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ASSESSING POTATO MORPHOLOGICAL AND PHYSIOLOGICAL TRAITS UNDER FERTILIZATION DURING THE FIRST YEAR OF FIELD ADAPTATION

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Abstract

*Potato, Solanum tuberosum L. represent one of the most important crop worldwide and from Romania. The general aim of the study was represented by the overall assessment of potato growth and development under specific field condition respectively Râșca county. Therefore, were chosen six potato germplasm (Red Fantasy, Bella Rosa, Dutch Red, Mauve, Captiva and Elfe), free of pest and diseased and the morpho-physiological features were evaluated. Different vegetative phenophases were assessed according to BBCH scale (**B**iologische **B**undesanstalt, Bundessortenamt und **CH**emische Industrie) with and without fertilization, productivity, chlorophyll content and chemical distribution of the most important chemical elements from potatoes. The results were differentiated between different principal growth stages pointed and also depending on potato variety. Râșca still remains one of the main location for obtaining high potato productivity.*

Key words*: BBCH scale, chemical distribution, chlorophyll content, Energy dispersive X-ray spectroscopy, Scanning electron microscopy, Solanum tuberosum L.*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most widely grown crops in the world (Birch et al., 2012), with a high contribution to food security (George et al., 2017). At the same time, it is an affordable crop (Zaheer & Akhtar, 2016) with high yields and can have a wide range of uses: as a source of food contribute to global food security (Birch et al., 2012), up to the industrial-scale production of by-products such as starch, alcohol and bioethanol (Thatoi et al., 2016).

Globally, potato production peaked in the last two decades in 2021 at around 376 million tons (FAO, 2022). The top potato producers worldwide are China, India and Ukraine (FAO, 2023). In Romania, in the year 2023, about 80000 ha were cultivated with potatoes, with a total production of more than one million tons (INSSE, 2024). More than 40% of the potato farms in the European Union come from Romania, and the annual national per capita potato consumption was about 36 kg/year in 2021 (Sterie et al., 2022). However, potato production and consumption has decreased notably in Romania since 2014 (Sterie et al., 2022). The underlying causes of this trend are represented by the increasing crop requirements for fertilizers and irrigation, especially in the context of climate change.

The use of fertilizers plays an important role in obtaining quality potato crops (Alva, 2004; Khan et al., 2012; Blecharczyk et al., 2023). The plant may have high fertilizer requirements due to its shallow root system (Iwama, 2008). Adequate fertilization of the potato crop requires a balanced supply of essential macro- and micronutrients, which play an important role in the physiological processes taking place in all organs of the plant (Tolesa, 2021). Potato fertilization can be achieved both by mineral or chemical fertilizers and by organic fertilization (manure) (Baniuniene & Zekaite, 2008). While manure increases soil quality in the long term (Edmeades, 2003), mineral fertilization is associated with a rapid supply of nutrients (Harris, 1992). Mineral fertilizers based on NPK represent the macronutrients needed by the potato plant (Tulung et al., 2021), nitrogen being responsible for the vegetative growth of the plant (Jenkins & Mahmood, 2003), phosphorus is associated with tuber initiation (Ekelöf, 2007), and potassium is involved in the crop resistance

to diseases (Zörb et al., 2014). Iron should not be neglected as it has a pivotal role in chlorophyll biosynthesis (Zhang et al., 2022). Magnesium and calcium play an important role in tuber starch content and cell wall structure resistance (El-Hadidi et al., 2017).

As an alternative to chemical formulas, the use of organic fertilizers, which gradually release nutrients (Shaji et al., 2021), improves soil structure and can positively influence the soil microbiome (Li et al, 2021). On the other hand, the use of mineral fertilizers in quantities that are not adapted to the crop and land area type can have undesirable effects on the soil by excess accumulation of salts, installing salinity, an increasingly common problem that endangers the quality of the substrate (Truşcă et al., 2023). The use of balanced fertilization is the long-term assurance of soil quality (Shah & Wu, 2019) and, implicitly, of plant health through the proper functioning of physiological processes associated with good crop yields and quality products (Brevik, 2015).

The aspects mentioned above require special attention, and testing the morpho-physiological responses of plants to different types of fertilization is an important step in establishing appropriate crop technologies for the needs of each plant and variety.

Advanced analytical methods, such as SEM-EDX (Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy), provide a detailed understanding of nutrient-plant interactions, allowing an accurate assessment of morphological and physiological changes following the application of different types of fertilization. This analytical technique not only measures the chemical composition of plant tissues, but also helps to identify variations in cell structure, which may be essential for understanding the processes of plant adaptation to environmental conditions (Bollavarapu et al., 2020).

This study focuses on the assessment of morphological and physiological traits of potatoes in the first year of field adaptation under the influence of different fertilization regimes, by using SEM+EDX as the main method of analysis and chlorophyll content. Through this research, we aim to contribute to a better understanding of the factors influencing potato development and to provide relevant information for the optimization of agricultural practices.

For this purpose, an experiment was proposed and carried out in which different parameters of six potato varieties were assessed in the presence of mineral and organic fertilizers. The objectives of the study were to monitor the chlorophyll content during three BBCH stages and to evaluate the levels of some chemical elements in the leaves. The yield for each variety was also determined according to the type of fertilization used.

MATERIALS AND METHODS

The experiment took place in the field in Dealu-Mare village, Rîsca, Cluj county, 46.7289772, 23.1306339. The testing started in 20 April 2024 and the harvest was collected in 25 September 2024. The experimental design consisted in six potato varieties respectively Red Fantasy (Rf), Bella Rosa (Br), Red Olanda (Ro), Purple (Mv), Captiva (Cap), Elfe (El) and two fertilization regime with required fertilization and without any fertilization. The potato for sowing was purchased from the Europlant SRL Research Center, in Avrig, Sibiu County and was stored between 15.10.2023-20.02.2024 in raschel net bags, in a place protected from humidity with a temperature between 8-10°C.

In the field with no fertilization, the seed potatoes were planted manually in nests with a depth of approximately 15-20 cm, at an air temperature at planting of 16-18°C.

The field with fertilization was previously plowed at 20-25 cm, with manure and chicken droppings applied. Afterwards the soil was milled one week before planting. On the planting day was also applied to the soil ammonium nitrate (NH_4NO_3) purchased from Azomures Producer (400 kg/ha). The seed potatoes were planted mechanically in nests approximately 15-20 cm deep. The pests and disease treatments were done at 3 weeks after planting on 17.04.2024, and an additionaly weed treatment was applied.

As for the foliar treatment, Polyfeed 20-20-20 from the manufacturer Haifa was used, 2 times before flowering, and 1 time after flowering, in a quantity of 4 kg/ha.

The experimental field consisted from 9000 m^2, to which a nested design was applied for the

monitoring of physiological parameters. The registered parameters for all analysis consisted in three replications, and each parameter was extracted from a $5m^2$ plot, randomly chosen from the experimental field.

Potato leaf chlorophyll content was recorded in three representative BBCH stage intervals for the crop. Therefore, the first assessments of the parameter were performed during the first interval, from leaf growth and development to tuber formation (C1-BBCH 1-4). The second set of assessments (C2) was performed from inflorescence emergence (BBCH 5) to fruit development (BBCH 7). Finally, chlorophyll content was also recorded for the late vegetative stages, i.e. in the interval BBCH 8 and 9 from fruit ripening to seedling senescence (C3). The parameter was assessed by a non-destructive method with the chlorophyll measurement instrument in SPAD units, i.e. chlorophyll meter MC100C from Apogee Instruments.

To determine the chemical composition of the leaves, the Scanning Electron Microscopy method was used with the Energy Dispersive X-Ray detector, using the equipment provided by the TermoFischer Scientific company.

To introduce the samples into the analysis chamber, they were placed on an aluminium foil on which a carbon tape was applied. The analysis was performed at different magnifications 400-1000 555X, using a Low Vacum Detector, the pressure in the analysis chamber was 100 Pa and the voltage varied between 15.00-25.00 kV, with a spot size of 4.5-5. The chemical analysis was performed using the EDX detector together with the Pathfinder application. The leaves subjected to SEM+EDX analysis were sampled on 15.07.2024 and then left at room temperature (21-23°C) for a period of 3 weeks.

RESULTS AND DISCUSSIONS

The relative chlorophyll content differed significantly depending on assessed phenophase C1-C3, betand based on the specific interaction of each potato varieties with fertilization regimes. Two-way ANOVA was performed for each individual assessment and highlighted significant differences in relative chlorophyll content between potato varieties (Figure 1).

In the phenophase of leaf development-tuber formation BBCH 1-4, the highest value was registered at purple potato variety (Mv) of 542±26.19 (SE-standard error) SPAD units from the control treatment. This value was significantly higher than the values obtained for Rf, with and without fertilization, and Mv with fertilization. The technology applied on potato only impacted significantly Mv variety, fertilization reduced significantly the relative chlorophyll content. This could be because of variety sensitivity to common potato technology. This sensitivity comes from physiological processes that happen especially in the vacuole where anthocyanins are present. Coloured potatoes contain anthocyanins in all the plant tissues including in tubers. The fertilization status due to osmosis process could alter anthocyanidin metabolism and biosynthesis (Zhang et al., 2024). In the control treatment with a low nitrogen dose, the relative chlorophyll content of purple potato was higher compared to the fertilized treatment where it appears that nitrogen presence inhibited anthocyanin synthesis process and relative chlorophyll content.

In the C2 assessment when the potatoes were in BBCH 5-7 inflorescence emergence-development of fruit, the only significant differences in relative chlorophyll content were registered at the varieties Br and Ro. The values were higher in the control treatment without fertilization 409±18.31SPAD units for Br and 416±11.65 SPAD units for Ro. The highest value of relative chlorophyll content registered in the second assessment was at Mv from the control treatment (449±20.65 SPAD units) with only 41.77 SPAD units more compared with the fertilized treatment. The Br variety registered the lowest value of relative chlorophyll content under fertilization of 233±24.23 SPAD units. Overall the relative chlorophyll content was higher in the treatment that lack fertilization. The only exception was El variety which registered similar value in both variants around 396 SPAD units at the second assessment.

In the last set of assessments (C3), corresponding to the final phenophases under evaluation, (fruit ripening and seed senescence - BBCH 8-9) (Figure 2), only in one potato variety a significant difference in relative chlorophyll content was observed with the application of fertilizers in the cultivation technology. The treatment produced a halving of

the parameter value, precisely in purple potato (Mv), where the maximum of 488±53.10 SPAD units was recorded in the control. The lowest leaf relative chlorophyll content was recorded for the Cap variety in the presence of fertilizers (205±15.26 SPAD units), a value with 110 SPAD units lower than the control. Altogether, the relative chlorophyll content evolution maintains the trend highlighted in C2, the values of the analysed parameter being higher in the absence of fertilizers.

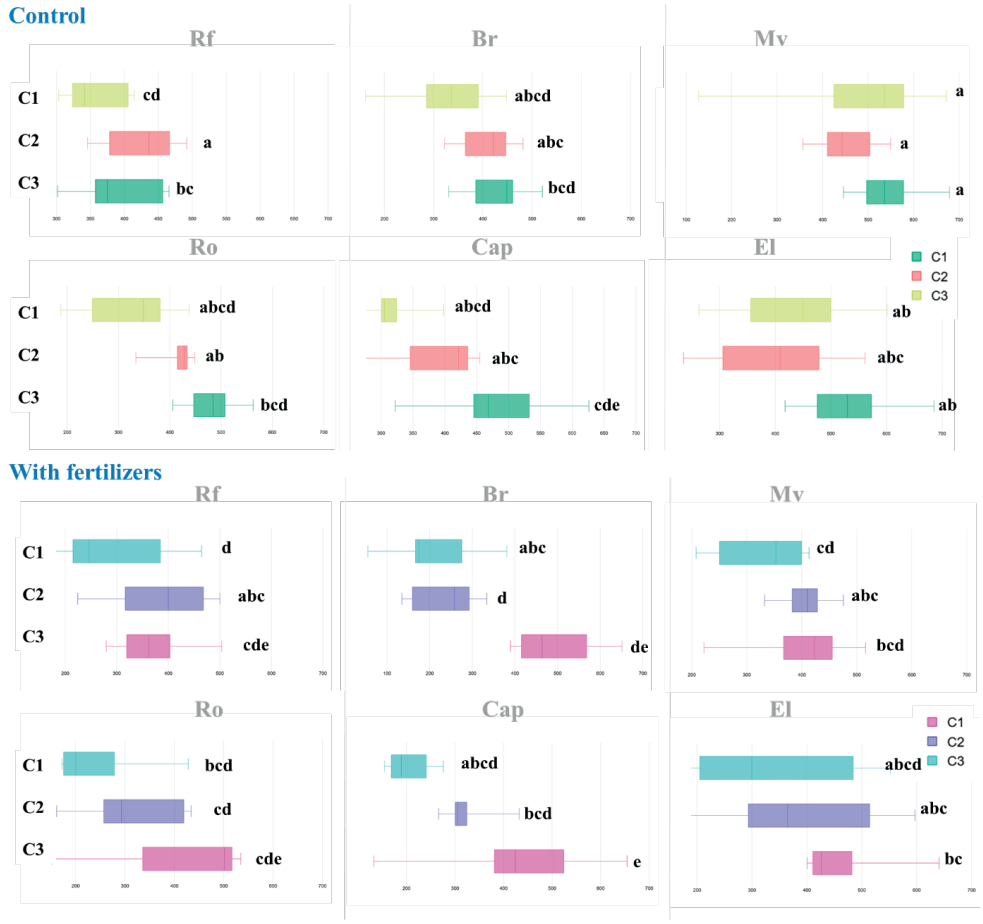

Figure 1. Chlorophyll content according BBCH scale C1 – BBCH 1-4 (leaf development-tuber formation), C2 – BBCH 5-7 (inflorescence emergence- development of fruit), C3- BBCH 8-9 (ripening of fruit and seed-senescence). Where ANOVA C1 var (StatF=3.16, p val=0.011), F (StatF=6.87, p val=0.010), var:F (StatF=2.31, p val=0.050), ANOVA C2 var (StatF=4.34, p val=0.001), F (StatF=21.43, p val<0.001), var:F (StatF=2.75, p val=0.023), ANOVA C3 var (StatF=7.70, p val<0.001), F (StatF=31.32, p val<0.001), var:F (StatF=0.51, p val=0.769), different letters from LSD test represent significant differences at p<0.05.

Figure 2. Potato (*Solanum tuberosum* L.) BBCH 1-4 (leaf development-tuber formation), BBCH 5-7 (inflorescence emergence- development of fruit) and BBCH 8-9 (ripening of fruit and seed-senescence).

The mass percentage analysis of the chemical elements of interest in the potato crop differed significantly depending on the potato variety and the chemical element evaluated (Table 1). The presence of leaf oxygen content did not vary with fertilizer application for all the varieties. However, except for Ro and El, no significant decreases in the mass percentage of oxygen were recorded for all other potato varieties. The highest value was recorded for Cap where the cultivation technology insignificantly increased the leaf oxygen proportion by 4%. Ro and El showed the opposite aspects to those observed for the other varieties. In the presence of fertilizers, the mass percentage of oxygen in the leaves decreased compared to the control values. The minimum percentages of oxygen were visible in Rf and Ro where decreases of 5.36 and 5.83% percent were recorded compared to the maximum in Cap.

Table 1. Leaves chemical properties of all six potato varieties studied with SEM+EDX (average±SE-standard error)

Var	F	O	C	K	Ca	Si	Mg	P
Rf	CE	50.79±0.60 **b**	40.42±1.10 **ab**	4.56±0.75 **abc**	1.71±0.26 **bc**	0.92±0.22 **ab**	0.44±0.09 **bcd**	0.2±0.05 **ab**
	F	54.22±0.85 **ab**	39.45±1.18 **ab**	2.59±0.38 **c**	1.37±0.04 **bc**	0.34±0.07 **b**	0.90±0.10 **ab**	0.13±0.01 **b**
Br	CE	52.07±0.74 **ab**	43.05±0.85 **a**	2.87±0.31 **c**	0.53±0.07 **bc**	0.36±0.09 **b**	0.09±0.01 **d**	0.14±0.03 **b**
	F	53.43±1.07 **ab**	38.94±1.23 **abc**	3.53±0.34 **bc**	1.96±0.13 **bc**	0.14±0.04 **b**	1.11±0.09 **a**	0.19±0.02 **ab**
Mv	CE	54.37±0.89 **ab**	40.06±1.08 **ab**	3.26±0.20 bc	0.91±0.10 **bc**	0.54±0.18 **ab**	0.35±0.06 **cd**	0.11±0.02 **b**
	F	54.67±2.56 **ab**	39.7±2.60 **ab**	3.44±0.37 **bc**	0±0 **c**	0.37±0.07 **b**	0.35±0.06 **cd**	0.11±0.01 **b**
Ro	CE	54.34±2.04 **ab**	26.79±5.16 **c**	10.35±1.93 **a**	2.19±0.44 **bc**	2.58±1.85 **a**	0.49±0.18 **bcd**	0.09±0.03 **b**
	F	50.38±1.36 **b**	43.86±1.45 **a**	1.98±0.1 **c**	1.70±0.33 **bc**	0.39±0.06 **b**	1.10±0.02 **a**	0.20±0.04 **ab**
Cap	CE	52.1±0.26 **ab**	40.01±0.08 **ab**	4.48±0.25 **abc**	1.76±0.03 **bc**	0.53±0.02 **ab**	0.51±0.08 **bcd**	0.20±0.01 **ab**
	F	56.15±1.05 **a**	29.06±7.56 **bc**	9.55±4.95 **ab**	2.97±1.80 **ab**	0.31±0.12 **b**	0.49±0.13 **bcd**	0.16±0.03 **ab**
El	CE	53.59±1.32 **ab**	38.82±1.85 **abc**	4.92±1.17 **abc**	1.22±0.19 **bc**	0.52±0.37 **ab**	0.30±0.11 **cd**	0.16±0.03 **ab**
	F	52.52±1.15 **ab**	29.07±3.40 **bc**	10.62±1.43 **a**	4.75±1.16 **a**	0.41±0.07 **ab**	0.62±0.27 **bc**	0.27±0.05 **a**
Var	StatF	0.94	2.11	3.05	3.75	1.28	3.42	2.62
	p val	***0.471***	***0.099***	***0.029***	***0.012***	***0.305***	***0.018***	***0.050***
F	StatF	0.83	0.72	0.05	3.93	3.29	32.37	2.25
	p val	***0.372***	***0.403***	***0.826***	***0.059***	***0.082***	***p<0.001***	***0.146***
Var:	StatF	2.59	5.40	4.84	3.32	1.06	5.31	2.83
	p val	***0.052***	***0.002***	***0.003***	***0.020***	***0.405***	***0.002***	***0.038***

Note: Var-potato variety; C- control field without fertilization, F-field with fertilization; O-oxygen, C-carbon, K-potassium, Ca-calcium, Si-silicon, Mg-Magnesium, P-phosphorus, different letters from LSD test show significant differences at $p<0.05$

Leaf carbon mass percentage can be associated with the carbon accumulation degree in the leaf. It is clearly visible that with the application of fertilizers, this parameter decreases but not significantly. There is a particular aspect in Ro in the presence of fertilizers, where the carbon mass percentage increases with about 17% compared to control. The mass percentage of leaf potassium varies both with changing cropping technology and potato variety. Its value more than doubles as a result of fertilizer application for Cap and El potato varieties. On the other hand, in Ro variety, where the minimum K percentage was recorded, the same cropping technology had an opposite effect on the parameter, its value being significantly reduced by 5 times compared to the control. An interesting aspect was observed for purple

potato, where the application of fertilizers did not influence the potassium mass percentage. For varieties Rf and Br, the values of the parameter varied insignificantly in the range 2.59-4.56, which can be concluded that fertilizer application does not noticeably influence the K percentage of leaf dry mass for the both varieties.

For half of the potato varieties tested, the calcium mass percentage decreases negligibly with the application of fertilizer cultivation technology. The Ca content in the plant is usually between 0.5 and 3% (Vâtcă, 2020). The Ro potato has the closest value within this normal range, however El has the highest value of 4.75% in the fertilization field, significant compared with the Ca content from other varieties and treatments. The lowest Ca content was recorded in purple potato, where fertilizer application totally reduced the Ca content in the leaf.

The mass percentage analysis of the chemical elements in the leaves shows insignificant decreases for silicon in the tested variants in the presence of fertilizers, with only one exception for Ro potato. This potato variety recorded the maximum value of Si in the control. Also in the control, but in the Rf potato, the value is almost double the maximum content of 0.5% Si that can enter in the composition of dicotyledonous and leguminous plants (Table 1).

The mass percentage of magnesium increased significantly in the presence of fertilizers in leaves of Br and Ro potatoes, where the maximum was recorded. In Br potato, the use of cultivation technology was associated with a 12-times increase in Mg content. Further, but not significant, increases were observed in Rf and El potatoes, where the element mass percentages doubled. On the other hand, two other potato cultivars, Mv and Cap, maintained a constant magnesium content in leaves, regardless of the cultivation technology, with values in the range 0.35-0.51%.

Within the same potato variety, fertilizer application does not significantly influence the mass percentage of phosphorus. Only two varieties, Rf and Cap, showed reduced decreases of the parameter in response to the cultivation technology. The maximum value of the parameter was recorded in El fertilized potatoes. At the opposite, the lowest percentage of this element was recorded in Ro control, however, the application of fertilizers to this potato variety was associated with a doubled value of the parameter. The cropping technology did not produce differences in the leaf phosphorus mass percentage in purple potato, maintaining the value of the parameter at the same level as that recorded in the control (Table 1).

Table 2. Average yield with standard error for all potato varieties from the field (t/ha)

No.	Var	C	F
1.	Br	7.67±0.33 **f**	36.67±0.88 **c**
2.	Cap	9.67±1.20 **f**	47.67±1.45 **a**
3.	El	7.00±1.15 **fg**	22.33±0.88 **d**
4.	Mv	3.33±0.33 **g**	16.33±1.45 **e**
5.	Rf	10.00±1.15 **f**	42.33±1.45 **b**
6.	Ro	6.33±0.33 **fg**	25.33±0.33 **d**

Note: Var-potato variety studied, C- control field without fertilization, F-field with fertilization, different letters from LSD test show significant differences at $p<0.05$

The average production recorded the highest values in the fertilized field for all potato varieties (Table 2). Although from a physiological point of view and relative chlorophyll content, the plants reported good functioning in the unfertilized field, in terms of production this was 5 times higher in Br, Cap, Mv, 4 times higher in Rf and Ro and 3 times higher in El. The maximum yield was registered to Cap potato variety from the fertilized field and significantly higher compared with all other yield values. In previous studies, Cap was classified as medium yielding sort with a medium-fast growing period around 92-94 days and also with around 17-27 t/ha productivity. On the other hand, El was set in the high yielding sort with medium growing time of around 95-97 days with a relative yield in the range of 12-17 t/ha (Eyvazov, 2025).

From the unfertilized field, Rf had the higher yield value significantly higher compared to Br, Cap and Rf.

Analyzing the correlation coefficients of the monitored parameters, significant correlations ($p<0.05$) are observed between different mass percentages of the chemical elements based on SEM+EDX assessment (Figure 3). A share of 36% from the leaves C mass percentage is negatively strong correlated with the oxygen. This could be explained by the biochemical reactions of amino-acids, water and other compound with oxygen in order to allocate carbon in the leaves (Figure 3).

Potassium has a weak positive correlation with oxygen, a share of 12% of K is influenced by O_2 and a negative strong correlation with carbon from leaves, a share of 88% of K is influenced by leaf compounds containing C. Potassium represent the principal microelement from the plants with the highest share (Vâtcă, 2020). It's role in growth and development of plants is was established. Furthermore, K have an important in the formation of bio-colloids and ensures their hydration, ensures good activity of plant enzymes. The plant leaves contain potassium around 10-15 mg/g dry matter (Vâtcă, 2020).

About 55% of calcium level is due the strong negative correlation with the leaves carbon content, and 64% of the level is sustained by its strong positive correlation with K. The presence of Si in the potato leaves is weak negatively correlated with C content (Figure 3). A share of 12% and 15% of P is based on the weak negatively correlation with O and the weak positively correlation with Mg. Phosphorus ester, phytin, is the Ca or Mg salt of phytic acid and represents 2% of the total phosphorus in the green organs of plants (Vâtcă, 2020). Phosphorus is a very important element for the composition of cellular structures with the role of energy carrier (Maciá, 2005; Pandey, 2018; Vâtcă, 2020).

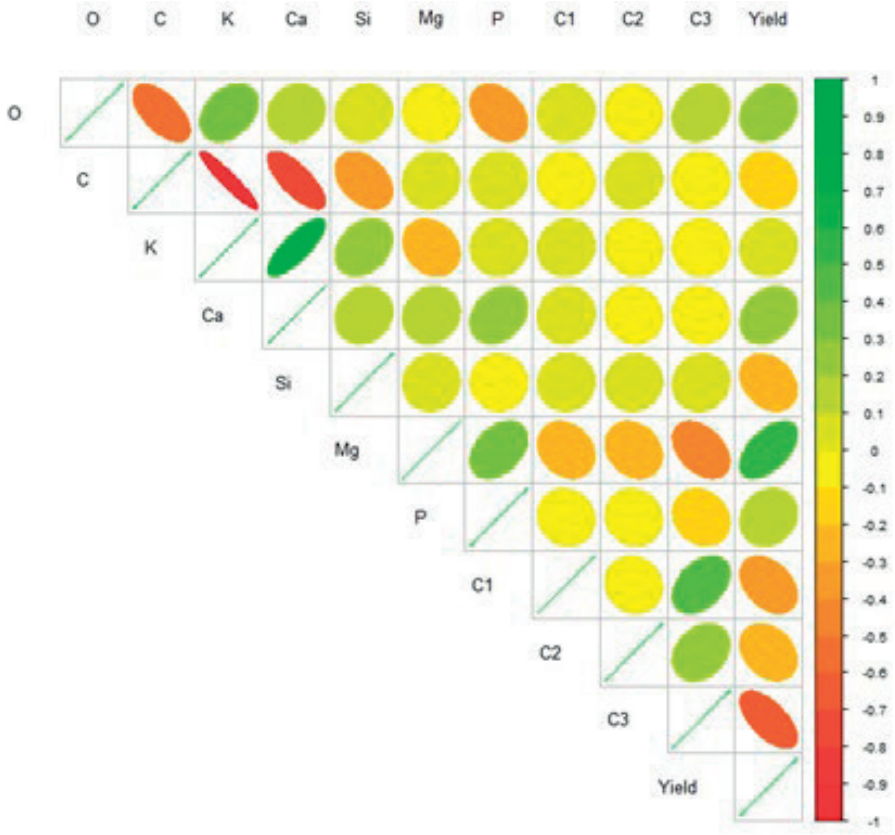

Figure 3. Correlation chart between all tested parameters physio-chemical and yield of six potato varieties

The values of relative chlorophyll content from the third assessments are weak negatively correlated with Mg in leaves content and weak positively correlated with the plant development during first assessment (Figure 3).
The obtained potato yield correlates positively around 30% with Mg content from the leaves, negatively weak determined by the relative chlorophyll content of potato leaves at the first assessment C1 (15%) and negatively strong determined (40%) by the relative chlorophyll content from the third assessment. A high chlorophyll content in the fertilized treatment could be explained by the fact that potato prolongs the vegetation period and invest again in the growth to the detriment of yield accumulation (Figure 3).

CONCLUSIONS

The relative chlorophyll content was higher at potato varieties from the field without fertilization.
The purple potato registered the highest relative chlorophyll content at all assessments.
The variety Cap has the lowest chlorophyll content at the end of the vegetation period.
The registered physiological parameters of potato are negatively correlated with the application of high fertilizer regimes for the majority of varieties tested.
The elements from potato leaves presented both convergent and divergent directions of correlations, indicating a variety specific response to treatment.

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