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Effect of growth regulators on the morphological traits of three potato cultivars in vitro

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Abstract

The experiments were conducted to study the effect of sub cultures (3 sub-cultures) of *in vitro* propagated plants of three cultivars of potato (Arnova, Riviera and Provento) and their interaction with Indole Acetic acid (IAA) at 0.0 , 1.0 mg L^{-1} and Benzyl Adenine (BA) at 0.0, 0.5, 1.0 and 2.0 mg L^{-1} concentrations on morphological traits of vegetative (plant height, number of shoots, leaves , nodes and length of shoots) and roots (number of roots and their lengths) system. The cultures were incubated under temperature of 25 ± 2°C and illumination for 16 h day^{-1}. The plants were transferred to the greenhouse and data were taken on the number of minitubers, their diameter and weight after 90 days. The results showed the effectiveness of IAA at 1 mg L^{-1} increasing in plant height (16.06 and 14.83 cm for Rivera and Provento cultivars, respectively), and in number of leaves and nodes (18.08 leaf $plant^{-1}$ for Rivera and 16.92 nod $plant^{-1}$ for Provento), and the number of roots and their length. (11.25 root $plant^{-1}$ and 11.25 cm for Rivera and Arnova cultivar, respectively). The results also showed the effectiveness of the combination of IAA (1 mg L^{-1}) + BA (0.5 mg L^{-1}) increasing the number and length of shoots (6.50 shoot $plant^{-1}$ and 4.83 cm for Provento and Rivera cultivar). A decrease in the number of roots was also observed with increasing concentrations of BA which added to the medium with or without of IAA. Results also recorded the highest average of diameter and weight of minitubers was 8.63 cm and 3.64 g for the mother plants of the Riviera and Provento cultivars, respectively, while the mother plants and *in vitro* propagated plants of three cultivars did not differ in the average number of minitubers.

Key words: Potato , Morphological traits, Sub cultures, IAA, Minitubers.

*Part of Mse. Thesis of the first author.

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I. INTRODUCTION

The potato crop *Solanum tuberosum* L. comes after rice, wheat and maize and is produced annually in a high proportion of the world's total area, and plays an important role in global food security (Wang et al., 2011). Potato was propagated in two methods, the first sexually through seeds, which is limited to breeding and improvement programs because of the genetic isolations that occur, which negatively affect the traits of the resulting tubers. The second method is asexual (vegetative propagation) by tubers (Al-Sharifi, 2020)), which is widely used in the propagation of most of the desired economically important potato cultivars. One of the most important problems facing potato cultivation in the world is the viral infection, which not only causes a decrease in yield, but also threatens the preservation of cultivars, either for commercial purposes or for breeding and improvement purposes, as obtaining high-quality potato tubers is critical in all potato production areas in the world. world (Morais et al., 2018). Therefore, many researchers have resorted to using the plant tissue culture technique due to high productivity within a small unit area under controlled conditions, as well as the wide production throughout the months of the year without waiting for rainy seasons or the appropriate season for the production, It is also characterized by the high speed of production, where it is possible to obtain hundreds of thousands of tissue plants in a short period of time, with ensuring that they are free from viral diseases. For more than two decades and to the present day this technique has been used successfully for the propagation of highly productive and virus-free genotypes (Otroshy, 2006) and the preservation of the mother plants and the mass production of the plant material of the potato crop (Smulders and de Klerk, 2011). Micrpropagation of potato includes two basic stages, the first one multiplication and the production of *in vitro* plantlets, and secondly the production of mini tubes in the greenhouse (Otroshy, 2006). The success of plant tissue culture requires the availability of medium, with its contents of salts, amino acids, vitamins, sugar and growth regulators (auxins, cytokines and gibberellins), whose presence is very necessary for plant growth, which is positively reflected in the continuity of micropropagation on a large scale .Auxins and Cytokines are the most widely used growth regulators in many studies (Al-Tikriti, 2001 Al-Husseini, 2016; Al-Sharifi, 2020) and they are usually added to the medium with specific concentrations either separately or in different combinations based on the gentotype, micropropagation stage, and the explants. Subculture may lead to phenotypic changes in *in vitro* propagated plants (Smulders and Klerk, 2011), which may affect their genetic stability, which is very necessary for obtaining plants identical to their mothers, which is one of the applications of plant tissue culture technique. Therefore, the aim of this article is to study the effect of sub cultures of *in vitro* propagated plants and their interaction with different concentrations of growth regulators indole acetic acid (IAA) and benzyl adenine (BA)) and its reflection on their morphological traits of the vegetative, root system and minitubers of three cultivars of potato and they are free from any morphological variation..

II. MATERIALS AND METHODS

The experiment was conducted in the Plant Tissue Culture Laboratory - Genetic Engineering Department - Food and Biotechnology Center in the Agricultural Research Department / Ministry of Science and Technology for the year 2021/2022. *In vitro* propagated plants of Potato cultivars (Arnova, Riviera and Provento) were subcultures for three times (3 Sub culture, each Sub culture is conducted every 4 weeks, Fig. 1) in MS medium (Murashige and Skooge, 1962) imported by Indian H Medium at 4.4 $g.L^{-1}$ without growth regulators and supplemented with sugar at 30 $g.L^{-1}$and agar 6

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-7 g.$L^{-1}$for the solidified medium. The cultures were incubated at a temperature of 25 ± 2 and an illumination of 16 h day $^{-1}$. In order to study the effect of Subcultures and its interaction with different growth regulators, *in vitro* propagated plants were cut into stem cuttings at length 1-1.5 cm which cultured jars (250 ml) (with an average of 3 stem cuttings jar $^{-1}$) containing 30 ml of the MS medium, with different concentrations of indole acetic acid (IAA) were added at 0.0 and 1.0 mg L^{-1} and overlapped with different concentrations of benzyl adenine. (BA) (0.0, 0.5, 1.0 and 2.0) mg L^{-1} with 5 replicates (jars) for each cultivar. The cultures were incubated under the previous conditions for 4 weeks. Data were taken on the morphological traits of the vegetative (plant height, number of shoots, length of shoots, number of leaves, number of nodes) and roots (number and length of roots).

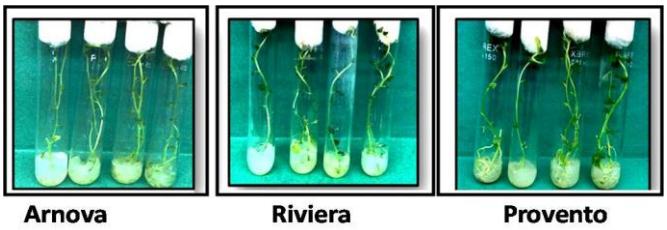

Figure (1). *in vitro* propagated plants after three sub cultures of three cultivars of Potato planted in MS medium free hormone.

field performance

In vitro propagated and mother plants of three cultivars of Potato were transferred to the greenhouse after acclimatization for two weeks under laboratory conditions (temperature 25 °C ± 2 and indirect lighting,).They were planted in nylon bags of 1 kg capacity, with 5 replications for each cultivars, then covered with plastic sheets to provide moisture for a period of two weeks, after which the covers were lifted, then watered and fertilized according to the fertilizer recommendation. Measurements were taken about the number, diameter and weight of the minitubers after 90 days of planting.

Statistical analysis

Factorial experiments were conducted using the Completely Randomized Design (CRD) with five replications, and the data were analyzed statistically using the statistical program Genstat. Means were compared using the Least Significant Difference (LSD) test at a probability level of 5%.

III. RESULTS AND DISCUSSION

The effect of growth regulators on the morphological traits of the vegetative and root system of *in vitro* propagated plants (after three sub cultures).

Results in Table 1 showed that the three cultivars interaction with IAA and BA significant effect in the average of plant height,. It seems clear that Rivera and Provento were significantly excelled on this trait when they were grown in the medium supplemented with 1 mg L^{-1} IAA at average lengths of 16.06 and 14.83 cm, respectively, which differed significantly from the rest of the interactions, followed by Arnova cultivar at 12.25 cm, which did not differ significantly about the two cultivars Rivera and Provento planted in the control treatment (without growth regulators) at rates 11.67

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and 11.58 cm, respectively (Figure 2) . On the other hand, the plant height decreased with an increase in BA concentrations when compared with the control treatment and the combination of 1 mg L^{-1}. IAA + 2 mg L^{-1} BA.

With regard to the interaction, the different behavior of the cultivars to add IAA and BA in the medium appears, where the excelled of the Provento cultivar in the medium with 0.5 mg L^{-1} BA was 6.50 shoots plant $^{-1}$ Which did not differ significantly in the stem cuttings of the same cultivar in the medium supplemented with 2.0 mg L^{-1} BA. This result may explain the role of cytokinin in increasing cell division, especially meristematic cells, and thus increasing the volume of different tissues of explants grown in medium (Delloloio, 2007) as well as the hormonal balance within the plant tissue (Singh and Kuar, 2011). As for the cultivar Arnova, its behavior differed in that trait, where it excelled in the control treatment (free hormone) with an average number of shoots 5.42 shoots plant $^{-1}$, which did not differ significantly when the medium with 2.0 mg L $^{-1}$ BA (4.67 shoot.plant^{-1}), and the cultivar Rivera gave average numbers of shoots 4.58, 4.42 and 3.75 shoot.plant^{-1} at 2, 0.5 and 1 mg L^{-1} BA, respectively. While the lowest average number of shoots was 1.17 plant $^{-1}$ in the comparison treatment (without addition) in which the cultivar Rivera and Provento were grown. As for the shoot length (table 1) showed susceptibility of the cultivars in that trait according to the addition of IAA and BA in the medium, where the cultivar Rivera gave the highest average length of 4.83 cm in the medium with 0.5 mg L^{-1} BA + 1 mg L^{-1} IAA .Followed by Provento cultivar at 0.5 mg L^{-1} BA, then the Arnova cultivar at 1 mg L^{-1} BA at rates of 4.58 and 4.08 cm, respectively. In the number of leave trait the results showed that excelled of the cultivar Rivera grown in the medium added 1 mg L^{-1} IAA at an average 18.08 leaf.plant^{-1}.It was followed by the Provento cultivar with an average of 17.00 leaf.plant^{-1}, which differed significantly from the rest of the interactions. Also, the Arnova cultivar at control treatment gave 12.67 leaf.plant^{-1}. The lowest number of leaves was 1.83 leaf .planat^{-1}at 0.5 mg L^{-1} BA of Arnova cultivar (table1). The same table showed that the triple interaction also had a significant effect on the number of nods, as the two cultivars Rivera and Provento were excelled at 1 mg L^{-1} IAA, with an average 16.92 nods .plant $^{-1}$, respectively, which differed significantly from the rest of the interactions. Whereas, the lowest rate of the number of nodes was 1.25 nods .plant $^{-1}$ at 1 mg L^{-1} IAA and 2 mg L^{-1} BA in which the cultivar Arnova was grown. With regard to the triple interaction on the number of root trait, the significant effect of adding IAA and BA to the medium is noted Whereas, the cultivars Rivera, Provento and Arnova gave rates of 11.25, 11.25 and 11.17 root plant $^{-1}$, respectively, in the medium supplemented with 1 mg L^{-1} IAA compared to the control treatment (6.42, 6.00 and 2.00 root plant^{-1}) from the side, the obvious decrease in the number of roots appears with an increase in the concentration of BA added to the medium, with or without IAA, In the length of roots trait the triple interaction had a significant effect on this trait, and it was noticed that the cultivars behavior differed in the addition of IAA and BA in the medium supplemented with 1 mg L^{-1} IAA (11.25, 10.42 and 10.42 cm) compared to the control treatment (4.46, 3.17 and 3.17 cm) for Arnova ,Rivera and Provento respectively, on the other hand, the obvious decrease in root length appears with an increase in the concentration of BA added to the medium, with or without IAA .

Table (1) Effect of cultivars and interaction of IAA and BA on vegetative and root morphological characteristics

IAA mg L $^{-1}$	BA mg L $^{-1}$	Cultivars	Plant height (cm)	NO. shoot	Shoot length (cm)	NO. leaves shoot^{-1}	NO. nodes shoot $^{-1}$	NO. Roots shoot $^{-1}$	Root length (cm)

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0.0	**0.0**	**Arnova**	٧,٦٧	٥,٤٢	٢,٢٨	٤,٤٢	٣,٩٢	٢,٠٠	٤,٤٦
		Riviera	١١,٦٧	١,١٧	١,٢٩	١١,٠٨	١٠,٠٠	٦,٤٢	٣,١٧
		Provento	١١,٥٨	١,١٧	٠,٩٦	١٠,٦٧	٩,٥٨	٦,٠٠	٣,١٧
	0.5	**Arnova**	٣,٣٣	١,٥٠	٠,٥٨	١,٨٣	١,٦٧	٢,٩٢	٢,٥٠
		Riviera	٤,٣٣	٣,٠٨	٢,٩٢	٧,١٧	٥,٩٢	٣,٥٠	٧,١٢
		Provento	٥,٧٩	٦,٥٠	٤,٥٨	٦,٩٢	٦,١٧	٢,٩٢	٣,٤٦
	1.0	**Arnova**	٣,٨٣	٣,٩٢	٤,٠٨	١٠,٧٥	٦,١٧	٥,٠٠	٥,٠٨
		Riviera	٤,١٧	٣,٧٥	١,٥٠	٦,٢٥	٤,٣٣	٢,٠٠	١,٥٠
		Provento	٣,٢٥	٢,٦٧	٢,٠٤	٣,١٢	٣,٠٠	٣,١٧	١,٩٦
	2.0	**Arnova**	٤,١١	٤,٦٧	٣,٠٠	٩,٥٠	٦,١٧	٣,٤٢	٣,١٠
		Riviera	٣,٤٢	٤,٥٨	٢,٧١	٩,٨٣	٧,٧٥	١,٥٨	٢,١٧
		Provento	٣,٢٥	٣,٥٠	٢,٩٦	٥,٩٢	٤,٦٧	٢,٠٠	١,٦٣
1.0	**0.0**	**Arnova**	١٢,٢٥	٢,٣٣	٢,٠٨	١٢,٦٧	١٠,٨٣	١١,١٧	١١,٢٥
		Riviera	١٦,٠٨	٣,٢٥	٣,٢٥	١٨,٠٨	١٦,٩٢	١١,٢٥	١٠,٤٢
		Provento	١٤,٨٣	٢,٥٠	٣,٢٥	١٧,٠٠	١٦,٩٢	١١,٢٥	١٠,٤٢
	0.5	**Arnova**	٢,٥٤	٢,٩٢	٠,٨٣	٢,٥٨	٣,٠٨	١,١٧	١,٠٠
		Riviera	٥,٨٣	٤,٤٢	٤,٨٣	١١,٠٠	٦,٢٥	٢,٢٥	١,٩٦
		Provento	٢,٤٢	١,٩٢	٢,١٢	٣,٥٨	٢,٧٥	١,٦٧	٠,٧٩
	1.0	**Arnova**	٢,١٧	٢,٥٠	١,٢٥	٥,٢٥	٢,٨٣	١,٠٠	١,٧١
		Riviera	٣,١٣	٣,١٧	١,٨٣	٥,٠٨	٢,٧٥	١,٢٥	٠,٩٦
		Provento	١,٣٣	١,٦٧	١,٢٥	٢,٠٨	١,٤٢	١,٦٧	١,٢٩
	2.0	**Arnova**	١,٠٨	١,٥٠	٠,٦٣	٢,٠٠	١,٢٥	١,٠٠	٠,٨٨
		Riviera	٣,١٣	٣,٢٥	٢,١٧	٥,٩٢	٣,٣٣	٢,٠٨	٢,٤٦
		Provento	١,٣٣	١,٦٧	١,٢٥	٢,٠٠	٢,٧٥	١,٦٧	٠,٧٩
	LSD 0.05		٢,٢١	٢,٥٤	١,٥٠	٣,٧٢	٢,٥٨	١,٥٣	٢,٥٠

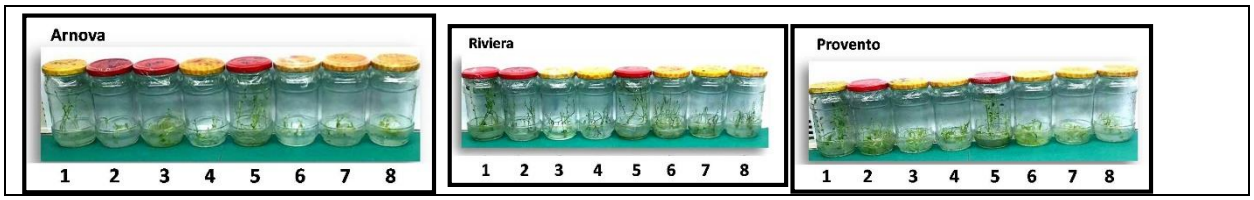

Figure 2. Response of stem cuttings of *in vitro* propagated after three sub cultures of three cultivars of Potato to different concentrations of BA (mg L-1) and their interaction with IAA (mg L-1). **1:** 0 + 0, **2 :**0 IAA + 0.5 BA, **3:** 0IAA + 1 BA, **4:** 0 IAA + 2 BA, **5:** 1 IAA + 0 BA, **6** :1 IAA + 0.5 BA, **7:** 1 IAA + 1 BA, **8:** 1 IAA + 2 BA.

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Morphological traits of minitubes.

The results of the statistical analysis in table 2, Figure 3 indicate that there is no significant difference between the *in vitro* propagated plants (after three sub cultures) and the mother plants of the three studied cultivars in the average number of minitubers. The results also showed significant differences in the average of the diameter and weight of minitubers resulting from *in vitro* propagated plants and the mother plants,, where the mother plants of the cultivar Rivera excelled by recording the highest tubers diameter of 8.63 cm followed by the Provento cultivar with a diameter of 8.29 cm, which did not differ significantly from the Provento cultivar. An average of 6.44 cm. In the weight trait of minitubers, the mother plants of the Provento cultivar excelled with an average 3.64 g, which differed significantly from the rest of the plants of the cultivars followed by the mother plants of the cultivar Rivera, with an average 2.69 g. It appears from the results of the same table that the cultivar Arnova gave the lowest average diameter of minitubers 2.29 cm for *in vitro* propagated and mother plants, and the lowest weight 0.18 g for in vitro propagated.

Table (2). Morphological traits of Minitubers of *in vitro* propagated after three sub cultures and mother plants of three cultivars of Potato cultured in green house.

Plants	Number (tuber plant-1)	Diameter (cm)	Weight (g)
***in vitro* propagated plants of Arnova**	٢,٣٣	٢,٢٩	٠,١٨
***in vitro* propagated plants of Riviera**	٢,٦٧	٣,٥٣	٠,٨٣
***in vitro* propagated plants of Provento**	٢,٣٣	٦,٤٤	١,١٥
Arnova - mother plants	٢,٦٧	٢,٢٩	١,٠٩
Riviera - mother plants	٣,٣٣	٨,٦٣	٢,٦٩
Provento - mother plants	٢,٣٣	٨,٢٩	٣,٦٤
	L.S.D. 0.05		
	Number	**Diameter**	**Weight**
	NS	٣,١٨٦	٠,٨٤١

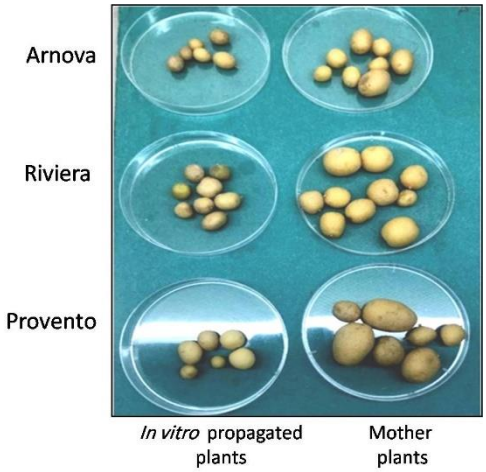

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Figure 3. Production Minitubers from *In vitro* propagated after three sub cultures and mother plants of three cultivars of Potato cultivars after 90 days under green house condition.

IV. DISCUSSION

It dealt with many previous studies (Al-Salji, 2022; AL-Hussaini; et al., 2015, Nadia and Adila, 2018; Al-Sharifi, 2020) the importance of studying the morphological traits of the vegetative and root system of *in vitro* propagated plants which gives an indication of the extent of the response of those plants to tissue culture technique and the effectiveness of the various types of growth regulators added to the medium in stimulating growth and division, which is reflected in these traits. Based on this, it became clear from the results that the effectiveness of IAA auxin at a concentration of 1 mg L^{-1} in increasing plants height (16.06 and 14.83 cm for Riviera and Provento cultivars) and number of leaves and nodes (18.08 leaves.plant^{-1} for Rivera cultivar and 16.92 node plant^{-1} for Provento cultivar and the number and length of roots (11.25 roots and 11.25 cm for Rivera and Arnova cultivars, respectively) This result can be explained by its effectiveness in promoting the vegetative shoots to root by stimulating the division the initial cells of root based on its internal or added in the medium. It encourages the emergence of adventitious roots through de-differentiation, in which cells divide to form the origin of the root, which continues to grow and develop until the roots primordium, which grows outer form the adventitious root. In addition to the role in increasing the number of meristem at the base of the vegetative shoot treated by de- differentiation process of specialized tissues and their converted into meristematic cells, which contributes to an increase the number of formed roots (Hartmann *et al*., 1997). Auxins also increase their size and speed the assembly of rooting compounds (Zahlout, 2006) and increase the flexibility of cell walls to cause an increase in their elongation and large size by stimulating and increasing the activity of many enzymes that are responsible for increasing the flexibility of cell walls and increasing their permeability, which is reflected in increasing the lengths of the roots (Taiz and Zeiger, 2010). The results also showed the effectiveness of the combination consisting of 1 mg L^{-1} IAA + 0.5 mg L^{-1} in increasing the number and length of shoots (6.50 plant shoot^{-1} and 4.83 cm for Provento and Rivera cultivar, respectively), as it seemed that the low concentration of BA was more appropriate in stimulating shoot number and length due to stimulatory action of the BA in inducing cell division and differentiation (Rezadost *et al*., 2013) As for the high concentrations, it adversely affected its presence with IAA compared to addition alone in the medium, and this explains its effect in reducing the role of endogenous auxin which responsible for elongating cells in the longitudinal axis and thus reducing the length of the vegetative shoots (Khazali, 2016). In addition, no abnormal cases appeared in plants in all of them except short plants which are due to the effect of high concentrations of benzyl adenine in cell division and reducing the role of auxin in the medium based on its chemical structure, length of the side chain and having three double bonds that increases its efficiency in stimulating cell division (George *et al*., 2008). With regard to field performance , the results showed that the plants, whether mother or *in vitro* propagated , did not differ in the number of minitubers, While the mother plants excelled in the traits of tuber diameter and weight, this result could be explained to the fact that the *in vitro* propagated plants have a different metabolism from those grown in the field and with throw feeding, which depends on the nutrients available in the medium containing salts, vitamins and amino acids and hormones, and thus they are completely dependent on those nutrients (Chandra *et al*., 2010), When transferred to the field, these plants must adapt to those conditions, which are stressful in multiple ways (Teixeira *et al*., 2017).These stresses may be the reason behind this, on the other hand, no

abnormal observation in the plants whether at the level of vegetative growth or minitubers resulting from *in vitro* propagated for third sub cultured in medium supplemented growth regulators.

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