ARBUSCULAR MYCORRHIZAL ROOT COLONIZATION OF TWO SPECIES FROM THE GENUS *PLANTAGO*

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Abstract

The percentage of arbuscular mycorrhizal root colonization was assessed for two species of *Plantago*: *Plantago lanceolata* and *Plantago major*, sampled from different locations. Ten samples were analyzed for each species by using the Trypan Blue staining technique. The study was intended to estimate the level of arbusculat mycorrhizal colonization (AM) of the two species of *Plantago* and compare the results obtained. The percentage of mycorrhizal colonization was on an average of 27.74% for *Plantago major* and 40.17% for *Plantago lanceolata*, the difference between the two species being of 12.43%. Trypan Blue staining provided a good contrast, fine mycorrhizal structures (hyphae and arbuscules) being emphasized on the microscope. The analysis of variance (ANOVA) shows that the means are significant for data distributions in the two species of *Plantago*. Kurtosis indicator had different values, the average distribution of the species being platikurtic for *Plantago lanceolata* (low variability in the data string) and leptokurtic for *Plantago majo*r (high variability).

Key words: arbuscular mycorrhiza, Trypan Blue, *Plantago lanceolata*, *Plantago major*, ANOVA

The study on the percentage of arbuscular mycorrhizal root colonization (AM) was performed on *Plantago lanceolata* L. and *Plantago major* L. (*Plantaginaceae* family), perennial, herbaceous plants, adapted to various climatic and soil conditions. They grow on all types of soil, meadows, roadsides, pastures and hayfields.

Even since 1918 it was reported that *Plantago* species are heavily colonized by arbuscular mycorrhizas. According to Gange and West (1994), plantain records low growths when roots are non-mychorrizal.

Symbiotic associations between plants and arbuscular mycorrhizas (AM) have beneficial effects on plants, ensuring proper absorption of nutrients, especially of phosphorus (Sharma, J. et al., 2007; Deacon, J. W., 2006. Thus, plants with mycorrhizas can reduce the amount of phosphorus in the soil, given that agriculture is a source of environmental pollution with phosphates (**Ruiz –** Lozano, J. M. et al., 1995). Also, mycorrhizas contribute to the increased resistance of plants to droughts, pathogens and insects attack (Phillips, J. M., Hayman, D. S., 1970; Borowicz, V. A., 2001; Gehring, C. A., Whitham, T. G., 2002; Ayres, R. L., et al., 2006).

Allen (1991) highlights the role of mycorrhizas in ecology and in stimulating plant growth. Mycorrhiza benefits cannot be neglected in terms of resource conservation in agriculture, because mycorrhizal plants absorb more nutrients and soil or substrate water.

As Hedlund states (2002), arbuscular mycorrhizal colonization of *Plantago lanceolata* roots is higher, in normal conditions, on abandoned lands compared to experimental fields.

Nicoleta Ianovici (2009), in her studies on the *Plantago* species (considered the pioneer in Romania), shows that mycorrhizal colonization is stronger in polluted areas. Intensity of mycorrhizal colonization in fragments of roots showed higher values for Zlatna (61.29%), Rizei Valley (53.78%) and Timişoara (39.29%).

The main objective of this study is to estimate the percentage of arbuscular mycorrhizal colonization of *Plantago lanceolata* and *P. major* roots, collected from an abandoned pasture and a park near Timişoara. Mycorrhizas were highlighted by Trypan Blue staining and the extent of colonization was determined by the gridline intersection method (Ianovici, Nicoleta, 2009; Giovannetti, M., Mosse, B., 1980).

MATERIAL AND METHOD

The percentage of root colonization with mycorrhizas was evaluated in two species of plantain, *Plantago major* and *Plantago lanceolata*. *Plantago lanceolata* was harvested from Timişoara Airport park, while *Plantago major* from an

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abandoned pasture belonging to Giarmata Vii – Timişoara village.

The plants root system was cut into segments of 2.5 cm. Thus, one sample was made of 40 segments, comprising 1 m of roots. 10 samples for each species of plantain were analyzed.

In order to detect and determine the percentage of root colonization, samples were processed, passing through the following stages: soil washing, cutting into segments of 2.5 cm, hot cleaning with KOH 10% (1 minute) and immersion into a solution of HCL 10% (15 minutes), staining with 0.2% Trypan Blue solution, lactic acid and glycerin (24 hours).

The percentage of root colonization was determined by the gridline intersection procedure (Giovannetti, M., Mosse, B., 1980), where roots are randomly dispersed into a 9 cm diameter Petri dish with gridlines. Samples were analyzed under the stereomicroscope by quantifying the intersections between lines and roots (horizontal and vertical), which either appear to be colonized (colored) or non-mycorrhizal (uncolored).

Colonized root segments were examined under a microscope, revealing the presence of hyphae and arbuscules into the cortical tissue.

Statistical analysis (ANOVA) was performed using the SPSS program.

RESULTS AND DISCUSSIONS

The percentage of root mycorrhizal colonization can be determined by different root cleaning and staining techniques, to highlight the structure of mycorrhizal fungi. The method used in this study for quantifying the colonized roots of *Plantago lanceolata* and *Plantago major* species is one of the most common in mycorrhizal research and involves the use of Trypan Blue staining the mycorrhizas. Trypan Blue staining technique was first described by Phillips and Hayman in 1970 (Gange, A. C. et al., 1999). Later on, this method proved to be the most used one, despite the fact that this type of dye is considered carcinogenic and we have to be particularly cautious when using it. Trypan Blue is suitable for assessing mycorrhizal colonization (Brundrett, M. C., et al., 1984; Brundrett, M. C., et al., 1996). According to Grace and Stribley (1991), 68% of researchers used Trypan Blue for root staining procedures.

Roots of the two plantain species considered for this study were cut into segments of 2.5 cm (1 sample = 1 m of roots). To obtain correct results, 10 root samples were analyzed for *Plantago lanceolata* and 10 for *Plantago major*. Samples thus prepared were thoroughly cleaned in a solution of KOH 10% for 1 minute. After cleaning, they were washed and filtered in a solution of HCL 10%, for 15 minutes. The next step consisted in washing the roots and transferring them in a lacto-glycerol solution to be stained (distilled water, lactic acid, glycerin – 1:1:1) and Trypan Blue (0.2%), where they remained for 24 hours. Good contrast was obtained, thus allowing the reading of samples under the stereomicroscope.

Quantification of mycorrhizal roots was done by the gridline intersection method (Giovannetti, M., Mosse, B., 1980). The same authors recommend the use of minimum 100 intersections to assess a sample.

After staining, roots were washed and read under binocular magnifier. Each sample was randomly dispersed in a 9 cm diameters Petri dish with gridlines on the backside. The level of mycorrhizal colonization was determined by quantifying the intersections between lines and roots (horizontal and vertical). Intersections considered to be colonized were colored while the non-mycorrhizal ones were uncolored.

The percentage of root colonization for *Plantago major* roots fluctuated between 16.77% and 53.65%, with a mean of 27.74%, while for *Plantago lanceolata* roots, colonization was between 32.82% and 52.65% with a mean of 40.17% (*table 1*). The results obtained are in agreement with Nicoleta Ianovici (2009), who shows in her studies on *Plantago* species that the percentage of mycorrhizal colonization in Timişoara area is of 39.29%.

Table 1

The extent of mycorrhizal colonization of *Plantago major* and *Plantago lanceolata* roots

Sample	Roots colonized by mycorrhizas (%)	
	Plantago major	*Plantago lanceolata*
1	53.65	52.08
2	26.87	32.82
3	28.76	37.75
4	23.40	36.20
5	21.23	35.29
6	28.77	42.37
7	16.77	44.44
8	28.94	37.43
9	29.97	42.55
10	21.08	40.77
Mean	**27.74**	**40.17**

Some root samples were analyzed under microscope, where the presence of mycorrhizal fungi structures (hyphae and arbuscules) could be noticed in both *Plantago lanceolata* and *Plantago major* roots.

Research results were statistically analyzed using SPSS. Indicators of central tendency, asymmetry and vaulting were calculated (*table 2).* For mean analysis, different levels of significance were used (confidence intervals): 0.05 (95%); 0.01 (1%) and 0.1 (10%).

In the case of *Plantago lanceolata*, the mean value is of 40.1700, with a deviation of ±5.55685 (*figure 1*). The given distribution is left-asymmetric – *Skewness = 0.956* and platikurtic – *Kurtosis = 1.231*. Platikurtic distribution shows that variability is low in the string of data because they are distributed throughout the variation field from minimum to maximum.

The mean for *Plantago major* is of 27.7440 with a deviation of ±10.00884 (*figure 2*). The given distribution is more left-asymmetric – *Skewness = 2.135* and leptokurtic – *Kurtosis = 5.862*. Leptokurtic distribution shows that variability in the string of data is higher and low and high values are concentrated in the middle.

Table 2

Indicators of central tendency

	N	Minimum	Maximum	Mean	Standard deviation	Variance	Skewness	Kurtosis
Plantago lanceolata	10	32.82	52.08	40.1700	5.55685	30.879	0.956	1.231
Plantago major	10	16.77	53.65	27.7440	10.00884	100.177	2.135	5.862

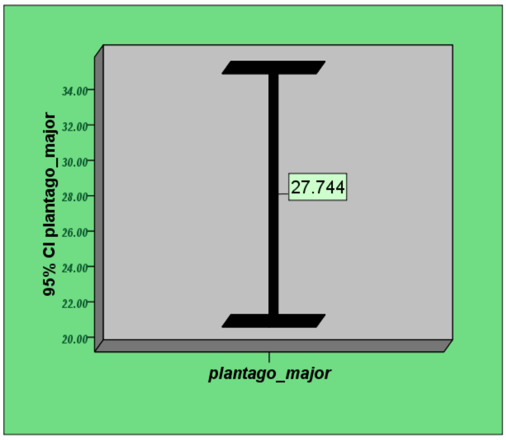

Figure 1 **Average value and screening interval for the species *Plantago major***

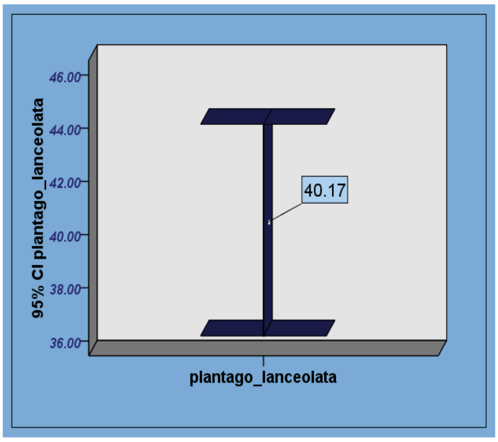

Figure 2 **Average value and screening interval for the species *Plantago lanceolata***

Table 3

Testing the distribution of the mean for the species *Plantago lanceolata* and *Plantago major*

	Mean = 27.74						
Plantago major	t			Mean Difference	95% Confidence Interval of the Difference		Tabular values $t_{0,05; 9}$
					Lower	Higher	
	8,766			27,74400	20,5841	34,9039	2,262
					90% Confidence Interval of the Difference		Tabular values $t_{0,1; 9}$
					21,9421	33,5459	3,25
					99% Confidence Interval of the Difference		Tabular values $t_{0,01; 9}$
					17,4580	38,0300	1,833
	Media = 40.17						
Plantago lanceolata	22,860			40,17000	95% Confidence Interval of the Difference		Tabular values $t_{0,05; 9}$
					36.1949	44.1451	2,262
					90% Confidence Interval of the Difference		Tabular values $t_{0,1; 9}$
					36.9488	43.3912	3,25
					99% Confidence Interval of the Difference		Tabular values $t_{0,01; 9}$
					34.4593	45.8807	1,833

Student – t test was used to determine the distribution of the mean. Distribution the mean for *Plantago major* species is of 27.74. The calculated value of the *Student* test is t_{calc}=8.766 (*table 3*). Levels of significance (confidence intervals of the mean with the level of significance) used for mean analysis were: 0.05 (95%), 0.01 (1%) and 0.1 (10%). The relationship $t_{calc} \succ t_{tab}$ is recorded for all levels of significance. This allows us to conclude that the mean calculated is of 27.74, significant for the given distribution.

Distribution of the mean in the case of *Plantago lanceolata* is 40.17. The calculated value of the *Student* test is t_{calc} = 22.86 (*table 3*). As for *Plantago major*, the calculated mean is significant for the given distribution.

Study results show that there is a difference of 12.43% between the calculated means of the two *Plantago* species regarding their degree of mycorrhizal colonization.

Trypan Blue staining offered a good contrast and mycorrhizal fungi structures were well emphasized under the microscope. Kurtosis indicator has different values, the distribution of the mean being platikurtic for *Plantago lanceolata* and leptokurtic for *Plantago major* (*table 2*).

Confidence intervals of the mean with levels of significance show that the calculated means are significant for the given distributions, at both *Plantago* species.

CONCLUSIONS

Results obtained indicate a mycorrhizal colonization of *Plantago major* L. and *Plantago lanceolata* L. roots, between 27.74% and 40.17%, with a 12.43% difference between the species. It can be noted that the degree of colonization for *Plantago lanceolata* species, collected from a park was higher compared to *P. major,* collected from an abandoned pasture. The recorded difference is due to the fact that plants come from sites with a different biodiversity.

Trypan Blue staining technique allowed a better visualization of mycorrhizal structures (hyphae and arbuscules).

ANOVA (analysis of variance), shows that the calculated means are significant for the given distributions, and indicators of central tendency (Skewness and Kurtosis) show a low variability in the string of data for *P. lanceolata* and a high variability for *P. major.*

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