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ARBUSCULAR MYCORRHIZAL FUNGI (AMF) AND SOIL CHEMICAL HETEROGENEITY SIGNIFICANTLY ALTERS NUTRITIONAL VALUE OF TOMATO FRUIT

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Abstract

Soil chemical heterogeneity critically alters colonization and diversity of Arbuscular Mycorrhizal Fungi (AMF) in roots of same host plant among different agro-ecological zones. AMF, being a natural soil bio-fertilizer, significantly affects the plant metabolome, changing the quality of its fruit. In present study, subsequent effect of soil chemistry and AMF on nutritional value of tomato fruit from place to place have been monitored. For this purpose, tomato plants along with their rhizospheric soil were sampled from different agro-ecological zones of Pakistan. Soil edaphic factors, AMF root colonization status, rhizospheric spore densities and diversity were estimated and also correlated. Also, the variation in secondary metabolites and antioxidant capacity of tomato fruit was examined. Out results indicated that agro-ecological zones having low soil phosphorus availability exhibits high AMF colonization and diversity, subsequently possessing high phenolic and flavonoid content in tomato fruits resulting in enhanced antioxidant capacity. The concluded correlative effect of spatial AMF diversity and soil chemistry on metabolomic profiles of host plant suggests detailed studies so that qualitative value of various food plants can be enhanced.

Key words: Soil chemistry, AMF, Nutritional value, Fruit quality.

Introduction

The quality of agricultural products in any country has considerable impact on its economy. The local economy expands when local purchase is competitive in price with the substituted commodity. This is only possible if quality and quantity of the local product is nutritious and adequate, respectively. Moreover, increasing population pressure results in increased demand for food production. In response, for enhanced production, the traditional farming practices utilizes chemical fertilizers which are expensive and also environmentally hostile. In addition, over-utilization of land for agricultural practices is leading to decline soil richness and environmental quality. Numerous programs have been designed and a number of farming techniques have been developed in the past years to reach the regional production potentials, allowing the regions to become more self-sufficient in the production and consumption of important agricultural products without affecting the environment. There is an impetus to develop more sustainable agricultural techniques, among them is exploitation of mycorrhizae, which designates the symbiotic association between fungi and the plant roots. Among several types, one is found associated with the vast majority of plants called as Arbuscular Mycorrhizal Fungi (AMF). Now it has been observed in all ecosystems, under all climates regardless of vegetation, soil type or growth conditions. Mycorrhizae influence plant stability and competition at community level and is important in nutrient retention and cycling at the ecosystem level (Tedersoo *et al.*, 2020). Due to improved nutrition, AMF colonized plants acquired increased resistance to environmental stresses and root pathogens. Apart from that, AMF may increase the establishment and biomass of host plants, also improving the quality of its fruit (Torres *et al.*, 2018). Thus, AMF may have a critical role in persuit of sustanible agriculture.

Soil chemistry critically affects the colonization status of arbuscular-mycorrhizal fungi in roots of host plant (Pollastri *et al.*, 2018). These also affect the diversity of arbuscular-mycorrhizal isotypes in the rhizospheric soil to a great extent. Over the past years, researchers have worked tiredlessly on the ecology of AMF interactions with plant and soil, which have provided a skylight into an underground world which is dynamic and diverse. Research focus on AMF influence on the physiology of fruit has been neglegted, which is the economically important part of plant. Previous studies revealed the significant impact of AMF on production of secondary metabolites during tomato fruit ripening, which influence nutritional value and flavor (Todeschini *et al.*, 2018). The correlation among AMF spatial diversity and soil chemistry and also with metabolism of tomato fruit is necessary to assess the actual reason behind the varying quality and nutritional value of fruit from place to place.

This study aimed to explore the subsequent effect of soil chemical heterogeneity on AMF spatial diversity and colonization on tomato fruit quality by affecting its secondary metabolites.

Materials and Methods

Tomato was the model plant under study as it was one of most common agricultural products in Asian countries and a profitable crop for majority of farmers. A comprehensive methodology was adopted, comprised of following stages.

Sampling strategy: Sampling was performed in ten agro-ecological zones of Pakistan confined by Pakistan Agriculture Research Council (PARC). One location from all zones was selected on the bases of tomato production. Selected sampling sites were Bahawalpur, Faisalabad, Gujranwala, Karachi, Sukkur, Swat, Charsadda, Waziristan, Pishin and Kilasaif (Fig. 1). Sampling was done randomly with a ten factorial treatment (10 sampling sites/ agro-ecological zones) replicated two times (2 locations at each sampling site) with three sub replicates from each location, resulting in a sum total of 60 samples. Plants were shipped to the lab at Center for Interdisciplinary Research in Basic Science, International Islamic university, Islamabad in polythene bags along with rhizospheric soil. Before sampling, the soil around plant and litter was removed. Upon arrival, all the samples were cleaned, air dried and later oven dried to make decoctions for estimation of secondary metabolites. Root samples were immediately washed, preserved in bottles containing FAA (Formaldehyde Acetic Acid) and refrigerated at $4^{o}C$.

Parameters studied

AMF composition and spatial diversity associated with tomato plant: Roots were processed and stained following a method as described by Dalpé & Séguin (2013). Root AMF colonization status was measured by Biermann and Linderman method (1981). Soil of all replicates from each agro-ecological zone was sieved and mixed to make a homogenous mixture and used to determine soil physic-chemistry and spore extraction. AMF spore densities were recorded using wet sieving and decanting technique (Gerdemann & Nicolson, 1963). Taxonomic identification of AMF species was performed followin International collection of arbuscular-mycorrhizal fungi (http://invam.caf.wvu.edu). The spores of each type was counted in all the samples and subjected to diversity indices and frequency of occurrence.

Plant biochemical analysis: Total phenolic content (TPC) and total flavonoid content (TFC) were determined by method as described by Clarke *et al*., (2013) and aluminum colorimetric method (Hussain *et al*., 2013), respectively. Total antioxidant capacity and reduction potential of the samples were investigated according to the procedure described by Aliyu *et al*., (2009). Antioxidant capacity of plants extracts was determined by using DPPH free radical scavenging assay (2,2-diphenyl-1-picrylhydrazyl) (Clarke *et al*., 2013) with slight modifications. As DPPH inhibition percentage was high in all samples, so IC50 was also estimated.

Agro-ecological zones	Latitude	Longitude
Bahawalpur	29° 34'	71° 68'
Faisalabad	31° 45'	73° 17'
Gujranwala	32° 14'	74° 24'
Karachi	25° 14'	67° 04'
Sukkur	27° 73'	68° 82'
Swat	34° 76'	72° 32'
Charsadda	34° 17'	71° 75'
South Waziristan	32° 32'	69° 83'
Pishin	30° 59'	67° 02'
Kilasaif	30° 63'	68° 39'

Fig. 1. Marked sampling sites from different agro-ecological zones of Pakistan and their coordinates.

Table 1. Physical and chemical composition of rhizospheric soil of tomato plant sampled from different agro-ecological zones.

Agro-ecological zone	EC (dSm^{-1})	pH	OC (%)	P (ppm)	K (ppm)	SP (%)	Texture
Bahawalpur	2.2 ± 1.47*	7.5 ± 0.07	0.35 ± 0.05	7.3 ± 0.67	206 ± 15.4	46 ± 1.02	Clay Loam
Faisalabad	3.8 ± 0.17	7.6 ± 0.04	0.70 ± 0.03	8.5 ± 1.66	187 ± 25.9	38 ± 1.20	Loam
Gujranwala	1.8 ± 0.52	7.3 ± 0.07	0.63 ± 0.02	8.3 ± 1.02	187 ± 10.0	40 ± 9.52	Loam
Karachi	2.4 ± 0.60	7.7 ± 0.04	0.56 ± 0.03	8.3 ± 2.20	168 ± 7.7	32 ± 1.02	Loam
Sukkur	2.8 ± 0.10	7.8 ± 0.03	0.63 ± 0.06	7.2 ± 0.48	150 ± 4.60	58 ± 1.41	Clay Loam
Swat	3.4 ± 0.22	7.3 ± 0.06	0.56 ± 0.04	4.5 ± 2.56	168 ± 10.0	44 ± 0.75	Loam
Charsadda	6.7 ± 0.04	6.8 ± 0.04	0.56 ± 0.05	5.3 ± 0.48	187 ± 5.31	42 ± 1.90	Loam
South Waziristan	1.7 ± 0.26	7.2 ± 0.02	0.63 ± 0.02	5.4 ± 3.33	206 ± 11.9	44 ± 1.17	Loam
Pishin	4.0 ± 0.17	7.5 ± 0.04	0.42 ± 0.03	6.8 ± 1.66	150 ± 25.9	34 ± 1.20	Loam
Kilasaif	3.4 ± 0.22	7.2 ± 0.06	0.56 ± 0.04	6.8 ± 2.27	131 ± 10.0	39 ± 0.75	Loam

*(Mean ± Standard error); (EC = Electrical Conductivity; pH = Negative Log of Hydrogen Ion; OC = Organic Carbon Content; P = Available Phosphorus Content; K = Available Potassium Content; SP = Saturation Percentage)

Statistics and computations: The descriptive statistics of AMF colonization status and distribution was performed by SPSS 17.0. Shannon-Weiner diversity index was determined for the assessment of diversity of AMF species in different zones, by the formula: $\Delta_{Sh} = - \sum(P_i/P_0) \log (P_i/P_0)$, where P_i is the spore abundance of one AMF species and P_0 is the total abundance of all AMF species (Uzma *et al.*, 2016). Further, computations were performed by using MS Excel 2013 and graphs were plotted to display the treatment affects and distribution of AM fungal mutualism.

Results

Colonization of arbuscular-mycorrhizal fungi varies in its response to physico-chemical composition of soil. Table 1 displays a detailed comparative analysis of soil physical and chemical properties in ten different agro-ecological zones and showed significant variation. The soil status was normal at all study sites except Charsadda where soil was saline and acidic. No comparable organic carbon difference was observed among different agro-ecological zones and was found lower, which can be attributed to common character of loamy soil condition in Pakistan. Phosphorus availability has always been an important character in AMF colonizing soils and thereby showed a greater diversity among different agro-ecological zones. Faisalabad exhibited highest phosphorus availability (i.e. 8.5 ppm) and Swat have 4.5 ppm phosphorus content minimum of all zones. Likewise, Potassium availability also varied significantly among different zones. However, all sampling sites were rich in potassium content, in general. Lastly water holding capacity of soil was found similar in all sampled zones, might be due to similar soil texture and same organic matter fraction range.

Along with seasonal variation in colonization of Arbuscular mycorrhizal structures in host plant roots, these also vary in their occurrence among spatially different areas even in same host. Figure 2 displays the colonization status of hyphae, vesicles, arbuscules and intra-radical spores of AMF and their variation among different agro-ecological zones. All three zones of KPK showed high AMF colonization status, Swat being the most AMF colonized agro-ecological zone. The percentage of intra-radical spores was generally lower. Interestingly, despite of strong rhizospheric colonization, no intra-radical spores were observed in roots of tomato plant in Bahawalpur, Gujranwala and South Waziristan. Along with root colonization status, AMF species diversity and spore densities in rhizospheric soil varied considerably among different agro-ecological zones (Table 2). Samples form Swat were not only highest in relative spore density (i.e. 21.24) but utmost in Shannon-Weiner diversity Index too, with a consortium of maximum AMF species.

Table 2. Relative spore density and Shannon-Weiner diversity Index of arbuscular-mycorrhizal fungi in ten different agro-ecological zones.

	Agro-ecological zones	Relative spore density	Shannon-Weiner diversity Index
1.	Bahawalpur	4.16	0.66
2.	Faisalabad	8.00	1.48
3.	Gujranwala	4.45	0.86
4.	karachi	9.75	1.52
5.	Sukkur	12.79	1.68
6.	Swat	21.24	3.00
7.	Charsadda	5.70	2.09
8.	South Waziristan	6.70	2.20
9.	Pishin	14.74	1.76
10.	Kilasaif	12.47	1.65

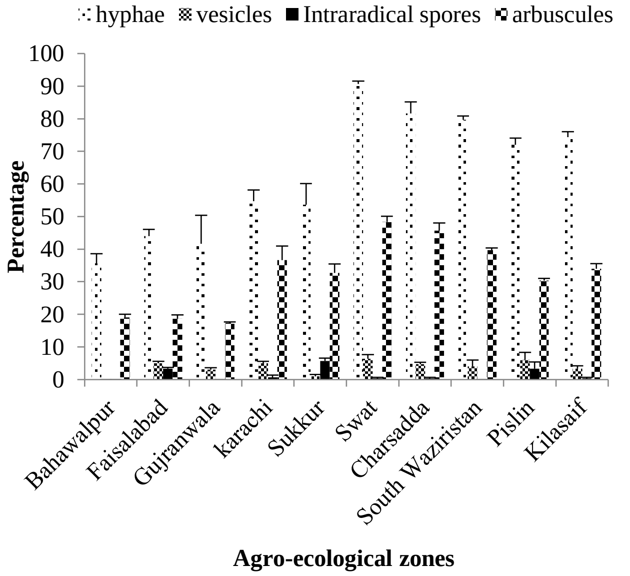

Fig. 2. Percentage of AMF propagules in tomato roots in ten different agro-ecological zones.

Table 3. Pearson's correlation among soil edaphic factors and colonization status of different mycorrhizal structures in tomato roots.

	Electrical conductivity	pH	Organic matter	Available phosphorus	Available potassium	Saturation percentage	Hyphal colonization	Vesicular colonization	Intra-radical spore colonization
pH	-0.514								
Organic matter	0.066	-0.005							
Available phosphorus	-0.349	**0.636***	0.115						
Available potassium	0.087	-0.286	0.309	-0.041					
Saturation percentage	-0.134	0.099	0.079	-0.264	-0.084				
Hyphal colonization	0.467	-0.544	0.092	**-0.873****	0.005	-0.062			
Vesicular colonization	0.402	-0.319	0.523	-0.304	0.234	-0.527	0.615		
Intra-radical spore colonization	0.112	0.549	0.537	0.287	0.013	0.503	-0.203	-0.088	
Arbuscular colonization	0.401	-0.424	-0.011	**-0.817****	-0.220	0.042	**0.898****	0.483	-0.180

*Correlation is significant at the 0.05 level (2-tailed)
**Correlation is significant at the 0.01 level (2-tailed)

Pearson's correlation among most soil parameters showed no correlation with any soil or AMF variable except available phosphorus and soil pH (Table 3). Soil phosphorus content showed a strong negative correlation with hyphal infection (r= -0.873, p<0.01) and arbuscules (r= -0.817, p<0.01). It showed that soils having low available phosphorus are more prone to AMF infection and possess high colonization status. Hyphal percentage and arbuscular abundance showed a strong positive correlation with each other (r= 0.898, p<0.01). It's obvious that roots possessing more intra-radical running hyphae possess more interface area (i.e. arbuscules). Available phosphorus content in soil also exhibited a significant positive correlation with soil pH (r= 0.636, p<0.05). It describes that reduction of phosphorus availability in soil also leads to soil acidity.

Results of nutritional variables in response to varying AMF and soil chemistry were found as expected. All agro-ecological zones of KPK attained maximum expression of studied variables except total flavonoid content (Fig. 3). Agro-ecological zone-Swat had the highest total phenolic content (128.0±4.1 µgGAE/mg D.W) antioxidant capacity (122.2±3.13 µgAAE/mg D.W) and reducing power (125.7±2.54 µgAAE/mg D.W), whereas Bahawalpur had the lowest. Highest flavonoid content was exhibited by South Waziristan (97.2 ± 2.26 µgQE/mg D.W). All the agro-ecological zones showed high DPPH free radical scavenging activity, Swat being highest exhibited 97.1% with IC50 value 67.8 µg/ml. Results showed that among ten agro-ecological zones, tomatoes grown in Swat are the most valuable and healthy. With an increase of phenolic content, total reducing power, DPPH free radical scavenging activity and IC50 value was increased significantly. Antioxidant activity in tomato fruit was also increased with increase in flavonoid content but the extent was low (Table 4). Our results showed that phenolic compounds are key contributing agents for the antioxidant activity of tomato fruit.

Discussion

Soil chemistry may generate a variety of mechanisms such as alterations in AMF colonization in roots and diversity of AMF associated with a host plant (Chaudhary *et al.*, 2015, Kumar & Ghose, 2008). The present study discovered variation in AMF colonization associated with tomato plant when sampled from different agro-ecological zones. Many authors have reported that differences in host species may alter levels of arbuscular-mycorrhizal fungi (Pepe *et al.*, 2016). Furthermore, a single plant species might also differ in colonization intensities when sampled from spatially different locations in the same season (Lekberg *et al.*, 2013). Our findings illustrate that soil chemical heterogeneity and inoculum potential can strongly influence AMF colonization (Lee *et al.*, 2013). The research also revealed qualitative differences in the distribution patters of AMF species, which might be due to ecological factors (Liu *et al.*, 2016).

The present study shows that spatial diversity (i.e. change in soil micro-environment) apparently has direct effect on mycorrhizal colonization in roots and occurrence of AMF species. The colonization status of AM fungal propagules and its diversity index showed a contradictory relationship with available phosphorus in rhizospheric soil of tomato plant. Previous studies revealed that varying

AMF diversity in host root is due to variation in amount of available P in rhizospheric soil (Liu *et al.*, 2016). However, relative AMF spore densities in host rhizosphere were not entirely correlated with soil phosphorus status in this study. Lin *et al*., (2020) stated that effect of phosphorus on spore density is different for different host species. Overall spore densities were low in all rhizospheric soils investigated in this study. Some authors associate low AMF spore numbers with semi-arid and arid lands (Mohammad *et al.*, 2003, Bencherif *et al.*, 2015).

Inspite of AMF infectivity, fluctuations also occurred in individual soil propagule correlations. Arbuscular and hyphal percentage in roots was positively correlated with each other and negatively correlated with soil phosphorus and pH. It might be of the fact that the exchange sites (arbuscules) are enhanced in stress alleviation modes (Aroca *et al.*, 2013). Positive correlation between soil pH and phosphorus availability can be explained by the fact that in highly acidic soils available phosphorus is strongly held by soil colloidal surfaces (Penn & Camberato, 2019).

AMF is an important variable in affecting the production of secondary metabolites in plants. Present study exhibits similar results. Tomato fruits sampled from Swat, the highest mycorrhizal inoculated zone, possessed high phenolic and flavonoid content among all agro-ecological zones. Increase in secondary metabolites of inoculated plants, which show no apparent increase in growth or productivity might be an indirect effect of AMF on plant metabolites through better nutrition (Lu *et al.*, 2015). AMF inoculation can be used to obtain plants enriched in chlorophylls, vitamins and carotenoids (Baslam *et al.*, 2013). They however, monitored that total phenolic content was not affected except in case of water stress. Contradictorily, some studies showed low (García-Macías *et al.*, 2007) or no correlation (Apostolou *et al.*, 2013) between phenolic content and antioxidant capacity. However, our results showed a positive correlation between these two variables (Rozpądek *et al.*, 2016, Torres *et al.*, 2016). The phenolic content in a plant is considered an important variable to maintain the antioxidant capacity (Goiris *et al.*, 2012). Increase in phenol content increases antioxidative activities (Rocchetti *et al.*, 2017). A significant positive correlation between phenolic content and the reducing power in present study have also been previously discussed (Qader *et al.*, 2011, Terpinc *et al.*, 2012). Flavonoid content is also positively correlated with antioxidative activities but not in all agro-ecological zones. This low correlation status has also been reported by other researches (Prasad *et al.*, 2009). The correlation status between all variables in present study confirmed that phenolic content is the main micro-constituent in contributing to the antioxidant activity of tomato fruits.

Understanding the effects of soil chemistry and spatial AMF diversity has direct role in improving plant nutritional value and may lead us to a new and better bio-fertility management designs. However, the relationship of cause and effect between AMF and plant and the correlation of both with the production of nutritionally rich agricultural products remained largely undemonstrated.

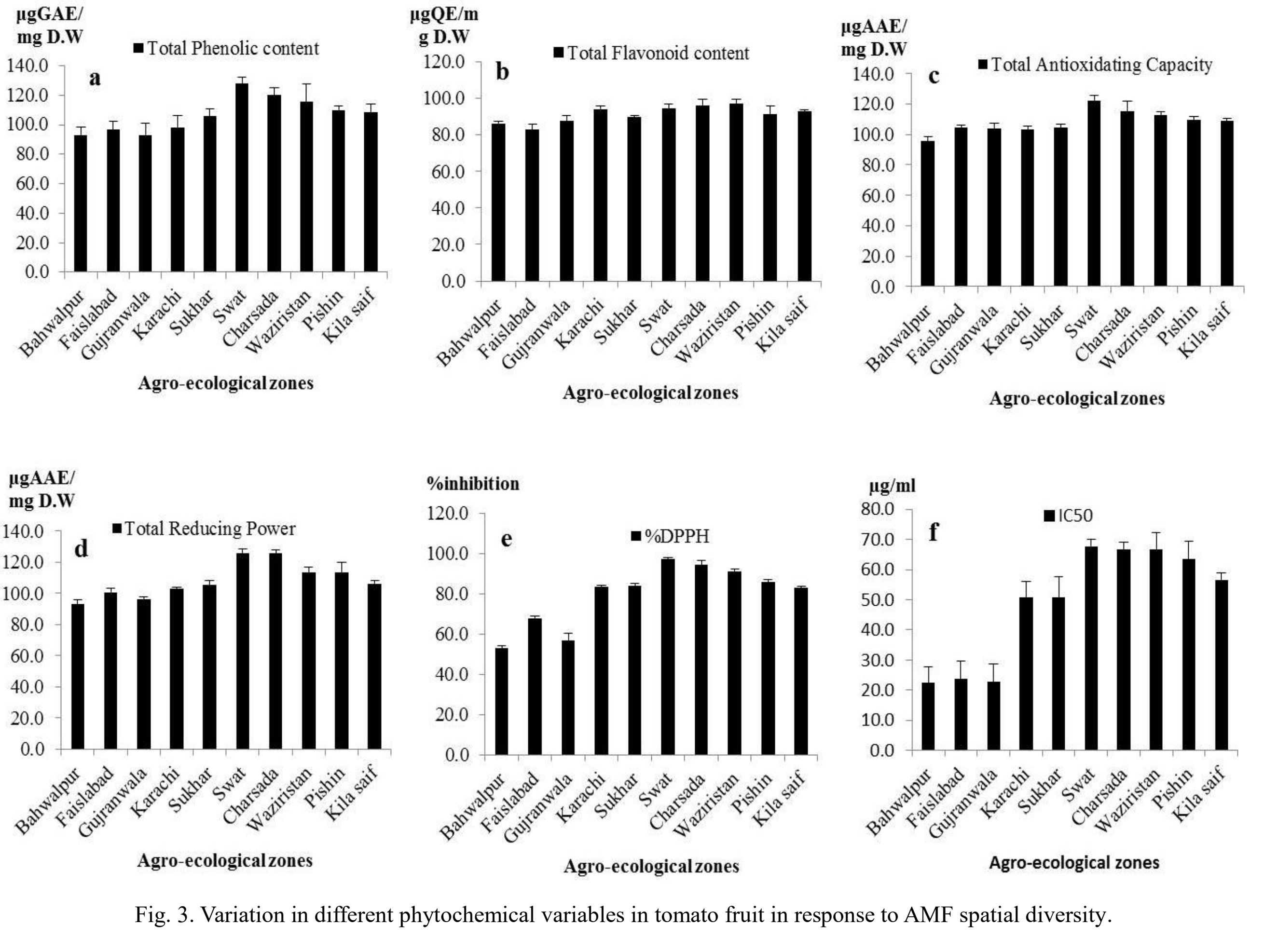

Fig. 3. Variation in different phytochemical variables in tomato fruit in response to AMF spatial diversity.

Table 4. Pearson correlation between different phytochemical variables in tomato fruit.

	Total phenolic content	Total flavonoid content	Total antioxidant capacity	Total reducing power	DPPH
Total flavonoid content	0.752*				
Total antioxidating capacity	0.943**	0.679*			
Total reducing power	0.965**	0.740*	0.937**		
DPPH	0.895**	0.823**	0.829**	0.896**	
IC50	0.888**	0.893**	0.790**	0.871**	0.961**

*Correlation is significant at the 0.05 level (2-tailed)
**Correlation is significant at the 0.01 level (2-tailed)

Conclusion

Soil chemistry especially available phosphorus content significantly affects AMF colonization and diversity in host roots and subsequently alters the nutritional value of fruit.

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