Current Trends in OMICS
Volume 1 Issue 2, Spring 2021
ISSN(P): 2221-6510 ISSN(E): 2409-109X
Journal DOI: https://doi.org/10/32350/cto
Issue DOI: https://doi.org/10/32350/cto.12
Homepage: https://journals.umt.edu.pk/index.php/CTO/index

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Journal QR

Author(s): Erum Dilshad, Ayesha Asif, Hina Arooj, Samra Hayat Khan, Syeda Marriam Bakhtiar

Affiliation: Department of Bioinformatics and Biosciences, Capital University of Science and Technology (CUST), Islamabad, Pakistan

Article History:
Received: September 14, 2021
Revised: October 27, 2021
Accepted: November 08, 2021
Available Online: December 31, 2021

Citation: Dilshad E, Asif A, Arooj H, Khan SH, Bakhtiar SM. Impact of BAP on in vitro regeneration of potato (*solanum tuberosum L.*). *Curr Trend OMICS.* 2021;1(2):67–79. https://doi.org/10/32350/cto.12/04

Article QR

Erum Dilshad

A publication of the
Department of Knowledge and Research Support Services University of Management and Technology, Lahore, Pakistan

Impact of BAP on *in vitro* Regeneration of Potato (*Solanum tuberosum L.)*

Erum Dilshad*, Ayesha Asif, Hina Arooj, Samra Hayat Khan, Syeda Marriam Bakhtiar

Department of Bioinformatics and Biosciences, Faculty of Health and Life Sciences, Capital University of Science and Technology (CUST), Islamabad, Pakistan

Abstract

The potato (Solanum tuberosum L.) plant is grown in about 150 countries of the world and is considered an important food crop. However, this crop is susceptible to different biotic and abiotic factors, which can affect its crop yield. This vulnerability can be reduced or eliminated by growing potatoes under sterilized conditions. Cytokinins, such as 6-Benzylaminopurine, are proven to show a significant role in the in vitro regulation of plants. In the current study, explants of Kuroda variety were/potato cv. Kuroda were grown using diverse concentrations of 6-Benzylaminopurine (BAP), which displayed varied results. BAP concentration of 0.01 mg/l showed a 10 cm shoot length with 41 shoots having 66.66% regeneration efficiency. Meanwhile, the explant grown in 0.25 mg/l BAP concentration showed 16 cm shoot length with 65 shoots having 83.33% regeneration efficiency. On the other hand, the explants that were grown using 0.05 mg/l and 1 mg/l BAP concentration showed 7 cm and 10 cm shoot length with 35 and 52 shoots having 63.33% and 76.66% regeneration efficiency, respectively. Therefore, it was concluded that 0.25 mg/L of BAP showed the best results with the highest number of shoots and shoot length as well as maximum regeneration efficiency among all the tested concentrations.

Keywords: 6-Benzylaminopurine, micropropagation, *Solanum tuberosum L.*, tissue culture

Introduction

The tissue culture technique has become a renowned substitute tool for the micropropagation of plants in recent years. Being an emerging technology,

*Corresponding Author: dr.erum@cust.edu.pk

the micropropagation of plants greatly influenced both the agricultural and industrial sectors of the world. It is also being used to meet the ever-growing world demand. Additionally, it has also greatly contributed to the field of agricultural sciences and has become an essential tool in contemporary cultivation. Using this technique, we can generate several clones and choose the required traits from a solitary seed or explants as well as determine the amount of space needed for their field trials. We can also eradicate plant bugs by using a wide array of sterile techniques. These biotechnological tools offer various valuable prospects for crop yield improvement under a sterile environment [1].

This technique encompasses a very tensile, rapid reproduction of plants due to a high proliferation rate in a very small period [2]. Tissue culturing has become a potential biotechnological tool of *in vitro* clonal propagation and is now a commercially viable method used to grow a wide range of herbaceous and woody plants [3]. For the first time, in 1960, Morel used this technique for orchid multiplication. With present-day technology, this technique is applicable to various plants and is proven to be an effective tool that speeds up the production of superior class disease-free plantlets, in terms of genetic and physiological consistencies [4]. The effect of cytokinins and auxins combination on *in vitro* regeneration of plants is reported to be effective [5]. BAP plays a variety of roles in plant development during the creation and functioning of shoot meristems. It is also involved in increasing shoot proliferation and elongation. Additionally, it is also known to promote the production of new shoots and dormant lateral buds [6].

These techniques have been practically and commercially demonstrated/applied on many diverse plant species, including fruit and vegetable plants. Potato is one of the most important non-cereal food crops globally and is considered a major food source for mankind. The proliferation of potatoes occurs asexually from tubers, which are used to cultivate potatoes in new areas. This form of proliferation threatens the upkeep of its genotypes for breeding or commercialization purposes. For this reason, the yield of this solanaceous plant is very low. Furthermore, since the use of the botanical seed exchange and conservation of germplasm

of potatoes is next to impossible, the utilization of orthodox approaches does not seem reasonable. In all potato-producing areas, the demand for high-quality tubers is steep since they all want to improve their crop yield Thus, the biotechnological techniques, grounded on tissue culture are imperative [7].

Micro-propagation can be a good substitute for the orthodox proliferation of potato since it can be used to produce uniform and identical potato seed material. Furthermore, large-scale clonal material can easily be produced in a short time duration by using a technique, which involves low-cost components. Micro-propagation used for commercial seed production has transferred potatoes from test tubes to the field. However, some viral, fungal, and bacterial diseases still hamper potato production. In this regard, researchers showed that some viruses can decrease the yield by 40% singly, and in combination with other viruses, the loss can range up to 90% [8].

Conversely, vegetative propagation of potatoes is done on a commercial scale. Though, this type of reproduction can instigate numerous viral, bacterial, and fungal infections in plants, causing yield and vigour losses. Thus, it is essential to use high phytosanitary, physiological, genetic quality, and virus-free propagative material to guarantee the maximum yield potential of the plant.

Hence, plant tissue culture can be useful to the potato production chain since it efficiently propagates the material of choice, upkeeps germplasm banks, and enables genetic exchange. It can also be used to study interactions with biotic and abiotic factors, which, in turn, can be used to yield genetically engineered plants and disease-free seed potatoes.

Methodology

This study aimed to identify the most optimized BAP concentration for *in vitro* shoot regeneration of *Solanum tuberosum*. The methodology of the research is given below:

Sterilization

Fungal, bacterial, and viral contaminations are the biggest hurdles standing in the way of establishing or optimizing the tissue culture protocol for potatoes. The growth of these microorganisms can be controlled by

removing the contaminated material by previously sanitizing the explants. We used Sodium Hypochlorite (NaOCl), which is a bleaching agent, as a sterilizing agent for the experiment. Apart from that, 70% ethyl alcohol was also utilized to accelerate the sterilization process [9]. Every empty piece of glassware including pipettes, test tubes, culture vessels, Petri dishes, and small instruments, such as scalpel and forceps, were autoclaved at 121° C under at least 15 psi of pressure for 20 minutes. Additionally, the work area, namely the laminar airflow cabinet, was switched on to sterilize the area. Then, it was wiped with the help of 70% ethyl alcohol. Next, it was sterilized using UV for 20 minutes. In this study, plant shoots were disinfested by immersing them in 70% alcohol for 20 seconds. Next, they were immersed in 1.5% bleach solution with 0.1% Tween-80 for 20 minutes. Afterwards, they were rinsed with sterilized deionized water [10].

Micropropagation Conditions

The potato plant "Kuroda"/potato cv. Kuroda was utilized as an explant. After sterilization, shoot tips were inoculated on MS medium supplemented with more cytokinin for differentiation into shoots. Afterwards, these multiplicated shoots were moved to the fresh medium containing cytokinin. Murashige and Skoog (MS) medium was used to tissue culture the potato (*Solanum tuberosum L.)* plants (Sigma Aldrich, M5519) [11]. The MS media composition was adjusted by adding the stock solutions that contained micronutrients, macronutrients, vitamins, and organic supplements. All of the components were added to the distilled water in a flask and mixed properly. The pH was adjusted to 5.8. Benzylaminopurine (BAP) phytohormone was used to regenerate shoots from the explant. It was prepared under sterilized conditions. For shoot regeneration media (SRM), BAP was added in the MS medium (pH 5.8) with the help of a micropipette. Then, it was autoclaved at 121° C under at least 15 psi of pressure for 20 minutes. After autoclaving, it was transferred into jars placed in a laminar flow hood. Four different concentrations were used as mentioned in Table 1. MS media without any BAP concentration was used as a control. At the establishment and multiplication stages, plants were kept in a growth chamber with a controlled temperature (25-27ºC) and light (14-16 h photoperiod) [7].

Table 1. Shoot Regeneration Media

S/No.	Medium	Concentration of BAP (mg/L)
1.	Control	0
2.	SRM1	0.01
3.	SRM2	0.25
4.	SRM3	0.05
5	SRM4	1

Inoculation of Explant and Sub-culturing

The Kuroda/potato cv. Kuroda was inoculated into the MS medium having different concentrations of BAP. This process was used to grow the micro plants along with a control group (plant group without BAP added in the media). The explant (a portion of shoots) was cut with the help of a surgical blade and was inoculated aseptically into the media with the help of forceps. Sterilized conditions were maintained in the laminar airflow cabinet throughout the experiment. The explants were carefully selected since it is difficult to cultivate small explants.

The size of the explant was 1-1.5 cm. A total of 3 explants per jar were inoculated. The jars were then sealed and kept in a growth room at 21±2˚C under light with a 16h photoperiod. After every two weeks, the explants were shifted to a fresh culturing medium. The growth and development of the tissue cultured shoots were monitored by observing the cultures at regular intervals in the culture room. Based on the observations under aseptic conditions, the explants were transferred to fresh media with the help of sterilized forceps in a laminar flow hood. After sub-culturing, they were placed back into the culture room. All the experiments were carried out in triplicate, while its data was obtained as mean value and standard deviation was calculated.

Shoot Regeneration Efficiency

Shoot regeneration efficiency of each medium was calculated after four weeks of inoculation of explant. The shoot's efficiency of regeneration was calculated by using the formula for Shoot Regeneration Efficiency [12].

$$Shoot\ Regeneration\ Efficiency = \frac{No.\ of\ shoot\ regenerated}{\text{Total no. of shoots inoculated}}\ x\ 100$$

Precautions for Making Sure the Aseptic Conditions

Every aseptic manipulation and inoculation was done in the laminar airflow cabinet to maintain aseptic conditions. All instruments including needles, forceps, and scapula were pre-sterilized. Then, the sterilization with the help of 70% ethyl alcohol was done. Afterwards, the flaming and cooling were performed in the laminar airflow cabinet. Hands were sterilized by wiping with 70% ethyl alcohol. All materials used for the experiment including the Petri dishes and the glass plates were sterilized in the autoclave. At this stage of the experiment, the flaming of the necks of the culture vessels was done before closing them. It was made sure to maintain aseptic conditions while doing every action so that all sorts of contamination can be avoided at any cost [9].

Results and Discussion

The conditions were optimized for potato shoot regeneration in the current study. The explants of potato cv. Kuroda were cultured on MS media using different concentrations of BAP. The data was analyzed after four weeks of culture. Experiments were carried out in triplicate to examine the explant's ability to form shoots. Four different types of regeneration media were used along with a control group. The media were supplemented with different concentrations of phytohormone, namely BAP. By keeping in view the days to shoot initiation and frequency of shoot regeneration, one simple but efficient shoot regeneration medium was selected from all four media so they can be used in the final experiments. A shoot tip can be generated from 500 to 1000 plants or even more, depending on the cultivar or the shoot tip. Bandinelli et al. (2013) reported that in Asterix, Macaca, and SMINIA793101-3 potato clones, a 50% reduction in the MS salt concentration during propagation raises the in vitro survival rate [13]. The growth of explants along with their shoot length, no. of shoots (inoculated and generated), and regeneration efficiency are given in Table 2 and Figure 2, 3 and 4. The explant cultured on MS basal medium was used to demonstrate the elongation of shoot and formation of delicate roots. It was determined that BAP concentrations caused varied effects on explant

multiplication. A maximum number of micro shoots were recorded at 0.25 mg/L of BAP. According to the results of the study, MS medium supplemented with 0.25 mg/L of BAP had the longest shoot length per explants in comparison with other concentrations. Figure 1 shows the growth of the explant on four different media supplemented with different concentrations of BAP. No shoot response was observed on control media.

Table 2. Growth of Explant on Different Shoot Regeneration

Media	BAP (mg/L)	Shoot Length (cm)	No. of Shoots Inoculated	No. of Shoots Regenerated	Regeneration Efficiency (%)
Control	0	0	0	0	0
SRM1	0.01	10	30	20	66.66
SRM2	0.25	16	30	25	83.33
SRM3	0.05	7	30	19	63.33
SRM4	1	9	30	23	76.66

Figure 1. Shoots Regeneration on SRM1 Medium (A), SRM2 (B), SRM3 (C) and SRM4 (D)

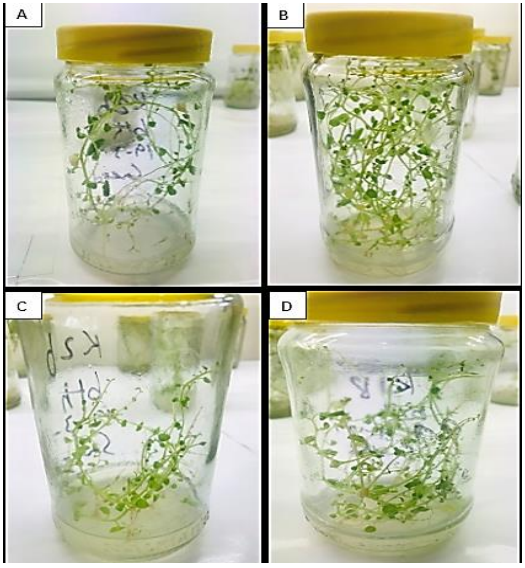
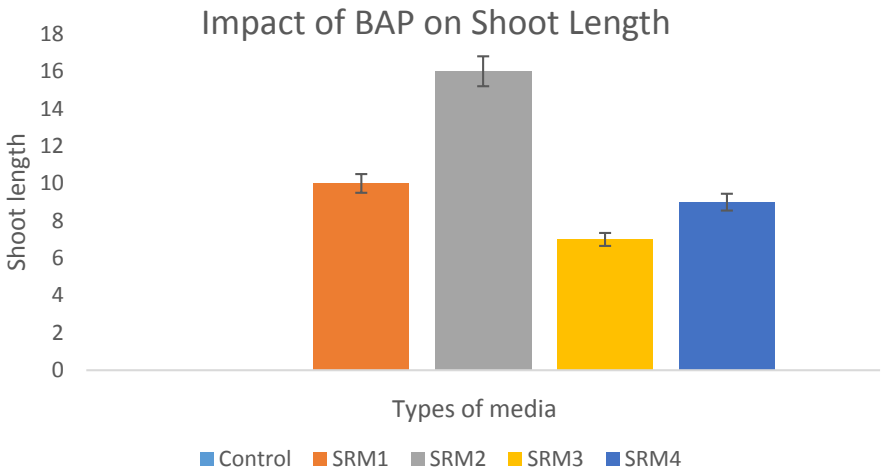
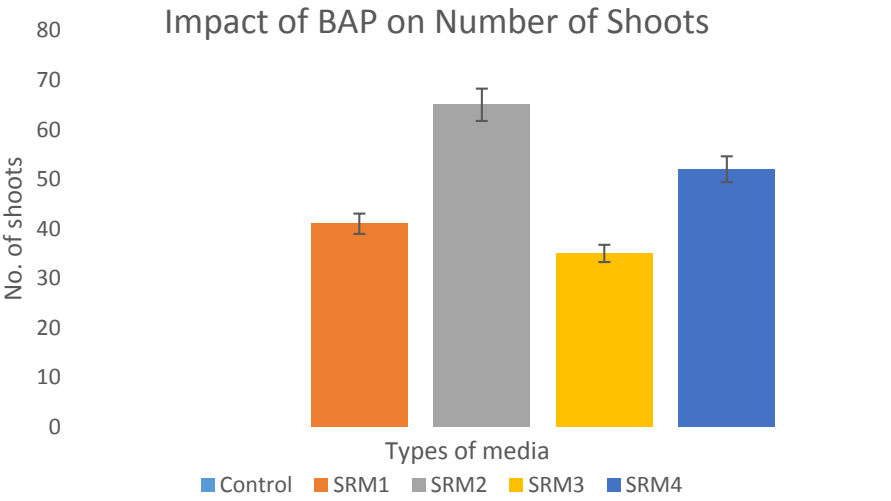


Figure 2. Shoot Length on Different Shoot Regeneration Media



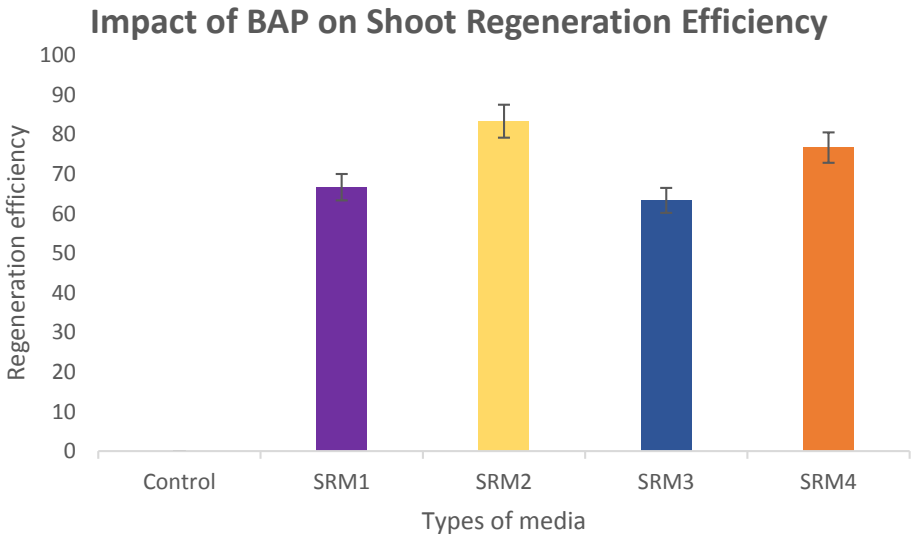
SRM stands for shoot regeneration media. Error bars indicate standard deviation as data was obtained in replicates.

Figure 3. Number of Shoots on Different Shoot Regeneration Media



SRM stands for shoot regeneration media. Error bars indicate standard deviation as data was obtained in replicates.

Figure 4. Shoot Regeneration Efficiency of Different Shoot Regeneration Media



SRM stands for shoot regeneration media. Error bars indicate standard deviation as data was obtained in replicates.

Cytokinins are a class of plant growth regulators that stimulate plant development. Benzyl amino purine is a first-generation synthetic cytokinin that stimulates fruit maturity and promotes plant growth as well as development by speeding up cell division. It plays an important function in plant development, regulation of shoot formation and multiplication as well as cell division and expansion [14]. In a previous report, the maximum number of shoots of potato (*Solanum tuberosum L.)* were induced by BAP alone, indicating that BAP at various doses enhances bud proliferation followed by shoot multiplication [15]. Another report states that the potato regeneration efficiency can be improved by using BAP concentrations ranging between 0-2.5 mg/L (9). Previously BAP at the concentration of 2.5 mg/L was found to be effective in initiating shooting response in *Artemisia carvifolia Buch* and *Artemisia annua* [16, 17]. Another report states that BAP at the concentration of 1mg/L displays the best shoot regeneration response in *Brassica oleraces* L. [18].

Conclusion

The production of disease-free plants from conventional methods has always been a challenge due to different abiotic and biotic factors. We can overcome this problem by producing disease-free potatoes using micropropagation. In this study, different concentrations of BAP were tested to find the most optimized concentration that produces the best results. It was concluded that 0.25 mg/l of BAP displayed 16 cm shoot length with 65 shoots having 83.33% regeneration efficiency. This proves that cytokinins play a very important role in the regulation of plant growth. Future researchers may utilize the above-optimized micropropagation to study the genetic transformation of potatoes having different genes. It can also be utilized to study the impact of different elicitors on the nutritious content of the potato plant.

Acknowledgement

We are thankful to the ORIC department and the competent authority of the Capital University of Science and Technology (CUST) for the funding of the current research project. We are also thankful to Dr. Muhammad Aish from NARC and Dr. Arshia Amin Butt from CUST for helping us to obtain the desired variety of potato plants.

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