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# EFFECT OF BAP ALONG WITH 2.4, D ON THE INDUCTION OF SHOOTS FORMATION ON POTATO CULTIVAR ARIZONA IN VITRO

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## Abstract

This research delves into the intricate interplay between two key growth regulators, BAP (6-benzylaminopurine) and 2,4-Dichlorophenoxyacetic acid (2,4-D), investigating their collective influence on shoot induction in the in vitro cultivation of the esteemed potato cultivar Arizona. The study is meticulously designed to unravel the nuanced impact of these regulators on shoot initiation within a controlled laboratory setting. Potato micropropagation stands as an essential facet of agricultural advancement, and the stimulation of efficient shoot formation serves as a pivotal step in this process. The unique growth characteristics of Cultivar Arizona provide an ideal platform for evaluating the synergistic effects of BAP and 2,4-D on the initiation and proliferation of shoots.

By meticulously observing and quantifying the response of potato explants to varying concentrations and combinations of BAP and 2,4-D, this study aims to discern optimal conditions conducive to enhanced shoot development. Through detailed analysis and observation, the research seeks to contribute valuable insights into refining in vitro cultivation methodologies for potato propagation, aiming at elevating efficiency and yield.

***Keywords: Potato micropropagation, BAP (6-benzylaminopurine),2,4-Dichlorophenoxyacetic acid (2,4-D) ,Shoot induction,Cultivar Arizona***

## I. INTRODUCTION

.loalkaloids, which are hazardous to humans and animals, although this information is not widely known. A glycoalkaloid called solanine is present in potatoes. When consumed in large enough quantities, it can irritate the gastrointestinal tract, affect the neurological system, and perhaps induce teratogenic or birth abnormalities. Some neurological symptoms may manifest as involuntary urination, weakening of the muscles, coma, convulsions, or ataxia.

Various glycoalkaloids are produced by potatoes. Of them, solanine is the most famous because of its association with food poisoning. Between thirty and eighty percent of the solanine in potato tubers forms on or near the epidermis. Solanine is a glycoalkaloid that has three sugars attached to its steroidik alkaloid nucleus. The presence of solanine in plants makes it the most potent cholinesterase inhibitor in food *(Amini, A., et al. 2019).*

Plantlet regeneration from potato callus, in vitro germination of seeds and sprouts as explants, and induction of callus from various components of germinated in vitro plantlets are all topics covered in this study. Cytokinin and auxin-supplemented media was used for in vitro cell proliferation of the regenerate plantlets. The cytokinin utilised for micropropagation of regenerated shoots was BA, while the auxin utilised was IBA. Root initiation was carried out in medium supplied with IBA, whereas multiplication was carried out in media supplemented with BA and 2,4-D. Using varying concentrations of soilless media

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in a polyhouse, shoots grown in a controlled environment were allowed to harden. The overall population's average survival rate was 60%( Chen et al. 2018).

- **Arizona America:** "The state of Arizona is one that is located in the countries southwest. Admitted to the Union on February 14, 1912, it is the 48th state. Arizona is renowned for having a variety of landscapes, such as mountains, canyons, forests, and deserts. Phoenix is Arizona's largest city and capitol. The state's hot summers and moderate winters are frequently attributed to its desert climate. Native American tribes can be found in Arizona, a state rich in culture that draws tourists with its natural wonders like the Grand Canyon and its cultural hotspots like Sedona.

- **Plant Hormone:** Plants produce a molecule called a "plant hormone" that controls a number of physiological functions and growth responses. These substances, often referred to as phytohormones, are essential for directing and coordinating many aspects of plant growth, such as cell division, elongation, differentiation, and responses to external stimuli.

- **2,4 D2:** 4-D belongs to the phenoxy herbicide family and is a synthetic herbicide. The compound known by its chemical name is 2,4-dichlorophenoxyacetic acid. It is a common tool for controlling broadleaf weeds in horticulture, forestry, and agriculture. Because 2,4-D has selective herbicidal activity, it mostly targets and suppresses broadleaf plants while largely ignoring grasses.

- **BAP:** It is an acronym for 6-benzylaminopurine, a synthetic member of the plant hormone class known as cytokinin. BAP is frequently utilized in agriculture and plant tissue culture. Being a cytokinin, BAP is essential for controlling several physiological functions in plants, such as organogenesis, shoot initiation, and cell division. BAP is frequently employed in plant tissue culture to promote cell division and the growth of shoots from explants, or plant tissues removed for culture.

## II. Literature Review

**Ahir et al. (2021)** researched the impacts of 2,4-D (2,4-dichlorophenoxyacetic corrosive) and BAP (6-benzylaminopurine) blends on Desiree cultivar in vitro shoot recovery. Their research, which was published, sheds light on the precise hormonal combinations that potatoes need in order to regenerate shoots effectively.

**Amini et al. (2019)** conducted research on the impact of medium composition and sucrose concentration on the production of microtubers and in vitro shoot regeneration in the Agria cultivar. Their research, which was published in *Plant Cell, Tissue, and Organ Culture*, provides important information regarding the best circumstances for shoot regeneration and the subsequent development of micro tuberculosis.

**Chaudhary et al. (2020)** Within the scope of the research project, the 'Kufri Himalaya' cultivar was subjected to an investigation into the impact that various medium compositions and light intensities had on the process of in vitro shoot regeneration. The findings of this study, which were published in the Journal of Applied Research on Medicinal and Aromatic Plants, shed light on environmental conditions that have an effect on the successful regeneration of shoots.

**Chen et al. (2018)** this study focused on the influence that the quality of the light had on the in vitro regeneration of shoots and the growth of plantlets in the Superior cultivar. The findings of their research, which were published in Plant Cell, Tissue, and Organ Culture, contribute to a better understanding of the ways in which particular light conditions influence the process of regeneration in potato cultivars.

**Dinesh & Singh (2020)** We investigated the effect that light intensity and photoperiod have on the development of plantlets and the regeneration of shoots in the Kufri Himalaya cultivar through in vitro experiments. Their study, which was published in the Potato Journal, makes a significant contribution to

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the optimisation of light conditions, addressing a crucial component in improving the effectiveness of shoot regeneration.

**Gupta et al. (2019)** An investigation of the process of shoot regeneration and multiple shoot induction from nodal explants of the Kufri Himalini cultivar was carried out. This investigation was carried out using genetic transformation that was mediated by Agrobacterium tumefaciens. This study, which was published in the Journal of Applied Research on Medicinal and Aromatic Plants, presents genetic transformation as an innovative method for the regeneration of shoots, hence broadening the scope of opportunities for crop development.

**Hasan et al. (2021)** Within the scope of their research, they evaluated the effects of varying concentrations of agar and amounts of sucrose on the in vitro regeneration of shoots and tuberization of the Cardinal cultivar. Their findings, which were published in Plant Cell, Tissue and Organ Culture, shed light on the function that agar and sucrose play in influencing shoot regeneration and tuberization, which contributes to the optimisation of tissue culture procedures.

# III. MATERIALS AND METHODS

**MS media preparation-** For the purpose of developing materials for in vitro research, a medium formulation referred to as MS medium, as described by Murashige and Skoog (1962), was used. All of the necessary stocks for making the medium were prepared and put in the fridge for later use. On hand are a variety of vitamins, hormones, inositol, MS major (10x), MS minor (200x), Fe-EDTA (100x), and other nutrients. This is described in detail below,

**Table 1:** MS major-10 X

| Name | Chemical Formula | Mass (g) |
|---|---|---|
| KNO3 | Potassium nitrate | 19 |
| NH4NO3 | Ammonium nitrate | 16.5 |
| CaCl2.2H2O | Calcium chloride | 4.4 |
| MgSO4.7H2O | Magnesium sulphate | 3.7 |
| KH2PO4 | Di hydrogen ortho | 1.7 |

Mix 100 millilitres of the solution with one litre of the medium.

**Hormones-**There was a 1 mg/ml stock concentration of all the growth hormones that were synthesised. The hormone was dissolved using either 0.1 N NaOH or 0.1 N HCl after an exact weighing. Using glass distilled water, the final volume was made up. We kept all of the growth hormones in the fridge at 40 degrees Celsius.

- **BAP (Benzyl amino purine)**

One hundred milligrammes of succinate benzyl amino acid Mix with 100 cc of water and add to the medium as needed.

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- **2,4-D**

1-methyl-2-chloro-phenoxyacetic acid 100 milligrammes Mix with 100 ml of water and add to the lit of medium as needed.

Rhizo Biotech of Ludhiana, Punjab, supplied the potato tubers of the Kufri Pokhran type. After half an hour of treatment with 20 ppm GA3 and 24 hours of covering with 70-micron clear polyfilm, the potato tubers were sprouted. It only takes three or four days for tubers to sprout. The experimental material was established using sprouts that were about 15-20 mm in size. The following items were brought to the laminar air flow hood: sterile distilled water, surgical blade, cotton chips, forceps, a 100 ml measuring cylinder and beaker, initiation medium, 70% alcohol, various concentrations of sodium hypochlorite, and different concentrations of HgCl2. To kill any residue particles or antifungal buildups, the explants were washed multiple times with sterile refined water that contained a drop of laboline cleanser to lessen surface strain. The first wash was with raw water.

**1.1. Callus Induction of Tomato**

**1. Impact of various explants and hormones pertaining to callus formation of potato**

**a.** The impact of utilising 2,4-D in potato varieties with shoot tip explants on callus induction Dear Pukhraj,

Media containing fluctuating groupings of 2,4-D were used to assess the impact of shoot tip explants on callus enlistment of potato (assortment Kufri pukhraj). The centralizations of 2,4-D used were 0.0, 1.0, 1.5, 2.0, 2.5, and 3.0 mgs/lit. In particular, ten culture containers containing in vitro-developed shoot tip explants were used for each treatment. The lifestyle were kept in an improvement chamber with a temperature of 28±2 ℃ and a photoperiod of 8 hours of light and 16 hours of shadowiness, with a light power of 2000 lux.

In order to induce callus formation, the cultures were documented 60 days later. Callus colour, callus texture, degree of development, and percentage of explants induced callus were the variables studied in the data.

**b.** Impact of Explant Type, 2, 4-D, and BAP on Kufri Pukhraj Potato Callus Induction.

This study aims to determine how the Kufri pukhraj potato variety responds to various explants, 2,4-D, and BAP in terms of callus acceptance. Separate synthetic compounds, as 2,4-D (2 mg/lit) and BAP (2 mg/lit), were added to the media. Ten culture holders were utilized for each treatment, and the explants utilized changed from root and shoot tips to leaf plates and internodes. The lifestyle were kept in an improvement chamber with a temperature of 28±2 ℃ and a photoperiod of 8 hours of light and 16 hours of lack of clarity, with a light force of 2000 lux. To instigate callus development, the way of life were reported 60 days after the fact. Explant kind, callus type, callus level of improvement, calli tone, and normal new weight of calli (mg) were the factors concentrated on in the information.

**1.2. Regrowth of plants from calluses**

**1. In vitro multiplication of regenerated plants of potato**

**a.** Effects of 2,4-D and BAP on in vitro shoot multiplication of potato variety (K. pukhraj)

Nodal cuttings of in vitro derived regenerated shoots were utilised as explants and put onto experimental media to evaluate the influence of varying doses of 2,4-D and BAP Regarding the expansion and maturation of plantlets during in vitro shoot multiplication of the potato variety Kufri pukhraj. Various quantities of 2,4-D (0.0, 1.0, 1.5, 2.0, 2.5 mgs/lit) and BAP (0.1 mg/lit) were added to the MS media. There were ten culture jars used for each concentration, and they were all different for the different treatments. Units of milligrammes per litamate denote all hormone concentrations. The samples were situated in a growth chamber kept at a temperature of 28±2 ℃, subjected to an 8-hour light and 16-hour

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darkness photoperiod, and exposed to a light intensity of 2000 lux. Number of shoots per calli, shot length, and percentage of shoot formation were reported 60 days following inoculation.

# IV. Result and discussion

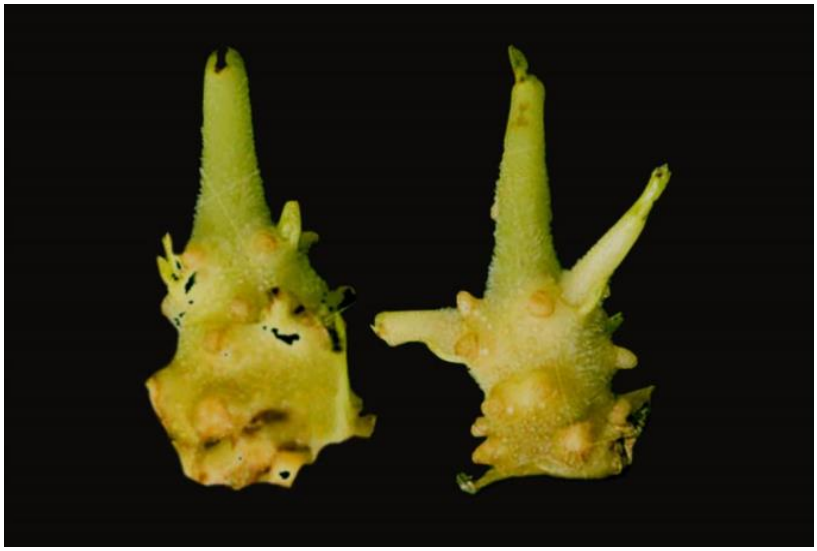

**Figure 1: Potato Sprouts Explants**

### 1.3. Callus Induction of Tomato

**1. Hormone and explant effects on potato callus induction**

**a.** The impact of a shoot tip explant on the induction of callus in a potato variety employing 2,4-D Kufri Pukhraj

To explore what different centralizations of 2,4-D meant for the callus acceptance interaction of the Kufri pukhraj potato assortment, scientists utilized media containing shoot tip explants at convergences of 0.0, 1.0, 1.5, 2.0, 2.5, and 3.0 mgs/lit. Information is displayed in table 7 (Plate-4), and in vitro developed shoot tip explants were used for each treatment.

Based on the data in the table, it is evident that 2, 2.5, and 3 mg/lit of 2,4-D were the most efficient concentrations for inducing huge callus with a loose, watery texture and greenish or white colour. The callus was moderate in size, loose in texture, and yellowish green in colour when the 2,4-D dosage was 1.5%. However, calluses with a compact texture and a yellowish hue were produced by very small amounts of 2,4-D, specifically 1.0%.

- **Plate 2**

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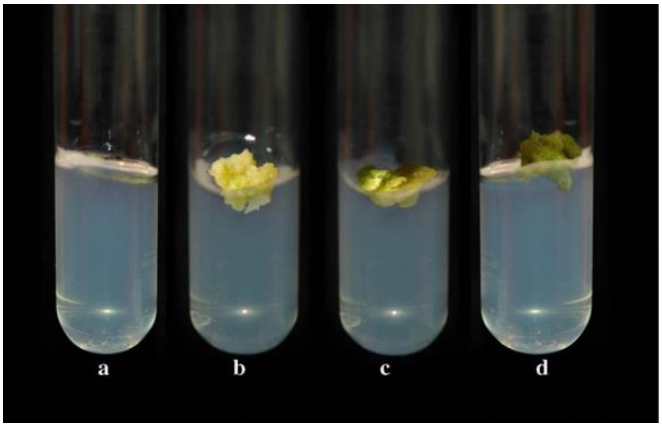


**Figure 2:** The effects of 2,4-D on the initiation of callus formation with various explants (a)Root, (b) Hypocotyle, (c)Cotyledon, (d)Internode

**Table 2:** impact of 2,4-D-induced callus induction on shoot tip explant in potato diversity Kufri Pukhraj

| Sr. no. | 2,4-D (mgs) | % explants formation of callus | Texture of callus | Callus color | Degree of callus formation |
|---|---|---|---|---|---|
| 1 | 0.0 | -- | -- | -- | -- |
| 2 | 1.0 | 60 | Compact | Y | + |
| 3 | 1.5 | 87 | Loose | YG | ++ |
| 4 | 2.0 | 100 | Loose Watery | G | +++ |
| 5 | 2.5 | 100 | Loose Watery | G | +++ |
| 6 | 3.0 | 100 | Loose Watery | GW | +++ |

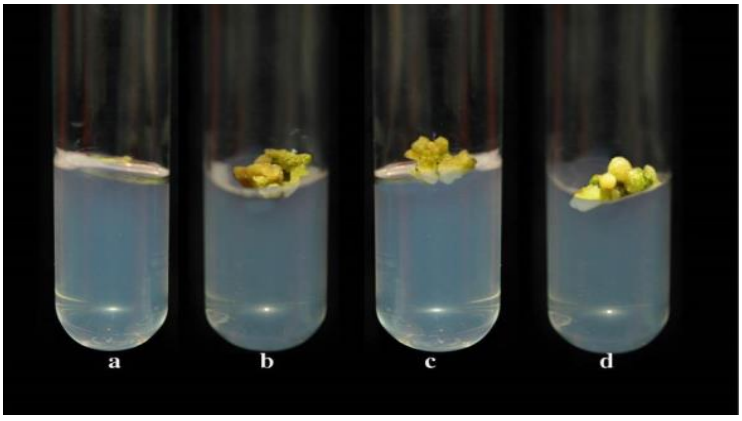


**Figure 3:** Effects of BAP on callus induction using different explants (a)Root, (b) Hypocotyle, (c)Cotyledon, (d)Internode

--=no callus, +=poor callus, ++=Moderate callus, +++=Massive callus Y-yellowish, G-greenish, GW-greenish white, W- whitish, YW- yellowish white

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**Table 3:** Influence of leaf disc explants using 2,4-D on the initiation of potato callus

| Sr. no | 2,4-D (mg) | Type / texture of callus | Callus weight (gm) |
|---|---|---|---|
| 1 | 0.0 | -- | -- |
| 2 | 1.0 | Compact | 0.67 |
| 3 | 1.5 | Compact | 0.72 |
| 4 | 2.0 | Loose | 0.64 |
| 5 | 2.5 | Loose Watery | 0.77 |
| 6 | 3.0 | Loose Watery | 0.81 |

**Shoot Regeneration of Potato**

- **Plate -12**

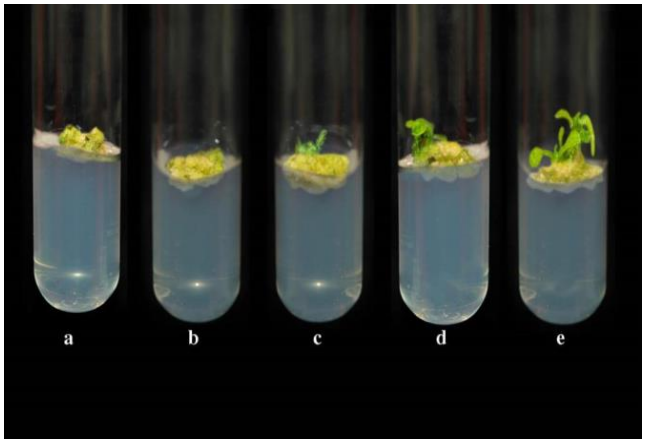


**Figure 4:** Effects of BAP on shoot regeneration from callus (a) 0.0 BAP, b) 1.0 BAP, c) 2.0 BAP, d) 3.0 BAP, d) 3.0 BAP, e) 4.0 BAP

**Table 4:** Impact of BAP on potato callus-induced regrowth of shoots

| BAP | Days of | Shoot | No. of | | Shoot |
|---|---|---|---|---|---|
| (mgs) | shoot | formation | | shoot per | length |
| | initiation | percent | calli | | (mm) |
| 0.0 | -- | -- | | -- | -- |
| 1.0 | -- | -- | | -- | -- |
| 2.0 | 60 | 12 | | 1 | 3 |
| 3.0 | 60 | 37 | | 1.6 | 4 |

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**Table 5:**

| Conc. Of 2,4-D (mgs/l) | Conc. Of BAP (mgs/l) | Av. Ht. of plantlets (mm) | Av. No of leaf/ plantlets | Degree of adventitious shoot formation | Degree of adventitious root formation |
|---|---|---|---|---|---|
| 0.0 | 0.1 | 32 | 7 | +++ | -- |
| 1.0 | 0.1 | 48 | 8 | + | -- |
| 1.5 | 0.1 | 63 | 10 | -- | + |
| 2.0 | 0.1 | 62 | 9 | -- | ++ |
| 2.5 | 0.1 | 67 | 9 | -- | +++ |

maximum at 2.5 2,4-D and 0.1 mg/lit BAP combo. A high leaf-per-plantlet ratio of 1.5 2,4-D and 0.1 mgs/lit BAP was observed. There was no accidental shoot formation at concentrations of 1.5 mg/lit, 2 mg/lit, and 2.5 mg/lit of 2,4-D.

+ = Poor; ++ = Moderate; +++ = Excellent

**Shoot Regeneration and shoot multiplication of Potato**

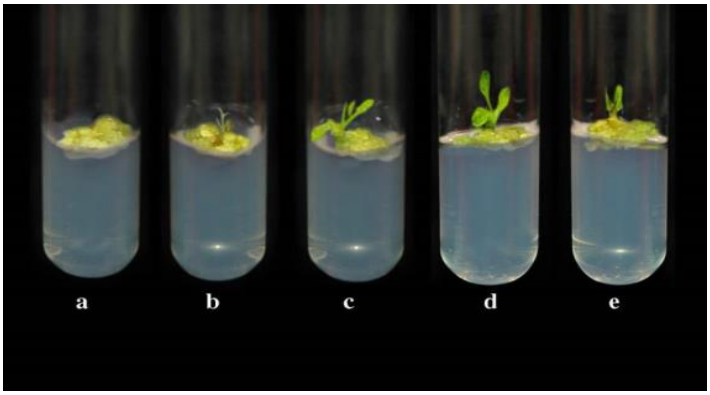


**Figure 5:** Effect of 2,4-D and BAP on the regeneration of shoots induced from callus (a) 0.0 BAP + 0.5 mgs 2,4-D, b) 1.0 BAP + 0.5 mgs 2,4-D, c) 2.0 BAP + 0.5 mgs 2,4-D, d) 3.0 BAP + 0.5 mgs 2,4-D, e) 4.0 BAP + 0.5 mgs 2,4-D

**Table 6:** Impact of 2,4-D and BAP on potato callus regeneration in shoots

| Sr. no. | BAP (mg) | 2,4-D (mg) | Days of shoot | Shoot formation | No of shoot/calli | Shoot length |
|---|---|---|---|---|---|---|

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| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | 0.0 | 0.5 | initiation | percent | -- | (mm) |
| 2 | 1.0 | 0.5 | 60 | 20 | 1.0 | 6 |
| 3 | 2.0 | 0.5 | 60 | 23 | 1.0 | 5 |
| 4 | 3.0 | 0.5 | 60 | 34 | 1.2 | 8 |
| 5 | 4.0 | 0.5 | 60 | 30 | 1.1 | 7 |

-- = No growth; + = Poor growth

## II. Discussion

Potatoes are semi-perishable and sensitive to illnesses and insects. In poorer countries, poor seed quality is the biggest problem restricting potato production. Tissue culture has greatly aided potato seed production by rapidly multiplying disease-free planting material. To obtain virus-free potato clones, mestem culture is working. Micropropagation and traditional multiplication technologies have made quick multiplication of these disease-free clones an essential aspect of seed production in many nations.

(BAP and 2,4-D) and other hormone combinations caused tomato regeneration and calluses. Selvi and Khader saw that as 0.2mg/l 2,4-D + 2.5mg/l BAP outlined the best shoots. Ouyang et al. discovered that tomato roots developed successfully on media boosted with 0.5 and 1.0 mg/lit auxin, indicating that tomato has high endogenous auxin levels. Roy, et al. produced callus from leaf explants in media was enhanced by 2,4-D (0.5 mg/lit) and Rao and Suvartha found that direct shoot recovery occurred at 1.0 mg/l of 2,4-D and 5.0 mg/l of BAP.

Comparative mix was best for shoot recovery in the chose assortment. After shoot recuperation, 0.5 mg/l 2,4-D rhizogenesis was achieved, and the plants were acquainted with develop soil. Raj et al.tracked down indistinct results with comparable improvement synthetics for shoot commencement and callus progression, yet at 2.0 mg/lit zeatin + 0.1 mg/lit 2,4-D for callus and 0.1 mg/lit for shoot inception. Devi et al. On a 2,4-D medium containing 0.5 mg/lit, no growth was observed.

## V. Conclusion

At 2, 2.5, and 3mgs/lit 2,4-D concentration, shoot tip explants formed enormous potato callii with loose watery texture and greenish and greenish white colour, while at 1.5%, moderate callus with loose texture and yellowish green colour was developed. Poor, compact, yellow callus was generated at 1.0% 2,4-D. The 1.0% and 1.5% 2,4-D concentrations produced compact potato callus with significant weight, while the 2.5% and 3.0% concentrations produced loose watery callus with high weight. 2.0% 2,4-D in the medium produced light, loose potato callus. 9. 2.0, 2.5, and 3.0 mgs/lit 2,4-D produced loose, watery potato callus with significant weights. At 1.0mgs/lit 2,4-D, compact callus with high weight was generated. 1.5mgs/lit2,4-D caused light, loose callus. 10. Potato explants were most induced into large callus with compact texture and yellowish green and greenish white colour with BAP (3 and 4 mgs/lit) and NAA. However, 2 mgs/lit BAP generated considerable callus with compact texture and yellowish green hue. BAP at 1 mgs/lit produced compact, yellowish green, poorly formed callii. 11. Potato shoot tip and internode on 2,4-D medium had huge loose callii and pale-yellow callus. Leaf disc-induced compact callus with modest development, greenish yellow hue, and significant weight. However, callus produced from leaf disc on BAP medium was compact and had a high average fresh weight.

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Potato shoot development % and length from callus were highest at 3mgs/lit BAP, followed by 4mgs/lit and 2mgs/lit. Interestingly, no observation was made at 1 mgs/lit BAP. BAP alone at 0.1 mgs/lit increased potato plantlet and leaf shoot development with significant height. Plantlet height, average leaf count, and adventitious root production were highest at 2.5 IAA and 0.1 mgs/lit BAP.

Leaf count per plantlet was high at 1.5 IAA and 0.1 mgs/lit BAP. In vitro shoot multiplication for tomato plantlet height, leaf count, and root count was better with IAA at 1.5mgs/lit and 2mgs/lit and BAP at 0.1mg/lit. However, such parameters were significantly induced at 1mg/lit and 2.5mg/lit IAA and BAP (0.1mg/lit). Most noteworthy shoot advancement, number of shoots per calli, and shoot length were impelled in vitro at 3mgs/lit and 4mgs/lit IAA with BAP (0.1mg/lit). IAA doses of 1mg/lit and 2mg/lit with BAP (0.1mg/lit) caused significant shoot multiplication in vitro.

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