Int. J. Agricult. Stat. Sci. Vol. 16, Supplement 1, pp. 1137-1141, 2020 www.connectjournals.com/ijass
DocID: https://connectjournals.com/03899.2020.16.1137 ISSN : 0973-1903, e-ISSN : 0976-3392
ORIGINAL ARTICLE

EFFECT OF BA ON INITIATION AND MULTIPLICATION OF *ATROPA BELLADONNA* L. SHOOT TIPS PRODUCED *IN VITRO* AND CONTENT PROTEIN

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Abstract : The study included the cultivation of shoot tips of belladonna *Atropa belladonna* L. resulting from tissue culture on the MS media with different concentrations of BA and protein content, and the results indicated that the highest average number of branches was 5.73 branches /explant of the shoot tipss on MS media supplied with 0.75 mg / L BA after 8 weeks of cultivation and obtained the highest protein content of 0.49 mg / g of shoot tips on MS media supplied with 1.00 mg / L BA after 8 weeks of implantation.

***Key words*:** *Atropa belladonna* L., Shoot tips, Protein content, Tissue culture.

Cite this article

Hiba Nawaf Ahmed (2020). Effect of BA on initiation and multiplication of *Atropa belladonna* L. shoot tips produced *in vitro* and Content Protein. *International Journal of Agricultural and Statistical Sciences.* DocID: https://connectjournals.com/03899.2020.16.1137

1. Introduction

Tissue culture technology is one of the biotechnologies that played and still plays an important role in humans lives, especially in the field of propagation of many types of plants because of the advantages of this method, perhaps the most important of which is obtaining huge number of plants without pathogens and similar to the mother plant at relatively short time and at any time of the year, in addition to using this technology in research and fields applications including plant breeding and improvement, the production of medicinal drugs and medicines and rapid cloning propagation, which is one of the applications of great importance that is followed by different methods of differentiation and morphogenesis such as the formation of adventitious buds, stimulation of the growth of axillary buds and the development of asexual embryos (somatic embryos) as well as the study of the primary aspects of plant growth and development and secondary metabolism [Kadim *et al*. (2019)].

Atropa belladonna L. known as Belladonna belongs to the Solanaceae family, which includes 90 genera and 2000 species of plants and is central and southern Europe and the original homeland of the plant and from it spread to Central and Western Asia, to the Himalayas and to Morocco and Algeria in the south, and cultivated in England, France and the United States of America. Belladonna is a plant known from ancient times, as the first diagnosis of the plant was in 1504 CE [Saadeddin *et al*. (2005)].

The belladonna plant is a perennial shrub herbaceous plant with thick roots and simple stalked leaves facing opposite at the top of the plant and reciprocating in the lower part of it is oval or cardiac shape with a sharp top and a smooth edge and a dark green color. The flowers are small, individually or double rarely, they come out from the simplest leaves and their shape is bell and the color is light crimson green, the fruits are small spherical cherry in color green to turn red, black at maturity and contain a lot of seeds with a gray-yellow color, the plant height is 100 cm and sometimes up to 150 cm.

Received *January 02, 2020* ***Revised*** *June 08, 202* ***Accepted*** *July 29, 2020*

Phytohormones are considered the most important endogenous substances for modulating physiological and molecular responses, a critical requirement for plant survival as sessile organisms [AL-Taey *et al*. (2010), AL-Taey *et al*. (2014)]. Cytokinines known to significantly improve the growth of crop plants, there are many researchers conducted about Cytokinines ability to improve the plant growth by enzyme activation [AL-Taey *et al*. (2018)].

The plant multiplies with seeds and the best date for sowing seeds is the beginning of spring in temperate regions and the beginning of summer for cold regions, and they are difficult to germinate due to the hardness of the seed cover and germination occurs during several weeks after planting [Al-Mukhtar and Ali (2019)].

The aim of this study is to obtain the highest average number of branches and the highest protein content from shoot tips culture resulting from tissue culture on MS media with different concentrations of BA.

2. Materials and Methods

The seeds of Belladonna plants obtained from the Medicinal and Aromatic Plants Unit of the College of Agriculture, University of Baghdad were used, as were placed under running water for 15 minutes, then washed with washing powder for 5 minutes with continuous stirring, then washed by placing them in a filter under running water for a 5 minutes, then transferred to the laminar hood and add 25% v : v NaCl solution for 15 minutes, then washed with distilled and sterile water for three consecutive times for 5 minutes each time. After the sterilization process was completed, the seeds were transferred to sterile Petri dishes in which filter paper was left for five minutes to dry, thus the seeds became ready for planting and then they were planted in free MS media. After seed germination and reaching the age of 3-4 weeks, shoot tips were cut for 1 cm in length for proliferation, a 250 ml glass vials were used to plant the vegetable parts with 40 ml of culture media at pH 5.7 and the bottle was covered with aluminium foil, sterilized. The culture media autoclaved at 121°C. and pressured 1.04 kg / cm^2 for 20 minutes, after planting the vegetable parts, the explants were transferred to the growth chamber under the intensity of illumination of 3000 lux and daily succession 16 hours of light followed by 8 hours of darkness equipped with white fluorescent tubes and a temperature of 25±2°C. Complete Randomized Design was used in a statement analysis T-tests were compared to the averages by polynomial Duncan's multiple range test below the level of Dunkin 5% probability [Alrawy and Khalafallah (1980)]. Each treatment consisted of ten replicates, and each duplicate contained one vegetable portion.

2.1 Proliferation and Multiplication Stage

The first stage of transplantation for 4 weeks was considered a proliferation stage, in which the explants (shoot tips) were cultivated on MS media with different concentrations of growth regulators and the these cultures (Reculture) were transplanted for another four weeks on the same MS as they were considered a multiplication stage and data was recorded. On the vegetative part and its development at the end of each stage. This stage included the study of the effect of adding BA to the MS media in concentrations (0.0, 0.25, 0.5, 0.75, 1.0) mg/L in the proliferation and multiplication of the shoot tips taken from seedlings resulting from tissue culture and their data were taken after 4 and 8 weeks of cultivation.

2.2 Estimating Protein Content in Vegetative System

The protein content was estimated according to the method of Lowry *et al*. (1951) and modified by Schacterle and Pollack (1973). This method is summarized by the interaction of the protein with the Folin reagent to give a blue complex as a result of the interaction of tryptophan and tyrosine with phosphomolybdo tungstic acid and read absorbance at a Spectro photometer. The wavelength is 650 nm and the intensity of absorption is proportional to the color concentration and BSA was used as standard solution.

Taking 1 gram of the vegetable total and crushing it in a ceramic mortar containing 5 ml of Trichloro acetic acid solution the samples were placed in 100 ml glass vials in a snow bath and placed in the rocking incubator at a speed of 100 rev/min/one hour and then harvested with a Hettich centrifuge (EBA, 35) centrifuge at a speed of 5,000 rpm for 5 minutes, the leachate is neglected and the remaining residue was washed 5 times using 5% TCA solution with resettlement each time under the same preceding conditions, then added to the precipitate 10 ml of 1 standard solution of sodium hydroxide, mix well and put in a rocker incubator for 24 hours, then harvested by centrifuge speed of 3000 rev/min for 10 minutes, then taken filtrate completes its size to 10 ml by adding sodium hydroxide solution

1n. Determine the total protein intake in the extract by taking 0.1 ml of filtrate + 0.9 ml distilled water and add 1 ml of the copper base reagent and mix well, then leave the tubes for 10 minutes at room temperature, then add 4 ml of Folin reagent, shake the tubes well and then it was placed in a water bath at 55°C for 5 minutes, after which the tubes were left to cool to room temperature, then the absorbance was measured at a wavelength of 650 nm with a spectrophotometer (1000 Sertescectil 1021) and the total amount of protein was determined using the standard curve resulting from use gradient concentrations between 0.0 - 400 g/mL of BSA.

3. Results and Discussion

Table 1 shows that the shoot tips of Belladonna grown on MS media equipped with different concentrations of BA in addition to the control treatment responded to the tissue cultivation by 100% and that cultivation when treating 0.75 mg/L BA resulted in obtaining the highest average number of branches 4.37 branch/explant was significantly superior to the rest of the treatments and cultivation when comparing treatment gave the highest average length of branches and number of leaves as it was 5.00 cm and 8.00 leaves / explant respectively for this stage (proliferation), when re-culturing another 4 weeks (multiplication stage) formed the shoot tips cultivated at the medium supplied with 0.75 mg/L BA, the highest mean number of branches, as it was 5.73 branches/explant were significantly superior to the rest of the transactions, and callus was created at 80% and with the highest amount of callus developed (Fig. 1) and when the comparison treatment gave the highest average length of branches and number of leaves, it was 5.17 cm and 8.30 leaves/ explant, respectively. The results indicate the rooting of the shoot tips when treating the comparison 100% at a rate of 15 roots/explant, as the table shows the protein content in the shoot tips cultivation on the solid MS medium equipped with different concentrations of BA 8 weeks after culture, where the explant parts were given at treatment 1. 00 mg/l BA. The highest protein content is 0.49 mg/g.

The results of Table 1 explain the multiplication of cultivated parts that BA is one of the cytokines that play a role in controlling apical dominance and consequently the number of branches increased and as a result of the state of balance between internal hormones and added growth regulators the highest values were obtained and that the increased

Table 1: Effect of BA on the proliferation and multiplication of shoot tips of Belladonna *Atropa belladonna* L. resulting from tissue culture on MS solid after 4 and 8 weeks of cultivation.

	Proliferation stage (4 weeks later)			**Multiplication stage (8 weeks later)**						
BA (mg.L)	**Av. Branches No.**	**Av. Length (cm)**	**Av. Leaves No.**	**Av. Branches No.**	**Av. Length (cm)**	**Av. Leaves No.**	**Callus formation (%)**	**Callus volume**	**Root formation**	**Av. Root no./ branch**	**Protein content (mg.g)**
0.0	1.50e	5.00a	8.00a	1.53e	5.17a	8.30a	0.0b	-	100	15.00	0.48
0.25	1.63d	2.00e	7.00b	1.71d	3.00d	7.30b	70a	++	0.0	0.0	0.22
0.5	4.00c	3.50b	6.00c	5.00b	5.00b	6.71c	70a	++	0.0	0.0	0.13
0.75	4.37a	2.73d	3.78e	5.73a	3.00d	5.30e	80a	+++	0.0	0.0	0.37
1.0	4.10b	3.23c	5.00d	c4.47	3.54c	5.40d	80a	++	0.0	0.0	0.49

* Values with similar characters for each factor or their interactions individually are not significantly different according to the Dunkin Multipliers test below the 5% probability level.

Fig. 1: Effect of BA on multiplication of shoot tips of Belladonna, *Atropa belladonna* L. resulting from tissue culture on MS solid after 8 weeks of cultivation.

concentration leads to a decrease. The values are for the number of branches because its effect becomes inversive [Smith (2000) and Hopkins and Hiiner (2004)] and the composition of the branches and leaves of the cultivated parts when comparing in the proliferation stage is due to the internal content in the tissues of the plant part of plant hormones [Murashige and Skoog (1962)] and explanation of increased lengths and number of sheets. Different factors indicate the effect of cytokines in the division and elongation of cells, which in turn is reflected in the characteristics of growth as well as its effect on building nucleic acids [Wasfy (1995)]. The appearance of callus in some treatments indicate that the development of callus on the plant piece depends on internal hormones, while some of the plant parts do not respond to the development of callus unless some growth regulators are added to the medium, mainly due to the internal level of their growth hormones and the growth regulators that regulate the creation of callus [Muhammed and Omar (1990)].

Acknowledgment

The authors are very grateful to the Alnoor University College for providing the facilities, which helped to improve the quality of this work.

References

Al-Mukhtar, Sarab Abdulhadi Mohamed Hussain and Moslim Abd Ali (2019). Effect of growth regulator in micropropagation of Catharanthus roses *in vitro*. *Journal of Physics: conference Series*. 1294 092031.

Al-Rawy, Khasha Mahmoud and Abdul Aziz Mohammed Khalaf Allah (1980). *Design and analysis of agricultural experiments*. Dar Al Kutub Printing Press, Mosul University, Iraq.

AL-Taey, D.K.A. and A.H. Saadoon (2014). Effect of treatment of Salicylic acid and water Salinity on the Growth and Nitrate Accumulation with nitrate reductase activity in the Leaves of Spinach, *Spenacia oleracea* L. *Journal of Babylon University, Pure and Applied Sciences*, **3(22)**, 1188-1203.

AL-Taey, D.K.A., S.S.M. Al-Azawi and M.H. Husien (2010). Effect of Spraying Acetyl Salicylic Acid on the Plant Tolerance for Salt Stress & Survival Percentage after Transplanting of Orange (*Citrus sinensis*). *Babylon Journal University-Pure and Applied science*, **18(4)**, 1513-1520

AL-Taey, D.K.A., A.K. Mijwel and S.S. Al-Azawy (2018). Study efficiency of poultry litter and kinetin in reduced effects of saline water in *Vicia faba*. *Research J. Pharm. and Tech.,* **11(1)**, 294-300.

Hopkins, W.G. and N.P.A. Hiiner (2004). *Introduction to Plant Physiology*. Third Edition. John Wiley and Sons. Inc

Kadim, Z.K., M.J.H. AL-Shareefi and M.L. Sabreen (2019). Effect of growth regulators on *in vitro* micropropagation of blue honeysuckle (*Lonicera caerulea* L.). *Res. on Crops*, **20(3)**, 635-641.

Lowry, O.H., N.J. Rosebruogh, A.L. Farr and R.J. Randall (1951). Protein measurements with the Folin reagent. *J. Biol. Chem*., **193**, 265-275.

Mohammed, Abdulmutallab Sayed and Mubashir Saleh Omar (1990). *Key concepts in plant cell and organ transplantation*. Directorate of Books House for Printing and Publishing, University of Mosul, Iraq.

Murashige, T. and F.A. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum*, **15**, 473-497.

Saad Eddin, Shorouq Muhammad Kazim, Adil Yousef Nasrallah and Medhat Al-Sahuki (2005). Growth, yield and alkaloids of belladonna. *Atropa belladonna* L. Effect of planting and seedling dates on the characteristics of growth and yield of plants in the open field. *Iraqi Agricultural Science Journal*, **36(1)**, 75-80.

Schacterle, G.R. and R.L. Pollack (1973). A simplified method for the quantitative assay of small amount of protein in biological materials. *Ann. Biochem*., **51**, 654-655.

Smith, R.H. (2000). *Plant Tissue Culture*. Academic Press. Har. S. and Tec. C., San Diego.

Wasfy, Imad Al-Din Hussein (1995). *Regulators of growth and flowers and their use in agriculture*. Academy. Cairo, Egypt.