MYCORRHIZAL NETWORKS IN A FERTILIZED WINTER WHEAT CROP

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Abstract

The simultaneous evolution of superior plants with mycorrhizian fungi has created effective and productive symbiotic systems. The enlarged transfer interface between the two partners and the hypal networks increases the plants resistance to stress factors and its effectiveness of exploiting the nutritive reserves. During the vegetation period, once the colonization and surface of radicular system decreased, phosphorus depletion may take place, leading to a deficiency instalment of this element. Mycorrhizal hyphal networks and symbiotic interface provides a realistic image of the nutritional efficiency of plants. The aim of this study is to asses the impact of mineral fertilization with NPK at the end of the vegetation period of winter wheat. A balanced colonization of the radicular system is stimulated by applying moderated doses, but for the development of a strong arbuscular circuit, it is preferred to apply quantities of maximum 100 kg/ha phosphorus in the autumn and at the end of winter. Mineral fertilization maintain the wheat's degree of colonization at a 20% level.

Key words: winter wheat, hyphal networks, fertilization, arbuscular circuit.

The simultaneous evolution of superior plants with mycorrhizian fungi has created effective and productive symbiotic systems, able to absorb and translocate mineral elements outside of the area directly accessible by the roots of plants (Smith, S.E., Read, D.J., 2010). The hyphal networks developed especially by the vesicular-arbuscular fungi are capable to mobilize the phosphorus from the soil and transfer it to the cellular level through the arbuscules formed in the radicular cortex (Dörmann *et al*, 2014, Hodge A., Storer K., 2014). The enlarged transfer interface between the two partners and the hypal networks increases the plants resistance to stress factors and its effectiveness of exploiting the nutritive reserves of the soil (Koltai H., Kapulnik Y., 2010). A high percentage of current agricultural systems make use of mineral fertilizers, their type and application partially supressing the mycorrhizian mechanism's ability to manifest and may decrease the colonization level (Alguacil *et al*, 2010; Wu *et al*, 2011). Thus, in radicular areas with high assimilation potential, the abundance of mineral elements reduces the extent of the radicular system, concomitantly inhibiting the need to develop symbiotic interfaces (Mao *et al*, 2014). During the vegetation period, once the colonization and surface of radicular system decreased, phosphorus depletion may take place, leading to a deficiency instalment of this element (Rai *et al*, 2013). Analysing the extensions of mycorrhizian hyphal networks and symbiotic interface provides a realistic image of the nutritional efficiency of plants, and also a series of parameters necessary for the prognosis techniques of final development for the cultivated species.

MATERIAL AND METHOD

The purpose of the current study is to asses the impact of mineral fertilization with NPK, applied at sowing (Na, Pa) and supplemented by nitrogen based fertilizers at the beginning of spring season (Nf1, Pf1, Kf1) and phasial – on vegetation (Nf2, Pf2), on the extent of mycorrhized hyphal network at the end of the vegetation period of winter wheat (Triticum aestivum). The collection of roots was performed from an experimental field with 6 fertilization variants (*table 1*), located in the Turda area, Cluj County, Romania, on a phaeosiom argic type of soil.

The percentage parameters of the mycorrhizial colonization were calculated according the formulas proposed by Trouvelot et al. (1986, quoted by Stoian H.V., 2011), frequency (freq), colonization intensity in the radicular system (int sys) and in root segments (int seg), arbuscules percentage in the radicular system (arb sys) and in mycorrhized segments (arb seg), completed with colonization degree (col deg - Stoian H.V., 2011). Data analysis was performed with the R Studio software (Team R., 2014). For highlighting the mycorrhiza's reaction to fertilization Tukey.HSD test was used from the "agricolae" package (de Mendiburu F., 2014). The tests were completed by calculating the Pearson's correlation between the colonization and fertilization parameters

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Table 1

Fertilization variants (kg/ha)

Variant	Total fertilization (Nt,Pt,Kt)	Autumn fertilization (Na,Pa)	Spring fertilization (Nf1, Pf1,Kf1)	Phasial fertilization (Nf2, Pf2)
G1	N120P120K48	N80P80K0	N40P40K48	N0P0
G2	N120P100K0	N80P80K0	N40P20K0	N0P0
G3	N120P80K0	N80P80K0	N40P0K0	N0P0
G4	N120P70K36	N40P40K0	N30P30K36	N50P0
G5	N120P95K0	N40P40K0	N30P30K0	N50P25
G6	N120P70K0	N40P40K0	N30P30K0	N50P0

RESULTS AND DISCUSSION

Separation based on periods of fertilization emphasizes the distinguishing influence of each mineral element on the mycorrhizal colonization parameters (*table 2*). The frequency is negatively correlated, but not significantly, with the application of complex fertilization networks in the autumn and beginning of spring seasons. However, this parameter is positively correlated with the nitrogen applied to vegetation and significantly positive with Phosphorus, in the same era of application.

The colonization intensity, for both the radicular system and the mycorrhized segments, is significantly positively correlated with the nitrogen applied during autumn or at the beginning of the spring, respectively to the phosphorus when applied during the autumn (*table 2*). This aspect indicates the yielding of abundant hyphal networks, with a role in absorption and stocking, especially of phosphorus from the soil's solution. At the other end, reduced quantities of phosphorus applied also on vegetation at the beginning of spring, along with the nitrogen applied on vegetation, are negatively correlated to the two parameters.

Table 2

Correlation of colonization frequency and intensity with applied fertilizers

	freq	int sys	int seg
Pt	-0.13	-0.22	-0.17
Kt	-0.06	-0.11	-0.14
Na	-0.32	0.57*	0.64**
Pa	-0.32	0.57*	0.64**
Nf1	-0.32	0.57*	0.64**
Pf1	-0.15	-0.80***	-0.79***
Kf1	-0.06	-0.11	-0.14
Nf2	0.32	-0.57*	-0.64**
Pf2	0.63**	-0.57*	-0.65**

Note: marked values significant at p<0.05; p<0.01**; p<0.001****

The colonization frequency of the radicular system is strongly fluctuating due to the applied fertilizers (*table 3*). The maximum value of this parameter (96.77%) is obtained for fertilization with N120P95 doses (G5), to which all the variants have recorded significantly negative differences. This aspect is owed to the continuous application of phosphorus in the fertilization networks in all vegetation stages, in equal amounts with nitrogen (autumn and beginning of spring). The differences between the experimental variants remain high, due to the potassium's insertion in the fertilization recipe or applying the entire phosphorus quantity during the autumn season.

Compared to the colonization frequency, the expansion of mycorrhizian hypae in the cortex, measured through the mycorrhization intensity in the system and in the segments, is oscillating in low limits (*table 3*). Standing out is the variant for which the total amount of phosphorus is applied, during autumn, with colonization intensity of 47.59% for the radicular system (51.17% for root segments), with significantly differences unto all the other tested variants. Applying the phosphorus all at once stimulates the development of a consistent mycorrhizal hyphal network, with a function in absorbing and stocking this element, preventing thus the occurrence of nutritional

unbalances. Only one experimental variant recorded the colonization intensity for the radicular cortex with a 17% value (G5). This phenomenon is explained by the high colonization frequency, owed to the much faster growth of the wheat's roots, in comparison with the colonization speed of the mycorrhized hypae. The other variants (G1, G2, G4 and G6) are maintained in a 25 – 28 % interval for the colonization intensity of systems (29-35% intensity in segments), with significantly differences compared to the minimum (G5) and maximum (G3) values obtained in the analysed experiment.

Table 3

Differences in colonization frequency and intensity due to fertilization'

freq			int sys			int seg		
variant	mean	significance	variant	mean	significance	variant	mean	significance
G1	86.21	cd	G1	27.17	b	G1	30.24	b
G2	75.84	e	G2	27.91	b	G2	35.25	b
G3	93.31	b	G3	47.59	a	G3	51.17	a
G4	87.30	c	G4	27.83	b	G4	30.00	b
G5	96.77	a	G5	17.01	c	G5	17.03	c
G6	84.02	d	G6	25.84	b	G6	29.46	b

Note: Different letters between parameters denote significant differences (Tukey HSD test, p < 0.05)

Table 4

Correlation of arbuscularity parameters and colonization degree with applied fertilizers'

	arb seg	arb sys	col deg
Pt	-0.42	-0.51*	-0.25
Kt	-0.10	-0.13	-0.11
Na	-0.35	0.10	0.48*
Pa	-0.35	0.10	0.48*
Nf1	-0.35	0.10	0.48*
Pf1	-0.12	-0.63**	-0.80***
Kf1	-0.10	-0.13	-0.11
Nf2	0.35	-0.10	-0.48*
Pf2	0.11	-0.34	-0.43

Note: marked values significant at p<0.05; p<0.01**; p<0.001****

Following the trend noticed for the colonization's frequency, the fertilizations applied in the autumn and beginning of spring periods reduce, non-significantly, the arbuscular percentage (arb seg) from the mycorrhized segments of the radicular cortex (*table 4*). At the complete radicular system level, the arbuscular percentage (arb sys) is negatively significantly correlated with the total amount of phosphorus and distinctive significantly with phosphorus the applied at the beginning of the spring. The correlations indicate the low potential of applying the fertilization in multiple phases, for establishing a strong arbuscular circuit.

The degree of colonization is highly negatively significantly to the phosphorus applied at spring's beginning, and negatively significant to the nitrogen applied to vegetation (*table 4*). An equal correlation to the nitrogen applied beginning of spring, but positively in this case, is signalled between the degree of colonization and nitrogen applied in the first two vegetation stages, under the aspect of influence by phosphorus from the beginning of the spring. The transfer of the soil solution directly into the radicular cortex's cells establishes the arbuscular circuit. The mycorrhized segments of the roots have a strongly oscillating arbuscular load among the experimental variants (*table 5*). The maximum value is obtained at G4 (35.57%), when applying phosphorus and nitrogen in equal quantities in the first two vegetation stages, respectively supplementing the fertilization network with potassium at the beginning of spring season. All the variants have recorded significantly differences to this variant, with the exception of G2 variant. The weakest experimental variants (G1, G3 and G6) recorded values under 20% for arbuscules in the mycorrhized root segments. This

phenomenon is owed to the investment of mycorrhizal fungi in the development of a strong intra-radicular hypal network, against the penetration of cortical cells and establishing an arbuscular circuit.

Table 5

Differences in arbuscularity parameters and colonization degree due to fertilization'

arb seg			arb sys			col deg		
variant	mean	significance	variant	mean	significance	variant	mean	significance
G1	10.32	e	G1	2.81	b	G1	23.43	b
G2	28.75	ab	G2	8.00	a	G2	21.18	bc
G3	18.89	cd	G3	8.99	a	G3	44.40	a
G4	35.57	a	G4	9.79	a	G4	24.34	b
G5	24.55	bc	G5	4.17	b	G5	16.46	c
G6	16.08	de	G6	4.15	b	G6	21.72	bc

Note: Different letters between parameters denote significant differences (Tukey HSD test, p < 0.05)

At the complete radicular cortex level, the arbuscular circuit values were recorded in the interval 2 – 10% (Table 5). The separation between the experimental variants is given by reaching the 8% value for arbuscules in the entire radicular system. In this mean, the G2, G3 and G4 variants recording significantly differences unto the other three experimental variants.

Established as a synthetic indicator, the degree of colonization plays the role of normalizing the differences between the components frequency – intensity of colonization in the radicular system (Table 5). The only variant with over 40% degree of colonization is G3, due to the high values of frequency and intensity. Compared to this variant, all the other variants have recorded negatively significantly differences. The degree of colonization for the other five experimental variants is oscillating in the 15 – 25% interval, with only one variant (G5) recording values under 20% for this specific indicator.

CONCLUSIONS

The balanced colonization of the radicular system is stimulated by applying moderated doses of mineral fertilizers during the vegetation period.

For the development of a strong arbuscular circuit, it is preferred to apply quantities of maximum 100 kg/ha phosphorus in the autumn and beginning of spring seasons.

For mineral fertilization cases, the wheat's degree of colonization is ranked at a 20% level, with growths determined by applying phasial nitrogen.

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