AGRONOMY SCIENCE AND BIOTECHNOLOGY

RESEARCH ARTICLE

Multivariate explanation of the establishment of soybean initial growth pattern via biostimulant seed treatment

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ABSTRACT

Given the search for answers that improve soybean plant development, this study aimed to analyze the multivariate explanation about the establishment of the initial soybean growth pattern through seed treatment. The study was conducted at Luiz Eduardo de Oliveira Sales Experimental Farm, in the municipality of Mineiros-GO. The soil of the experimental area was classified as NEOSSOLO Quartzarenico. The experimental design was randomized blocks in factorial 10x5 corresponding to 10 soybean genotypes (Flecha, Bonus, TEC7548, M7739, 36B31, W791, M7198, M6210, Power and 48B32) and 5 seed treatments (Water, Sprint-Alga, Booster, Acorda and Stimulate), in 4 repetitions. The obtained data was submitted to the assumptions of the statistical model, verifying the normality and homogeneity of the residual variances, as well as the additivity of the model. Afterwards, the analysis of variance was performed in order to identify the interaction between soybean genotypes x seed treatment, applying uni and multivariate tests. The summary analysis of variance revealed significant interaction between cultivar x seed treatment. The seed treatment influenced the morphological components of soybean seedlings, showing their correlation with the fresh aerial and root mass, as well as different patterns that were observed according to the genetic variation.

Keywords: correlations, *Glycine Max*, canonical variables, genetic dissimilarity, UPGMA, Artificial Neural Networks.

Citation: Ferreira, L. L., Resende, J. M., Carvalho, I. C., Carnevale, A. B., Fernandes, M. S., Santos, N. S. C., Batista, P, F., Pereira, A. I. A., Curvêlo, C. R. S., Amaral, U., Silva, R. V., & Loro, M. V. (2022). Multivariate explanation of the establishment of soybean initial growth pattern via biostimulant seed treatment. *Agronomy Science and Biotechnology*, 8, 1-11. https://doi.org/10.33158/ASB.r161.v8.2022

Received: December 22, 2021.
Accepted: February 26, 2022.
Published: April 12, 2022.

English by: Luiz Leonardo Ferreira.

INTRODUCTION

Soybean is most grown major crop in planted area in the country, and is currently consolidated as one of the most prominent products of Brazilian agribusiness (Zago *et al*., 2018). As the development of soybean has become important for the Brazilian economy, this sector is being one of the main pillars of the the national economy.

The Brazilian territorial area corresponds to approximately 851.95 million hectares. The potential for national arable land is estimated at 152.5 million hectares, or 17.9% of the national territory. However, 62.5 million hectares are being used for agriculture, in which the soybean cultivation area represents 38 million hectares, the largest among the crops planted in the country (Empresa Brasileira de Pesquisa Agropecuária (Embrapa, 2018). In the 2020/2021 crop year, Brazil had an area of 38 milion hectares of soybean planting, and production of 135 milion tons.

According to Kryzanowshi et al. (2008) seed quality is defined as a series of genetic, physical, physiological and sanitary attributes that interfere with the ability to produce a uniform crop, consisting of representative vigorous plants and free from invasive or undesirable plants. Seed quality can be affected by several factors during the production process, as well as by the interaction between genotypes x environments (Carvalho et al., 2015). An example of this factor would be sowing under adverse environmental conditions such as inadequate temperatures, soil water deficit related to low quality seeds used in sowing.

The use of quality seeds and the use of products that improve their field performance are important elements for high agricultural production. In this case, biostimulants are components that favor plant growth and can act alone or in combination, interfering with various physiological and / or morphological processes, such as germination, vegetative growth, flowering, fruiting, senescence and abscission. In beans, Ferreira et al. (2021) showed that the use of foliar biostimulants enhances characteristics such as number of pods, seed weight and grain yield.

According to Binsfeld et al. (2014), soybean seed treatment with fungicides has been widely adopted allows the germination of infected seeds, control seed-borne pathogens and protect seeds from soil fungi, enabling greater potential for early crop development and initial stand establishment at reduced costs.

The use of biostimulants increases the components of plant development, even though there are few studies addressing the physiological characteristics of soybean related to the application of these products. Seed application may give rise to plants that are more vigorous, longer lengths, increased dry matter, and percentage of emergence in sand and soil adjusted for increased product doses in various crops (Marques *et al*., 2014). Kolling et al. (2016) points out that the results reported in the literature regarding the use of biostimulants indicate that the responses to the application of these products depend on the genotype used, the composition of humic substances present in the products, and the environmental conditions (Ferreira et al. (2019) and Silva et al. (2020).

Given the search for answers that improve soybean plant development, the objective of this study was to analyze the multivariate explanation about the establishment of the initial soybean growth pattern via seed treatment with biostimulants.

MATERIAL AND METHODS

The study was conducted at Luiz Eduardo de Oliveira Sales Experimental Farm, in the municipality of Mineiros-GO, located between the geographic coordinates of 17°34'10 '' South latitude and 52°33'04 '' West longitude, with an average altitude of 760 m. The average temperature is 22.7°C, the average annual rainfall is 1695 mm occurring mostly in spring and summer. The experimental area is classified as Aw (hot dry) climate. The soil of the experimental area was classified NEOSSOLO Quartzarenic, with medium texture, gently undulating to flat topography and good drainage (Embrapa, 2013).

Prior to the installation of the experiment, soil analysis was performed in the 0-20 cm layer and the following characteristics were observed: hydrogen potential 5.7; calcium 3, magnesium 0.8, aluminum 0.2, hydrogen + aluminum 2, cation exchange capacity 5.9, in $cmol_c.dm^3$; potassium 53, phosphorus 59, sulfur 1.7, boron 0.2, copper 1.4, iron 51, manganese 23, zinc 8.3, sodium 1.5, in mg dm^{-3}; clay 223, silt 50, sand 728, organic matter 20 and organic carbon 12, in g dm^{-3}. Data was taken according to Embrapa's methodology (2009).

The experimental design was randomized blocks in factorial 10x5 corresponding to 10 soybean genotypes (Arrow, Bonus, TEC7548, M7739, 36B31, W791, M7198, M6210, Power and 48B32) and 5 seed treatments (Water, Sprint-Alga, Booster, Acorda and Stimulate), in 4 repetitions, totaling 50 treatments and 200 experimental units. Each plot was sized with five rows spaced 0.5 m apart with and 1 m long. The main morphoagronomic characteristics of soybean genotypes are described in Table 1.

Table 1. Main morphoagronomic traits of soybean genotypes. Mineiros-GO, UNIFIMES, Brazil, 2020.

Cultivar		Weight of a Thousand seeds (g)	Genetics	Maturation group	Architecture	Cycle (days after emergency)
Commercial	Common					
Bonus 8579 RSF IPRO	Bonus	190	Bramax	7.9	Undetermined	105 a 122
TEC7548 IPRO	TEC7548	180	Bayer	7.5	Undetermined	116 a 124
Flecha 6266RSF IPRO	Flecha	190	Bramax	6,6	Undetermined	95 a 105
M 7739 IPRO	M7739	175	Monsoy	7.7	Undetermined	105 a 115
CZ 36B31 IPRO	36B31	170	Bayer	6.6	Undetermined	105 a 112
W 791 RR	W791	185	Bayer	7.7	Undetermined	105 a 120
M7198 IPRO	M7198	175	Monsoy	6.8	Undetermined	99 a 107
BMX Power IPRO	Power	170	Bramax	7.3	Undetermined	105 a 115
M6210 IPRO	M6210	168	Monsoy	7.2	Undetermined	105 a 115
CZ 48B32 IPRO	48B32	140	Bayer	7.9	Determined	115 a 120

The tillage system was carried out with harrowing and plowing of the area on

11/23/2017. The seeds were treated one day before sowing in polyethylene bags, with the recommended label doses of each product (Table 2). Sowing was performed on 12/08/2017, manually distributing 15 seeds per meter in the furrow (300.000 plants ha^{-1}). The cultural treatments pertinent to weed control were performed whenever necessary. The main characteristics of the products used as seed treatments were described in Table 2.

Table 2. Main characteristics of the products used as seed treatment. Mineiros-GO, UNIFIMES, Brazil, 2020.

Nomenclature		Active Ing.	Dose (ml or g ha^{-1})
Technic	Initial		
Water	AGU	Distilled water H_2O	800
Sprint-Alga	SPR	Seaweed Extracts	40
Booster	BOO	Citric Acid + Potassium Hydroxide	500
Acorda	ACO	Organic carbon	500
Stimulate	STI	Cytokine + Gibberellin + Indolalcanoic Acid	600

For the measures were taken on 20 seedlings per experimental unit and the following data was recorded: stand (STD) in units per linear meter, by counting vigorous plants; (COR) root length in cm, stem diameter (DIC) in cm, hippocotyl height (ALH) in cm, height of epicotylus (ALE) in cm and plant height (ALP) in cm, using a tape measure; aerial fresh matter (MFA) in g, and root fresh matter (MFR) in g.

The obtained data was submitted to the assumptions of the statistical model, verifying the normality and homogeneity of the residual variances, as well as the additivity of the model. Afterwards, the analysis of variance was performed in order to identify the interaction between soybean genotypes x seed treatment. Subsequently, the variables were subjected to linear correlation in order to understand the tendency of association, and their significance was based on 5% probability. The canonical correlations were estimated between group 1 (MFA and MFR) and group 2 (STD, COR, DIC, ALH, ALE and ALP), with significance between the character groups evaluated based on chi-square statistics. After genetic dissimilarity by the Mahalanobis algorithm where the residual matrix was weighted, after the phylogenetic tree of the distances was constructed through the UPGMA grouping, the biplot canonical variables method was used to visualize the general variability. of the experiment and multivariate trends. Character matrix data were subjected to unsupervised computational learning through Artificial Neural Networks using the K-means and Kohonen Map algorithms (Carvalho, 2018). The analyzes were performed in Rbio R interface (Bhering, 2017), in addition to Software Genes (Cruz, 2016).

RESULTS AND DISCUSSION

The summary analysis of variance with the mean square QM and significance by the F test revealed significant interaction between G genotypes x TS seed treatment, in the variables STD stand, DIC stem diameter, height of epicotyl ALE, fresh air mass MFA and root fresh mass MFR ($p < 0.01$) and the variables of root length COR and height of the hypocotyl ALH ($p < 0.05$) (Table 3). Results found corroborate Carvalho et al. (2015), Toledo *et al*. (2015) and Tatto *et al*. (2018).

Table 3. Summary of analysis of variance (QM and CV (%)) for STD stand, COR root length, DIC stem diameter, ALH hypocotyl height, ALE epicotyl height, ALP plant height, MFA fresh air mass and mass fresh root MFR of G soybean genotype seedlings, submitted to different treatments of TS seeds. Mineiros-GO, UNIFIMES, Brazil, 2020.

Factors	GL	STD	COR	DIC	ALH	ALE	ALP	MFA	MFR
G x TS	36	260.16^{**}	7.64^{*}	0.00^{**}	0.09^{*}	0.41^{**}	12.98^{ns}	14.87^{**}	0.27^{**}
G	9	969.79^{**}	13.18^{**}	0.00^{**}	0.36^{**}	4.42^{**}	185.49^{**}	12.89^{**}	0.38^{**}
TS	4	456.05^{**}	3.63^{ns}	0.00^{ns}	0.05^{ns}	0.76^{**}	10.21^{ns}	3.10^{ns}	0.40^{*}
Blocks	3	101.55^{ns}	111.29^{**}	0.07^{**}	0.65^{**}	0.10^{ns}	38.97^{**}	38.64^{**}	0.63^{**}
Residue	147	106.32	5.00	0.00	0.06	0.13	9.51	2.62	0.13
CV (%)	-	23.30	11.02	8.81	12.21	11.89	9.09	16.44	15.49

** significant at 1% probability by F test; * significant at 5% probability by F test; nsignificant at 5% probability by F test.

Understanding the magnitude of existing associations between agronomic traits is important to help obtain improved genotypes that meet market expectations of high performance (Nardino et al., 2016). Pearson's correlation coefficients arranged in the correlation networks revealed 7 positive correlations between soybean variables, among the different seed treatments. Correlations were diagnosed in pairs (DICxMFA) in the AGU; (ALPxALE and MFAxMFR) in SPR; (DICxMFA) for BOO; (MFAxALP) present in ACO; and (ALExALP and ALPxMFA) in TS STI (Figure 1). The use of correlation networks can increase the effectiveness of selection in soybean breeding, once it allows us to quickly identify the pairs of traits that present higher magnitude correlations; and determine which groups of variables most significantly influence the most important characters for the breeding program and identify the correlated variable groups.

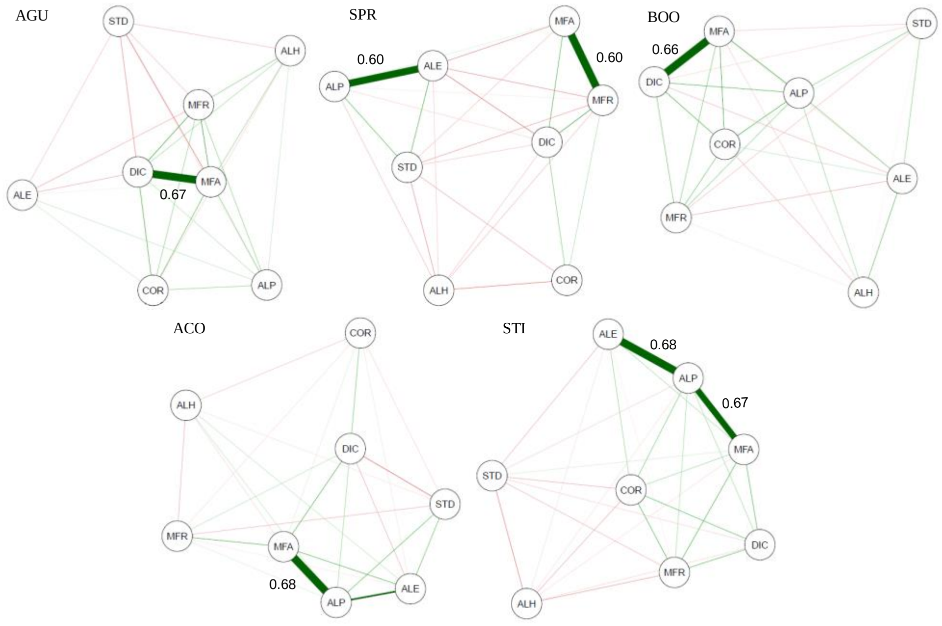

Figure 1. Simple linear correlation applied to STD stand, COR root length, DIC stem diameter, ALH hypocotyl height, ALE epicotyl height, ALP plant height, MFR root fresh mass and MFA aerial fresh mass of soybean genotype seedlings of soybean genotypes submitted to different seed treatments. Mineiros-GO, UNIFIMES, Brazil, 2020.

According to the results obtained in the canonical correlation analysis, it was observed the formation of two significant canonical pairs ($p \leq 0.01$), by the chi-square test for each seed treatment, with high canonical correlation ($r \geq 0.99$), corroborating with Carvalho *et al*. (2015), where the high magnitude of the canonical correlation coefficients shows high dependence between the two trait groups. The information was described taking into consideration only the first canonical pair in the AGU, SPR and BOO seed treatments. However, the performance of the variables in OAC and STI were described with the two canonical pairs (Table 4).

Table 4. Canonical loads of fresh mass (group 1) and morphological (group 2) traits in the canonical correlations (r) between soybean genotype groups submitted to different seed treatments. Mineiros-GO, UNIFIMES, Brazil, 2020.

Character [1]	Canonical pair		Canonical pair		Canonical pair		Canonical pair		Canonical pair	
Group 1	1º	2º	1º	2º	1º	2º	1º	2º	1º	2º
	AGU		SPR		BOO		ACO		STI	
MFR	0.59	0.21	0.63	-0.10	0.47	0.19	-0.17	-0.34	-0.32	-0.53
MFA	0.73	-0.09	0.53	0.20	0.78	-0.06	-0.81	-0.10	-0.82	-0.04
Group 1	1º	2º	1º	2º	1º	2º	1º	2º	1º	2º
STD	-0.42	0.51	-0.33	0.22	-0.20	-0.24	-0.10	0.58	-0.14	0.40
COR	0.53	-0.21	0.22	-0.54	0.71	0.10	-0.13	0.25	-0.24	-0.71
DIC	0.92	0.11	0.92	-0.14	0.83	-0.09	-0.55	-0.19	-0.55	-0.49
ALH	0.38	0.35	-0.17	0.41	-0.13	0.05	0.03	0.56	0.07	0.41
ALE	-0.03	-0.80	-0.47	-0.22	-0.14	-0.70	-0.61	0.44	-0.26	0.22
ALP	0.43	0.08	-0.01	0.41	0.68	-0.25	-0.86	0.18	-0.82	0.09
r	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
p	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Group 1: MFA aerial fresh mass and MFR root fresh mass; Group 2: DIC stem diameter, ALH hypocotyl height, ALE epicotyl height, ALP seedling height and CRA root length.

It was observed that the increase of MFR and MFA variables in soybean seedlings were explained by the increase in COR and DIC for all seed treatments in question, as well as for ALT except SPR, following a trend for all reduction in STD. In other words, it has been described that seedlings with higher COR, DIC and ALT and low seedling population contribute to the increase in MFR and MFA (Table 4).

The interaction sequence between the 10 soybean genotypes and 5 seed treatments were arranged for the phylogenetic tree construction identifying the formation of 7 significant clusters with emphasis on I (48B32 Stimulate and M7739 Booster), IV (36B31 Stimulate and Bonus Stimulate), V (48B32 Wake), VI (48B32 Booster) and VII (48B32 SprintAlga) (Figure 2). According to Cruz et al. (2004), the establishment of groups containing genotypes with homogeneity within and heterogeneity across groups is the starting point for a more thorough evaluation of them, in order to make their use in the breeding programs. The 48B38 genotype had its performance highly influenced by seed treatment management, being present in 5 distinct and possible groups. Its sensitivity and expressiveness contributed to the formation of 3 clusters (V, VI, and VII). STI and BOO participated in 4 distinct groups each, showing potential change in the performance of the soybean genotypes under study, opening new possibilities for further studies with these active ingredients for further work (Figure 2).

The canonical axes add up to a total explanation equivalent to 67.9, 76.8, 73.2, 76.9 and 77.3% of the total data variation in the AGU, SPR, BOO, ACO and STI seed treatments, respectively. The traits that most influenced the genotype distinction among seed treatments were MFA and DIC (Figure 3).

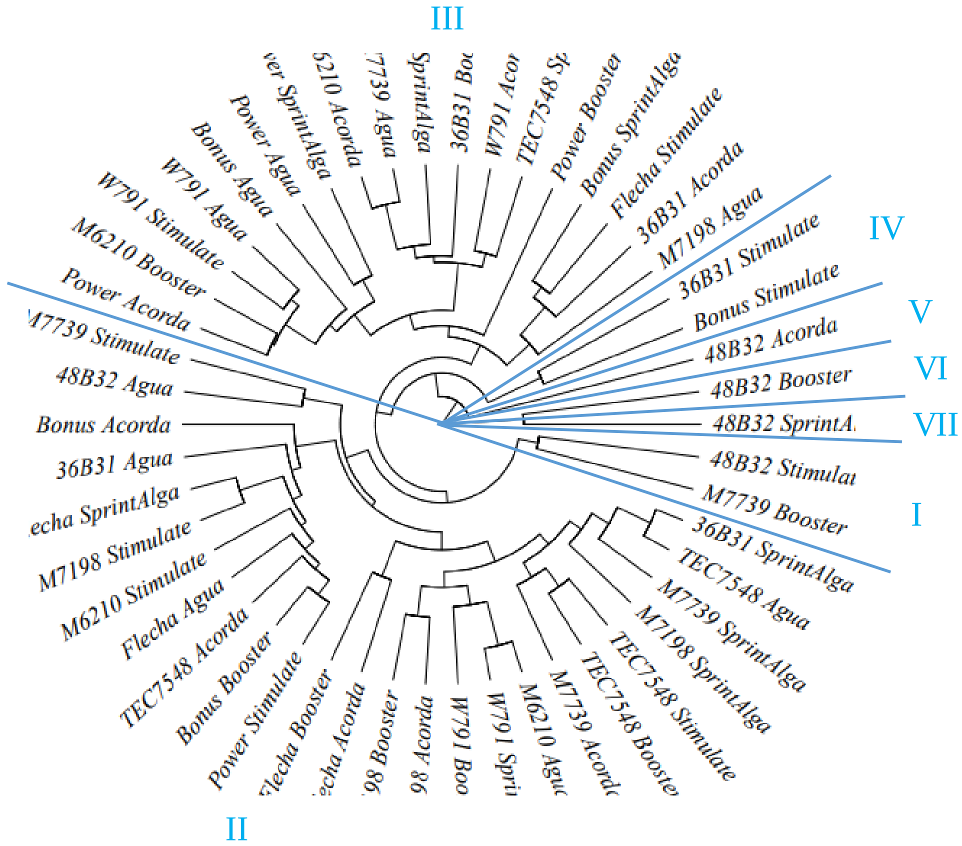

Figure 2. Phylogenetic tree of dissimilarity of soybean genotypes submitted to different seed treatments, obtained by UPGMA clustering method, using the generalized Mahalanobis distance. Mineiros-GO, UNIFIMES, Brazil, 2020.

Similarities were addressed between ALP, MFA, MFR, ALH and DIC, as well as for MFA, MFR and DIC in the treatments of AGU and SPR seeds, respectively, which were positively expressive in both canonical variables in the Flecha genotype. The Bonus genotype presented high means in MFA, MFR and DIC traits in BOO seed treatment, as well as the TEC7548 genotype for MFA, ALP and DIC traits (Figure 3). Silva *et al*. (2015) stated that multivariate analysis techniques are efficient to verify similarities or differences in yield variability based on chemical and physical soil attributes in study.

For this study, the Kohonen Map was employed using 50 inputs (neurons) represented by soybean cultivar interaction and seed treatment. The phenotypic matrix was submitted to interactive procedures that defined a neural network with nine centroids topologies, establishing associative patterns among the tested characters (Figure 4). The standards set by the traits (Centroid I) UL-CR and UL-FO, (Centroid II) BK-CR, BO-ST and EX-FO, (Centroid II) EX-ST, BO-FO, BK-ST and BO-FI, (Centroid IV) BK-FI, (Centroide V) UL-FI, EX-CR and UL-AG, (Centroide VI) BK-AG and EX-AG, (Centroide VII) BO-AG, BK -FO and EX-FI and (Centroide VIII) UL-ST and BO-CR (Figure 4).

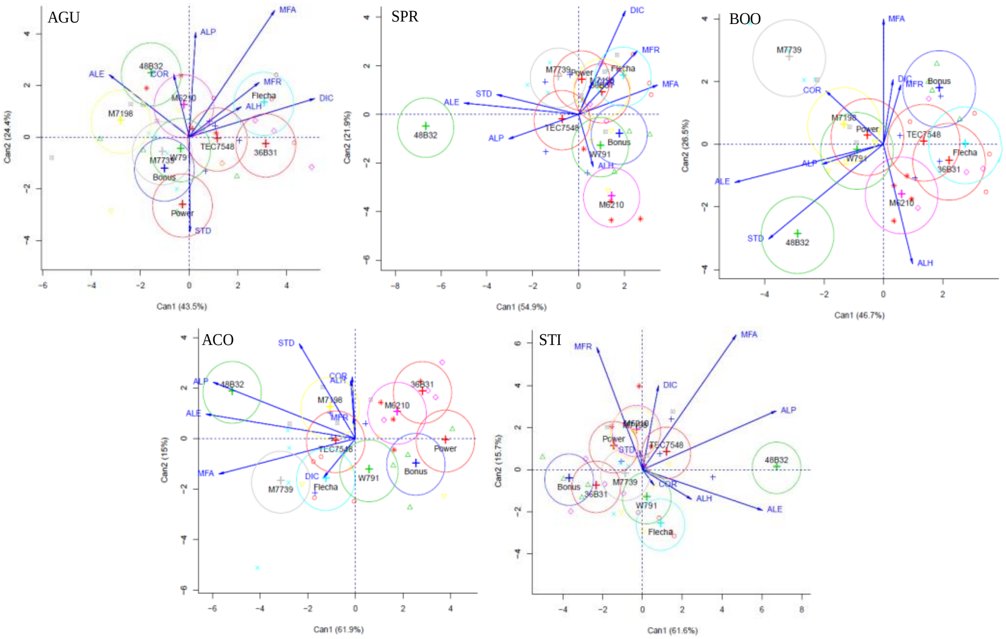

Figure 3. Analysis of canonical variables based on Mahalanobis distances for STD stand, COR root length, DIC stem diameter, ALH hypocotyl height, ALE epicotyl height, ALP plant height, MFR root fresh mass; and fresh aerial mass MFA of soybean genotype seedlings of soybean genotypes submitted to different seed treatments. Mineiros-GO, UNIFIMES, Brazil, 2020.

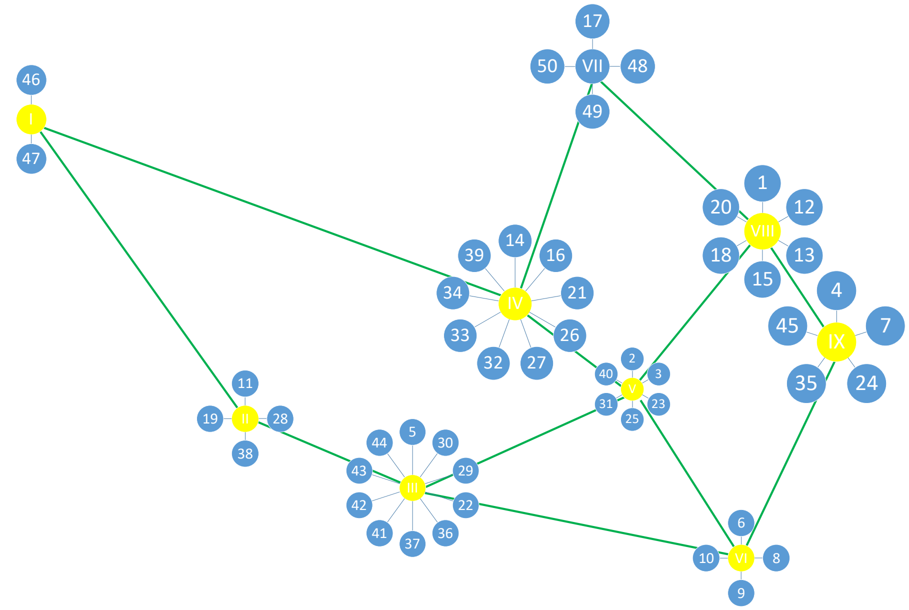

Figure 4. Artificial Neural Networks obtained by Kohonen Map defining centroids (blue) and neurons (yellow) and synaptic links (blue lines), as follows: 1-Flecha_APR, 2-Flecha_BOO, 3-Flecha_ACO, 4-Flecha_AGU, 5-Flecha_STI, 6-Bonus_APR, 7-Bonus_BOO, 8-Bonus_ACO, 9-Bonus_AGU, 10-Bonus_STI, 11-TEC7548_APR, 12-TEC7548_BOO, 13-TEC7548_ACO, 14-TEC7548_AGU, 15-TEC7548_STI, 16-M7739_APR, 17-M7739_BOO, 18-M7739_ACO, 19-M7739_AGU, 20-M7739_STI, 21-36B31_APR, 22-36B31_BOO, 23-36B31_ACO, 24-36B31_AGU, 25-36B31_STI, 26-W791_APR, 27-W791_BOO, 28-W791_ACO, 29-W791_AGU, 30-W791_STI, 31-M7198_APR, 32-M7198_BOO, 33-M7198_ACO, 34-M7198_AGU, 35-M7198_STI, 36-

M6210_APR, 37-M6210_BOO, 38-M6210_ACO, 39-M6210_AGU, 40-M6210_STI, 41-Power_APR, 42-Power_BOO, 43-Power_ACO, 44-Power_AGU, 45-Power_STI, 46-48B32_APR, 47-48B32_BOO, 48-48B32_ACO, 49-48B32_AGU e 50-48B32_STI. Mineiros-GO, UNIFIMES, Brazil, 2020.

CONCLUSIONS

Multivariate analyzes efficiently express the correlations between variables and interactions on the establishment of initial soybean growth pattern via seed treatment with biostimulants.

The seed treatment influenced the morphological components of soybean seedlings, showing their correlation with the fresh aerial and root mass, and different patterns were observed according to the genetic variation.

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