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FIRST RESULTS REGARDING RELATION BETWEEN GIBBERELLINS AND OTHER GROWTH HORMONES IN MICROPOPAGATION PROTOCOLS OF TWO ECONOMICALLY IMPORTANT SPECIES: *Solanum tuberosum* and *Ipomoea batatas*

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Abstract

Potato is the world's most important non-cereal food crop, one of the major sources for humankind food. Conventional propagation asexual by tubers, can disseminate pathogens to new cultivation areas which can threatens the maintenance of genotypes of these specie. Ipomoea batatas as well, is a hard climate conditions plant, with a major role in food worldwide battle and have similar response to viruses or diseases In this work we analyzed varieties of Ipomoea batatas, 'Ro-Ch-M', 'KSH' and 'KSP1', two varieties of Solanum tuberosum L. with purple flesh, 'Violet Queen' and 'Purple Majesty'. The stydy compare the influences of gibberellic acid GA3, along with another two hormones, cytokinins (6-benzylaminopurine BAP), and α-naphthaleneacetic acid (NAA), the culture duration and response to tuberization of those varieties. Optimal proliferation was observed when shoots were cultured on MS medium that was supplemented with 1.5 mg/L GA3 and a variation of another two hormones. In this medium, the greatest number of shoots (4.1) and total number of nodes (12.2) per explant were observed.

Key words*: auxins, cytokinins, gibberellins, Ipomoea batatas, micropropagation, Solanum tuberosum.*

INTRODUCTION

Roots and tubers are an important source of food, nutrition and income for a large part of the worlds (Diaconu et al., 2018). Potato (*Solanum tuberosum* L.) is a crop of high biological value for its amount of vitamins, minerals trace elements and valuable protein (Fiergert et al., 2000). It is the fourth most cultivated food crop exceeded only by wheat, rice, and maize (Zaheer et al., 2016). The edible part of potato is the tuber, which is used as cheap food, industrial raw material, animal feed, and seed tuber for crop production.

Sweet potato is considered the seventh most important food crop in the world and is ranked fourth in developing countries (FAO, 1997). It is cultivated in more than 100 countries (Gastelo et al., 2014) as a valuable source of food for humans, animals and industrial raw material (Devaux et al., 2021). However, pests and viral diseases prevent the crop from reaching its maximum agricultural potential. Viruses and shortage of good quality seeds limiting potato and sweet potato production, and that why tissue culture techniques are an alternative of vegetative plant propagation for those two species (Zine et al., 2008). Potato micropropagation methods are used on large scale now due to plant capacity of multiplication on culture medium supplemented with hormones. That allows multiplication on large scale of asexual virus free plants. The potato plants are multiplied with a range of different techniques, such as nodal segments apex culture or meristem culture and hormonal and nutritional composition of media promotes rapid development of new plantlets (Murphy, 2003)

Diseases like bacterial wilt, scab, anthracnose, stem and root rot are the common challenges in sweet potato multiplication, and that why micropropagation is the most potential technique to achieve the goals of quality virus free planting material (Dewir et al., 2020).

Gibberellins commonly known as gibberellic acids first appear in 1950s, but they had been discovered much earlier in Japanese rice culture (Jones et al., 1994). In 1950s scientists of Tokyo University identify and stabilized 3 different gibberellins, gibberellin A1, gibberellin A2 and gibberellin A3 and nowadays we use in plant micropropagation especially GA3 (Lang, 1970). Gibberellic acid has been reported to inhibit meristematic grows in tobacco or enhanced the grows if the cassava (*Manihot esculenta*) callus culture derived from medium contain GA in addition to N6-benzylamionopurine (BAP) (Jansson et al., 2009). Gibberellins are involved in a wide range of plant responses, include promotion of elongation in stems and grass leaves, induction of hydrolytic enzymes such as α-amylase and protease facilitating endosperm mobilisation in grass and cereals, or promote seed germination, sex determination, fruit development and juvenility control (Salem and Hassanein, 2017). Addition of growth regulators to the culture media has been reported to improve the growth and development of shoots (Rabbani et al., 2001), though they are genotype dependant. Using higher concentrations of GA3 supplemented with 1-naphthaleneacetic acid (NAA) and vitamins has increase number of nodes (Zaman et al., 2007). Rabbani (2001) recommended the use of higher concentrations of GA3, supplemented with other phytohormones like NAA/BAP and vitamins in order to increase the multiplication capacity of potato. For a rapid multiplication addition of GA3 to MS media is shown improving explants growth and shoots development (Muller & Lipschutz, 1984). Since each hormone has it's unique signal on regeneration (Vreugdenhil et al., 2007), it's important to determine the combined effects of these on *in vitro* regenerative processes. Even GA is essential in adventitious shoots starting with various *in vitro* culture types like potato discs, meristem culture or shoots (Vinterhalter et al., 1997), the exact role of this hormone in shoot formation process is not fully determined yet (Ehsanpour & Jones, 2000).

MATERIALS AND METHODS

1. **Plant material and study area**

Two *Solanum tuberosum* purple cultivars, 'Violet queen' and 'Purple majesty' and three *Ipomoea batatas* cultivars, 'RO-CH-M', 'KSP-1' and 'KSH' were analysed.

Solanum tuberosum 'Violet queen' (previous 'Hot Purple') - a potato with deep purple skin and high concentration of anthocyanins and flesh descended from ancient Peruvian varieties. Originated from a cross made in 2000 between the selection designated 'VG3CAE 5' as the female parent and 'Charmante' as the male parent at the HZPC 'Research & Development Centre' in Metslawier, The Netherlands.

Solanum tuberosum 'Purple majesty'- Peruvian cultivar, probably the most intensely from all purple potatoes. The origin of 'Purple Majesty' (experimental designation CO94165-3P/P) is the result of the cross made in 1994 between 'All Blue'

Figure 1. *Solanum tuberosum* 'Violet queen'

and ND2008-2 at the San Luis Valley Research Centre, Colorado State University; it is a beautiful purple colour potato with exceptional flavour and texture. Is an early-maturing potato variety that is typically ready to harvest in 85-90 days after planting.

Ipomoea batatas - 'KSP-1', matures in 3.5-4 month, well grow in sandy soil of 'Research and Development Station for Plant Culture on Sands Dabuleni', Romania, being drought-resistant with vigorous growth and high productivity (Draghici, 2018).

2. **Micropropagation**

For *Solanum tuberosum* 'Violet Queen', ex-plants were initial cultivated from flesh biolo-

gical material, on MS medium without hormones for 3 months with 3 sub cultivations in order to obtain diseases free explants (Figure 1).

Figure 2. *Solanum tuberosum* 'Purple Majesty'

After primary sterilization (fungal decontamination with Aliette 80WG, 0.4% for 20 minutes), the explants were treated with 70% ethanol for 35 seconds, rinsed with sterile distilled water, dipped in 0.2 mg/l $HgCl_2$ (mercuric chloride) for 4.5 minute and washed for 4 times with sterilised distilled water.

For *Solanum tuberosum* 'Purple majesty', explants were generated from *in vitro* stabile culture, multiplicated and conserved on MS medium without hormones for over 6 months (Figure 2).

For *Ipomoea batatas* 'KSP-1' cultivar, tests were started with sterile unimodal segments obtain form rooted shoots in vitro cultivated on MS medium without hormones (Figure 3).

Figure 3. *Ipomoea batatas* 'KSP-1'

KSP-1 culture were stared with nodal segments, immersed in 0.4% Aliette 80WG for 10 minutes, rinsed with distilled water, treated for 20 seconds with 70% ethanol and rinsed again. Decontamination was made with 0.2 mg/l $HgCl_2$ (mercuric chloride) for 3 minutes and washed for 3 times with sterilised distilled water.

3. **Culture initiation**

Cultures for both species were initiated with 0.5-0.8 cm uninodal segments for all tested varieties from aseptic *in vitro* pre-culture on MS medium with different concentration of BAP (V variant), GA_3 (X variant) (Table 1) and ANA (Y variant). Each treatment had five repetitions and five replications. The culture media was pH 5.75, 30 g/l agar and 7 g of sucrose in 30 ml container for each repetion. Media was autoclaved for 21 minutes at 121°C. Cultures were inoculated in the laminar flow bench and incubated at 24±1 °C under 14 h of light. All measurement was done at 7-12-19-26 and 33 days from inoculation.

Table 1. Media combination in different treatments on variant X

Variant	BAP mg/l	GA_3 mg/l	ANA mg/l
X0	0	0	0
X1	0.25	0	0.03
X2	0.25	0.5	0.03
X3	0.25	1.0	0.03
X4	0.25	1.5	0.03
X5	0.25	2.0	0.03

The GA_3 impact on growing, number of leaves, height of the plants, number of *in vitro* roots and callus proliferation were analysed. This paper is part of a complex study on both species regarding interaction between three important hormones (BAP, GA_3, NAA) and here we test the method that we applied on *Solanum tuberosum* and discuss only two parameters – height and roots.

The obtained experimental data were statistically processed using the software Jasp 0.16.1. ONE WAY ANOVA tests were used to study the influence of different variants during the time. Also, we used POST-HOC Test to identifying the significant differences between samples (p value less 0.05).

RESULTS AND DISCUSSIONS

Results were obtained after 33 days of culturing and metric observations.

1. *In vitro* shoot induction and viability of explants. Purple potato cultures were established from uninodal segments of *Solanum tuberosum* L. 'Purple majesty' (PM) and 'Violet queen'(VQ) varieties. All cultures remain sterile after 10 days after inoculation and allow metric observation. One explant from X5_PM were exhausted starting with day 12.

2. Shoot length

2.a. PM_X variant. Variation of GA_3 plus NAA and BAP affected shoot length variability on both varieties among them. The longest shoots were observed on PM variant starting with day 26, where we found a significant differences between Control_PM and X5_PM ($p=0.016$). The trend is confirmed at 33 days with a 4.18 cm average (Figure 4) for Control_PM height. The Post Hoc Test comparisons show a significant differences only between control_PM and X5_PM (2 mg/l GA_3), where *p* value is slightly below 0.05)

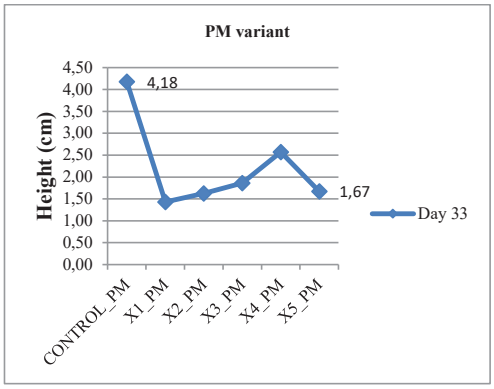


Figure 4. Height distribution on PM variety on day 33

2.b. VQ_X variant. For VQ variety, ANOVA shoes that are significant differences between the height for variant on day 12 from inoculation. Post Hoc test shoes that variant X1_VQ, X2_VQ, X5_VQ doesn't have significant differences between them, but they have significant difference form the control, and variant X3_VQ (1.5mg/l GA_3) show significant higher results then variants X1_VQ, X2_VQ, X5_VQ

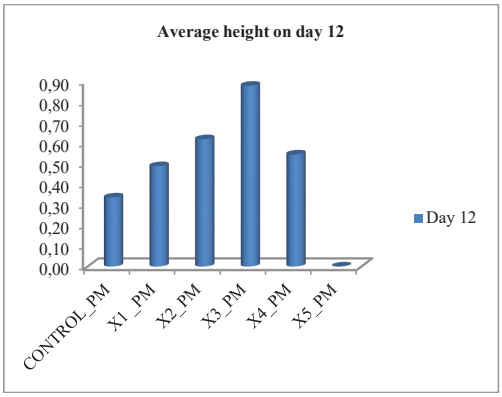
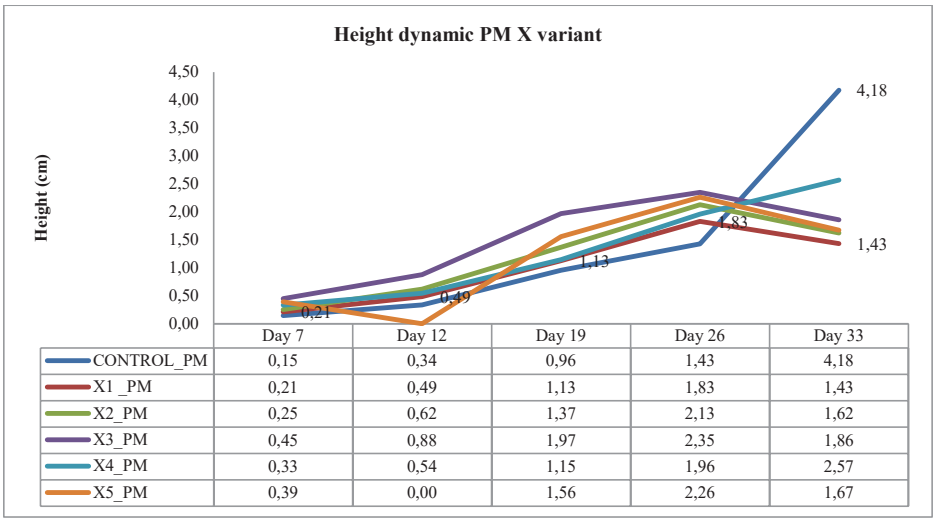


Figure 5. Height distribution on PM variety on day 12

2.c. *Dynamics of shoot height.* Regarding dynamic of height growing on PM variant, there are no significant differences between X1, X2,..X5_PM. Between day 1 and day 26, height parameter had a similar evolution both on control and variants. Starting with day 26, the Control_PM (without hormones) show a rapid growth rate, with an 4.18 cm average.

The lowest height rate with 1.43 cm we observe on X1_PM variant (with 0.25 mg/l BAP, 0.03 mg/l NAA and no GA_3) (Figure 5).

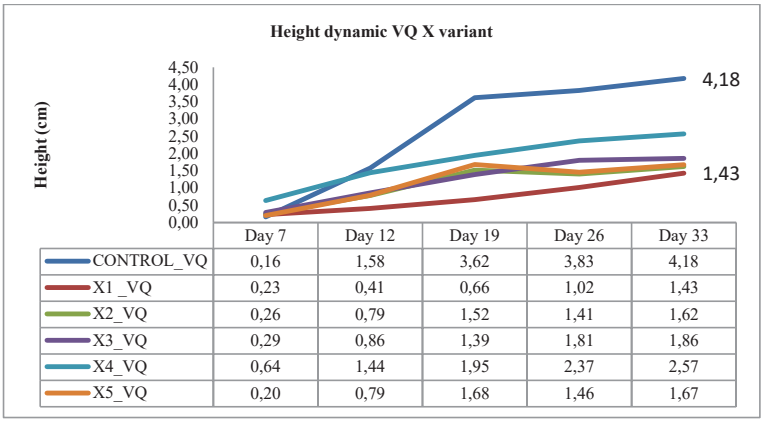
Regarding dynamic of height of VQ variant, the significant rise is shown on Control_VQ (without hormones), starting with day 12, continuing ascension until day 33, at an 4.18 cm average (Figure 6). The other variants X2_VQ, X3_VQ, X4_VQ and X5_VQ don't had any important dynamic, instead of X1_VQ (with 0.25 mg/l BAP, 0.03 mg/l NAA and no GA_3). Even it had a continuous ascending height curb, the value at day 33 is the lowest from all range.



	Day 7	Day 12	Day 19	Day 26	Day 33
CONTROL_PM	0,15	0,34	0,96	1,43	4,18
X1 _PM	0,21	0,49	1,13	1,83	1,43
X2_PM	0,25	0,62	1,37	2,13	1,62
X3_PM	0,45	0,88	1,97	2,35	1,86
X4_PM	0,33	0,54	1,15	1,96	2,57
X5_PM	0,39	0,00	1,56	2,26	1,67

Figure 6. Height dynamic for PM_X variant

The dynamic of both variant (PM and VQ) show that the minimum and maximum ranges are similar on PM and VQ (1.43 cm; 4.18 cm), but the growth dynamics on each variant are different as shown in (Figure7).

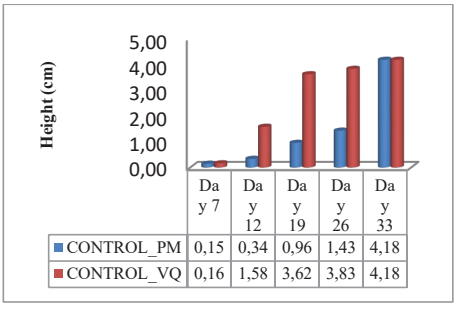


	Day 7	Day 12	Day 19	Day 26	Day 33
CONTROL_VQ	0,16	1,58	3,62	3,83	4,18
X1 _VQ	0,23	0,41	0,66	1,02	1,43
X2_VQ	0,26	0,79	1,52	1,41	1,62
X3_VQ	0,29	0,86	1,39	1,81	1,86
X4_VQ	0,64	1,44	1,95	2,37	2,57
X5_VQ	0,20	0,79	1,68	1,46	1,67

Figure 7. Height dynamic for VQ_X variant

As we see in Figure 8, we have the following significant differences on Control_PM vs. Control_VQ: day 12- very significant diference ($t = 4.458$; $df=54$; $p < 0,001$); day 19 - very significant diference ($t = 6.262$; $df=54$; $p < 0,001$; day 26 - very significant diference ($t = 4.948$; $df=58$; $p < 0,001$).

Also, we observed on day 26 that we have significant difference between X2_PM and X2_VQ, both with 0.5 mg/l GA_3 ($t = 2.878$; $df=58$; $p = 0.005$) (Figure 8).



	Day 7	Day 12	Day 19	Day 26	Day 33
CONTROL_PM	0,15	0,34	0,96	1,43	4,18
CONTROL_VQ	0,16	1,58	3,62	3,83	4,18

Figure 8. Relation between height from Control_PM and Control_VQ

3. Root number.

3.a. PM_X variant. ONE WAY ANOVA show significant difference between variants (p = 0.016 < 0.050) and Post Hoc Test show us there are significant difference between X1_PM (no GA_3) and X4_PM (1.5 mg/l GA_3) (Figure 9)

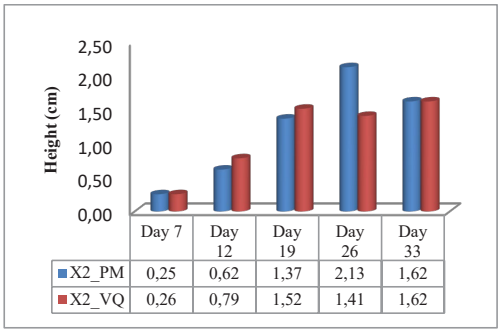


Figure 9. Root number distribution on PM variety on day 19

In day 26 we obtain the similar results as in day 19 (Figure 10), ONE WAY ANOVA show significant difference (F=3.874, p= 0.003) between variants and Post Hoc Test show us difference between X1-X2, X1-X4, X3-X4.

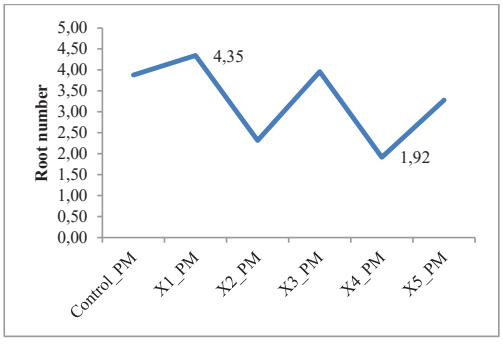


Figure 10. Number of roots PM_X on day 19

For day 33, ONE WAY ANOVA show significant difference (F=7.033, p< 0.001) and from Post Hoc Test we obtain a significant diference (p=0.008) between Control_PM (no hormones) and X3_PM (1.0 mg/l GA_3) (Figure 11)

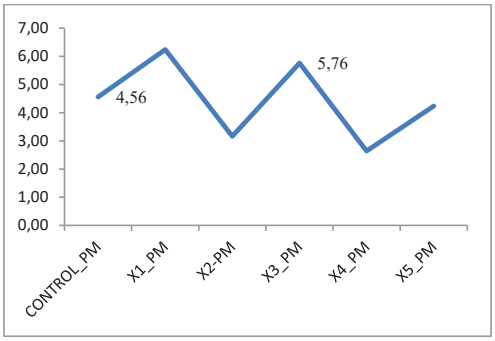


Figure 11. Number of roots PM_X on day 26

3.b. VQ_X variant. In day 7, the p value for ONE WAY ANOVA show very significant difference (p<0.001) but the Post Hoc Test show significant difference only between Control_VQ and X1_VQ (0.25 mg/l BAP, 0.03 mg/l NAA but no GA_3) (p=0.029).

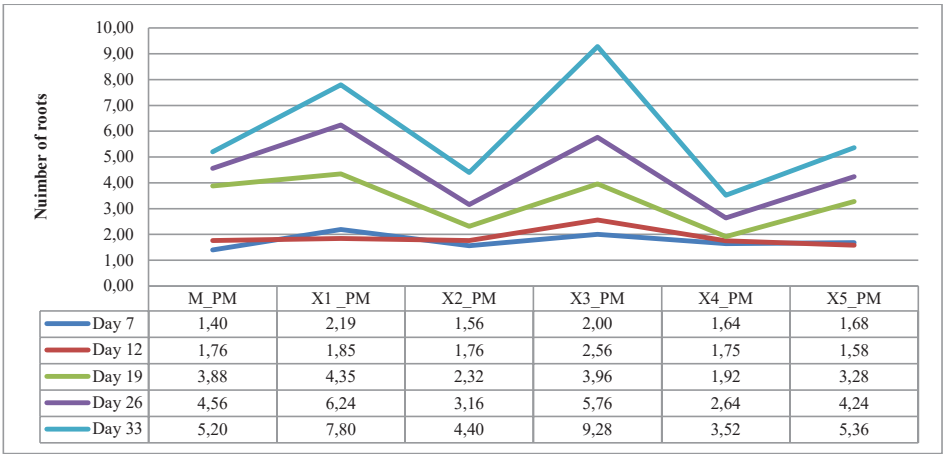
For VQ variant with day 12 to day 33 we have significant difference between variants and Post Hoc Test sow us significant difference between Control and the rest of variants (Table 2).

3.c. *Dynamics of root number*

Regarding dynamic of the roots number on PM_X variant, we observe that we have a sinusoidal evolution of roots number (Figure 12). Regarding dynamic of the number of the roots for VQ_X variant, for the Control we have a logarithmic grows and for the rest we observe a three stage of dynamics (Figure 13).

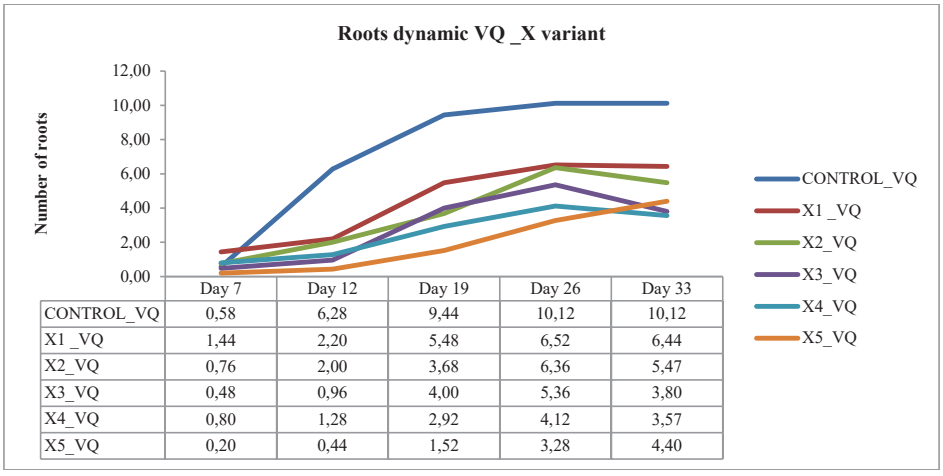
Table 2. Root number distribution for VQ_X

		Day 7	Day 12	Day 19	Day 26	Day 33
	ANOVA	<0.001	<0.001	<0.001	<0.001	<0.001
Post Hoc Test	C-X1	0.029	<0.001	<0.001	0.03	0.02
	C-X2	0.987	<0.001	<0.001	0.02	0.003
	C-X3	0.999	<0.001	<0.001	0.001	<0.001
	C-X4	0.968	<0.001	<0.001	<0.001	<0.001
	C-X5	0.76	<0.001	<0.001	<0.001	<0.001



	M_PM	X1 _PM	X2_PM	X3_PM	X4_PM	X5_PM
Day 7	1,40	2,19	1,56	2,00	1,64	1,68
Day 12	1,76	1,85	1,76	2,56	1,75	1,58
Day 19	3,88	4,35	2,32	3,96	1,92	3,28
Day 26	4,56	6,24	3,16	5,76	2,64	4,24
Day 33	5,20	7,80	4,40	9,28	3,52	5,36

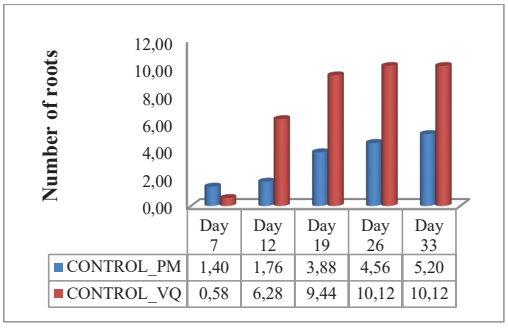
Figure 12. Roots dynamic PM_X variant



	Day 7	Day 12	Day 19	Day 26	Day 33
CONTROL_VQ	0,58	6,28	9,44	10,12	10,12
X1 _VQ	1,44	2,20	5,48	6,52	6,44
X2_VQ	0,76	2,00	3,68	6,36	5,47
X3_VQ	0,48	0,96	4,00	5,36	3,80
X4_VQ	0,80	1,28	2,92	4,12	3,57
X5_VQ	0,20	0,44	1,52	3,28	4,40

Figure 13. Roots dynamic PM_X variant

Regarding number of roots compared on both cultivars PM and VQ, ONE WAY ANOVA show us two distinctive type of differences:
a) between Controls: day 12 – Control_PM vs Control_VQ ($t = 4.462$; $df=48$; $p < 0.001$ /very significant differences; day 19 – Control_PM vs Control_VQ ($t = 4.813$; $df=48$; $p < 0.001$/very significant differences; day 26 – Control_PM vs Control_VQ ($t = 4.623$; $df=48$; $p < 0.001$/very significant differences; day 33 – Control_PM vs Control_VQ ($t = 3.989$; $df=48$; $p < 0.001$/very significant differences (Figure 14)



	Day 7	Day 12	Day 19	Day 26	Day 33
CONTROL_PM	1,40	1,76	3,88	4,56	5,20
CONTROL_VQ	0,58	6,28	9,44	10,12	10,12

Figure 14. Roots number for Control_PM and Control_VQ

b) between variants: day 19 - X5_PM vs. X5_VQ (t = 2.952; df=48; p = 0.005/distinct significant differences); day 26 - X2_PM vs. X2_VQ (t = 2.894; df=49; p = 0.005/distinct significant differences); day 33 - X3_PM vs. X3_VQ (t = 5,269; df=48; p < 0.001/very significant difference (Figure 15)

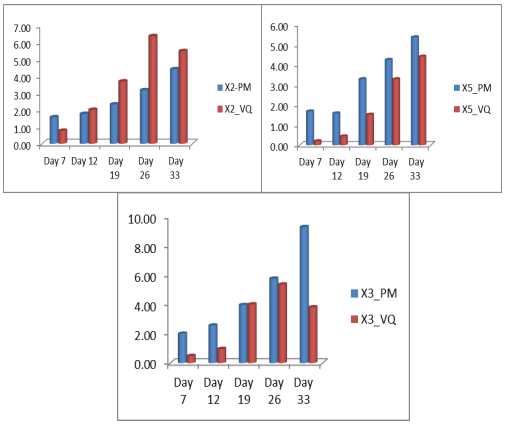


Figure 15. Differences between PM and VQ variants on day 19, day 26 and day 33

CONCLUSIONS

All parameters analysed show us a synergic relation between GA_3, NAA (with 0.03 mg/l) and BAP (0.25 mg/l) and even Control on both varies show better metric values, X3 medium, with 1 mg/l GA_3 had a good average for height for PM cultivar and X4 medium, with 1.5 mg/l GA_3 had a good impact on height average for VQ cultivar. All results will be correlate with other parameters that we follow in out thesis: leaf, callus and secondary shoots (data not shown at this moment).

On the roots experience, X3 variant (0.25 mg/l BAP, 1 mg/l GA_3, 0.03 mg/l NAA) on PM cultivar reached an 9.28 cm average at day 33. For VQ, no variants of GA_3 made significant rates against Control. Regarding roots, comparing dynamics reveal two different type of root evolution, one sinusoidal for PM_X and one logarithmic, for VQ_X, information that will be compared with other two linked experience: V (variation of BAP) and Y (variation of NAA) - data not shown at that moment.

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