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VARIATION OF AUXINS AND CYTOKININES IN MICROPROPAGATION PROTOCOLS OF TWO WORLWIDE IMPORTANT SPECIES: SOLANUM TUBEROSUM AND IPOMOEA BATATAS

Oana VENAT[1], Adrian-George PETICILĂ[2], Cătălina-Ioana NICOLAE[1], Cristian-Mihai POMOHACI[3], Dorel HOZA[2]

[1]Research Centre for Studies of Food and Agricultural Products Quality, University of Agronomic Sciences and Veterinary Medicine, 59 Marasti Blvd, District 1, 0111464, Bucharest, Romania
[2]Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, 0111464, Bucharest, Romania
[3]Faculty of Land Reclamation and Environmental Engineering, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, 0111464, Bucharest, Romania

Corresponding author email: catalinaioana.nicolae@gmail.com

Abstract

Solanum tuberosum and Ipomoea batatas are the third and the seventh world ranking species in terms of yield and consumption and they are relatively cultivated based on micropropagation techniques for limiting viruses spreading and other 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' and their response to variation of auxins and cytokinins on in vitro cultivation. The study compared the effects of basal MS medium containing various concentrations of α-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP) in combination with gibberellic acid (GA3) in micropropagation of those species, using material from a starter culture in vitro induced as well. For Solanum tuberosum, the shoot induction ranged between 4–5 days with variation among NAA concentrations, the longest shoots (9,8 cm), maximum number of nodes (4-5), and maximum number of leaves (10.00) were recorded on 'Purple Majesty' on variant containing 0,25 mg/L BAP + 0.03 mg/L NAA+0,05 mg/L GA_3. For Ipomoea batatas, the shoot induction ranged between 5-6 days with with variation among NAA concentrations, the longest shoots (7,8 cm), maximum number of nodes (3.8 cm), and maximum number of leaves (12.00) were recorded on 'KSP-1' on variant containing 0,25 mg/L BAP + 0.05 mg/L NAA+0,05 mg/L GA_3. The results showed that the combined effect of various concentrations of NAA between 0.01 mg/l and 0,05 mg/L, BAP between 0,25 mg/L and 1.00 mg/L and GA3 could provide solution for extend in vitro production of Solanum tuberosum tubers and potatos for Ipomoea batatas as base materials for industrial cultivation.

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

INTRODUCTION

Potato and sweet potato are major food and non-food crops among tubers and roots countries (Basera et al., 2018). Potato is cultivated in over 150 countries and they have outstanding importance for commercial purposes, the demanding on the market for those two species is higher than last century (statista.com, 2022).

Potato has high nutrition value (Devaux et al., 2021) and roots of sweet potato, behind nutraceutical value, contain a high amount of starch, used as raw material, for animal feeding and beverage industry (Danci et al., 2011; Kapinga et al., 2007). Usually vegetative propagated by shoots, roots, steam cutting, sweet potato propagated like this can be viral or bacterial contaminated and seriously impact mass production (Wondimu et al., 2012).

Propagate vegetative as well, potato can accumulate over 40 viral diseases, bacteria and systemic fungi that can impact production worldwide (Gastelo et al., 2004).

Now days, researches focuses on eradicate viruses from seed material, develop of new cultivars, new breeding lines and stabile techniques for long term storage (Gaba and Singer, 2009).

Plant tissue culture can be use in potato and sweet potato propagation in order establish a virus free material, to retain germplasm lines, offer answers regarding species resistance to all type of stress, or to promote material for breeding (Devaux et al., 2021).

Among plant tissue, micropropagation has the main role and through it is possible to develop protocols for *in vitro* culture establishment (Vinterhalter et al., 1997).

The plant hormones, mainly auxin and cytokinin, are critical for plant regeneration in micropropagation and especially cytokines playing an vital role in shoot organogenesis (Bidabadi and Jain, 2020). For potato, the success of *in vitro* multiplication depends on the presence of a balanced combination or auxins (0.01 mg/L NAA) and gibberellic acid (0.25 mg/L) (Badoni and Chauhan, 2009). Danci (2011) show that best result for regenerate shoots from meristems was achieved with auxins and Gibberellins (1 mg/L IAA, 1 mg/LIBA and 0.3 mg/L GA3) and the lowest results were obtained with cytokines (1 mg/L kinetin and 1 mg/L N6-benzyladenine). Rabbani et al., (2001) studied effects of different concentrations of GA_3 (between 1, 2, 3, 4, 5 mg/L) and BAP (between 0.5, 1, 1.5, 2, 2.5 mg/L) and maximum shoot length was obtained with 4 mg/L GA_3 and maximum number of shoots was obtained with 2 mg/L BAP.

Zang et al. (2005) and Dewir et al. (2020) suggest that increased concentration of indole-3-acetic acid (IAA) increased shoot length and the trigger of IAA action was addition of GA_3 into the medium.

In 2021, Hajare propose a full strength MS medium with variable concentration of BAP (0, 0.5, 1, 1.5 and 2 mg/L with an optimum of 1.5mg/L) combined with different concentration of NAA (0, 1, 2, 3 and 4 mg/L with an optimum of 3 mg/L) was report for shoot initiation; for shoot multiplication medium was supplemented with BAP (1, 1.5, 2, 2.5 mg/l) and kinetin (1, 1.5, 2, 2.5 mg/L). For rooting, same Hajare, combined IBA (0.5, 1, 1.5, 2 mg/L with best results on 1 mg/L) and IAA (0.5, 1, 1.5, 2 mg/l with best results on 0.5 mg/L).

MATERIALS AND METHODS

1. **Plant material and study area**

We used two *Solanum tuberosum* purple cultivars, 'Violet queen' and 'Purple majesty' and three *Ipomoea batatas* cultivars, 'RO-CH-M', 'KSP-1' and 'KSH' Results that will be discussed here refers only on *Solanum tuberosum*, both varieties, data regarding on *Ipomoea batatas* remain unpublished until confirming.

Solanum tuberosum 'Violet queen' (first name 'Hot Purple') – is a Peruvian potato variety, with deep purple skin and high concentration of anthocyanins in the flesh.

Solanum tuberosum 'Purple majesty' is a Peruvian cultivar as well, with remarkable purple flesh colour probably the most intensely colour from all purple potatoes, with exceptional flavour and texture.

2. **Micropropagation**

Solanum tuberosum 'Violet Queen'. Explants were initial cultivated from fresh biological material for 3 months. In order to obtain diseases free explants, we sub cultivativated explants for 3 times on Murashige & Skoog medium without hormones (Figure 1).

First sterilization was fungal decontamination with Aliette 80WG, 0.4% for 20 minutes. Explants were immerse in 70% ethanol for 35 seconds, rinsed with sterile distilled water, dipped in 0.2 mg/L $HgCl_2$ (mercuric chloride) for 4.5 minute. Then we washed for 4 times with sterilised distilled water.

Figure1. Fresh material and *in vitro* cultivate plant material of *Solanum tuberosum* 'Violet queen'

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

Figure 2. Stored *in vitro* material and sub cultivate plant of *Solanum tuberosum* 'Purple majesty'

3. **Experience design**

Cultures were initiated with uninodal segments between 0.5-0.8 cm for all tested varieties from aseptic *in vitro* pre-culture on MS medium supplemented with different concentration of BAP (V variant), GA_3 (X variant) and NAA (Y variant) (Table 2) and control. Variant V has different concentration of cytokine - BAP, between 0 and 1 mg/l (Table 1) and variant Y was designed with different concentration of auxin - NAA, between 0.1-0.5 mg/l.

Table 1. Media combination in different treatments on variant V

Variant	BAP mg/l	GA_3 mg/l	ANA mg/l
control	0	0	0
V1	0	0.05	0.03
V2	0.25	0.05	0.03
V3	0.5	0.05	0.03
V4	0.75	0.05	0.03
V5	1.0	0.05	0.03

Each treatment had five repetitions and five replications. We used culture medium with pH 5.75-5.78, 30 g/l agar and 15 g/l of sucrose in 30 ml container for each.

Table 2. Media combination in different treatments on variant Y

Variant	BAP mg/l	GA_3 mg/l	ANA mg/l
control	0	0	0
Y1	0.25	0.05	0
Y2	0.25	0.05	0.01
Y3	0.25	0.05	0.02
Y4	0.25	0.05	0.03
Y5	0.25	0.05	0.05

Media was autoclaved at 121°C, for 21 minutes. Cultures were inoculated in the laminar flow bench and incubated at 24±1°C under 14h of light. All measurement was done at 7-12-19-26 and 33 days from inoculation.

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

RESULTS AND DISCUSSIONS

Results for this experiment were obtained after 33 days of culturing and dynamic metric observations and present in this paper only cytokines variant.

1. *In vitro* shoot induction and viability of explants. Cultures were initiate from uninodal segment of *Solanum tuberosum* L. 'Purple majesty' (PM) and 'Violet queen' (VQ) varieties. All experiments remain sterile after 10 days post inoculation and allow metric observation until day 33.

2. PM_VQ_V variant. Variation of BAP plus NAA and GA_3 affected shoot number and length of five variants. Significant differences ($p < 0.001$) was observed on variants V2 (0.25 mg/l), V3 (0.5 mg/l) and significant was only V4 (0.75 mg/l) with $p = 0.003$ (Figures 3 and 4).

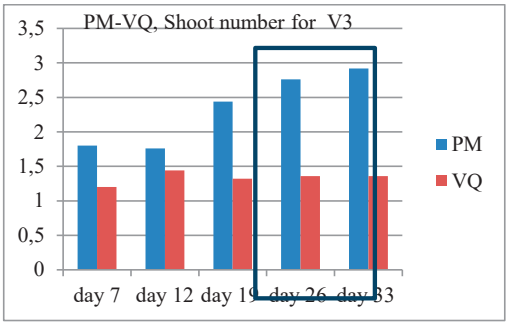


Figure 3. PM-VQ comparative shoot number on V2 (0.25 mg/l)

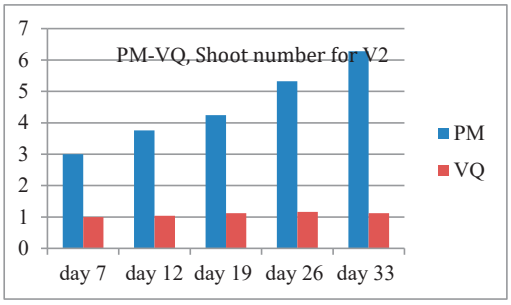


Figure 4. PM-VQ comparative shoot number on V3 (0.5 mg/l)

Regarding the height of explant exposed on BAP variation, Post Hoc test show us significant differences ($p < 0.001$***), starting with day 12: for variant V2 (0.25 mg/l) in day 26 ($p < 0.057$***), and for variant V3 (0.5 mg/l) in day 26 with $p < 0.001$*** (Figure 5).

Regarding number of leaves, ONE WAY ANOVA show us significant differences between PM and VQ cultivars on V2 variant (0.25 mg/l) with $p < 0.001$***, starting with day 19 of observation. The trend is maintained until day 33 (Figure 6).

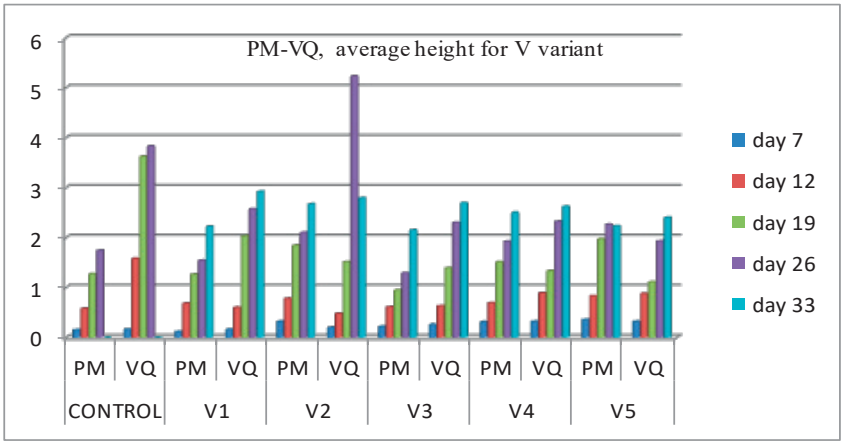


Figure 5. PM-VQ average height for V variant

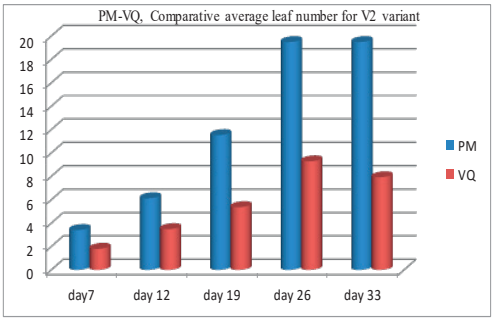
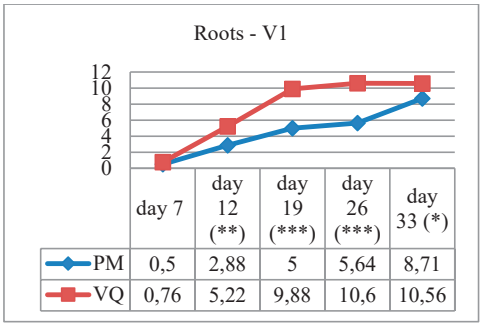


Figure 6. PM-VQ average leaf number for V2 variant

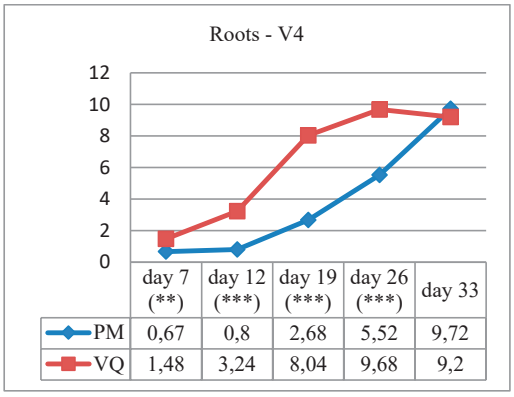
Regarding root system of explants, biometric readings show that on variant V2 (0.25 mg/l) there wasn't any differences between PM and VQ. Instead, significant differences appeared on V1, V3, V4 and V5.



	day 7	day 12 (**)	day 19 (***)	day 26 (***)	day 33 (*)
PM	0,5	2,88	5	5,64	8,71
VQ	0,76	5,22	9,88	10,6	10,56

Figure 7. PM-VQ average roots number for V1 variant

Significant differences ($p < 0.001$***) was recorded starting with day 12: for V1 (0 mg/l) in days 19 and 26 (Figure 7), for V3 (0.5 mg/l) in days 12 and 19 (VQ); and for V4 (0.75 mg/l) for days 12/19/26 (VQ).



	day 7 (**)	day 12 (***)	day 19 (***)	day 26 (***)	day 33
PM	0,67	0,8	2,68	5,52	9,72
VQ	1,48	3,24	8,04	9,68	9,2

Figure 8. PM-VQ average roots number for V4 variant

Regarding auxins experience or variant Y, meta data show us as that the impact of NAA in combination with BAP and GA_3 on the development of explants is major when we compare PM and VQ varieties. Post Hoc test doesn't express any significant difference from Y1(with 0.25 mg/l BAP; 0.05 mg/l GA3; 0.0 mg/l NAA) and Y2 (with 0.25 mg/l BAP; 0.05 mg/l GA3; 0.01 mg/l NAA) on number of shoots, and for Y4 (with 0.25 mg/l BAP; 0.05 mg/l GA3; 0.03 mg/l NAA) and Y5 (with 0.25 mg/l BAP; 0.05 mg/L GA3; 0.05 mg/l NAA) for number of leaves. Instead of this, significant difference ($p < 0.001$***) appear in Y1 (with 0.25 mg/l BAP; 0.05 mg/l GA3; 0.0 mg/L NAA), Y2 (with 0.25 mg/l BAP; 0.05 mg/l GA3; 0.01 mg/l NAA) and Y3 (with

0.25 mg/l BAP; 0.05 mg/L GA3; 0.02 mg/l NAA) regarding the number of roots and biometric measurement confirm this evolution starting even with day 7 (data not show at this moment).

CONCLUSIONS

In this part of study we observe the influence of BAP and NAA on *Solanum tuberosum* cultivars development and grows though micropropagation. Parameters analysed reveal that V2 (0.25 mg/l BAP; 0.05 mg/l GA_3; 0.03 mg/l NAA) increase the number of leaves and V4 (0.25 mg/l BAP; 0.05 mg/l GA_3; 0.03 mg/l NAA) for number of shoots, but discussion need to be done only in relation with cultivar, because thre are different responses on different stimulus and in relation with control.
A discussion need to be done on variant V5 (1 mg/l BAP; 0.05 mg/l GA_3; 0.03 mg/l NAA). Comparison between PM and VQ and statistical analyses show that only on number of roots we can discuses about a significant difference on day 19 and day 26. All other measurable parameters, like number of leaves, height, or number of shoots don't show any difference between development of our cultivars.

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