

## Importance of Mycorrhization under Heat Stress Conditions: Technological Tools to Stimulate Mycorrhizal Colonization

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### Abstract

Agriculture is considered to be one of the most vulnerable sectors to climate-change. Crop production, particularly in tropical regions is facing increasing stresses due to natural and anthropogenic factors. Increased incidence of abiotic and biotic stresses has become a major cause for stagnation of productivity in main crops. There is evidence of yield declines in crops in many parts of the world due to increasing water stress, reduction in number of rainy days and increased air temperature. The symbiosis between arbuscular mycorrhizal fungi and plants has been studied for many decades. This symbiosis is recognized as the interaction with the greatest economic impact on agriculture due to the reduction of environmental stresses. The effects of mycorrhization on plants grown under heat stress conditions and the effect of Penegetic technology in increase of the mycorrhization percentage was evaluated in this study. It was observed that plants in symbiosis with mycorrhizae showed greater tolerance to heat stress and that the use of Penegetic in the crops enabled a significant increase in the mycorrhization percentage and it promote to be efficient to improve the conditions of maintenance and productivity of plants under stress conditions.

**Keywords:** Microbial stimulation; Stress tolerance; Plant development; Grain production.

### 1. Introduction

The demand for technologies, management and agricultural practices that can reduce the impacts of the climate increases every year. Climate changes are causing serious negative impacts on crop growth and yields and they impose severe pressure on the land use and on management for reducing these effects. Heat and drought are the most worrying abiotic stress that may reduce the productive areas of the planet in the near future (Challinor et al. 2009; Abhilash et al. 2016; Dubey et al. 2016; Suneel and Manjeet 2020; Sharma et al. 2020). We know that the climate is constantly changing and that forecasts indicate that science and new technologies will be responsible for maintaining the productivity levels of different crops. We also know that nature is efficient in providing conditions for plants to develop and produce even in adverse environments.

Soil biology and the interactions that occur in the soil ecosystem are fundamental to provide a favorable environment for plant development. Among these biological interactions, the symbiosis of the roots with arbuscular mycorrhizal fungi (AMF) represents the cooperation between plants and microorganisms of greater importance in agriculture (Huisman et al. 2020). Arbuscular mycorrhizal fungi are key microorganisms that make up a large part of the microbial biomass of cultivated soils. About 80% of plants are symbiotically associated with these fungi, which are notably important agents for improving soil quality and crop performance. Bean, soybean, corn, sunflower and wheat can form symbiosis with 40 species of arbuscular mycorrhizal fungi.

Farmers that are getting high levels of grain productivity, especially soybeans, beans, corn and sorghum, are currently working with managements and technologies that preserve soil biology, ensuring the maintenance of biological interactions in the system. Many of these managements aim at maintaining and increasing mycorrhizal activity in the soil (Gutjahr and Parniske 2013). These effects can be confirmed through laboratory analysis by comparing the biological quality of the soil in an integrated management system aiming at increases biological activity. There is greater growth and health quality of the plants that form mycorrhizal associations in the field. These effects are related to the fact that mycorrhizal plants are able to explore a much larger volume of soil in search of water and nutrients, as the set of fungi hyphae acts as an extension of the root volume of plants, in addition to synthesize compounds that provide forms of nutrients that would be inaccessible to plants were it not

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for the action of fungi. This association is mutualistic, positive for both individuals, as it provides nutritional benefits, disease protection and tolerance to abiotic stresses (Rodriguez et al. 2008; Duc et al. 2018; Pavithra and Yapa 2018). Tolerance to abiotic stresses, especially heat stress, is a fundamental benefit resulting from the symbiosis between cultivated plants and soil microbiology.

Penergetic technology has attracted attention among farmers and the scientific community in agricultural systems. Although the use of technology in agriculture is recent, research has already demonstrated the effect of the product on wheat crop (Kadziulienė et al. 2005; Perkarskas et al. 2011; Pekarskas 2012a; Pekarskas and Sinkevičienė 2015; Pekarskas et al. 2017), soybean (Souza et al. 2017), coffee (Franco Júnior et al. 2018; Franco Júnior et al. 2019), bean (Brito et al. 2012; Cobucci et al. 2015), potato (Jakiene et al., 2008) and barley (Pekarskas, 2012b). Steffen et al. (2016) observed an increase in the biological activity of Penergetic treated soils in soybean and wheat crops. The authors observed that the feeding activity of fauna and microorganisms in the sub superficial layer was intensified with the use of Penergetic technology.

The aim of this study was to evaluate the effects of mycorrhization on plants grown under heat stress conditions and the potential effect of Penergetic technology in the increase the mycorrhization percentage.

## 2. Methodology

Damage from lost productivity by heat stress was evaluated between 2018 and 2020. The percentage of mycorrhizal colonization in corn, bean, soybean, sunflowers (summer crops) and wheat (winter crops) plants was analyzed during this period. In this way, commercial areas of grain production were chosen, in regions where there was a history of stresses caused by excess heat (high temperatures for summer and winter crops). Farmers allowed the monitoring of crop development, from sowing to harvest, in these areas.

Simultaneously, the efficiency of Penergetic technology in increasing symbiosis between plants and arbuscular mycorrhizal fungi was evaluated. Periodic collections were performed whenever the plants were under heat stress conditions for these evaluations. The evaluations aimed to demonstrate the effect of stimulating the formation of mycorrhizal symbiosis and the benefits that this symbiosis provides.

It was decided to use soybean as a culture for assessing mycorrhization and photosynthetic efficiency among the agricultural crops studied. The percentage of root colonization (mycorrhizal symbiosis) and the quantum yield of photosynthesis ( $f_v/f_m$  ratio) were evaluated during the flowering period of the soybean. The percentage of mycorrhizal colonization was determined according to the methodology proposed by Koske and Gemma (1989). The quantification of the maximum yield of photosynthesis was determined according to the methodology proposed by Leuchaudel et al. (2010).

Analysis of variance (ANOVA) was measured by using the SISVAR software (Ferreira 2014) as well as mean values in each treatment were compared by the t-test. The correlation between  $f_v/f_m$  ratio and mycorrhizal colonization was evaluated by Pearson's correlation coefficients.

## 3. Results and discussion

The results showed the efficiency of Penergetic technology in promoting conditions for the establishment of symbiosis between the root system of plants and arbuscular mycorrhizal fungi (Figure 1). Plants in association with these fungi have an increased water and nutrient absorption capacity. This increase is due to the extension of the fungi hyphae that are able to explore a soil volume much higher than the volume that the plants roots are able to explore (Figure 2). Some studies show that plants in symbiosis with mycorrhizal fungi are able to absorb up to twice more water compared to plants that are not in symbiosis with these fungi.

There is a consensus among researchers studying the benefits that mycorrhizal fungi provide to plants: the symbiosis between plants and arbuscular mycorrhizal fungi results in the reduction of losses due to stress factors. Arbuscular mycorrhizal fungi may alter plant growth and productivity under conditions of drought and heat stress.

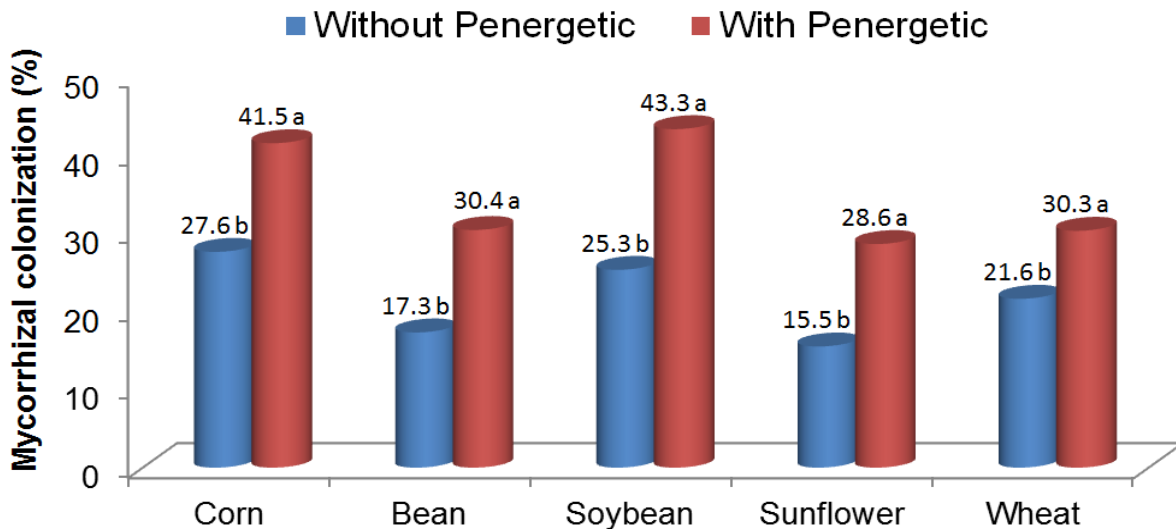


Figure 1: Percentage of mycorrhizal colonization in different crops with and without the use of Penergetic technology under heat stress conditions. Assessments carried out in southern Brazil. Columns followed by the same letter in the same crops do not differ by Tukey's test at 5% probability of error.

The response of plants to colonization by these fungi depends on the severity of these stresses. Thus, symbiosis with mycorrhizal fungi may reduce stress by some mechanisms: increased water absorption from the soil by hyphae; alteration of hormonal levels causing changes in stomatal conductance; increased leaf turgor and reduced osmotic potential; and improving the nutrition of the host plant. Regardless of what mechanisms are taking place in the plant, one observation is true: mycorrhizal fungi provide better conditions for plants to produce more.

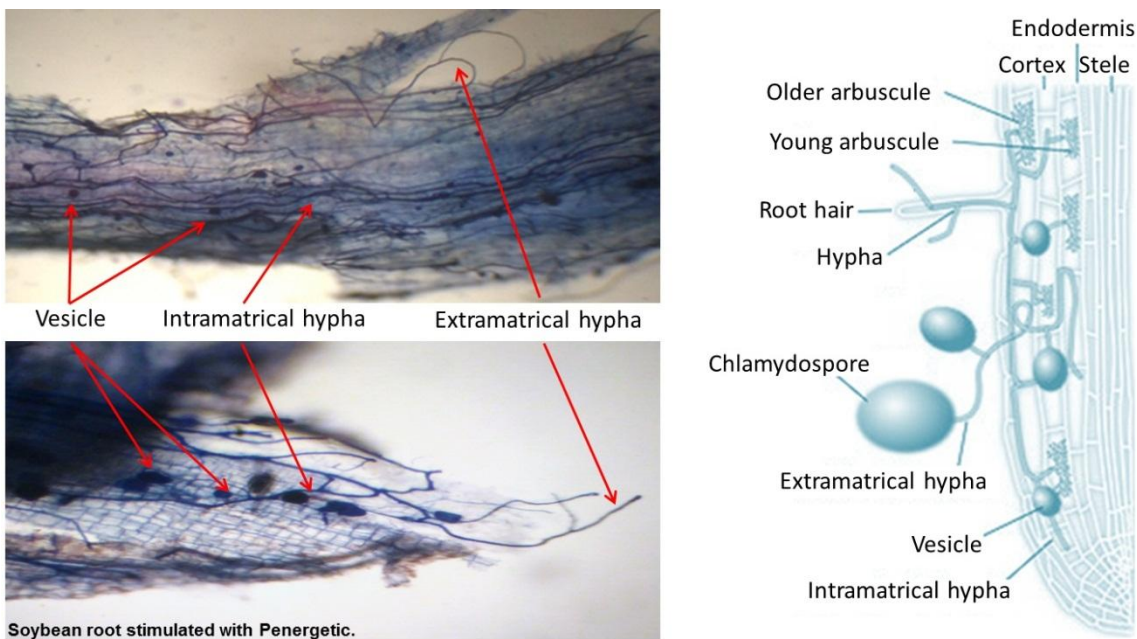


Figure 2: Structure of arbuscular mycorrhizal fungi in soybean roots. Scheme of symbiosis between root and mycorrhizal fungi published by Monk et al. (2015).

Plant adaptation to the environment and increasing tolerance of plants to heat stress are significant effects of the mycorrhizal association. The results obtained in this study corroborate with results presented in the literature (Ahmad et al. 2010; Birhane et al. 2012; Latef et al. 2016; Duc et al. 2018).

It was observed that the use of Penergetic in soybean caused an increase in the volume of roots (Figure 3). This result is essential for maintaining plants under stress conditions.

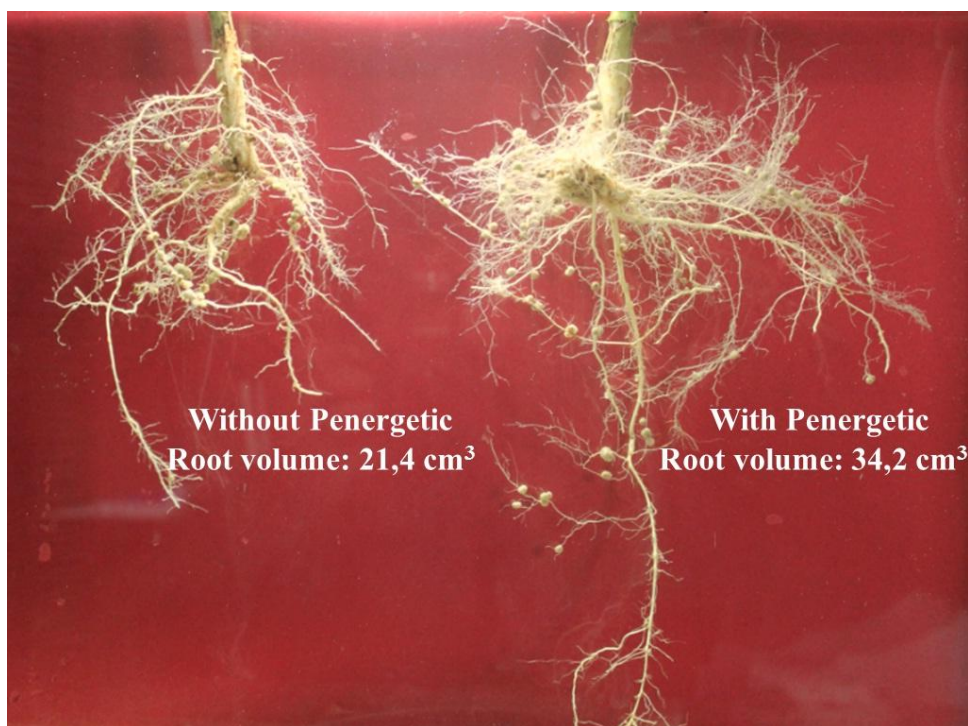


Figure 3: Volume of soybean roots (grain filling period) in areas with and without the use of Penergetic technology under heat stress conditions.

Wu et al. (2010) and Wang et al. (2011) observed that the plant-microorganism interaction promotes an increase in plant roots while studying the effects of symbiosis between cultivated plants and arbuscular mycorrhizal fungi.

Inhibition of photosynthesis may occur if the air temperature is above the optimum. Under these conditions the photosynthesis begins to decrease gradually and reversibly, at first, and irreversibly if the stress occurs for a long period of days. The reversible inactivation of photosynthesis is a result of damage in the reactions of chloroplasts, which persists for some time after returning to favorable temperature conditions. It is known that the increase in temperature results in the blocking of the reaction centers of photosystem II (PSII). Then it causes enzyme inactivation, pigment discoloration, lipid peroxidation and other damage. Under these conditions, it is necessary that there is no water limitation for the plant. Mycorrhizal colonization shows us the quantity (in percentage) of the roots that are effectively forming an association with arbuscular mycorrhizal fungi and the evaluation of the quantum yield of photosynthesis shows the capacity that the plant has in using thermal energy in the photosynthetic process (Figure 4). The quantum yield evaluation was carried out on days when the air temperature was above the limit for the photosynthetic process of the plant.

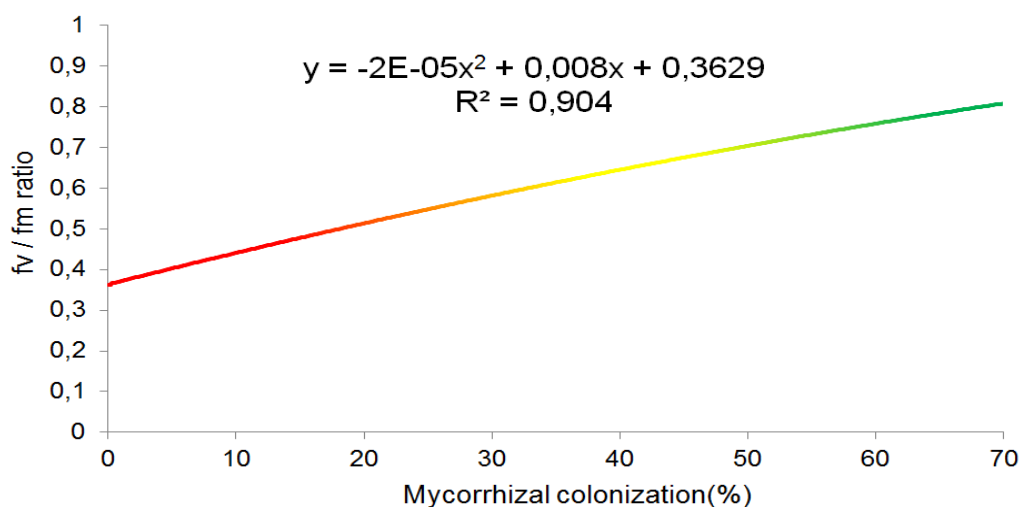


Figure 4: Correlation between the percentage of mycorrhizal colonization and the quantum yield of photosynthesis under heat stress conditions. Result obtained in soybeans during the flowering period with air temperature above 35°C.

Water is essential to allow the stomata of the leaves to remain open and gas exchange continues to occur. However, excess heat is associated with reduced water availability in the soil in most cases. It is precisely in these conditions that the symbiosis of plants with mycorrhizal fungi is fundamental to enable the plant to have a quantum yield of photosynthesis capable of promoting development and production conditions even under stress conditions.

The association of plants with arbuscular mycorrhizal fungi results in a significant increase in the soil volume explored by the roots. The expansion of fungal hyphae in the soil causes greater absorption of water and nutrients. This greater supply of water to the plant results in conditions of adaptability to heat stress conditions, keeping the process of photosynthesis active during the stress period. The results obtained in this study corroborate those presented by Pimior et al. (2005) and Mathur and Jajoo (2020), who describe the action of arbuscular mycorrhizal fungi in maintaining photosynthesis II photosynthesis activity in periods of heat stress. It was possible to observe a direct correlation between the percentage of mycorrhizal colonization (percentage of roots in symbiosis) and the maintenance of the quantum yield of photosynthesis. It is essential that plants are in symbiosis to ensure productivity even under heat stress conditions. So, stimulating the activity of mycorrhizal fungi in the soil and the symbiosis of these fungi with plants is a smart strategy for producing food in regions with a history of heat stress.

It was observed an increase in productivity of the cultures that were managed using the treatment with Pengergetic technology in the studied areas (Figure 5). This management resulted in an increase in mycorrhizal colonization. This increase in mycorrhizal colonization certainly resulted in an increase in the water absorption capacity and reduction of the plant's physiological stresses in high temperature periods during the flowering stage.

According to technical information, the Pengergetic product is manufactured using the bioprogramming process, in which extremely low electromagnetic fields (EMF) are generated to create energy potentials (Prade 2009).

Among these is the oxidation-reduction potential, stimulating several biological processes in plants, animals, soils and water. The consequence is the ability of microbial activation and regulation of biological processes, resulting in improved quality of the agricultural environment and, consequently, of crops.

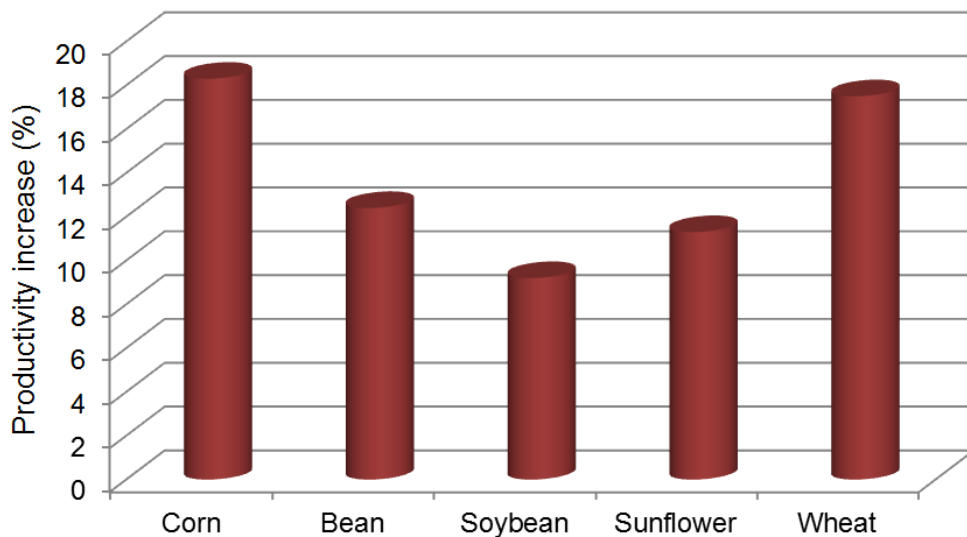


Figure 5: Productivity increase (%) of the crops in relation to areas that did not use of Pengergetic technology and suffered the same kind of heat stress.

According to Beretta et al. (2019), the results support the possibility of using this technology on a large scale although evaluations regarding the effects of the use of different electromagnetic scales on microbial activity are scarce. The different agricultural practices, usually used in production systems, directly interfere in the growth and multiplication of arbuscular mycorrhizal fungi in the soil and in their symbiosis with the plant. Practices such as: tillage (no tillage), pH adjustment, fertilization, annual crop rotation, cover crops and crop-pasture integration, increase the mycorrhizal fungi community in the soil and the occurrence of mycorrhiza and optimize the effects of symbiosis, benefiting agricultural production. On the other hand, when soil and crop management is inadequate, there may be a reduction in this community and a consequent reduction in the response of plants to the inputs used.



It has been observed that crop rotation among the various agricultural practices, can significantly increase the mycorrhiza symbiosis with plants. So, it is important and necessary to program the appropriate crops and cultivars and the type of rotation to be used for each production system. Some annual and cover crops as well as some pastures have a high degree of mycorrhizal dependence. When used in a rotation system, these plants increase the community of native arbuscular mycorrhizal fungi in the soil and benefit from mycorrhiza, as well as the subsequent crops.

So, if we know that crops of great agricultural interest such as soybean, bean and corn are dependent on mycorrhizal associations, it is easy to understand that we have to prioritize management that stimulates biological balance in soils. These managements have to, fundamentally, use tools such as: crop rotation, efficient cover plants (presence of bioactive compounds), adequate and precise fertilization programs and phytosanitary control.

The soil must be seen as a production unit all year round. Not only in the harvest period. This is why it is so important to plan and understand the processes that take place in the soil and the interaction with the different forms of life that inhabit the same system. It is possible to improve the quality and productivity of crops, ensuring longevity and sustainability of the soil when we know about these interactions.

#### 4. Conclusion

The microbial stimulus provided by Penegetic technology resulted in significant increase in productivity in relation to plants that were not stimulated. Thus, stimulating soil microbial communities represents an efficient and fundamental strategy to improve the quality and performance of agricultural crops, especially in areas or crops that are sensitive to environmental stresses.

#### 5. References

- Abhilash, P. C., Dubey, R. K., Tripathi, V., Gupta, V. K. & Singh, H. B. (2016). Plant growth-promoting microorganisms for environmental sustainability. *Trends in Biotechnology*, 34:847–850.
- Ahmad, P., Jaleel, C. A., Salem, M. A., Nabi, G. & Sharma, S. (2010). Roles of enzymatic and non-enzymatic antioxidants in plants during abiotic stress. *Critical Reviews in Biotechnology*, 30, 161–175.
- Beretta, G., Mastorgio, A. F., Pedrali, L., Saponaro, S., & Sezenna, E. (2019). The effects of electric, magnetic and electromagnetic fields on microorganisms in the perspective of bioremediation. *Reviews in Environmental Science and Bio/Technology*, 18, 29-75.
- Birhane, E., Sterck, F. J., Fetene, M., Bongers, F. & Kuyper, T. W. (2012). Arbuscular mycorrhizal fungi enhance photosynthesis, water use efficiency, and growth of frankincense seedlings under pulsed water availability conditions. *Oecologia*, 169, 895–904.
- Brito, R.O., Dequech, F. K., & Brito, R. M. (2012). Use of penegetic products P and K in the snap bean production. *Annual Report of the Bean Improvement Cooperative*, 55, 277-278.
- Challinor, A. J., Ewert, F., Arnold, S., Simelton, E. & Fraser, E. (2009). Crops and climate change: progress, trends, and challenges in simulating impacts and informing adaptation. *Journal of Experimental Botany*, 60:2775–2789.
- Cobucci, T., Nascente, A. S. & Lima, D. P. (2015). Phosphate fertilization and penegetic application in the yield of common bean. *Revista Agrarum*, 8, 358-368.
- Duc, N. H., Csintalan, Z., & Posta, K. (2018). Arbuscular mycorrhizal fungi mitigate negative effects of combined drought and heat stress on tomato plants. *Plant Physiology Biochemistry*, 132, 297–307.
- Dubey, P. K., Singh, G. S. & Abhilash, P. C. (2016). Agriculture in a changing climate. *Journal of Cleaner Production* 113:1046–1047.
- Ferreira, D. F. (2014). Sisvar: A Guide for Its Bootstrap Procedures in Multiple Comparisons. *Ciência e Agrotecnologia*, 38, 109-112.
- Franco Junior, K. S., Terra, A. B. C., Teruel, T. R., MantovanI, J. R. & Florentino, L. A. (2018). Effect of cover crops and bioactivators in coffee production. *Coffee Science*, 13, 559 - 567.
- Franco Junior, K. S., Florentino, L. A., Dias, M. S. & Franco, T. C. (2019). Influence of the use coverage plants and the bioactivator in the physical-biological characteristics of soil cultivated with coffee. *Coffee Science*, 14, 116-122.
- Gutjahr, C., & Parniske, M. (2013). Cell and developmental biology of arbuscular mycorrhiza symbiosis. *Annual Review of Cell and Developmental Biology*, 29, 593–617.
- Huisman, R., Hontelez, J., Bisseling, T. & Limpens, (2020). E. SNARE Complexity in Arbuscular Mycorrhizal Symbiosis. *Frontiers in Plant Science*, 11, 354.
- Jakiene, E., Venskutonis, V. & Michevicius, V. (2008). The effect of additional fertilization with liquid complex fertilizers and growth regulators on potato productivity. *Sodininkystei Daržininkyste*, 27, 259- 267.
- Kadziuliene, Z., Feiziene, D. & Semaskiene, R. (2005). Peculiarities of some legumes and cereals under organic farming system. *NJF Report*, 1, 103-106.

- Koske, R. E. & Gemma, J.N. (1989). A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research*, 92, 486-505.
- Latef, A. A. H. A., Hashem, A., Rasool, S., Allah, E. F. A., Algarawi, A. A., Egamberdieva, D., Jan, S., Anjum, N. A. & Ahmad, P. (2016). Arbuscular mycorrhizal symbiosis and abiotic stress in plants: A review. *Journal of Plant Biology*, 59, 407–426.
- Lechaudel, M., Urban, L. & Joas, J. (2010). Chlorophyll fluorescence, a nondestructive method to assess maturity of mango fruits (Cv. 'Cogshall') without growth conditions bias. *Journal of Agricultural and Food Chemistry*, 58, 7532- 7538.
- Mathur, S & Jajoo, A. (2020). Arbuscular mycorrhizal fungi protects maize plants from high temperature stress by regulating photosystem II heterogeneity. *Industrial Crops and Products*.143, 111934.
- Monk, J., Tauschke, M., Eulenstein, F. & Behrendt, A. (2015).Mycogro AG and MycogroHort – mycorrhizal products mad in New Zealand. Conference: Moving farm systems to improved nutrient attenuation, Massey University, New Zealand. Report No. 28.
- Pavithra, D., and Yapa, N. (2018). Arbuscular mycorrhizal fungi inoculation enhances drought stress tolerance of plants. *Groundwater for Sustainable Development*. 7, 490– 494.
- Perkarskas, J., Vilkenyte, L., Sileikiene, D., Cesoniene, L. & Makarenko, N. (2011).Effect of organic nitrogen fertilizers provita and fermentatorPenergetic-K winter wheat and on soil quality. *Environmental Engineering*, 8, 248-254.
- Pekarskas, J. (2012). Effect of growth activator Penergetic-P on organically grown spring wheat. *Žemės ūkio mokslai*, 19, 151-160.
- Pekarskas, J. (2012). Influence of growth activator Penergetic-P on yield and chemical composition of barley in organic farm. *Žemės ūkio mokslai*, 19, 249-256.
- Pekarskas, J. & Sinkevičienė, J. (2015).Effect of biopreparations on seed germination and fungal contamination of winter wheat. *Biologija*, 61, 25–33.
- Piniór, A., Grunewaldt, S. G., Von Alten, H. & Trasser, R. J. (2005). Mycorrhizal impact on drought stress tolerance of rose plants probed by chlorophyll a fluorescence, proline content and visual scoring. *Mycorrhiza*, 15:596.
- Prade, E. (2009). *A vision becomes reality*. Bio Energetik, 200p.
- Rodriguez, R. J., Henson, J., Van Volkenburgh, E., Hoy, M., Wright, L., Beckwith, F., et al. (2008). Stress tolerance in plants via habitat-adapted symbiosis. *International Society for Microbial Ecology*. 2, 404–416.
- Sharma, L., Priya, M., Kaushal, N., Bhandhari, K., Chaudhary, S., Dhankher O. P., Prasad, P. V. V., Siddique, K. H. M. & Nayyar, H. (2020). Plant growth-regulating molecules as thermoprotectants: functional relevance and prospects for improving heat tolerance in food crops. *Journal of Experimental Botany*, 71, 569–594.
- Souza, A. A. de., Almeida, F. Z. de. & Alberton, O. (2017).Growth and yield of soybean with Penergetic application. *Revista Scientia Agraria*, 18, 95-98.
- Steffen, G. P., Maldaner, J., Morais, R. M. DE., Saldanha, C. W., Steffen, R. B., Antonioli, Z. I. & Jacques, R. J. S. (2016). Degradation of residues and food activity in soils with biological activation. *International Journal of Current Research*, 8, 42784-42789.
- Suneel, S. & Manjeet. (2020) Heat Stress Effects in Fruit Crops: A Review. *Agricultural Reviews*, 41, 73-78.
- Wang, X., Pam, O., Chen, F, Yan, X. & Liao, H. (2011). Effects of co-inoculation with arbuscular mycorrhizal fungi and rhizobia on soybean growth as related to root architecture and availability of N and P. *Mycorrhiza*. 21, 173-181.
- Wu, O. S., Zou, Y. N. & He, X. H. (2010). Contributions of arbuscularmycorrhizal fungi to growth, photosynthesis, root morphology and ionic balance of citrus seedlings under salt stress. *Acta Physiologiae Plantarum*. 32, 297-304.