICON, MYCORRHIZA AND PHOSPHORUS BACTERIA APPLICATION ON INCREASING PHOSPHORUS UTILIZATION EFFICIENCY AND STEM RESISTANCE IN SUNFLOWER (*Helianthus annuus*)

Aysen AKAY

University of Selcuk, Agricultural Faculty, Konya, Türkiye

Corresponding author email: aakay@selcuk.edu.tr

### Abstract

Today, the intensive use of chemical fertilizers causes various environmental problems. Inoculation of microbial preparations as an alternative to chemical fertilizers to plants can be an effective method for sustainable production efforts. Silicon application shows potential to increase nutrient uptake by roots and nutrient availability in the rhizosphere through complex mechanisms, can increase P availability in the soil. In this study, the effects of silicon application and inoculation of phosphorus solubilizing bacteria and mycorrhiza on sunflower plant growth and phosphorus utilization efficiency were investigated. Oil sunflower seed, *Glomus etunicatum*, mycorrhiza complex and with phosphorus bacteria were inoculated. Si application was also made at doses of 0-30-60 kg/da. After the sowing of the experimental plants, mycorrhiza and P bacteria were inoculated. The experiment was terminated when 50% of the flowers bloomed. According to the results, average plant height, flower wet and dry weights showed significant differences with Si treatments, Si with mycorrhiza and P bacteria treatments. 30 Si kg/ha dose had more positive effects on plant growth.

**Key words:** silicon, phosphorus, mycorrhiza, phosphorus bacteria.

### INTRODUCTION

Silicon, which is not classified as an absolutely essential element for plants, has actually been observed to have beneficial effects in various plant species and environmental conditions. It has been reported that Si has the potential to increase the uptake of nutrients in the rhizosphere and root zone through complex mechanisms (Pavlovic et al., 2021). Si has been described by various researchers as an “agriculturally essential element” due to its ability to increase plant resistance to plant diseases and pests and stressful conditions such as drought, metal toxicity, salinity and sodium (Detmann et al., 2012; Klotzbücher et al., 2018; Liang et al., 2015). Some plant species are minimally affected by Si fertilization compared to other plants (Coskun et al., 2019).

Silicon has been reported to play an important role in P nutrition as well as in the uptake of other plant nutrients (Schaller et al., 2021; Pavlovic et al., 2021). Also, Si has been shown to have a mitigating effect in wheat (Kostic et al., 2017; Neu et al., 2017), tomato (Zhang et

al., 2019), paddy (Pati et al., 2016; Hu et al., 2018), maize (Owino-Gerroh and Gascho, 2005), potato (Soltani et al., 2017; Soratto et al., 2019) plants under conditions of limited availability of phosphorus. Si has been reported to be effective in alleviating P deficiency by increasing root uptake and utilization of phosphorus within plant tissues (Neu et al., 2017; Zhang et al., 2019).

Although the use of phosphorus fertilizers is recommended to eliminate P deficiency in soil, there are factors that reduce the efficiency of phosphorus use in acidic and calcareous soils. In recent years, it has been reported that arbuscular mycorrhizal fungi (AMF), phosphate solubilizing bacteria (PSB) and Si application can be effective and economical solutions to increase the availability and efficiency of phosphorus in soil (Etesami et al., 2021). In this study, the effects of inoculation of sunflower plants with different doses of Si, a phosphorus solubilizing bacterium and two different mycorrhizae on plant growth parameters were investigated.

## MATERIALS AND METHODS

The experiment was conducted as a pot experiment in greenhouse conditions in the spring of 2024. Before the experiment was established, leek seeds were inoculated (500 spore/pot) with the existing *Glomus etunicatum* mycorrhiza species obtained from previous projects and mycorrhiza isolated from natural plant roots from the mining area under greenhouse conditions. When the inoculated seeds develop as plants and reach the height of 30-40 cm, the underground part of the plant was taken along with the soil, mixed thoroughly and stored at +4°C until use. Thus, the propagation of these mycorrhizae from leek roots was carried out.

The experimental soil was collected from a depth of 0-30 cm, sieved through a 4 mm sieve, and filled into the pots by weighing (4 kg of

soil pot<sup>-1</sup>) as an oven-dried basis. The experiment was conducted in a factorial design with three replications and three factors:

Mycorrhiza: *Glomus etunicatum* and natural mix mycorrhiza inoculation (500 spores/pot to a depth of 10 cm from the soil surface) and without inoculation (M0).

Phosphorus bacteria were applied from a commercial preparation (*Bacillus pumilus*) with inoculation (B+) and without inoculation (B-).

Silicon application: Si(OH)<sub>4</sub> containing 26% water soluble silicon from a commercial product (Agrisilica) was used. Application doses were 0 (Si0), 30 (Si30) and 60 (Si60) kg Si/da.

Sunflower seeds (MAY M96CL02 oil sunflower seed) were sown 8 seeds in each pot and diluted to 5 plants after emergence.

The analysis results of the soil used in the experiment are presented in Table 1.

Table 1. Initial physicochemical properties of selected soil used in pots

| Parameters                   | Results | Commentary      | Literature of analysis method |
|------------------------------|---------|-----------------|-------------------------------|
| pH (1:2.5 soil:water)        | 8.8     | Strong alkaline | (Richards, 1954)              |
| EC (1:2.5 soil:water)        |         | Lightly Salt    | (Richards, 1954)              |
| ( $\mu$ S cm <sup>-1</sup> ) | 428     |                 |                               |
| CaCO <sub>3</sub> (%)        | 38.23   | High            | (Bayraklı, 1986)              |
| Organic matter (%)           | 1.45    | Little          | (Walkley and Black, 1934)     |
| Texture class                | Loam    |                 | (Gee and Bauder, 1986)        |
| Ca (mg kg <sup>-1</sup> )    | 3415    | Sufficient      | (Thomas, 1982)                |
| Mg (mg kg <sup>-1</sup> )    | 368     | Sufficient      | (Thomas, 1982)                |
| K (mg kg <sup>-1</sup> )     | 210     | Sufficient      | (Carson, 1980)                |
| Na (mg kg <sup>-1</sup> )    | 114     |                 | (Thomas, 1982)                |
| P (mg kg <sup>-1</sup> )     | 34.7    | More            | (Olsen and Sommers, 1982)     |
| Fe (mg kg <sup>-1</sup> )    | 3.86    | Medium          | (Lindsay and Norvell, 1978)   |
| Zn (mg kg <sup>-1</sup> )    | 3.75    | Sufficient      | (Lindsay and Norvell, 1978)   |
| Mn (mg kg <sup>-1</sup> )    | 1.03    | Deficiency      | (Lindsay and Norvell, 1978)   |
| Cu (mg kg <sup>-1</sup> )    | 0.72    | Sufficient      | (Lindsay and Norvell, 1978)   |
| B (mg kg <sup>-1</sup> )     | 2.65    | More            |                               |

After sowing, the experimental plants were irrigated according to the field capacity of the soil, and the plants were waited until the formation of the flower plate to see the inoculation and effectiveness of mycorrhiza and P bacteria. The experiment was finished when 50% of the flowers opened after four months of development. N-P-K fertilizer was applied to the plants once during the growing period. During harvesting, the plants were cut above the soil, the above-ground parts and root parts of the plants were taken separately and brought to the laboratory. Wet weights of above-ground stems and flowers and dry

weights after drying at 65°C were taken on a precision balance. Mycorrhizal inoculation status of plant roots was determined in "Nikon ECLIPSE E 100" (Koske and Gemma, 1989; Giovenetti and Mosse, 1980). Chlorophyll was measured twice and plant height was measured three times at one month intervals throughout the experiment.

Statistical analysis of the values obtained as a result of the research, it was carried out with the Minitab 18 Statistical Software package program. All data (Si, Mycorrhiza, and P bacterium interactions) were subjected to the Tukey's multiple comparison test to determine

the statistical significance of the treatment effects.

## RESULTS AND DISCUSSIONS

The results showed that silicon application had a significant effect on plant height, flower weight and mycorrhizal hyphae formation. Similarly, Si\*Mycor., Si\*P bac. and Si\*Mycor.\*P bac. interactions also showed significant effects on plant height, plant top and flower weights and mycorrhizal hyphae

formation (Table 2). Leaf chlorophyll contents varied between 21.62-32.66 on average (Figure 1), plant height values varied between 36.67-48.80 cm, 49.87-63.33 cm, 77.87-89.73 cm in three different periods and the highest values were in Si60-Mmix-PBac+ treatment (Figure 2). In ornamental sunflower, silicon treatment resulted in the formation of thick and straight stems, increased flower and stem diameters and height, and improved sunflower quality compared to untreated controls (Kamenidou and Cavins, 2008).

Table 2. Analysis of the variances for the measured traits

| Applications      | Leaf Chlorophyll |    | Plant height measurements |    |    | Plant weights |            | Flower weights |            | Mycorrhiza   |           |            |
|-------------------|------------------|----|---------------------------|----|----|---------------|------------|----------------|------------|--------------|-----------|------------|
|                   | 1                | 2  | 1                         | 2  | 3  | wet weight    | dry weight | wet weight     | dry weight | Arbüscul (%) | Pouch (%) | Hyphae (%) |
| Si doses          | ns               | ns | **                        | ** | ns | ns            | ns         | **             | **         | ns           | ns        | *          |
| <i>Mycorrhiza</i> | ns               | ns | ns                        | ns | ns | **            | **         | **             | **         | **           | **        | **         |
| P bac.            | ns               | ns | ns                        | ns | ns | ns            | ns         | ns             | ns         | ns           | ns        | ns         |
| Si*Myc            | ns               | ns | *                         | *  | ns | **            | **         | **             | **         | ns           | ns        | ns         |
| Si*P bac.         | ns               | ns | **                        | ** | *  | **            | **         | **             | **         | ns           | ns        | ns         |
| Myc*P bac.        | ns               | ns | ns                        | ns | ns | ns            | *          | **             | *          | ns           | ns        | *          |
| Si* Myc*P bac.    | ns               | ns | *                         | *  | ns | **            | **         | **             | *          | ns           | *         | **         |

ns, nonsignificant; \*, significant at  $P \leq 0.05$  \*\*, significant at  $P \leq 0.01$ .

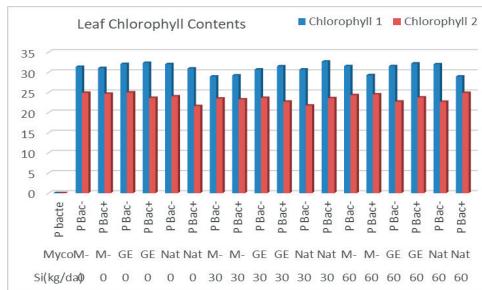


Figure 1. The effects of Si (Si0; Si30; Si60 kg/da), Mycorrhiza (with, *G. etunicatum*; Natural mix; without, M-) and P Bacteria (with, P Bac+; without, P Bac-) on the leaf chlorophyll contents

Wet and dry weight of the above-ground part of the plant increased significantly with mycorrhiza application; this increase was especially higher in Si30-Mycorrhiza treatments. Similar situation was also observed in mycorrhiza and P bacteria co-application. In Si30-*G. etunicatum*-P Bac+ treatments, the wet weight of the above-ground part was 144.62 g and the dry weight was 32.71 g, while the wet weight was 113.81 g and the dry weight was 27.74 g in the control treatment (Figure 3).

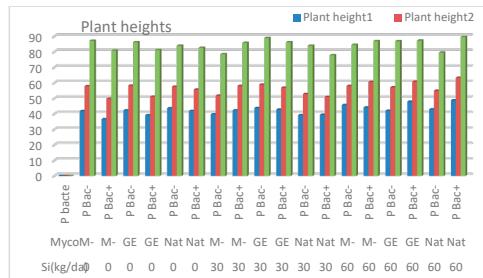


Figure 2. The effects of Si, Mycorrhiza and P Bacteria on the plant heights (cm)

When the effect of soaking the seed in different concentrations of nano-silicon solutions on the germination characteristics of sunflower was investigated in the pre-sowing revitalization processes, it was observed that germination time was significantly reduced and root length, average daily germination, seedling vigor index and final germination percentage were better (Janmohammadi and Sabaghnia, 2015). In another study, it was observed that wheat root length and root relative yield increased with 100 mg/kg silicon application (Mali and Aery, 2008).

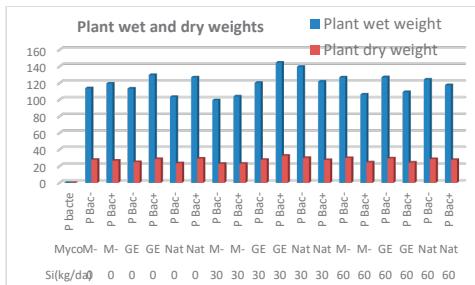


Figure 3. The effects of Si, Mycorrhiza and P Bacteria on the plant wet and dry weights (g)

While flower wet and dry weight did not change with phosphorus bacteria application, it increased significantly with mycorrhiza application. This increase was especially in Si30-Natural mix mycorrhiza treatment. The highest flower wet and dry weight values were again in Si30-*G. etunicatum* and natural mix mycorrhiza treatments (Figure 4).

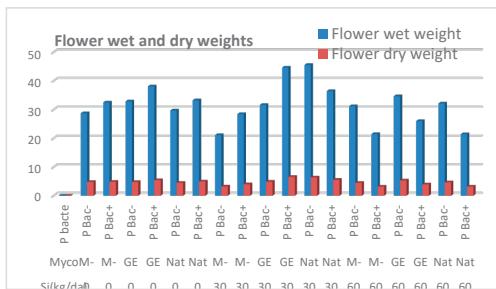
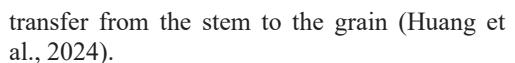


Figure 4. The effects of Si, Mycorrhiza and P Bacteria on the flower wet and dry weights (g)

It has been reported that the interaction between Si and other nutrients such as N, P, K in the soil-plant system has a positive effect on paddy yield, especially through increasing Si availability in the soil and increasing nutrient

Table 3. The effects of Si (Si0; Si30; Si60 kg/da) and Mycorrhiza (with, *G. etunicatum*; Natural mix; without, M-) on the plant values



In our study, it was observed that the effect of silicon in combination with mycorrhiza inoculation increased both plant aboveground parts and flower weight.

Arbuscule, hyphae and pouch (%) values indicating mycorrhizal inoculation in plant roots showed significant increases with mycorrhizal inoculations, while phosphorus bacteria treatment did not cause significant differences in these values.

The highest inoculation to the plant root was especially in the Natural mycorrhiza mix treatment compared to the control and *G. etunicatum*.

In the triple interactions, these values did not change with silicon doses.

In this case, it can be said that mycorrhizae isolated from the natural environment are more effective than *G. etunicatum* and have a higher positive effect on plant growth (Figure 5, Table 3).

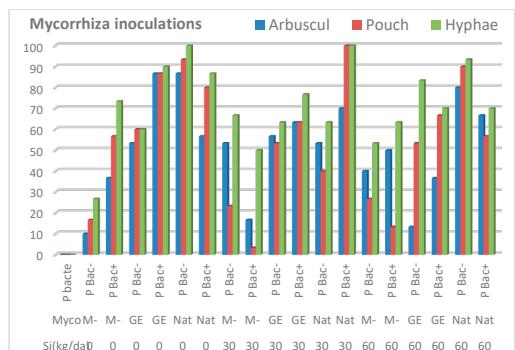


Figure 5. The effects of Si, Mycorrhiza and P Bacteria on the mycorrhiza inoculations (%)

| Si (kg/da) | Myco.    | Leaf Chlo. 1 | Leaf Chlo. 2 | Plant height 1(cm) | Plant height 2(cm) | Plant height 3(cm) | Plant wet weight (g) | Plant dry weight (g) | Flower wet weight (g) | Flower dry weight (g) | Arbü. (%) | Pouch (%) | Hyph. (%) |
|------------|----------|--------------|--------------|--------------------|--------------------|--------------------|----------------------|----------------------|-----------------------|-----------------------|-----------|-----------|-----------|
| 0          | M-       | 31,2         | 24,8         | 39,3               | 53,9               | 84,0               | 116,7                | 27,2                 | 30,6                  | 4,8                   | 23        | 37        | 50        |
| 0          | G. etun. | 32,2         | 24,3         | 40,8               | 54,7               | 83,8               | 121,6                | 26,9                 | 35,4                  | 5,1                   | 70        | 73        | 75        |
| 0          | Nat.     | 31,5         | 22,8         | 42,8               | 56,7               | 83,3               | 115,2                | 26,4                 | 31,5                  | 4,7                   | 72        | 87        | 93        |
| 30         | M-       | 29,1         | 23,4         | 41,1               | 54,9               | 82,2               | 101,7                | 23,0                 | 24,8                  | 3,6                   | 35        | 13        | 58        |
| 30         | G. etun. | 31,1         | 23,2         | 43,3               | 57,9               | 87,6               | 132,5                | 30,2                 | 38,1                  | 5,7                   | 60        | 58        | 70        |
| 30         | Nat.     | 31,7         | 22,7         | 39,3               | 52,0               | 80,9               | 130,7                | 28,7                 | 41,0                  | 6,0                   | 62        | 70        | 82        |
| 60         | M-       | 30,4         | 24,4         | 45,0               | 59,4               | 85,8               | 116,6                | 27,2                 | 26,3                  | 3,8                   | 45        | 20        | 58        |
| 60         | G. etun. | 31,9         | 23,3         | 45,0               | 59,0               | 87,1               | 118,1                | 26,9                 | 30,3                  | 4,6                   | 25        | 60        | 77        |
| 60         | Nat.     | 30,5         | 23,8         | 45,9               | 59,2               | 84,6               | 120,9                | 28,1                 | 26,8                  | 3,9                   | 73        | 73        | 82        |

## CONCLUSIONS

Overall, this study showed that silicon in combination with mycorrhiza had a significant effect on all measured traits; this increase was especially in the Si30-Natural mix mycorrhiza treatment.

This study showed that silicon and natural mycorrhiza inoculation together have significant and positive effects on sunflower plant growth parameters; further studies should be carried out by examining plant nutrient uptake in this regard. In previous studies, it has already been stated that silicon is an agronomically necessary element due to its positive effects on plant resistance mechanism; however, it is understood that new studies should be carried out on the mechanism of action of this element and its effect on different plants should be investigated.

## ACKNOWLEDGEMENTS

This research work was carried out with the support of Selcuk University BAP Coordinator ship.

## REFERENCES

Bayraklı, F. (1986). Toprak ve Bitki Analizleri (Çeviri). 19 Mayıs Üniversitesi Ziraat Fakültesi S:77-79, Samsun.

Carson, P.L. (1980). *Recommended potassium test*. Recommended chemical soil test procedures for the North Central Region. North Dakota Agric. Exp. Stn. Bull. 499, 17-18.

Coskun, D., Deshmukh, R., Sonah, H., Menzies, J. G., Reynolds, O., Ma, J. F., et al. (2019). The controversies of silicon's role in plant biology. *New Phytol.* 221, 67–85. doi: 10.1111/nph.15343

Detmann, K. C., Araujo, W. L., Martins, S. C., Sanglard, L. M., Reis, J. V., Detmann, E., et al. (2012). Silicon nutrition increases grain yield, which, in turn, exerts a feed-forward stimulation of photosynthetic rates via enhanced mesophyll conductance and alters primary metabolism in rice. *New Phytol.* 196, 752–762. doi: 10.1111/j.1469-8137.2012.04299.x

Etesami, H., Jeong, B. R., & Glick, B. R. (2021). Contribution of arbuscular mycorrhizal fungi, phosphate-solubilizing bacteria, and silicon to P uptake by plant. *Frontiers in plant science*, 12, 699618.

Gee, G.W. & Bauder, J.W. (1986). Particle-Size Analysis 1. Methods of Soil Analysis: Part 1 - Physical and Mineralogical Methods (methodsofsoilanal1), 383-411.

Giovannetti M, Mosse B. (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist* 84: 489-500

Hu, A. Y., Che, J., Shao, J. F., Yokosho, K., Zhao, X. Q., Shen, R. F., et al. (2018). Silicon accumulated in the shoots results in down-regulation of phosphorus transporter gene expression and decrease of phosphorus uptake in rice. *Plant Soil.* 423, 317–325. doi: 10.1007/s11104-017-3512-6

Huang, S., Pu, L., He, G., Wang, X., Chen, D., Xie, X., ... & Lu, Y. (2024). Silicon in soil and its interaction with nitrogen, phosphorus, and potassium nutrients on rice yield: A case study of paddy fields in the Taihu Lake region, China, without a history of silicon fertilization. *Soil and Tillage Research*, 238, 106027.

Janmohammadi, M. & Sabaghnia, N. (2015). Effect of pre-sowing seed treatments with silicon nanoparticles on germinability of sunflower (*Helianthus annuus*). *Bot Lith.* 21(1), 13-21.

Kamenidou, S., Cavins, T. J. & Marek, S. (2008). Silicon supplements affect horticultural traits of greenhouse-produced ornamental sunflowers. *HortScience*, 43(1), 236-239.

Klotzbücher, A., Klotzbücher, T., Jahn, R., Xuan, L. D., Cuong, L. Q., Van Chien, H., ... & Vetterlein, D. (2018). Effects of Si fertilization on Si in soil solution, Si uptake by rice, and resistance of rice to biotic stresses in Southern Vietnam. *Paddy and Water Environment*, 16, 243-252.

Koske, R. E. & Gemma, J. N. (1989). A modified procedure for staining roots to detect VA mycorrhizas.

Kostic, L., Nikolic, N., Bosnic, D., Samardzic, J. & Nikolic, M. (2017). Silicon increases phosphorus (P) uptake by wheat under low P acid soil conditions. *Plant and Soil*, 419, 447-455.

Liang, Y., Nikolic, M., Bélanger, R., Gong, H. & Song, A. (2015). *Silicon in Agriculture: From Theory to Practice*. Dordrecht: Springer.

Lindsay, W.L. & Norvell, W.A. (1978). Development of a DTPA Soil Test for Zinc, Iron, Manganese, and Copper 1, *Soil Science Society of America Journal*, 42 (3), 421-428.

Mali, M., & Aery, N. C. (2008). Influence of silicon on growth, relative water contents and uptake of silicon, calcium and potassium in wheat grown in nutrient solution. *Journal of Plant Nutrition*, 31(11), 1867-1876.

Neu, S., Schaller, J. & Dudel, E.G. (2017). Silicon availability modifies nutrient use efficiency and content, C:N:P stoichiometry, and productivity of winter wheat (*Triticum aestivum* L.). *SCI REP-UK* 7 (1), 40829. <https://doi.org/10.1038/srep40829>.

Olsen, S. R., & Sommers, L. E. (1982). *Phosphorus*. Pp: 403-430. *Methods of soil analysis, Part, 2*.

Owino-Gerroh, C. & Gascho, G. J. (2005). Effect of silicon on low pH soil phosphorus sorption and on uptake and growth of maize. *Commun. Soil Sci. Plant Anal.* 35, 2369–2378. doi: 10.1081/lcss-200030686

Pati, S., Pal, B., Badole, S., Hazra, G. C. & Mandal, B. (2016). Effect of silicon fertilization on growth, yield, and nutrient uptake of rice. *Commun. Soil Sci. Plant Anal.* 47, 284–290. doi: 10.1080/00103624.2015.1122797

Pavlovic, J., Kostic, L., Bosnic, P., Kirkby, E. A., & Nikolic, M. (2021). Interactions of silicon with essential and beneficial elements in plants. *Frontiers in Plant Science*, 12, 697592.

Richards, L.A. (1954). Diagnosis and Improvement of Saline and Alkaline Soils. U.S. Dep. Agr. Handbook, Washington.

Schaller, J., Puppe, D., Kaczorek, D., Ellerbrock, R. & Sommer, M. (2021). Silicon cycling in soils revisited. *Plants* 10:295. doi: 10.3390/plants10020295

Soltani, M., Kafi, M., Nezami, A. & Taghiyari, H. R. (2017). Effects of silicon application at nano and micro scales on the growth and nutrient uptake of potato minitubers (*Solanum tuberosum* var. Agria) in greenhouse conditions. *BioNanoScience* 8, 218–228. doi: 10.1007/s12668-017-0467-2

Soratto, R. P., Fernandes, A. M., Pilon, C. & Souza, M. R. (2019). Phosphorus and silicon effects on growth, yield, and phosphorus forms in potato plants. *J. Plant Nutr.* 42, 218–233. doi: 10.1080/01904167.2018.1554072

Thomas, GW. (1982). Exchangeable Cations, Pgs 159–165 In: Methods of Soil Analysis. Part II (page A. L Miller, R. H., and Keeney, D. R., eds.). 2nd Edition. America Society of Agronomy and Soil Science of America. Madison. Wisconsin, USA.

Walkley, A., Black, I.A. (1934). An Examination of the Degtjareff Method for Determining Soil Organic Matter, and a Proposed Modification of the Chromic Acid Titration Method. *Soil Science*, 37(1), 29-38.

Zhang, Y., Liang, Y., Zhao, X., Jin, X., Hou, L., Shi, Y., et al. (2019). Silicon compensates phosphorus deficit-induced growth inhibition by improving photosynthetic capacity, antioxidant potential, and nutrient homeostasis in tomato. *Agronomy* 9:733. doi: 10.3390/agronomy9110733