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Original Article

Effect of foliar application of salicylic acid and mycorrhiza on quantitative and qualitative traits of maize

Efeito da aplicação foliar de ácido salicílico e micorrizas em características quantitativas e qualitativas do milho

M. P. Younespour[a], M. Samdeliri[a], P. Mazloom[a*], A. M. Mirkalaei[a] and M. Moballeghi[a]

[a]Department of Agronomy, Chalus Branch, Islamic Azad University, Chalus, Iran

Abstract

An experiment was performed to investigate the effect of mycorrhizal symbiosis and foliar application of salicylic acid on quantitative and qualitative traits of maize during 2018 and 2019 in the research farm of Islamic Azad University, Chalous Branch. Split plot in a randomized complete block design with three replications was used. Experimental factors included mycorrhiza species of (*G. mosseae*), (*G. geosporum*) and (*G. intraradices*) at two levels (no consumption and consumption of mycorrhiza) and salicylic acid at two levels (no consumption and consumption of 1 mµ of salicylic acid). Results of interaction effects of mycorrhiza and salicylic acid on the measured traits revealed that the maximum 1000-grain weight, grain yield, biological yield, phosphorus, potassium, nitrogen percentage and yield of maize grain protein were observed in G. mosseae treatment under foliar application of salicylic acid. Foliar application of salicylic acid increases the root length and provides the necessary conditions for increasing water and nutrient uptake alongwith increase in photosynthesis and thus allocates more photosynthetic substance for development of reproductive organs. Hence, it increases maize grain weight and accordingly grain yield. In general, the results revealed that mycorrhiza and foliar application of salicylic acid increase growth indicators, yield and yield components. It also improved the quality traits of the maize plant. Based on results, the interaction effect of *G. mosseae* treatment and foliar application of salicylic acid yielded better results than other treatments. Mycorrhiza increases the number of grain in the ear, the number of rows in the ear, increases the plant's ability to absorb phosphorus, and the increase of mycorrhiza along with salicylic acid shows the maximum grain yield in maize. Finally, it can be concluded that the use of mycorrhiza and salicylic acid can be effective in increasing grain in the plant.

Keywords: salicylic acid, growth indices, grain yield, mycorrhizal symbiosis.

Resumo

Um experimento foi realizado para investigar o efeito da simbiose micorrízica e aplicação foliar de ácido salicílico em características quantitativas e qualitativas do milho durante 2018 e 2019 na fazenda de pesquisa da Universidade Islâmica Azad, Chalous Branch. Foi usada uma parcela dividida em um delineamento de blocos casualizados com três repetições. Os fatores experimentais incluíram espécies de micorrizas (*G. mosseae*, *G. geosporum* e *G. intraradices*) em dois níveis (sem consumo e com consumo de micorrizas) e ácido salicílico em dois níveis (sem consumo e com consumo de 1 mµ de ácido salicílico). Os resultados dos efeitos da interação de micorriza e ácido salicílico nas características medidas revelaram que peso máximo de 1.000 grãos, rendimento de grãos, rendimento biológico, fósforo, potássio, porcentagem de nitrogênio e rendimento de proteína de grão de milho foram observados no tratamento *G. mosseae* sob aplicação foliar de ácido salicílico. A aplicação foliar de ácido salicílico aumenta o comprimento da raiz e fornece as condições necessárias para aumentar a absorção de água e nutrientes juntamente com o aumento da fotossíntese e, assim, aloca mais substância fotossintética para o desenvolvimento dos órgãos reprodutivos. Assim, aumenta o peso do grão de milho e, consequentemente, o rendimento de grãos. Em geral, os resultados revelaram que a micorriza e a aplicação foliar de ácido salicílico aumentam os indicadores de crescimento, rendimento e componentes do rendimento. Também melhoram as características de qualidade da planta de milho. Com base nos resultados, o efeito de interação do tratamento *G. mosseae* e aplicação foliar de ácido salicílico produziu melhores resultados do que outros tratamentos. A micorriza aumenta o número de grãos na espiga, o número de fileiras na espiga e a capacidade da planta de absorver fósforo, e o aumento da micorriza junto com o ácido salicílico mostra o rendimento máximo de grãos no milho. Por fim, pode-se concluir que o uso de micorriza e ácido salicílico pode ser eficaz no incremento de grãos na planta.

Palavras-chave: ácido salicílico, índices de crescimento, rendimento de grãos, simbiose micorrízica.

*e-mail: P_mazloom@iauc.ac.ir, pooriamazloom4@gmail.com
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1. Introduction

Maize is one of the most important crops and after wheat and rice and is the third most common grain crop (Ahmed, 2015). Increased maize production in Iran as well as high nutrient needs and excessive chemical inputs have led to increased costs and environmental risks. (Karami et al., 2018). Nowadays, the use of biological fertilizers to reduce the use of chemical fertilizers and increase crop yields is a crucial issue in moving towards sustainable agriculture (Arbab, 2014; Karami et al., 2018). Mycorrhiza fungi have symbiotic relationships with roots of most crops that improve growth and yield of host plants in sustainable agricultural systems by increasing the uptake of nutrients such as phosphorus and some trace elements, increasing water uptake, reducing the negative impact of environmental stresses and increasing resistance to pathogens (Sharma et al., 2002; Karami et al., 2018; Rani et al., 2018; Rani et al., 2023).

Parsa Motlagh (2010) stated that in general, mycorrhizal fungi increased the dry weight of maize root, because these fungi had many fibers that these fibers enter plant root system and increase root weight on one hand and increase root growth by uptake of phosphorus and water on the other hand. Plant hormones are known as a powerful and sustainable tool in reducing unfavorable effects of biotic and abiotic stresses on plants. Salicylic acid or benzoic ortho hydroxy acid is a phenolic compound that is produced by root cells in many plants and as an hormone-like substance, plays a major role in plant growth (Khan et al., 2015). Salicylic acid plays a major role in regulating various physiological processes such as plant growth and development, ion uptake, photosynthesis and germination, maturation and defense responses (Miura and Tada, 2014; Pooja, 2019; Yadav et al., 2020; Singh et al., 2023; Mousavi et al., 2019). Shakirova et al. (2003) reported that the application of salicylic acid at a concentration of 0.5 mM increased the number of grains per ear row in maize. A study conducted by Khodary (2004) on maize revealed that foliar application of salicylic acid increased growth indicators, pigment content and photosynthesis rate. Mehrabian Moghaddam et al. (2011) reported that salicylic acid caused a significant increase in forage dry weight in maize. This study was carried out to achieve the goals of sustainable agriculture to reduce the use of chemical fertilizers and also to achieve greater efficiency in the use of resources.

Various researchers used graphical analysis(Correlation diagram, principal component diagram) to investigate the morphological and physiological traits of maize in order to investigate yield and yield components and showed the correlation of most traits with grain yield trait (Mousavi et al., 2023, Mousavi et al., 2016; Shojaei et al., 2023, Szabó et al., 2022).

2. Materials and Methods

This experiment was performed during kharif season of 2018 and 2019 in the research farm of Islamic Azad University, Chalous Branch, as a split plot in a randomized complete blocks design with three replications. Experimental factors included mycorrhiza species of (*G. mosseae*), (*G. geosporum*) and (*G. intraradices*) at two levels (no consumption and consumption of mycorrhiza) and salicylic acid at two levels (no consumption and consumption of 1 mM of salicylic acid). To determine the physical and chemical properties of soil, in the first year and before planting, soil samples were randomly taken from the ground of the experiment site at a depth of 0-30 cm and its results are presented in Table 1. Each plot with dimensions of 4.5 × 6 m including 6 planting lines and a distance of 75 cm between rows was considered that the distance between each plant on a row was considered at 20 cm. Seed depth of 3-5 cm was created by making a groove on the stack. Nitrogen fertilizer was applied to soil in three stages (planting, stemming and flowering). In treatments of seed inoculation with mycorrhizal fungus, 20 grams of inoculation substance was in direct contact with the seed simultaneously with planting. The first irrigation was performed immediately after planting to improve the emergence of seedlings. Three days later, for better germination, the second irrigation was performed. To achieve proper density, the plant was thinned in one stage and after complete establishment in the four-leaf stage. Foliar application of salicylic acid was performed in a 12-leaf stage at a rate of 1 mM. Weed control was performed manually early in the growing season. Harvesting operations were performed at full maturity and manually for both years. To measure the traits of maize, two lines from each experimental plot were sampled from both sides after removal of the margin effect and sent to laboratory, and finally the yield of each plot was generalized to kg/ha. To determine the yield and yield components of grain, by removing the side rows and 50 cm from the beginning and end of each plot as a margin effect, one square meter of the middle part of each plot was selected and sent to laboratory. Simultaneously with harvesting, five plants from each plot were selected separately and the number of grains per row, 1000-grain weight was measured.

Kjeldahl method was used to determine the percentage of grain nitrogen (Jackson and Volk, 1969). The amount of grain protein was obtained by multiplying the grain nitrogen percentage by 6.25. Grain protein yield was obtained by multiplying grain yield by the amount of grain protein. Data were analyzed using Excel, Excel Stat 2019 and SAS 9.3 softwares. LSD test at 5% probability level was used to compare the means of desired traits.

Table 1. Some physical and chemical properties of experiment site soil.

Depth (cm)	Soil texture	PH	Electrical conductivity	Organic carbon (%)	Total nitrogen (%)	Absorbable phosphorus ppm	Absorbable Potassium ppm
0-30	Loam- sandy clay	7.2	1.13	1.3	0.09	11	314

3. Results and Discussion

Number of rows per ear: based on the results of analysis of variance, the effect of application of mycorrhiza and salicylic acid on the number of rows per ear showed that the triple effect of year × mycorrhiza × salicylic acid on the number of rows per ear was significant at the level of one percent (Table 2). Results of comparing the means of triple effect of year × mycorrhiza × salicylic acid on the number of rows per ear showed that the highest number of rows per ear was observed in G. geosporum and G. intraradices in the second year and under foliar application of salicylic acid, respectively, at the rate of 18.57 and 18.03 rows. Minimum number of rows per ear was obtained in the control treatment in the second year and in the absence of foliar application of salicylic acid (11.60 rows) (Table 3). Shakirova et al. (2003) reported that with the application of salicylic acid at a concentration

Table 2. Results of compound analysis of the effect of mycorrhiza and salicylic acid application on quantitative traits of maize.

Source of variations	degree of freedom	Mean of squares					
		Number of rows per ear	Number of grains per ear	1000-grain weight	Grain yield	Biologic yield	Harvest index
Year	1	6.27 ns	11689.69ns	6813.94 ns	10902308.38 ns	1536456.43 ns	182.81 ns
Replication (year)	4	0.22	2712.40	1220.39	1416884.43	1495281.49	41.81
mycorrhiza	3	2.49 ns	9104.55 ns	1807.07ns	2891305.16 ns	5529093.56 ns	42.75 ns
year × mycorrhiza	3	2.16 ns	9832.12*	5886.15**	9417845.89**	3944936.17*	132.66 ns
Salicylic acid	1	109.91*	3839.48 ns	1549.50 ns	2479201.24 ns	24594455.87*	28.00 ns
Year × Salicylic acid	1	0.53 ns	2137.48 ns	667.60 ns	1068161.77 ns	107088.07 ns	46.68 ns
Mycorrhiza × salicylic acid	3	2.22 ns	4713.38*	2210.89*	3537416.72*	12238588.95*	87.27 ns
Year × Mycorrhiza × Salicylic acid	3	4.06**	449.08 ns	238.78 ns	382052.74 ns	1024318.54 ns	33.72 ns
Total error	28	0.76	2236.50	1191.83	1596289.73	979152.00	48.59
Coefficient of variations (%)	-	15.42	16.38	14.36	13.14	15.13	13.93

*, ** and ns are significant at the level of 5 and 1% and non-significant (ns), respectively.

Table 3. Comparison of the mean effect of mycorrhiza, salicylic acid and year on the number of rows per ear.

Mycorrhiza	Salicylic acid	Year	Number of rows per ear
G. geosporum	Lack of foliar application	First	15.38 b
	Lack of foliar application	Second	15.37 b
	foliar application	First	17.67 ab
	foliar application	second	18.57 a
G. intraradices	Lack of foliar application	First	14.49 b
	Lack of foliar application	second	14.73 b
	foliar application	First	17.29 ab
	foliar application	second	18.03 a
G. mosseae	Lack of foliar application	First	15.43 b
	Lack of foliar application	second	15.50 b
	foliar application	First	17.73 ab
	foliar application	second	17.73 ab
control	Lack of foliar application	First	11.60 c
	Lack of foliar application	second	15.03 b
	foliar application	First	17.16 ab
	foliar application	second	17.57 ab

In each column, the means that have the same letters are not significantly different at the level of 5% according to the LSD test.

of 0.5 mmol, the number of grains per ear row in maize increased. This increase may be due to role of salicylic acid in induction of flowering. Also, with salicylic acid foliar application, stronger plants produce larger ears that have more grains per row. By increasing the absorption of water and nutrients, especially phosphorus, Mycorrhiza fungus has increased the rate of photosynthesis and production of assimilates to a certain extent to be genetic function. As a result, it has increased the number of rows of grains in ear to a certain number that determines the genetic characteristics of ear. In general, fungus and foliar application of salicylic acid increased the number of rows in maize ears.

Number of grains per ear: The results of analysis of variance showed the effect of mycorrhiza and salicylic acid application on the number of grains per ear showed that the interaction of year × mycorrhiza and mycorrhiza× salicylic acid on the number of grains per ear was significant at the level of 5% (Table 2). Results of comparing the mean effects of year× mycorrhiza on the number of grains per ear show that the highest number of grains in each ear in G. intraradices in the first year was 339 grains. The lowest number of grains per ear was obtained in the control and G. mosseae treatment in the first year (Table 4). In general, the use of fungus increased the weight of maize ears. Rahmani (2015) stated that different species of mycorrhiza caused a significant increase in the number of grains per row of maize, which is consistent with the results of the present study. Based on researchers, phosphorus is an important factor in maize pollination, so that its deficiency delays plant pollination and it is done incompletely. As a result, phosphorus deficiency leads to lack of grain formation in the ear (Ghorchiani et al., 2011). By dissolving insoluble soil phosphates, Mycorrhiza increases the plant ability to uptake phosphorus and increases the number of grains in ear row. The results of interaction of mycorrhiza and salicylic acid showed that maximum number of grains per maize ear was obtained in the treatment of G. intraradices in the absence of foliar application of salicylic acid (337 grains). The minimum number was observed in the control treatment and in the absence of foliar application of salicylic acid (223 grains). In control and treatment with G. mosseae groups, foliar application of salicylic acid increased the number of grains per maize ear compared to non-foliar application (Table 5). The number of grains per plant is obtained by multiplying the number of grains per row and the number of rows per ear. Since mycorrhiza and salicylic acid increased the number of grains per row and the number of rows per ear, increasing the number of grains per plant as a result of using mycorrhiza and

Table 4. Comparing the mean effects of mycorrhiza application and year on quantitative traits of maize.

Mycorrhiza	Year	Number of grains per ear	1000-grain weight (g)	Grain yield (kg/ha)	Biological yield (kg/ha)
G. geosporum	First	275.97 bcd	233.48 bc	9339.05 bc	19485.63 b
	second	321.28 ab	245.48 bc	9819.20 bc	19226.38 b
G. intraradices	First	339.50 a	262.71 ab	10508.48 ab	19527.27 b
	second	299.37 abc	236.92 bc	9476.83 bc	18581.78 bc
G. mosseae	First	243.30 d	208.37 c	8334.80 c	19403.11 b
	second	331.28 ab	289.34 a	11573.79 a	20825.06 a
Control	First	233.34 d	209.28 c	8371.39 c	17877.32 c
	second	265.04 cd	237.41 bc	9496.56 bc	19091.40 b

In each column, the means that have the same letters are not significantly different at the level of 5% according to the LSD test.

Table 5. Comparing the mean effects of mycorrhiza and salicylic acid application on quantitative traits of maize.

Mycorrhiza	Salicylic acid	Number of grains per ear	1000-grain weight (g)	Grain yield (kg/ha)	Biological yield (kg/ha)
G. geosporum	Lack of foliar application	293.40 ab	243.75 ab	9749.84 abc	20133.82 b
	Foliar application	303.84 ab	235.21 ab	9408.42 abc	18587.20 cde
G. intraradices	Lack of foliar application	337.06 a	257.20 a	10288.06 ab	17899.55 de
	Foliar application	301.81 ab	242.43 ab	9697.26 abc	20209.50 b
G. mosseae	Lack of foliar application	264.55 bc	228.66 ab	9146.29 bc	18671.12 cd
	Foliar application	310.04 ab	269.06 a	10762.30 a	21557.05 a
Control	Lack of foliar application	223.76 c	209.17 b	8366.81 c	17441.25 e
	Foliar application	274.62 bc	237.53 ab	9501.14 abc	19527.47 bc

In each column, the means that have the same letters are not significantly different at the level of 5% according to the LSD test.

salicylic acid is justified. Since salicylic acid is known as a hormone-like substance, it seems that this substance to increase the growth of shoots and plants by affecting the vegetative and reproductive meristems and increased leaf area increases the metabolism as a s a result the number of grains increases.

1000-grain weight: The interaction effect of mycorrhiza × year and interaction effect of mycorrhiza × salicylic acid on the 1000-grain weight of maize was significant at the level of 1% and 5%, respectively (Table 2). The results of comparing the mean effects of Mycorrhiza on 1000-grain weight showed that the highest 1000-grain weight was observed in G. mosseae in the second year at the rate of 289.34 g. The lowest 1000-grain weight was obtained in the control treatment in the first year (Table 4). In general, application of fungus increased the 1000-grain weight of maize. Although the mycorrhizal fungi from plants are used assimilates, their positive role in absorbing water and nutrients, especially phosphorus, and transporting it to host plants, can be noted. This symbiotic relationship with maize enhances growth, increases photosynthesis, assimilate production, and results in larger grains with an acceptable weight, increasing the weight of 100 grains. Results of interaction effect of mycorrhiza and salicylic acid showed that the maximum 1000-grain weight of maize in the treatment of G. mosseae under foliar application of salicylic acid and G. intraradices under its non-foliar application was obtained at 269.06 and 257.20 g, respectively. Minimum weight was observed in the control treatment and in the absence of foliar application of salicylic acid at the rate of 209.17 g (Table 5). The increase in grain weight as a result of foliar application may be attributed to the increase in photosynthetic production that forms a storage source for destination and increase of tank capacity, which results in increased grain weight and increased yield (Pooja and Sharma, 2016). Foliar application also produces stronger plants and these plants produce larger grains with more weight. Ebrahimi et al. (2012) reported that salicylic acid had a significant positive effect on 1000-grain weight of maize.

Grain yield: Based on the results of analysis of variance, only the interaction effect of year × mycorrhiza on grain yield was significant at the level of one percent and the interaction effect of mycorrhiza × salicylic acid on grain yield was significant at the level of five percent (Table 2). The results of comparing the mean effects of mycorrhiza × year on grain yield show that maximum grain yield was observed in G. mosseae in the second year at the rate of 11573 kg / ha. The minimum grain yield was obtained in the control treatment in the first year (Table 4). In general, the use of fungi increased maize grain yield (Chen et al., 2004). Rahmani (2015) reported that with application of mycorrhiza, the grain yield increased significant and among the various species of mycorrhiza, Glomus species led to higher grain yield. It seems that foliar application of salicylic acid increases root length and provides the necessary conditions for increasing water and nutrient uptake and increasing photosynthesis and thus allocating more photosynthetic substance for development of reproductive organs, so it increases grain weight of maize and grain yield (Jirani et al., 2009). Mycorrhizal symbiosis through proper nutrition increases the dry weight of roots and shoots and finally increases yield and yield components, since mycorrhizal plants can absorb the inaccessible phosphorus of plants that are farther away from their roots through mycelium, and as a result, cause more nutrients to be absorbed by the roots. In the present study, mycorrhiza with a significant increase of grain yield components such as 1000-grain weight and number of grains per row led to an increase in grain yield, which is the result of yield components. Also, results of interaction effect of mycorrhiza and salicylic acid showed that maximum maize grain yield was obtained in of G. mosseae treatment under foliar application of salicylic acid at the rate of 10762 kg / ha. The minimum maize grain yield was also observed in the control treatment and in the absence of foliar application of salicylic acid at the rate of 8366 kg/ha (Table 5). Rapid establishment of plant by application of salicylic acid treatment and symbiosis of mycorrhizal fungi that enhance growth, increases uptake of nutrients and ions required by plant, increases maintaining of photosynthetic sources during the growing season, and increases receiving radiant energy and transporting photosynthesis substances towards grain, which in turn will increase the grain yield in the plant.

Biological yield: Results of analysis of variance of the effect of mycorrhiza and salicylic acid on biological yield showed that the interaction effect of year × mycorrhiza and mycorrhiza × salicylic acid on biological yield was significant at the level of 5% (Table 2). Results of comparing the mean effects of year× mycorrhiza on the biological yield of maize showed that the maximum biological yield was observed in G. mosseae treatment in the second year at the rate of 20825 kg / ha. The minimum biological yield was obtained in the control treatment in the first year (Table 4). Mycorrhizal fungi can increase nutrient uptake, leading to increased growth and weight of the host plant. Also, an increase in hormone levels has been observed in mycorrhizal symbiosis. With increasing these hormones, specially, cytokinin, photosynthesis rate increases through factors such as opening of pores, effect on ion transport and regulation of chlorophyll content, the effect on ion transport and regulation of chlorophyll content and finally increased rate of photosynthesis can increase the production of biomass in mycorrhizal plants by stimulating growth (Kafkas and Ortas 2009). Mycorrhiza fungi increase the activity of some enzymes such as phosphatase, alkaline phosphatase and accordingly, they help plant growth and increase vegetative growth (Zhang et al. 2011). Also, results of interaction effect of mycorrhiza and salicylic acid showed that the maximum biological yield of maize was obtained in G. mosseae treatment under foliar application of salicylic acid at the rate of 21557 kg/ha. The minimum biological yield of maize was also observed in the control treatment and in the absence of foliar application of salicylic acid at the rate of 17441 kg/ha (Table 5). In general, it can be concluded that salicylic acid as a plant growth regulator stimulates cell division in root and stem meristems and thus increases the production of biomass and plant dry matter and finally increases yield. Mehrabian Moghaddam et al. (2011) reported that salicylic acid caused a significant increase in forage dry weight in maize. It seems that salicylic acid increases the growth of vegetative organs through cell division and elongation, increases leaf area and increases photosynthetic area. It also increases biological yield by affecting the growth of reproductive organs such as number of grains, 1000-grain weight and thus grain yield.

Harvest index: Based on the results of analysis of variance, the effect of application of mycorrhiza and salicylic acid on harvest index showed the main effect and interaction effect of treatments had no significant effect on harvest index (Table 2).

Grain phosphorus: Results revealed that only the interaction effect of year ×mycorrhiza and mycorrhiza× salicylic acid on grain phosphorus was significant at the level of 1% and 5%, respectively. Other effects on grain phosphorus were not significantly different (Table 6). The results of comparing the mean effects of year ×mycorrhiza on grain phosphorus are presented in Table 7. The results show that the minimum rate of phosphorus was observed in G. mosseae in the second year at the rate of 0.60%. The minimum grain phosphorus was also obtained in the control treatment in the first year (Table 7). In general, the use of mushrooms increased the phosphorus of maize grain. Rahmani (2015) stated that the use of G. mosseae fungus increased the rate of phosphorus in maize, which is consistent with results of our study. Mycorrhiza inoculation significantly increased the phosphorus rate of chickpea. In most cases, increased uptake of nutrients and improving the nutrition of phosphorus are the first signs of increased growth and yield in mycorrhizal plants (Parsa Motlagh, 2010). Phosphorus in the soil is a sedentary element so that even if phosphorus is added to soil as a solution, it is quickly stabilized in the form of calcium phosphate or other forms and becomes immobile, so mycorrhizal fungi increase uptake of minerals especially phosphorus and accumulation of dry matter accumulation of many crops in soils with low phosphorus. Mycorrhizae can also influence plant growth through indirect mechanisms including effects on rhizosphere characteristics such as pH change and root system pattern. Also, the results of interaction between mycorrhiza and salicylic acid showed that the maximum grain phosphorus was obtained in the treatment of G. mosseae under foliar application of salicylic acid at the rate of 0.60%. The minimum rate was observed in the

Table 6. Results of analysis of variance of the effect of mycorrhiza and salicylic acid application on qualitative traits of maize grain.

Source of variations	degree of freedom	Mean of squares				
		Grain phosphorus	Grain potassium	Grain nitrogen	Protein	Protein yield
Year	1	0.09 ns	10.96 ns	0.39 ns	15.26 ns	14310.23 ns
Replication (year)	4	0.01	0.26	0.05	1.98	1925.02
Mycorrhiza	3	0.03 ns	2.79 ns	0.10 ns	4.05 ns	4180.20 ns
Year × Mycorrhiza	3	0.09**	8.70**	0.34**	13.18**	13489.34**
Salicylic acid	1	0.27 ns	1.92 ns	0.09 ns	4.37 ns	2278.18ns
Year × Salicylic acid	1	0.00 ns	0.66 ns	0.04 ns	1.49 ns	647.99 ns
Mycorrhiza × salicylic acid	3	0.03*	3.13*	0.13 *	4.95 *	4952.46*
Year × Mycorrhiza × Salicylic acid	3	0.00 ns	0.25 ns	0.01 ns	0.53 ns	568.33 ns
Total error	28	0.01	0.74	0.06	2.23	2254.19
Coefficient of variations (%)	-	19.19	18.26	13.14	13.15	20.54

*, ** and ns are significant at the level of 5 and 1% and non-significant (ns), respectively.

Table 7. Comparison of the mean effects of mycorrhiza application and year on quality traits of maize grain.

Mycorrhiza	Year	Grain phosphorus (%)	Grain potassium (%)	Grain nitrogen (%)	grain protein (%)	Protein yield (kg/ha)
G. geosporum	first	0.39 cde	4.40 cde	1.76 bc	11.05 bc	166.11 bc
	second	0.44 bc	4.92 bc	1.85 bc	11.62 bc	185.59 bc
G. intraradices	first	0.51 b	5.45 b	1.98 ab	12.43 ab	211.79 ab
	second	0.40 cde	4.52 bcde	1.79 bc	11.21 bc	170.10 bc
G. mosseae	first	0.32 de	3.59 de	1.57 c	9.86 c	134.65 c
	second	0.62 a	6.77 a	2.18 a	13.69 a	255.36 a
Control	first	0.31 e	3.55 e	1.58 c	9.90 c	133.96 c
	second	0.41 bcd	4.60 bcd	1.79 bc	11.23 bc	173.59 bc

In each column, the means that have the same letters are not significantly different at the level of 5% according to the LSD test.

control treatment and in the absence of foliar application of salicylic acid (0.26%) (Table 8). Rahmani (2015) reported that using G. mosseae increased the rate of phosphorus in maize, which is consistent with the results of our study. One of the most obvious effects of mycorrhiza inoculation is to increase uptake and improvement of phosphorus by mycorrhizal plants. In this study, it was found that the rate of phosphorus uptake in mycorrhiza-inoculated treatments increased, indicating more uptake of phosphorus by the fungal hyphae and its transport to maize plant.

Grain potassium: based on results of analysis of variance, only the interaction effect of mycorrhiza×year and mycorrhiza ×salicylic acid on grain potassium was significant at the level of 1% and 5%, respectively (Table 6). The results of comparing the mean effects of mycorrhiza × year on grain potassium showed that the highest grain potassium was observed in G. geosporum in the second year at the rate of 6.77%. The lowest grain potassium was obtained in the control treatment in the first year (Table 7). Results of the study conducted by Ghorbanian et al. (2014) also showed that the effect of fungus on grain potassium was significant. There is no reliable information on the symbiosis role of mycorrhizae in the uptake of potassium by the host plant because most of the results are inconsistent with each other and indicate the indirect effect of increasing phosphorus uptake in the host plant. However, it can be stated that about 40% of the total potassium absorbed by the host plant is due to activity of extra-root hyphae of arbuscular mycorrhizal fungi (Marschner and Dell, 1994; Rahmani, 2015). Also, the results of the interaction effect of mycorrhiza and salicylic acid showed that the highest grain potassium was obtained in the G. mosseae treatment under foliar application of salicylic acid at the rate of 5.92% and its minimum rate was observed in the control treatment and in the absence of foliar application of salicylic acid (3.55%) (Table 8). Due to an increase in stomatal conductance in fungus-treated plants, root growth and nutrient uptake increase, leading to increased potassium uptake in the plant. The study conducted by Amin et al. (2008) showed that salicylic acid increased the uptake of potassium in wheat grains.

Grain nitrogen: based on the results of analysis of variance, the effect of application of mycorrhiza and salicylic acid on grain nitrogen showed that the interaction effect of year × mycorrhiza and mycorrhiza × salicylic acid on grain nitrogen was significant at the level of 1% and 5%, respectively (Table 6). The results of comparing the mean effects of mycorrhiza × year show that the maximum grain nitrogen was observed in G. geosporum in the second year at the rate of 2.18. The minimum grain nitrogen was obtained in treatment of G. mosseae in the first year (Table 7). In general, the use of fungus increased maize nitrogen. Mycorrhiza fungus increases the element phosphorus in the plant. Phosphorus plays a major role in providing energy in the structure of ATP, and since high energy is required to stabilize nitrogen, the presence of phosphorus is essential in increasing grain nitrogen (Zubillaga et al., 2002). Also, the results of interaction of mycorrhiza and salicylic acid showed that the maximum nitrogen of maize grain was obtained in G. mosseae fungus treatment under foliar application of salicylic acid at the rate of 2.03% and the minimum rate was observed in the control treatment and in the absence of foliar application of salicylic acid (1.58%) (Table 8). The results of other studies have also shown that application of mycorrhiza induces the activity of nitrogenase enzyme in the roots of inoculated plants and as a result, it provides nitrogen for the plant (Okon and Kapulnik, 1986). The present study revealed that G. mosseae fungus increased the uptake of water and nutrients in the maize plant by increasing the chlorophyll content, and as a result, it increased nitrogen content in the grain. Protein content and grain protein yield: Based on the results of analysis of variance, the effect of mycorrhiza and salicylic acid application on the content and yield of grain protein showed that only the interaction of year × mycorrhiza and mycorrhiza × salicylic acid on grain protein was significant at the level of 1% and 5%, respectively (Table 6). The results of comparing the mean effects year × mycorrhiza on grain protein content and yield showed that the maximum grain protein content and yield was observed in G. mosseae in the second year and the minimum grain protein yield was obtained in control and G. mosseae treatment in the first year (Table 7). In general, the use of fungus increased content and yield of grain protein. It seems that in this experiment, the positive effects of mycorrhizal fungi increased photosynthesis by increasing the uptake of water and nutrients, and it

Table 8. Comparing the mean effects of mycorrhiza and salicylic acid application on quality traits of maize grain.

Mycorrhiza	Salicylic acid	Grain phosphorus (%)	Grain potassium (%)	Grain nitrogen (%)	grain protein (%)	Protein yield (kg / ha)
G. geosporum	Lack of foliar application	0.38 cd	4.87 b	1.84 abc	11.53 abc	183.62 abc
	Foliar application	0.46 bc	4.45 bc	1.78 abc	11.13 abc	168.08 abc
G. intraradices	Lack of foliar application	0.43 bcd	5.25 ab	1.94 ab	12.17 ab	203.84 ab
	Foliar application	0.48 b	4.72 b	1.83 abc	11.47 abc	178.05 abc
G. mosseae	Lack of foliar application	0.34 de	4.43 bc	1.73 bc	10.82 bc	166.51 bc
	Foliar application	0.60 a	5.92 a	2.03 a	12.73 a	223.50 a
Control	Lack of foliar application	0.26 e	3.55 c	1.58 c	9.90 c	134.04 c
	Foliar application	0.47 bc	4.60 b	1.79 abc	11.24 abc	173.51 abc

In each column, the means that have the same letters are not significantly different at the level of 5% according to the LSD test.

resulted in production of more assimilates and improved growth, which finally increased the content of plant protein compared to the non-inoculated treatment. It can also be stated that with application of mycorrhiza species in the plant, the production of growth hormones of gibberellin and cytokinin increases (Raei et al., 2015) and accordingly it provides better conditions for growth and development and increased protein content. Mobasser et al. (2014) reported that mycorrhizal fungus increased the content of maize protein. Also, the results of interaction effect of mycorrhiza and salicylic acid showed the highest content and yield of grain protein was observed in the G. mosseae treatment under foliar application of salicylic acid at the rate of 12.73% and 223.50 kg/ha and the minimum was observed in the control treatment and in the absence of foliar application of salicylic acid (Table 8). The study conducted by Amin et al (2013) also showed that the use of salicylic acid increased maize protein. Abdel-Wahed et al. (2006) reported that foliar application of salicylic acid at low concentrations caused significant increases in sugar, protein and oil in maize grains.

3.1. Correlation between traits

In order to evaluate the correlation of the examined traits, the graphical analysis of the correlation between the traits was used (Figure 1). In this biplot diagram, the cosine of the angle between trait vectors indicates the intensity of correlation between traits. If the angle between the vectors is less than 90 degrees, the correlation between the vectors is equal to +1, if the angle between the vectors of the traits is 90 degrees, the correlation between the vectors of the traits is equal to zero, and if the angle between the vectors is 180 degrees, it indicates a correlation of -1 (Yan and Kang, 2002).

In examining the correlation diagram of quantitative traits, Number of grain per ear, Biological yield, Grain yield and Harvest index traits had a positive correlation, Grain yield, Harvest index and 1000-grain weight traits also had a positive correlation. Two traits Grain yield and Number of rows per ear also had a negative correlation with each other due to the 180 degree angle between the two vectors (Figure 1A). In examining the correlation diagram of qualitative traits, positive correlation was observed between Grain nitrogen and Protein yield traits and Protein and Grain phosphorus traits together. Also, two traits Protein yield and Protein showed a positive correlation with each other. Grain potassium and Protein traits also had a negative correlation. Considering that Grain nitrogen and Grain potassium traits had an angle of 90 degrees between their vectors, the correlation between them was estimated to be zero (Figure 1.B). Shojaei et al. (2022, 2023) used the correlation chart to detect positive and negative correlation between traits in their research (Shojaei et al., 2022, 2023). Based on the correlation intensity map drawn on the test data, which is respectively based on red color (highest correlation intensity), brown to green color (medium correlation intensity), blue color (lowest correlation level) and white color (absence of Correlation) separates traits and compares the degree of correlation of traits based on this (Figure 2). In the examination of the correlation intensity map in the quantitative traits evaluated in the experiment, the highest correlation intensity was found between Grain yield trait with Harvest index, 1000- grain weight and Number of grain per ear, and Harvets index trait had the highest correlation intensity with 1000- grain weight, Number of grain per ear and Number of rows per ear traits and 1000- grain weight trait with trait Number of rows per ear showed the highest intensity of correlation (Figure 2A). In the analysis of qualitative traits, Protein yield trait with Grain nitrogen and Grain phosphorus traits and Protein trait with Grain potassium trait showed the highest intensity of correlation (Figure 2B). Semeskandi et al. (2023) used this type of graph in their experiment to investigate morphological traits and investigated the intensity of correlation between traits (Semeskandi et al., 2023).

3.2. Principal Components Analysis (PCA)

In order to analyze the main components, quantitative and qualitative traits were put together and based on the

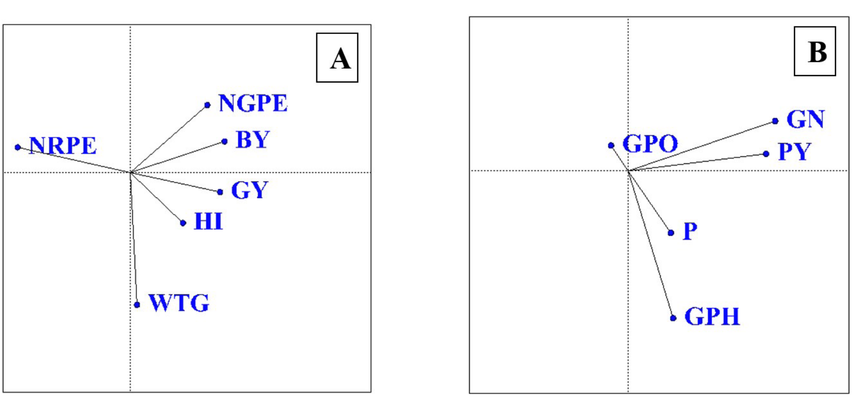

Figure 1. Correlation diagram between traits studied in the experiment. (A) Investigating the correlation of traits in terms of quantitative traits; (B) Examining the correlation of traits in terms of qualitative traits. (NRPE: Number of rows per ear, NGPE: Number of grain per ear, WTG: 1000-grain weight, GY: Grain yield, BY: Biological yield, HI: Harvest index, GPH: Grain phosphorus, GPO: Grain potassium, GN: Grain nitrogen, P: Protein, PY: Protein yield).

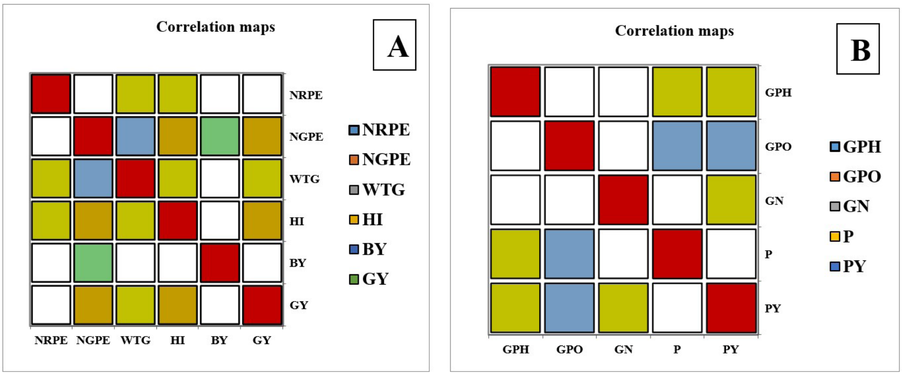

Figure 2. Examining the intensity of correlation between traits. (A) Examining the intensity of correlation between quantitative traits; (B) Examining the intensity of correlation between qualitative traits. (NRPE: Number of rows per ear, NGPE: Number of grain per ear, WTG: 1000-grain weight, GY: Grain yield, BY: Biological yield, HI: Harvest index, GPH: Grain phosphorus, GPO: Grain potassium, GN: Grain nitrogen, P: Protein, PY: Protein yield).

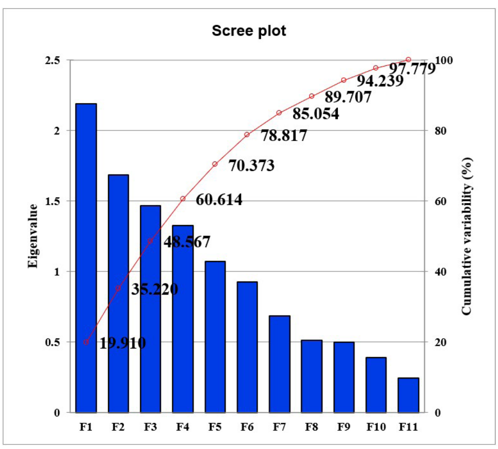

Figure 3. Eigenvalue diagram of quantitative and qualitative traits investigated in the experiment.

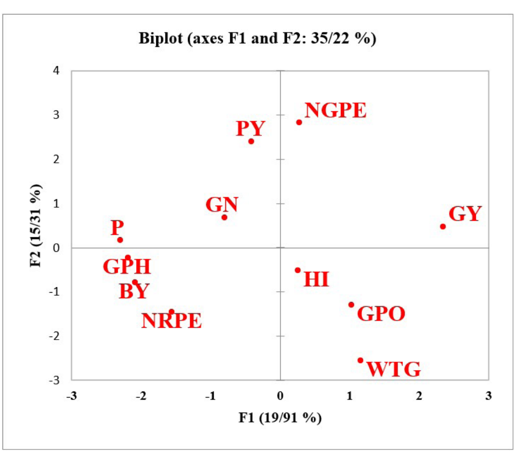

Figure 4. Investigating quantitative and qualitative traits evaluated in terms of the first and second main components. (NRPE: Number of rows per ear, NGPE: Number of grain per ear, WTG: 1000-grain weight, GY: Grain yield, BY: Biological yield, HI: Harvest index, GPH: Grain phosphorus, GPO: Grain potassium, GN: Grain nitrogen, P: Protein, PY: Protein yield).

Eigenvalue diagram (Figure 3), the first 5 components accounted for more than 70% of the variance of the total data. 19.9% was related to the first component, 15.31% was related to the second component, 13.3% was related to the third component, more than 12% was related to the fourth component and 9.75% was related to the fifth component. In the comparison graph of traits in terms of the first and second main components, Number of grain per ear and Grain yield traits had positive coefficients in terms of the first and second components. Harvest index, Grain potassium and 1000- grain weight traits had positive coefficients in terms of the first component and negative coefficients in terms of the second component. Protein yield, Grain nitrogen and Protein traits also had negative coefficients in terms of the first component and positive coefficients in terms of the second component. Finally, Grain phosphorus, Biological yield and Number of rows per ear traits had negative coefficients in terms of the first and second components (Figure 4).

4. Conclusion

In general, the results showed that mycorrhiza and foliar application of salicylic acid increase the indicators of growth, yield and yield components. It also improved the quality traits of maize plant. Based on the obtained results, the combination of G. mosseae treatment and foliar application of salicylic acid had better results than other treatments and this combination is recommended for the study area.

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