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TESTING THE INFLUENCE OF THE ENVIRONMENTALLY FRIENDLY PHYTOHORMONE GIBB A3, UPON MAIZE MACRO- AND MICROELEMENTAL CONTENT

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Abstract

Ourdays, the identification of friendly solutions in order to enhance physiological development of cereals is one of the main priorities of agriculture. This approach, also contributes to the promotion of the practices of the sustainable agriculture. A practice, which occupies more and more space is the use of endogenous type substances, as phytohormones, in order to increase cereals' physiological development, and nutritional traits. This is the context where the aim of our study is placed, meaning that we focused on determining the effects of GIBB A3 phytohormone on two maize hybrids content in macro-, and microelements, within specific environmental conditions of Transylvania. Two maize hybrids were studied, Turda Star, and Turda 200, respectively, and their elemental content was quantified by AAS, and raw data were processed with IBM SPSS Version 20. The results of our trial show the influence of GIBB A3 hormone upon the analyzed macro- (K, P, S, Cl, Mg, Ca), and microelemental (Fe, Mn, Zn) content of studied maize hybrids, mainly K, and P, which may also be considered as indicators of physiological status of the plant.

Key words: *AAS spectrometry, hybrid, indicator, physiology, statistics.*

INTRODUCTION

In time, it has been shown that plants need for their growth and development, besides exogenous factors (soil, water, mineral substances, temperature, light, etc.), also some substances, which result from internal metabolic mechanisms, and who are generically named growing substances, of phytohormones, growth stimulators or regulators (Kefeli and Kalevitch, 2013).

Starting from the concept, introduced since 1910 by Fitting, we must underline that phytohormones or growing regulators are organic compounds, which, in low, and very low concentrations, stimulate, inhibit, or qualitatively modify the plant growing and development, also playing role in stress regulation (Iqbal et al., 2014; Pandey et al., 2017; Tripathi et al., 2017).

The physiological efficiency of a certain hormone depends on its concentration, and sensitivity of the cells. This sensitivity, is, on its turn, determined by the number of the receptor molecules, their affinity against the hormones, and efficiency of the transduction of the signal initiated by receptor - hormone complex.

New trends are reported in engineering phytohormones for enhancing abiotic stress in crops (Weyers and Paterson, 2001; Wani et al., 2016).

The phytohormones play an important role in control of plant growing ad development mechanisms, and, as consequence, plays an important role in reproduction processes (Lambers et al., 2008).

The most important classes of vegetal hormones described by literature, are: auxins, gibberellins, cytokines, ethylene, abscisic acid, jasmonate and brassinolides, which can be identified using a large diversity of methods (Durgbansi et al., 2005; Kazan, 2015; Liu et al., 2013; Mori et al., 2017; Yin et al., 2015).

According to literature, the vegetal hormones are classified as follows (Lambers et al., 2008; Liu et al., 2013; Rajkumar Biology: A guide for 11^{th}&12^{th} CBSE Students):

- primary hormones: auxins, gibberellins, cytokinins, ethylene, abscisic acid;

- secondary hormones: jasmonic acid and brassinosteroids, juglone, salicylic acid, polyamides;
- other hormones: peptide hormones, oligosaccharides, phospholipids, ARNm or protein; strigolactones.

The gibberellins are natural phytohormones, isoprene derivatives, widely spread in nature, which stimulated both division and elongation of the vegetal cells. Because of their important action upon plant growth and development, they have numerous practical uses in plant improvement, for obtaining new pant lines, hybrids, and varieties. They may also be used for intensive development of agriculture, horticulture, forestry, medicinal plants, etc. (Davière and Achard, 2013; Lambers et al., 2008).

The gibberelin was discovered in Japan, in 898, when Hotoaro Hori realized that excesive long rice plants were exposed to a *Gibberella fujikuroi* fungus. The fungus water extract produced similar symptoms in tested plants, and this led to the idea that fungus conains a substance responsible of this effect. The first identified gibberelin was the gibberelinic acid, or G3. This first discovery was fallowed by the discovery of other gibberellins, until in plants and fungi were identyied, in total, about fifty gibberellins. In practice, the used gibberellins are purifired products of synthesis. The most used gibberellin is G3, and less used are the mixture GA4+GA7 or GA7. The synthetic gibberellins were obtained in the '80s, and they are environmentally friendly solutions for plants development (Gupta and Chakrabarty, 2013; Hedden and Sponsel, 2015; Lambers et al., 2008).

The main alterations appeared in the metabolism of plants due to gibberellin actions are: the intensification of transpiration, and increase of the water consumption, the intensification of photosynthesis, the stimulation of the seed respiration during germination, the delay of the ageing process of the vegetal tissues, the correction of the negative effects produced by virosis and Botrytis, decrease of the starch content in plants and germinated seeds (Gupta and Chakrabarty, 2013; Hedden and Thomas, 2012; Hedden and Sponsel, 2015; Lambers et al., 2008; Zawaski and Busov, 2014).

Macro- and microelements content of plants, generally speaking, and of maize hybrids, in particular, play important role in enzymes activity, but may also be considered as real indicators of their physiology.

This study was carried out with the aim of identifying the action of an environmentally friendly solution of fertilization, with the GIBB A3 phytohormone on several macroelements (K, P, Ca, Mg, S, Cl) and microelements (Fe, Mn, Zn) from two maize hybrids reared in specific conditions of Transylvania.

MATERIALS AND METHODS

The study area

The trial was carried out in pedo-climatic conditions of Transylvania, meaning the experimental field of the Station of Research and Development for Agriculture Jucu (46º 51'18'' N and 23º 47' 35'' E), County of Cluj, during spring and summer of 2016.

The biological material

Two maize hybrids Turda Star and Turda 200 are used.

Turda Star is a semi-early triple hybrid, recorded in 1976, FAO group 370, with good resistance to low temperatures encountered in the first period of vegetation, and also to mechanical damages (falling, and/or frightening), draught, high temperatures, mites, and diseases. In 2000, it was introduced again in the Official Register. The grain is yellow, dentate, 310 g MMB, 79 – 92% ratio grain/cob, rich in starch, about 70 – 71%, and 10,000 – 12,800 kg/ha, yield potential.

Turda 200 is an early double hybrid, also recorded in 1976, and introduced again in the Official Register, in 2000. The plant is of moderate size, with 13 -15 leaves, semi-erected to inclined. The cob is of cylinder - conic shape, with moderate length, and rachis of intense red color, with 14 – 6 layers of grains. The grain is dentate, golden yellow, 260 – 33 g and MMB. The grain has the following chemical composition: 11.8 – 14.4% protein, 4 – 5.4% fat, and 67.7 – 70.3% starch.

The chemical and biological materials

The chemical materials used for seed treatment are the fungicides Royal Flo 42 S (Arysta) and Vitavax 200 (Chemtura). Royal Flo 42 S (active ingredient: Tiuram 480 g/L) was in doses of 3 L/t (http://www.agrovet1.ro/royal-flo-42-s-arysta/), and Vitavax 200 (active ingredients: Carboxin 200 g/L+ tiram 200 g/L), in doses of 2.5 L/t (http://www.pestcontrol-expert.ro/chemtura-vitavax-200-ff.html).

The GIBB A3 is a commercial product destined to fertilization. It is presented as pills, with 5g x 20% content in gibberellin acid, and 8 minutes time of solving in solution.

In order to obtain maximum product efficacy, the solutions must be applied on cultures in maximum time interval of 36 hours from preparation, otherwise the ”hardness” of used water will affect the quality of product.

The product administration on cool weather (in the morning or in the evening) is recommended. The administration when sun is strong must be avoided.

When in term of 8 hours from administration, strong rains are reported, the product action may be affected.

In this case, the administration of another treatment, with 50% concentration of previous, is recommended.

The GIBB A3 must be foliar administered in 2 - 3 treatments, as solutions in doses of 500 L/Ha by each treatment (www.giberelina.ro).

The methodology

Both cultures were sowed in 28.04.2016, with beans as pre-emergent culture.

Before sowing, herbicidation was performed with Glyphosate, in doses of 3L/Ha.

The seed used within our experiment, is delivered by the crops of the previous year, 2015.

The seed treatments against mites and diseases was performed with Royal Flo 42 S in Turda 200, and with Vitavax 200 in Turda Star. The treatments with GIBB A3 was performed in two phases

- First treatment was applied in phase with 3 – 4 leaves (after two weeks from sowing, 13.05.2015), in doses of 0.5 g/20 L water.
- The second treatment was applied after two weeks from the first treatment, in doses of 1 g/20 L water.

Four groups are organized, as follows:

- Control group 1 (Turda Star hybrid, no phytohormone treatment),
- Experimental group 1 (Turda Star hybrid treatment with phytohormone GIBB 3A),
- Control group 2 (Turda 200 hybrid, no phytohormone treatment),
- Experimental group 2 (Turda 200 hybrid treatment with phytohormone GIBB 3A).

The laboratory analysis

The maize leaves samples were conditioned and analyzed in the Laboratory of the Control of the Environmental Quality and Plant Protection, from the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. The macro- (K, P, Ca, Mg, S, Cl), and microelemental (Fe, Mn, Zn) content of foliar tissue of maize samples was quantified by AAS spectrometry with graphite furnace.

The statistical analysis

The software package IBM-SPPS Statistics 20 was used for performing descriptive statistics (means, standard deviations and standard errors) establishing correlations (Pearson’s correlations) between microelements, and implementing the Principal Components Analyze (PCA) for emphasizing the principal components (factors) of our study, and correspondent factor loadings, variables, respectively.

RESULTS AND DISCUSSIONS

In Turda Star maize hybrid Control Group 1, K is the macroelement with higher concentration (1.51%), while if analyzing microelements, biggest mean concentration is reported for Mn (0.23%). Bigger concentrations, compared to Control Group 1, are reported in Experimental Group 1, where Turda Star hybrid was treated with gibberellin phytohormone, GIBB A3, in K, and all microelements, Zn, Fe, Mn, respectively (Table 1).

Except the differences between S, Zn and Mn contents, quantified in studied, groups that are statistically not significant ($p > 0.05$), those between other analyzed macro-and microelements are statistically assured at different thresholds of significance (Table 1).

Table 1. The basic statistics for the macro- and micronutrients content (%) identified in Turda Star maize hybrid untreated (Control group 1) and treated with GIBB A3 (Experimental group 1)

Issue	N	Control group 1			Experimental group 2			p
		$\bar{X}$	s	$s_{\bar{X}}$	$\bar{X}$	s	$s_{\bar{X}}$	
K	10	1.50	0.04	0.01	1.40	0.09	0.03	0.025
P	10	0.67	0.08	0.03	0.98	0.33	0.11	0.011
S	10	0.43	0.19	0.06	0.23	0.03	0.01	0.055
Mg	10	0.33	0.22	0.07	0.12	0.02	0.01	0.006
Cl	10	0.31	0.29	0.09	0.06	0.03	0.01	0.014
Zn	10	0.16	0.18	0.06	0.17	0.01	0.00	0.062
Fe	10	0.07	0.04	0.01	0.58	0.56	0.18	0.011
Mn	10	0.23	0.45	0.14	0.25	0.37	0.12	0.491
Ca	10	0.05	0.01	0.00	0.48	0.04	0.01	0.038

N – number of cases; $\bar{X}$ – mean; s – standard deviation; $s_{\bar{X}}$ – standard error of mean; probability value.

The same analysis conducted in Turda 200 maize hybrid, also emphasizes that K records bigger concentrations, of all analyzed macro- and microelements, in both, Control Group 2 (1.51%), and Experimental Group 2 (1.20%), the differences being statistically significant ($p < 0.05$). Fe is the microelement with biggest mean concentration in both groups, control (0.54%), and experimental (0.90%), respectively (Table 2). Contrarily to results obtained in Turda Star maize hybrid, in Turda 200 hybrid, except K, all macro-and microelements had bigger concentrations in Experimental Group 2, compared to control (Table 1). The similarity with situation identified in Turda Star hybrid consists in lack of statistically significance of differences ($p > 0.05$) between S, Zn and Mn contents, quantified in recorded in compared groups (Table 1).

Table 2. The basic statistics for the macro- and micronutrients content identified in Turda 200 maize hybrid untreated (Control group 2) and treated with GIBB A3 (Experimental group 2)

Issue	N	Control group 2			Experimental group 2			p
		$\bar{X}$	s	$s_{\bar{X}}$	$\bar{X}$	s	$s_{\bar{X}}$	
K	10	1.51	0.18	0.06	1.20	0.39	0.12	0.033
P	10	0.49	0.08	0.02	1.04	1.40	0.44	0.029
S	10	0.30	0.21	0.07	0.29	0.04	0.01	0.496
Mg	10	0.12	0.03	0.01	0.18	0.02	0.01	0.079
Cl	10	0.11	0.05	0.01	0.20	0.29	0.09	0.065
Zn	10	0.05	0.01	0.00	0.11	0.21	0.07	0.126
Fe	10	0.54	0.54	0.17	0.90	0.87	0.28	0.012
Mn	10	0.09	0.01	0.00	0.13	0.31	0.10	0.069
Ca	10	0.30	0.23	0.07	0.44	0.34	0.11	0.044

N – number of cases; $\bar{X}$ – mean; s – standard deviation; $s_{\bar{X}}$ – standard error of mean; probability value.

Concerning macro- and microelemental contents of maize resulted from our trial, we find that, our results are consistent with those cited by literature, according to whom the administration of gibberellin phytohormone influence their ration in plants (Kaya et al., 2006; Suge et al., 1986).

As shown, in both maize hybrids (Table 1, and Table 2) K and P, which have well-known role in decreasing respiration in plants, are decreased, in terms of concentration, in experimental groups treated with gibberellin GIBB A3 phytohormone.

In Turda Star hybrid, simple correlations, positive, and negative, of different degrees of intensity are reported (Table 3). Antagonist evolutions are identified between K, P, Mg, and Cl concentration in studied groups.

If those between Mg and Cl may be neglected, because they are very weak, the correlations, moderate identified for K ($R = -0.463$), and weak to moderate for P ($R = -0.297$), confirm once again the role of gibberellin GIBB A3 phytohormone in promoting plant transpiration, phenomenon reflected by decreased K, and P concentrations, compared to control (Table 3).

Table 3. The correlation matrix between the macro- and micronutrients content identified in Turda Star maize hybrid untreated (Control group 1) and treated with GIBB A3 (Experimental group 1)

Control/ Experimental	K	P	S	Mg	Cl	Zn	Fe	Mn	Ca
K	-0.463								
P		-0.297							
S			0.212						
Mg				-0.095					
Cl					-0.091				
Zn						0.441			
Fe							0.873		
Mn								0.607	
Ca									0.603

Because correlations are identified between studied parameters in Turda Star hybrid, we may implement the Principal Components Analysis (PCA).
The factor reducing emphasize the presence of 2 main factors, Factor 1 – the technology of culture, and Factor 2 – biostimulation approach.

While Factor 1 explains 53.28% of variance, Factor 2 explains only about 18.17% (Table 4). Stronger correlations are revealed between the Factor 1 (technology of culture) and experimental variables (macro- and microelemental content of Turda Star maize hybrid), compared to those resulted between no biostimulating practice (Factor 2), and the same variables (Table 5).

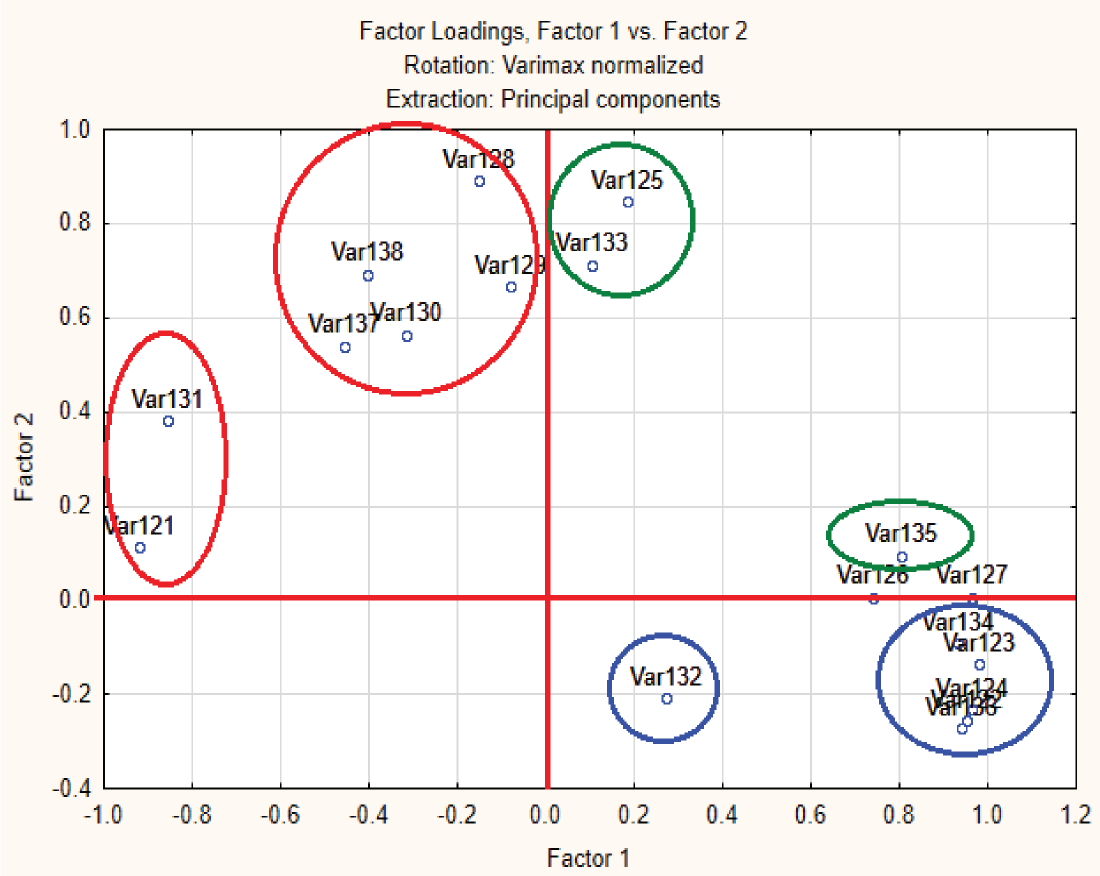
Table 4. The total variance explained for Turda Star maize hybrid

Issue	Eigenvalue	% Total - variance	Cumulative - Eigenvalue	Cumulative - %
Factor 1	9.590	53.281	9.590	53.281
Factor 2	3.272	18.179	12.862	71.460

Table 5. The factor loadings in Turda Star maize hybrid

Issue	Factor – 1 Technology	Factor – 2 Biosimulation	Issue	Factor – 1 Technology	Factor – 2 Biosimulation
Var121	0.930104	-0.172381	Var141	-0.552429	-0.185375
Var122	-0.983212	-0.018388	Var142	-0.954307	-0.039950
Var123	-0.981154	0.115799	Var143	0.393854	-0.499895
Var124	-0.993875	-0.002027	Var144	-0.198676	-0.806977
Var125	0.011230	0.968878	Var145	0.836721	-0.114616
Var126	-0.774575	0.063597	Var146	0.755269	-0.566412
Var127	-0.948085	0.164455	Var147	0.928971	-0.007254
Var128	0.350953	0.918269	Var148	-0.707243	-0.069726
Var129	0.270132	0.466006	Var149	-0.685963	-0.549811
Explained variance	5.477346	2.073661	Explained variance	4.514730	1.578233

The projections of the variables on PC1 x PC2 plane of the PCA (Figure 1) shows that K, Mn and Ca concentrations emphasized in Turda Star maize hybrid in both biostimulated and no-biostimulated experimental variants, and P concentration in experimental variant (biostimulated with GIBB A3 phytohormone) are mainly influenced by the biostimulation practice (Factor 2). The evolutions of the Cl concentrations, in both experimental variants (with and no biostimulation), Mg, and Zn concentrations in experimental variant where the phytohormone GIBB A3 was administered, are influenced by both principal factors, technology of culture and biostimulation practice, respectively (Figure 1).



Var 121 – K Control Group 1; Var 122 – P Control Group 1; Var 123 – S Control Group 1; Var 124 – Mg Control Group 1; Var 125 – Cl Control Group 1; Var 126 – Zn Control Group 1; Var 127 – Fe Control Group 1; Var 128 – Mn Control Group 1; Var 129 – Ca Control Group 1; Var 130 – K Experimental Group 1; Var 131 – P Experimental Group 1; Var 132 – S Experimental Group 1; Var 133 – Mg Experimental Group 1; Var 134 – Cl Experimental Group 1; Var 135 – Zn Experimental Group 1; Var 136 – Fe Experimental Group 1; Var 137 – Mn Experimental Group 1; Var 138 – Ca Experimental Group 1.

Figure 1. The projections of the variables (on PC1 x PC2 plane) of the Principal Components Analysis applied to Turda Star maize hybrid content in macro- and microelements within experimental conditions

Weak to very strong simple correlations, positive, and negative are emphasized between studied macro- and microelements concentrations in Turda 200 hybrid, simple correlations (Table 6). Similarly with data resulted from analyze performed in Turda Star hybrid, negative moderate, and weak to moderate correlations resulted between K ($R = -0.534$), and P ($R = -0.279$). Here, it also results that gibberellin GIBB A3 phytohormone promotes decrease of K, and P concentrations, compared to control (Table 6).

The simple correlations resulted between studied macro- and microelements studied in Turda 200 maize hybrid allow us to apply the Principal Components Analysis (PCA). The same as situation reported in Turda Star hybrid, two main factors, Factor 1 – the technology of culture, and Factor 2 – biostimulation are identified.

Factor 1, the technology of culture, respectively explains 51.12% of variance, while Factor 2, biostimulation practice, explains only 15.13% of variance (Table 7).

Also, similarly to results of the PCA analysis conducted in Turda Star hybrid, stronger correlations are reported between the technology of culture - Factor 1, and experimental variables (macro- and microelemental content of Turda 200 maize hybrid), while between no biostimulating practice - Factor 2, and macro- and microelemental content of Turda 200 maize hybrid, they are weaker (Table 8).

Table 6. The correlation matrix between the macro- and micronutrients content identified in Turda 200 maize hybrid untreated (Control group 2) and treated with GIBB A3 (Experimental group 2)

Control/ Experimental	K	P	S	Mg	Cl	Zn	Fe	Mn	Ca
K	-0.534								
P		-0.279							
S			0.192						
Mg				0.254					
Cl					0.211				
Zn						0.233			
Fe							0.966		
Mn								0.657	
Ca									0.692

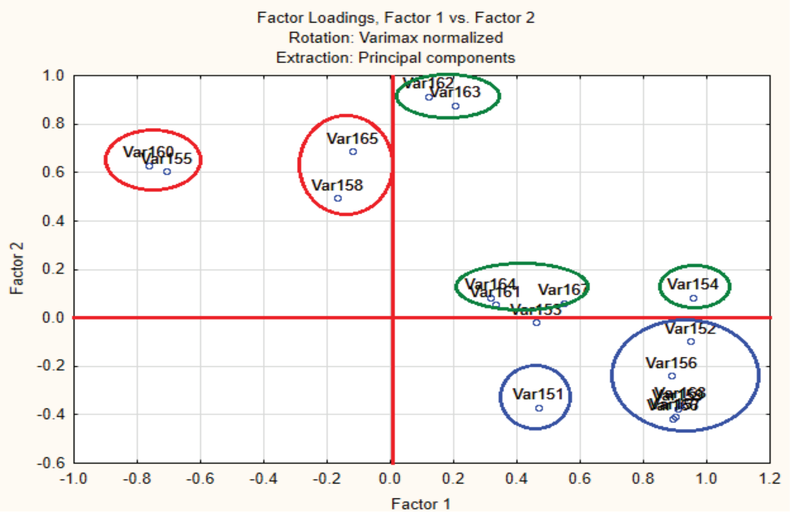
The PC1 x PC2 plane of the PCA (Fig. 2) reveals that Cl and Mn content of Turda 200 maize hybrid that receive no biostimulation, K, and Zn concentrations in Turda 200 maize hybrid that receive biostimulation with GIBB A3 phytohormone are mainly influenced by the biostimulation practice (Factor 2), while Turda 200 maize hybrid concentrations of Mg in both experimental variants, P, S, Cl and Mn, in experimental variant biostimulated, are influenced by technology of culture and also biostimulation practice (Figure 2).

Table 7. The total variance explained for Turda 200 maize hybrid

ssue	Eigenvalue	% Total - variance	Cumulative - Eigenvalue	Cumulative - %
Factor 1	9.203	51.128	9.203	51.128
Factor 2	2.724	15.136	11.927	66.264

Table 8. The factor loadings for Turda 200 maize hybrid

Issue	Factor – 1 Technology	Factor – 2 Biosimulation	Issue	Factor – 1 Technology	Factor – 2 Biosimulation
Var121	0.602741	0.553260	Var141	0.969620	-0.011768
Var122	0.934739	-0.214311	Var142	-0.326305	-0.894258
Var123	0.478382	-0.631391	Var143	0.518196	-0.664544
Var124	0.866911	0.126160	Var144	0.496141	-0.186586
Var125	-0.879318	-0.086397	Var145	-0.298415	-0.906257
Var126	0.912178	0.337125	Var146	0.646876	-0.251975
Var127	0.973873	-0.051860	Var147	-0.901819	-0.046954
Var128	-0.335501	0.689975	Var148	-0.372569	0.187739
Var129	0.972559	-0.043151	Var149	-0.892006	-0.085991
Explained variance	5.829545	1.368331	Explained variance	3.816583	2.205909



Var 151 – K Control Group 2; Var 152 – P Control Group 2; Var 153 – S Control Group 2; Var 154 – Mg Control Group 2; Var 155 – Cl Control Group 2; Var 156 – Zn Control Group 2; Var 157 – Fe Control Group 2; Var 158 – Mn Control Group 2; Var 159 – Ca Control Group 2; Var 160 – K Experimental Group 2; Var 161 – P Experimental Group 2; Var 162 – S Experimental Group 2; Var 163 – Mg Experimental Group 2; Var 164 – Cl Experimental Group 2; Var 165 – Zn Experimental Group 2; Var 166 – Fe Experimental Group 2; Var 167 – Mn Experimental Group 2; Var 168 – Ca Experimental Group 2.

Figure 2. The projections of the active variables (on PC1 x PC2 plane) of the Principal Components Analysis applied to Turda 200 maize hybrid content in macro- and microelements within

CONCLUSIONS

According to results of our study, result several conclusions:

- In both studied maize hybrids, Turda Star and Turda 200, K and P are the studied macroelements with highest concentration in both variants (without, and with biostimulation), with smaller concentration in biostimulated variants, compared to control, which is no treated with GIBB A3 gibberellin phytohormone. The differences between macro- and microelements concentrations in both studied maize hybrids (Turda Star and Turda 200) are statistically assured at different significance thresholds, for majority of macro- and macroelements analyzed in this study.
- Simple correlations, of different intensities, from very weak to very strong, are emphasized in studied maize hybrids, Turda Star and Turda 200. Negative moderate correlations are reported between K concentrations in biostimulated and no biostimulated experimental variants in both Turda Star (R = -0.463) and Turda 200 (R = -0.534) hybrids, while between P concentrations, also negative but weak to moderate correlations are established in Turda Star (R = -0.297) and Turda 200 (R = -0.279).
- The PCA shows, in both studied maize hybrids, Turda Star, and Turda 200, respectively that the same two principal factors are identified. In both cases, Factor 1 is represented by the technology of culture, explains the biggest part of variance (53.28% in Turda Star hybrid, and 51.12% in Turda 200 hybrid), and it is stronger correlated to experimental variables (studied macroelements – K, P, S, Mg, Cl, Ca, and microelements – Fe, Mn, Zn), compared to the other principal factor, Factor 2. This last one, Factor 2, represented by the biostimulation practice, performed in our case with GIBB A3 gibberellin phytohormone, explains 18.17% of variance in Turda Star maize hybrid, and 15.13% in Turda 200 Maize hybrid. Whatever maize hybrid, the biostimulation practice influences at greater extent the K concentrations in maize. Our results, also show the influence of biostimulation with GIBB A3 gibberellin phytohormone on plant physiology, reflected by decrease of K, and P concentrations, with role in plant transpiration.
- Further research is needed in order to establish correlations, and explain interrelationships between maize hybrids development, plant macro- and microelemental content, and physiological traits, and also.

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