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BIOFORTIFICATION AND SHOOT: ROOT RATIO IN WHEAT SEEDLINGS UNDER THE INFLUENCE OF CERTAIN MINERAL ELEMENTS

Florin SALA[1], Hamzeh RAWASHDEH[1,2], Marius BOLDEA[1]

[1]Banat University of Agricultural Sciences and Veterinary Medicine, "Regele Mihai I al României", Timisoara, Soil Science and Plant Nutrition Department, Calea Aradului 119, 300645, Timișoara, Romania, Phone +40256 277280, Fax: +40256 200296, Email: florin_sala@usab-tm.ro, marius_boldea@usab-tm.ro
[2]Water Management and Environment Research Department, National Center for Agricultural Research and Extension, P.O. Box 639, Baqa 19381, Jordan; hamz_rawashdeh@yahoo.com

Corresponding author email: florin_sala@usab-tm.ro

Abstract

Researching the way to strike a balance for the shoot : root ratio is beneficial for the normal growth and development of wheat plants even from the early stages of vegetation. The aim of this research was to study the influence of some mineral elements, with a structural and nutritional value, on shoot and root biomass and on the shoot : root ratio in wheat seedlings. The fertilizer used for providing the mineral elements was Lithovit; this is a product based on calcium carbonate and magnesium, with a balanced content of nutrients (Ca, Mg, Fe, Si, K, Na, P and Mn). The biological material was represented by Alex wheat cultivar, species Triticum aestivum L. ssp vulgare. The mineral elements were applied on the shoots when the wheat seedlings were in development stage 12-13 BBCH, through three concentrations of Lithovit (0.35%, 0.7% and 1%). One variant was treated with water, thus it became the control variant, for reference. The treatments applied had a different influence on the quantity of fresh and dry biomass at root level and at shoot level; the results presented statistical significance ($p < 0.01$; Fcrit <<F, for Alfa = 0.001, ANOVA test). The values of the shoot : root ratio for fresh weight (S/R_{Fw}) ranged from 1.006 to 1.015 in the treated variants, while for the control variant the result was 0.970. As for the dry weight (S/R_{Dw}), the results yielded by the treated variants were between 1.010 and 1.033, and the result for the control was 0.977.

Key words: *balanced growth, dry weight, fresh weight, shoot : root ratio, mineral elements, wheat.*

INTRODUCTION

The critical nutrition stages in straw cereals are associated with the first stages of vegetation (10 - 20 BBCH code stage), the deficit of nutrients in these stages being reflected afterwards in the vegetation state of crops and in the quality and quantity of yields (Mengel and Kirkby 2001).
The root and shoot systems of wheat have been studied in different stages of vegetation, in order to assess the nutrition and vegetation state of plants, plant state in relation with soil works, plants – nutrient relation (Erenoglu et al., 2010), plant tolerance to temperature and water stress (Essemine et al., 2010; Wasson et al., 2012), plant tolerance to salinity (Bahrani and Joo 2012; Tammam et al., 2008).
In the study of the foliar and root systems, or of plants in their entirety, different parameters and indices have been used, of different types: morpho-physiological (Bi et al., 2011; Rapaport et al., 2014), biochemical (Moayedi et al., 2011), enzymatic (Amirjani and Mahdiyeh 2013), genetic (El Sidding et al., 2013; Rashidi 2011) for the characterization of the momentary state of wheat plants under the influence of specific factors or of the relations of plants with different bioactive components in the environment.
Nutrients in the soil or in applied fertilizers play a major role in plant development; their influence on the root and foliar systems in wheat is impacted by a series of factors (De Giorgio and Fornaro 2012; Ehdaie et al., 2010; Pedersen et al., 2010; Raza et al., 2013).
Nutrients have different, specific contributions to balanced formation and growth of the root and foliar systems. A well-developed root system, starting with the early stages of

vegetation, will explore a larger volume of soil to provide water and nutrients to plants.
The balanced formation and development of the plant highly depends on the main macronutrients (N, P, K) as well as on elements with a structural role, Ca and Si (Ali et al., 2009; White and Broadley 2003), which contribute to the formation of the resistance structure at cell and tissue level. Micronutrients are associated with different enzymatic systems, and they also take specific action towards the development of the root system or the foliar system (Anongo et al., 2014).
The present study focused on the influence of certain mineral elements with a structural and nutritive role on wheat plants. The aim of this study was to harness the accumulation of dry and fresh matter and their balance ratio in the root system and in the foliar system on wheat seedlings.

MATERIALS AND METHODS

The research was carried out at the Faculty of Agriculture within Banat University of Agricultural Sciences and Veterinary Medicine from Timisoara, Romania, in the years 2013-2014. The study included an investigation of the influence of certain mineral elements with nutritive and structural roles on the content and ratio of fresh matter and dry matter at shoot and root level in wheat seedlings, for harnessing their growth and development.
The experiments were organized in laboratory conditions, in germinators with controlled conditions. Each variant was isolated, in order to make the best out of the applied treatments, with no external influences. The biological material was represented by Alex wheat cultivar, species *Triticum aestivum* L. ssp. *vulgare*, with specially selected seeds for ensuring uniformity to the seed material. Twenty seeds were used in each treatment, the average weight of seeds per variant being 1.094 ± 0.007 g.
A natural product based on calcium and manganese carbonate was used to provide the mineral elements; this belongs to group Lithovit, and has the following composition: 75% $CaCO_3$, 4% $MgCO_3$, 0.5% Fe, 5% Si, 0.1% K_2O, 0.015% Na, 0.015% P_2O_5, 0.01% Mn. There was a control variant (V_1) and other three variants with different Lithovit concentrations (0.35% - V_2, 0.7% - V_3 and 1% - V_4).
The treatments, in the form of a solution, were applied by spraying on the shoots of wheat seedlings when these were in growth stage 12-13 BBCH. Immediately after treatment, the variants were isolated and were placed individually under a hand glass, in order to avoid potential outside influences and to observe the contribution of each treatment to the realization of the parameters under study.
In order to consider the effects of elements with nutritional and structural roles on the biofortification of wheat seedlings, some parameters were determined on the dry and fresh weight at root level (R_{Fw} - root fresh weight, R_{Dw} - root dry weight) and at shoot level (S_{Fw} - shoot fresh weight, S_{Dw} - shoot dry weight). In addition, the balance ratio was determined between foliar and root mass in what dry and fresh weight were concerned (S/R_{Fw} - shoot : root fresh weight, S/R_{Dw} - shoot : root dry weight). The determinations were made with Axis scales, with an accuracy of ±0.001 g.
The experimental data on the root and foliar systems in each variant were processed through analysis of variance (ANOVA) using the mathematical module in EXCEL 2007. Correlation coefficients, regressions and Principal Component Analysis (PCA) were determined using the software PAST (Hammer et al., 2001). The symbols *, ** and *** used in the paper represent statistical significance at 99.9% ($LSD_{0.01\%}$), 99% ($LSD_{0.1\%}$) and 95% ($LSD_{0.5\%}$) probability level.

RESULTS AND DISCUSSIONS

Various concentrations of minerals applied on wheat seedlings in 12-13 BBCH code stage growth had different influences on dry and fresh matter accumulation rate in the root system and in the foliar system of wheat seedlings.
At root level, the quantity of fresh weight accumulated by seedlings ranged from 1.918±0.087* g in variant V_4 (Lithovit 1%) and 1.955±0.021* g in variant V_2 (Lithovit 0.35%), while in the control variant the measurements showed 1.760±0.027 g. The dry weight in the root system was 0.199±0.004 g in variant V_4 and 0.227±0.002*** g in variant V_2, while the

control variant gave the results 0.196±0.002 g, Table 1.

At the level of the foliar system of wheat seedlings, fresh weight varied between 1.699±0.059 g in the control variant and 2.028±0.036** g in variant V_2 (Lithovit 0.35%), while, for the same variants, dry weight presented values between 0.191±0.003 g and 0.235±0.006*** g, Table 1.

According to ANOVA statistical analysis, the experimental results presented high statistical significance, p< 0.01, $F_{calculated} > F_{theoretical}$ for Alfa = 0.001 (Table 2).

Both in the root and shoot systems, small concentrations of minerals among those tested via Lithovit determined better growth rhythm and better accumulation of fresh and dry matter, although in all treated variants the values recorded were higher than those in the control variant.

Table 1. Variation in the fresh and dry matter contents under the influence of Lithovit

Variant	Lithovit Concentration	Root		Shoot	
		R_{Fw}	R_{Dw}	S_{Fw}	S_{Dw}
	(%)	Average values (g) and SE			
V_1 (Mt)	0	1.760±0.027	0.196±0.002	1.699±0.059	0.191±0.003
V_2	0.35	1.955±0.021*	0.227±0.002***	2.028±0.036**	0.235±0.006***
V_3	0.7	1.933±0.043*	0.220±0.001***	1.945±0.029**	0.223±0.002**
V_4	1	1.918±0.087	0.199±0.004	1.917±0.031*	0.201±0.005
Limits of significance for the differences (LSD)		$LSD_{5\%}$ = 0.162 $LSD_{1\%}$ = 0.245 $LSD_{0.01\%}$ = 0.394	$LSD_{1\%}$ = 0.005 $LSD_{1\%}$ = 0.007 $LSD_{0.1\%}$ = 0.011	$LSD_{5\%}$ = 0.158 $LSD_{1\%}$ = 0.240 $LSD_{0.1\%}$ = 0.385	$LSD_{5\%}$ = 0.017 $LSD_{1\%}$ = 0.026 $LSD_{0.01\%}$ = 0.041

R_{Fw} – root fresh matter; R_{Dw} – root dry matter; S_{Fw} – shoot fresh matter; S_{Dw} – shoot dry matter; SE – standard error

Table 2. Evaluation of variation source, ANOVA test

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	33.96301	5	6.792603	998.9	9.17E-61	4.692334
Within Groups	0.448352	66	0.006793			
Total	34.41137	71				

Alfa = 0.001

An interdependence relation was identified between the fresh and dry matter in the shoots of wheat seedlings, with a high degree of significance and statistical certainty (p<0.01; R^2 = 0.900), it is described by equation (1) with graphical representation in Figure 1.

$$S_{Dw} = 0.257x^2 - 0.8243x + 0.8472 \quad (1)$$

where:
S_{Dw} - Shoots dry weight on wheat seedlings;
x - Shoot fresh weight on wheat seedlings - S_{Fw}

In addition, an interdependence relation was identified between the dry weight content in the shoots and the dry weight content in the roots of wheat seedlings (p<0.01; R^2=0.874), equation (2).

$$S_{Dw} = 38.39x^2 - 14.95x + 1.651 \quad (2)$$

where:
S_{Dw} - Shoot dry weight on wheat seedlings;
x - Shoot dry weight on wheat seedlings - R_{Dm}

Dry weight distribution between shoot and root systems in wheat seedlings depends on a series of external factors which influence the growth medium of the two biological systems, as well as the plant as a whole.

Chapin et al. (1988) considered that the root : shoot ratio is often increased by soil factors which influence specific root activities, such as low water potentials and low availability of nutrients.

Changes in the root : shoot ratio are determined also by environmental factors, which increase specific shoot activity (i.e. enzymatic activity, photosynthetic rate) such as high light intensity (Mahall et al., 1981) or CO_2 concentration (Larigauderie et al., 1991).

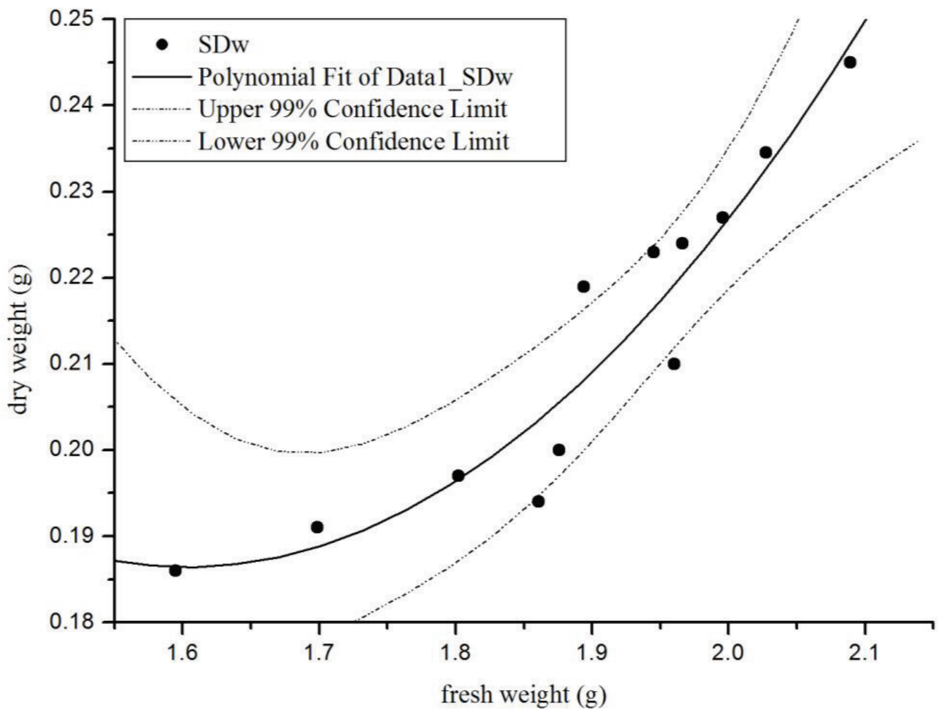

Figure 1. Particular relation between fresh and dry weight in the shoots of wheat seedlings under the influence of Lithovit

Based on studies and research in this direction, an empirical and mechanistic model was considered between shoot and roots (Wilson, 1988). In functional equilibrium models, it is proposed that dry matter is partitioned between shoot and roots to maintain a balance between root activity and shoot activity. It was presumed that the root : shoot equilibrium was maintained by redistributing the biomass between root and shoot rather than by a change in the intensity specific for these.

Based on other studies, another model was proposed, according to which dry matter partitioning between shoots and roots is regulated by amounts of carbon (resulting from shoot activity) and nitrogen (resulting from root activity) within the plant (Hilbert and Reynolds 1991). According to these models, surplus carbohydrates should favour biomass allocation towards the roots and surplus of nitrogen should favour biomass allocation towards the shoot.

Engels (1994) studied the influence of various thermal values on the root system and on the shoot : root ratio in wheat. He found a different reaction of the dry matter content and of the respective equilibrium ratio to the thermal factor. He drew the conclusion that dry matter partitioning between shoot and roots at low RZT (root zone temperature) is not causally related to the internal nitrogen or carbohydrate status of the plants.

Furthermore, balanced activity between shoot and roots is maintained by adaptations in specific shoot and root activity, rather than by an altered ratio of biomass allocation between shoot and roots.

After extensive research, Gaj (2010) noticed the influence of different potassium fertilization levels on the nutritional status of winter wheat and on yield during critical growth stage, highlighting the importance of a balanced state of nutrition of wheat plants, even in the early stages, for the normal evolution of the crop and for obtaining high yields.

Allard et al. (2013) found the genetic variability in biomass allocation to roots in wheat is mainly related to crop tilling dynamics and nitrogen status. Their results indicate that there is little prospect for breeding strategies specifically aiming at optimizing wheat root biomass allocation and N remobilization for improving NUE and GPC for elite genotypes at least in highly productive conditions.

Other studies (Ashagre et al., 2014) traced the influence of boron on seed germination and seedling growth in wheat (*Triticum aestivum* L.), observing the differentiated effect of boron in relation to the concentration on the parameters under analysis: germination percentage and rate, shoot and root lengths, shoot and root fresh and dry weights, root number, root - shoot ratio and seedling vigour index.

The favourable influence of boron and iron administered on the shoots in early growth parameters of wheat was shown also by Rawashdeh and Sala (2013), Rawashdeh et al., (2014).

Based on the correlations identified in this research between the root system and the shoot system in wheat seedlings, comparative analysis of experimental data highlighted a change in shoot : root ratio for fresh weight (S/R_{Fw}) and dry weight (S/R_{Dw}) under the influence of the treatment with Lithovit.

In the control variant, the shoot : root ratio was 0.961±0.020 g for fresh weight (S/R_{Fw}) and 0.979±0.006 g for dry weight (S/R_{Dw}). In the variants treated with Lithovit a balancing was observed in the shoot : root ratio, with values that ranged from 0.998±0.07 - 1.037±0.019 g for fresh weight (S/R_{Fw}) and 1.010±0.04 - 1.033±0.018 gfor dry weight (S/R_{Dw}), Table 3.

Table 3. Shoot : root ratio in wheat seedlings

Variant	Lithovit concentration (%)	S/R_{Fw}	S/R_{Dw}
V_1 (Mt)	0	0.961±0.020	0.979±0.006
V_2	0.35	1.037±0.019	1.033±0.018
V_3	0.7	1.008±0.037	1.014±0.013
V_4	1	0.998±0.07	1.010±0.04

S/R_{Fw} - shoot : root fresh weight; S/R_{Dw} - shoot : root dry weight

The research found improper shoot : root ratio (S/R) with low Lithovit concentrations. Between the values of the balance ratios shoot : root mass for fresh weight (S/R_{Fw}) and dry weight (S/R_{Dw}), positive correlation was identified, with a high degree of statistical significance and certainty ($p<0.01$; $R^2 = 0.915$), described by equation (3) with graphical representation in Figure 3.

$$S / R_{Dw} = 0.377x^2 - 0.1972x + 0.8268 \quad (3)$$

where:
S/R_{Dw} - shoots : roots dry weight;
x - shoots : roots fresh weight - S/R_{Fm}

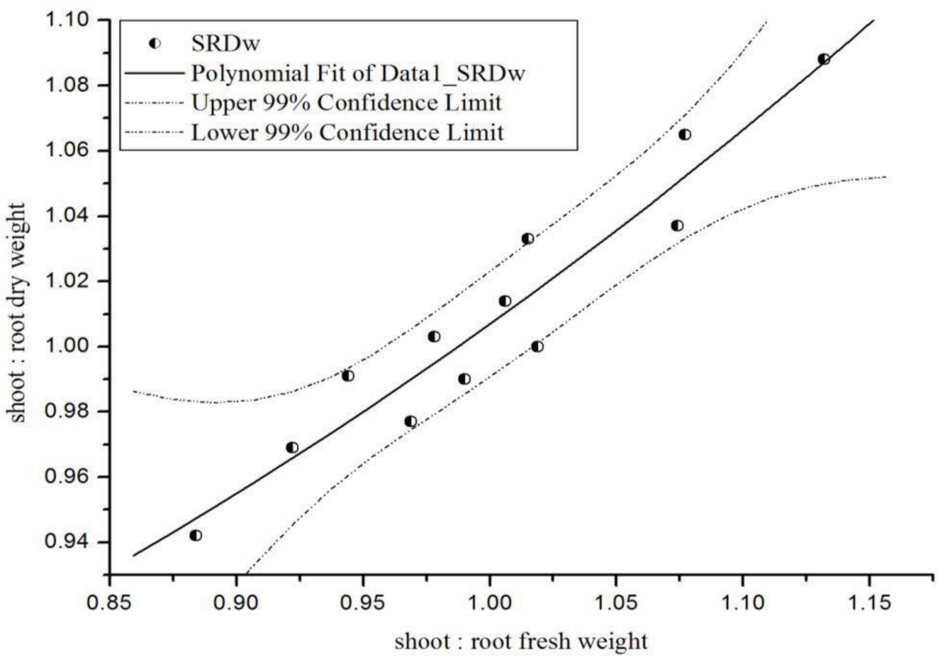

Figure 2. Distribution of the particular values on the shoot : root ratio for fresh weight (S/R_{Fw}) and dry weight (S/R_{Dw}) on wheat seedlings

Principal Component Analysis (PCA) of the experimental data facilitated the orientation and grouping of the variants in relation to the parameters analysed and the contribution to generating the variance.

With high statistical certainty (94.079% of variance in PC1 and 5.167 % of variance in PC2), experimental data were grouped and oriented based on their affinity for parameters that generated the variance, with distinct separation of variants V_2 and V_3 for dry weight in the root system (R_{Dw}) and shoot system (S_{Dw}), Figure 3.

Higher Lithovit concentrations influenced the fresh weight content, variant V_4 presenting affinity for this category of parameters. In the context of this research, the results generated by the control variant placed this variant in an independent position, with the smallest values for the parameters under analysis.

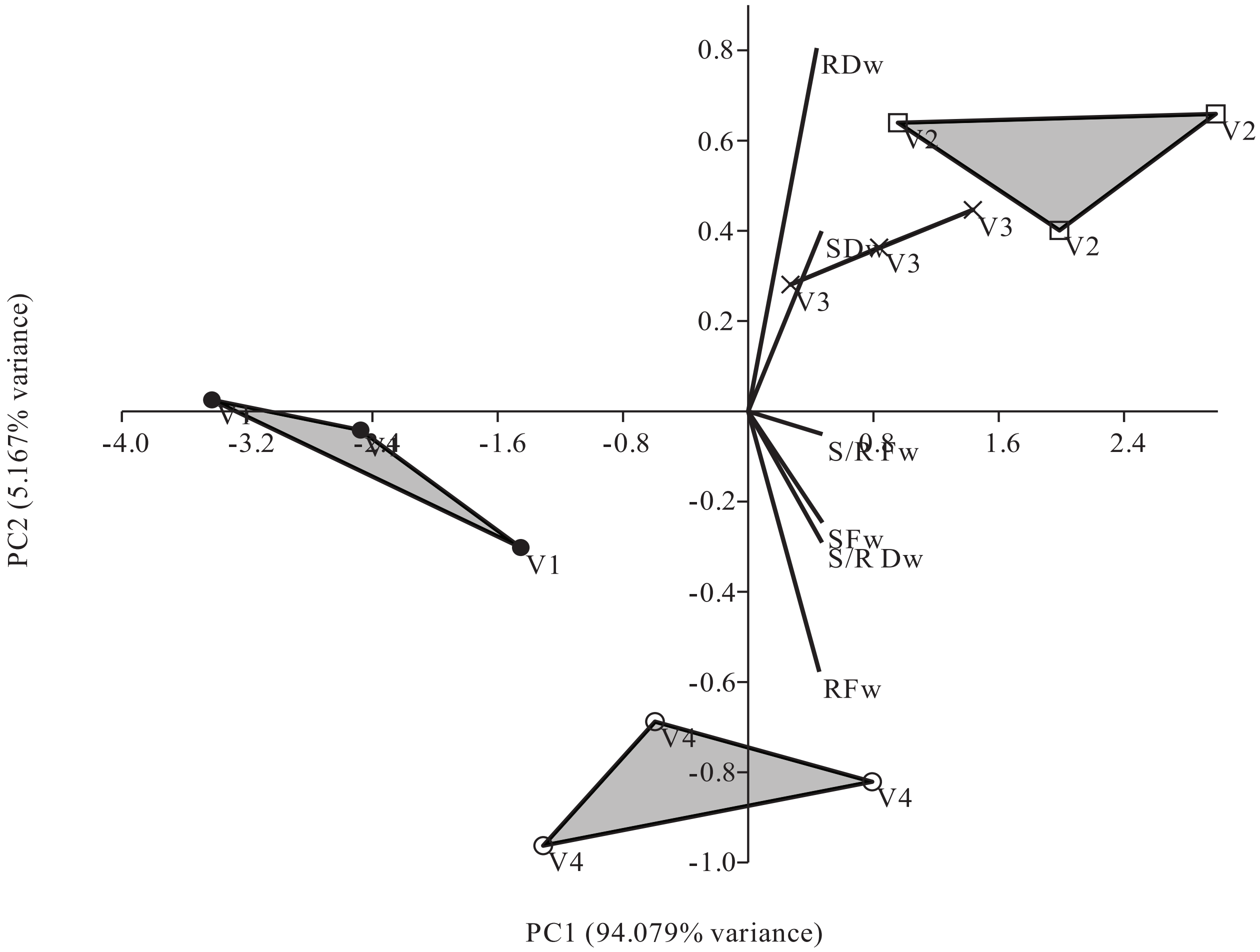

Figure 3. Variance within group through PCA analysis and orientation and grouping of trial variants depending on the parameters measured (R_{Fw} – root fresh weight; R_{Dw} – root dry weight; S_{Fw} – shoot fresh weight; S_{Dw} – shoot dry weight; S/R_{Fw} – shoot : root fresh weight; S/R_{Dw} – shoot : root dry weight)

CONCLUSIONS

The intake of minerals with a nutritive and structural role provided through Lithovit by foliar application on wheat seedlings influenced the synthesis and accumulation of fresh matter and dry matter in wheat shoots and roots. Rebalancing of the shoot : root ratio was recorded for fresh weight (S/R_{Fw}) and dry weight (S/R_{Dw}). The low concentrations among those tested (0.35% - 0.5%) had a more positive effect on the indices regarding fresh and dry weight in wheat shoots and roots. Based on PCA analysis, it was possible to evaluate the source of variance in the experimental results, low concentrations of Lithovit being associated to dry weight in shoots (S_{Dw}) and roots (R_{Dw}) and the S/R_{Dw} ratio, while the higher concentrations were associated with fresh weight.

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