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BIOTECHNOLOGY

# INFLUENCE OF PLANT GROWTH REGULATORS ON IN-VITRO MICROPROPAGATION OF POTATO VARIETIES

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## ABSTRACT
Potato (*Solanum tuberosum* L.) is a tuberous, starchy and dicotyledonous plant of the Solanaceae family. After rice and wheat, it is the third most important food crop. Cytokines and auxins together are reported to be effective for plants in-vitro regeneration. The IBA was more effective than IAA in stimulating earlier root development. The Central objective of the current investigation was to evaluate the role of plant growth stimulants over potato variety (Asterix and Bartina). The Present investigation was performed at Allama Iqbal Open University, Islamabad during 2023. A Completely randomized design (CRD) with three repetitions was adopted for its conductance. Least significant Difference (LSD) test have been performed for testing significant mean differences between them. In the present study indole-acetic acid (IAA) and benzyl amino purine (BAP) were used for shoot proliferation and multiplication while Indole -Butyric Acid (IBA) was used for root development. The meristem tips of the chosen varieties were excised under sterile conditions and cultured on Murashige and Skoog (MS) basal medium with various treatments of BAP (0, 1.5,3, 4.5mg/l) and IAA(0,0.5,1mg/l) for shoot multiplication while IBA with different concentrations (0,1,0.5 and 2mg/l) were used for root initiation. The result indicated that at the concentration of 4.5mg/l BAP + 1mg/l IAA, the Asterix variety showed the best response as a mean number of shoots was 9.2, the highest shoot length was 10.2 cm and a number of leaves was 7.0. Similarly, the Bartina variety showed their best response at the same concentration as mean of a number of shoots was 7.8, The highest shoot length measured 9.9 cm, and the number of leaves was 8.9, in comparison to the other treatments. The results also indicated that at l mg/l IBA was best for both varieties, as the number of roots were 9.53 and root length was 9.86 cm in the Asterix variety. Similarly, in Bartina variety the number of roots was 8.60 and the root length was 7.60cm.

**KEYWORDS**: *Solanum tuberosum;* asterix; bartina; MS media; BAP; IBA; IAA; Pakistan

## INTRODUCTION
The Potato (*Solanum tuberosum L.*) is a dicotyledonous, tuber-bearing, starchy plant belonging to the Solanaceae family. It ranks fourth in most important food crops in the globe as well as most broadly developed tuber crop that contributes greatly to food security and human nutrition (Karim *et al.,* 2010; Chemeda *et al.,* 2014). It is, after wheat and rice, the third most important food crop (Janasky *et al.,* 2019). Potatoes are farmed on 19.2 Mha of land worldwide annual yield of 376, with an.8 tons (FAO, 2021; Tiwari *et al.,* 2021). In 2008, Pakistan produced 2.5 million metric tons of potatoes (FAO, 2008). The country cultivates potatoes over a vast area, yielding approximately 4.1 million tons. They are primarily grown in the middle and northern plains of Punjab and KPK. Pishin, Saifulla, Kalat, Killa and the Gilgit district in Gilgit-Baltistan are some of the western Baluchistan districts that support potato farming. In comparison to neighboring India and Bangladesh, the production of potatoes has not yet reached its full potential in Pakistan (Majeed and Muhammad, 2018). Potatoes are an important crop in Pakistan, cultivated over 0.31 million hectares of land and producing approximately 7.9 million tons per year (Economic Survey of Pakistan, 2021-2022). With the world's population approaching 8 billion, there are fewer resources available, which limits the amount of food crops that can be produced. In order to address this problem, scientists are using a variety of methods, such as in vitro culture, which can produce plants with high productivity rapidly and that are resistant to drought (Farhatullah *et al.,* 2007). Both industrialized and developing nations are increasingly using culture of tissues and rapid growth techniques to produce potatoes (Khanna and Raina, 2021). In addition to boosting rates of proliferation, tissue culture techniques can alter the germplasm itself while preserving current resources. Through germplasm preservation, pathogen-free breeding development, and micropropagation, tissue culture has long been

utilized to increase potato production (Zimnoch-Guzowska *et al.,* 2021). In developing countries lack of quality seed is recognized as the most important factor limiting potato production. Fortunately, potatoes benefited from the advances in traditional and modern biotechnology early on, and they are used to solve the world's problems related to potato production and enhancement. Meristematic tissue culture may be the first biotechnological strategy for effectively removing viruses from contaminated potato clones (Singh *et al.,* 2017). This method has been successfully combined with micropropagation over time to produce potato seeds free of disease (Kumari *et al.,* 2021).

Researchers are working on various potato cultivars such as Carrera, Belete, Magic, Riviera, Gudene and Tresor to increase their production (Alemayehu *et al.,* 2018). A first-generation synthetic cytokine, benzoyl amino purine stimulates cell division and expansion, controls shoot growth and multiplication, and increases plant growth (Carimi *et al.,* 2003). The growth and survival of the meristem explant and virus-free production of plantlet is greatly affected by BAP. Meristem-derived explant cannot survive or grow in the presence of $GA_3$ alone (Ullah *,* 2012). Shoot growth may be aided by the independent addition of zeatin, kinetin, or benzyl aminopurine (BAP) to the nutritional medium. Reports vary on how efficiently zeatin promoted shoot initiation, with some suggesting it was less, equal, or even more effective than BA as the cytokinin component (Anjum and Ali, 2005). Although BAP encourages shoot elongation and proliferation, it is also known to encourage the formation of new shoots and latent lateral buds (Ashraf *et al.,* 2014). When growing kuroda at different concentrations of 6-Benzylaminopurine (BAP), the greatest results were obtained with 0.25 mg/L of BAP in terms of shoot length, number, and regeneration efficiency (Dilshad *et al.,* 2021). Cytokines and auxins together were reported to be effective for plants in vitro regeneration (Koleva *et al.,* 2012). Indole butyric acid (IBA) is a synthetic auxin that regulates plant growth and development. IBA is frequently used in plant tissue culture to promote root formation and boost the success rate of in vitro propagation (Khandaker *et al.,* 2022). The objective of the current study is to rise the protocols for the production of potato varieties (Asterix and Bartina). Thus, the current study assesses how plant growth regulators, specifically BAP, IAA, and IBA, affect the potato cultivars Asterix and Bartina.

## MATERIALS AND METHODS

***Plant material:*** The experiment was carried out in 2023 at the Allama Iqbal Open University's plant tissue culture lab in the Agricultural Sciences department in Islamabad, Pakistan. The shoot tips from the Asterix and Bartina potato plant kinds that were used as explants were gathered at NARC Islamabad. True potato seeds and mini tubers were initially used to grow the plants. Plants were taken to a lab for additional treatments after reaching a specific height.

***Explants sterilization***: For shaking explants, the harvested explant was placed in tween-20, then placed in an autoclaved jar and shaken for 20 minutes. This process was carried out under laminar flow hood. Then, for 20 to 30 minutes, all of the jars were held under tape while water ran to get rid of any extra foam (this was done to get rid of any contaminants the explant had before going to the lab). The explants were sterilized with Clorox. After being sterilized with 20% Clorox, the two types of transplants were rinsed three times for five minutes each time with distilled water. A laminar air flow hood was used for the sterilization process.

***MS media supplements:*** The MS medium supplemented with MS salt 4.41g/L and 30g/L sucrose was used. To induce shooting, varying concentrations of PGRs such as BAP (0,1.5mg/L, 3mg/L, 4.5mg/L) and IAA (0,0.5mg/L,1mg/L) were used alone or in combination. For rooting IBA alone used in different concentrations such as (0, 0.5 mg/L, 1mg/L,1.5mg/L,2mg/L). Gallen gum 2.25ml/L was used as an alternative to agar for the hardening of media.PH Ranges from 5.6-5.8. Before being added to test tubes or jars, the media were autoclaved for 20 minutes at 121oc.

***Inoculation and incubation:*** After preparation, the media was transferred to the laminar hood for inoculation of explants. Meristem tips were used as in vitro regeneration of potato. The cutting of shoot tips from explants were carried out under a laminar flow hood in an aseptic condition. After cutting, the shoot tips were carefully taken up by sterilized forceps and inoculated into autoclaved jars and test tubes. The number of explants per jar were 5. Keep the cultures in room at 25±2oc under 16/8 hours' photoperiod with light intensity of 3000lux. Observation was carried out in first month of cultivation for contamination and growth rate. After 2 weeks, the proliferated shoots were separated from the explants and moved to MS medium containing varying concentrations of PGRs for multiplication.

***Sub-culturing and acclimatization:*** After 2 weeks, the proliferated shoots were separated from explants and moved to MS medium containing varying concentrations of PGRs for multiplication. After the proliferation of shoots, the plantlets were moved to the other medium containing varying concentrations of IBA for rooting. After shoots were transferred to rooting media, the number of roots was examined after 6 weeks

Rooted plantlets are gradually acclimatized to ex-vitro conditions by transferring them to pots containing a sterile potting mix and gradually reducing humidity in a controlled environment (Gamborg *et al.,* 1968).

***Experimental design and statistical analysis:*** This experiment was carried out following a completely randomized design (CRD). In this study, 12 treatments consisting of different concentrations of BAP and IAA were applied in three replications to potato varieties (Asterix and Bartina) for the proliferation of shoots (Table 1) and 5 treatments of IBA in different concentrations for proliferation of roots (Table 2). The main variables studied were shoot numbers and length, leaves number, roots numbers and length. The collected data were imported into a computer and analyzed using SPSS software for further study. Two-way ANOVA at P≤0.05 was used to identify significant differences in response variables between treatments and varieties. The LSD test was used to identify significant differences between means.

**Table 1:Treatments of BAP and IAA for shoots development of both varieties**

| Treatments | BAP (mg/l) + IAA (mg/l) |
|---|---|
| I | 0 + 0 |
| Ii | 1.5 + 0 |
| Iii | 3 + 0 |
| Iv | 4.5 + 0 |
| v | 0 + 0.5 |
| vi | 0 + 1 |
| vii | 1.5 + 0.5 |
| viii | 3 + 0.5 |
| ix | 4.5 + 0.5 |
| x | 1.5 + 1 |
| xi | 3 + 1 |
| xii | 4.5 + 1 |

**MS (Murashige and Skoog) basal media was fortified with 30 g/l sucrose, 2.25 g/l Gellan gum powder and different concentration of BAP and IAA**

**Table 2: Treatments of IBA for roots initiation of both varieties**

| Treatments | IBA (mg/l) |
|---|---|
| i | 0 |
| ii | 0.5 |
| iii | 1 |
| iv | 1.5 |
| v | 2 |

**MS (Murashige and Skoog) basal media was fortified with 30 g/l sucrose, 2.25 g/l Gellan gum powder and different concentrations of IBA.**

## RESULTS AND DISCUSSION

***BAP and IAA influence on shoots:*** For both potato varieties, the data showed that the control group with no BAP and IAA does not show the shoot formation. The addition of 1.5mg/l BAP in Asterix variety resulted in 3.5 ± 0.62 shoots while 4.5mg/l BAP further enhanced the results to 3.9 ± 0.8 shoots. It was observed that Asterix gives the maximum shoots number (3.9 ± 0.8) with BAP alone at 4.5 mg/l concentration (Table 3). Adding IAA to BAP in different combinations enhanced shoot proliferation significantly. Specifically, the treatment of 4.5 mg/l BAP + 1 mg/l IAA resulted in 9.2 ± 1.06 shoots for Asterix, making it the optimal treatment for this variety (figure 1). In the second variety, Bartina, the application of 1.5 mg/l of BAP resulted in 2.1 ± 0.41 shoots followed by 3.6 ± 0.52 shoots numbers at 3mg/l of BAP. Combined effect of BAP with IAA in Bartina variety significantly increase the shoot formation as were noted in Asterix variety (Table 3). In this case, the maximum shoots number (7.8 ± 1.83) were recorded at 4.5mg/l BAP + 1 mg/l IAA in Bartina variety (Figure 1).

The effect of varying concentrations of BAP and IAA on the shoot length of Asterix and Bartina varieties was also assessed. The alone effect of IAA and BAP did not improve shoot length in both Varieties, while combined effect of BAP and IAA improve the shoot length of both potato varieties. The highest shoot length observed in Asterix was 10.2 ± 0.61 and in Bartina was 9.8 ± 0.23 at 4.5 mg/l BAP + 1 mg/l IAA respectively (Table: 4). These findings reveal that shoot length is pointedly affected by the combination of BAP and IAA (figure 1), with different treatments providing the best results for different varieties.

The number of leaves generated by Asterix and Bartina varieties under different concentrations of BAP and IAA was examined. The alone effect of BAP significantly increases the number of leaves. The highest leaves 3.9 ± 1.15 were recorded at 4.5mg/l BAP in Asterix variety and in Bartina variety 3.2 ± 1.40 leaves were recorded at 3mg/l BAP. Similarly, the combined effect of BAP and IAA were also recorded. The highest mean number of leaves observed in Asterix was 7.0 ± 1.73 and in Bartina was 8.9 ± 1.50 at 4.5 mg/l BAP + 1 mg/l IAA respectively (Table 5). Result indicated that BAP and IAA together have a substantial effect on leaf count, with different treatments working best for different varieties.

BAP is a cytokinin that is essential for the development and growth of shoots (Buah *et al.,* 2000). The present study reveals that BAP was most effective when it combined with IAA, for optimum shoot proliferation of Asterix and Bartina variety. It has previously been studied how BAP and IAA work together to promote shoot propagation in various potato varieties, and

**Table 3: Influence of BAP and IAA on shoot number of both varieties**

| Treatments | | Number of shoots | |
|---|---|---|---|
| BAP | IAA | Asterix | Bartina |
| 0 | 0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 1.5 | 0 | 3.5 ± 0.62 | $2.1^{j}$ ± 0.41 |
| 3 | 0 | 3.4 ± 1.05 | 3.6 ± 0.52 |
| 4.5 | 0 | 3.9 ± 0.8 | 2.4 ± 0.89 |
| 0 | 0.5 | 2.7 ± 0.75 | 3.2 ± 0.70 |
| 0 | 1 | 2.4 ± 0.45 | 4.3 ± 0.61 |
| 1.5 | 0.5 | 5.8 ± 0.76 | 4.4 ± 0.6 |
| 3 | 0.5 | 4.5 ± 0.55 | 4.6 ± 1.15 |
| 4.5 | 0.5 | 5.6 ± 0.85 | 4.4 ± 0.52 |
| 1.5 | 1 | 5.2 ± 0.72 | 4.7 ± 0.49 |
| 3 | 1 | 7.1 ± 2.10 | 5.2 ± 0.62 |
| 4.5 | 1 | 9.2 ± 1.06 | 7.8 ± 1.83 |

**The values next to ± sign indicated the standard deviation. Each data is an average of three replicates, where n=5.**

**Table 4: Influence of BAP and IAA on shoot length of both varieties**

| Treatments | | Shoot length (cm) | |
|---|---|---|---|
| BAP | IAA | Asterix | Bartina |
| 0 | 0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 1.5 | 0 | 3.2 ± 1.06 | 2.5 ± 2.30 |
| 3 | 0 | 4.0 ± 1.40 | 1.9 ± 1.05 |
| 4.5 | 0 | 5.1 ± 1.44 | 3.4 ± 1.99 |
| 0 | 0.5 | 5.8 ± 1.75 | 2.7 ± 1.07 |
| 0 | 1 | 5.6 ± 2.77 | 2.7 ± 1.25 |
| 1.5 | 0.5 | 5.3 ± 2.13 | 9.0 ± 0.73 |
| 3 | 0.5 | 5.0 ± 1.86 | 3.5 ± 2.60 |
| 4.5 | 0.5 | 5.5 ± 0.60 | 3.1 ± 1.68 |
| 1.5 | 1 | 5.2 ± 1.31 | 5.0 ± 2.11 |
| 3 | 1 | 3.8 ± 2.89 | 5.7 ± 2.21 |
| 4.5 | 1 | 10.2 ± 0.61 | 9.8 ± 0.23 |

**The values next to ± sign indicated the standard deviation. Each data is an average of three replicates, where n=5.**

the results of the current study interrelated with the findings of Mollika *et al*. (2020) the authors indicated that Asterix and Diamant varieties developed shoots on MS medium having 4.0 mg/l BAP and 1.0 mg/l IAA.

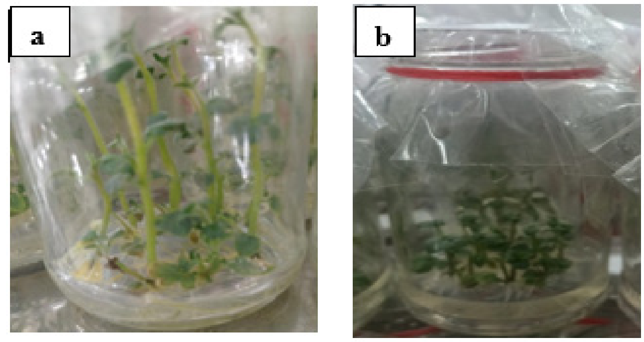


**Fig. 1 shoot number and length Asterix(a) Bartina (b)**

**Table 5: Influence of BAP and IAA on number of leaves of both varieties**

| Treatments | | Number of leaves | |
|---|---|---|---|
| BAP | IAA | Asterix | Bartina |
| 0 | 0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 1.5 | 0 | 2.7 ± 0.75 | 1.5 ± 0.50 |
| 3 | 0 | 2.3 ± 0.57 | 3.2 ± 1.40 |
| 4.5 | 0 | 3.9 ± 1.15 | 2.1 ± 0.80 |
| 0 | 0.5 | 2.3 ± 0.58 | 0.4 ± 0.55 |
| 0 | 1 | 2.2 ± 0.25 | 2.4 ± 0.60 |
| 1.5 | 0.5 | 3.0 ± 0.90 | 2.1 ± 1.00 |
| 3 | 0.5 | 3.7 ± 1.48 | 2.2 ± 0.46 |
| 4.5 | 0.5 | 4.0 ± 0.90 | 2.1 ± 0.20 |
| 1.5 | 1 | 3.5 ± 1.28 | 2.9 ± 0.97 |
| 3 | 1 | 4.3 ± 2.33 | 3.2 ± 1.26 |
| 4.5 | 1 | 7.0 ± 1.73 | 8.9 ± 1.50 |

**The data in the table indicated the mean of number of leaves. Each data is an average of 3 replicates, where n=5.**

For Asterix each nodal segment produced an average of 9.88 shoots with a shoot length of 8.90cm and each microtuber disc produced 5.63 shoots with a shoot length of 10.30 cm. On the other hands, Diamant produced an average of 8.82 shoots per nodal segment and 5.31 per microtuber disc after 30 days, with shoot lengths of 9.80 cm and 9.30 cm, respectively, after six weeks. The current study is further supported by the results reported by (Borna *et al.,* 2019), who detected efficient shoot regeneration from the peripheral region of micro tuber disc explants on MS medium fortified with 4.0 mg/L BAP and 2.0 mg/L IAA.The largest length of shoots observed was an average of 10.30 ± 0.56 cm from micro tuber discs and 8.90 ± 0.74 cm from nodal segments. Together, these studies suggest that MS medium containing BAP and IAA is highly effective

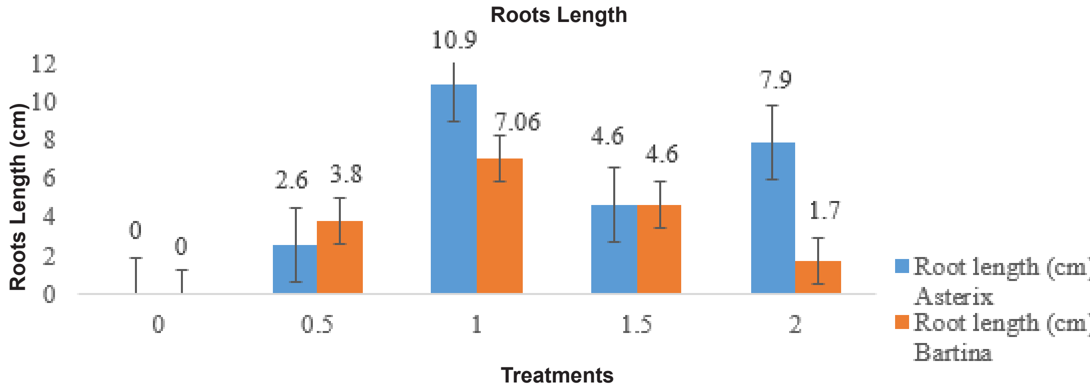


**Fig. 2: Effect of different concentration of IBA on roots length of both varieties. The bar represents mean root length.**

for promoting shoot proliferation and elongation in potato micro propagation. In this study, our findings concluded that MS basal media containing BAP and IAA in a concentration of (4.5 mg/l + 1 mg/l) gave the highest length of shoots, number of shoots and number of leaves in Asterix and Bartina, respectively.

**Table 6: Effect of different concentration of IBA on number of roots**

| Treatments | Number of roots | |
|---|---|---|
| **IBA mg/l** | **Asterix** | **Bartina** |
| **0** | 0.00 ± 0.00 | 0.00 ± 0.00 |
| **0.5** | 2.73 ± 0.23 | 3.80 ± 2.84 |
| **1** | 9.53 ± 0.30 | 8.60 ± 0.20 |
| **1.5** | 4.60 ± 2.35 | 1.70 ± 0.81 |
| **2** | 7.86 ± 1.17 | 3.80 ± 2.84 |

**The data in the table indicated the mean of roots. Each data is an average of 3 replicates, where n=5. The value next to ± sign indicated the standard deviation.).**

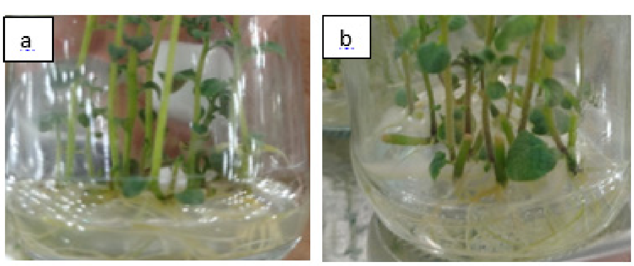


**Fig. 3: Root number and length Asterix (a) Bartina (b)**

### Effect of IBA on root numbers and root length

In the Asterix variety, the treatment with 1mg/l IBA resulted in the highest number of roots (9.53 ± 0.30), followed by the treatment with 2 mg/l IBA (7.86 ± 1.17). The control group (0 mg/l IBA) resulted no roots. Similarly, for the Bartina variety, yielded the highest number of roots (8.60 ± 0.20) at treatment with 1 mg/l IBA were recorded (Table 6). These data show the significant impact of IBA on root induction. For the Asterix variety, the highest root length (10.9 ± 1.02 cm) was recorded with the treatment of 1mg/l IBA, followed by 2 mg/l IBA (7.9 ± 1.28 cm) (figure 3). Bartina variety treated with 1 mg/l IBA gives the highest root length (7.9 ± 1.28 cm). Likewise, the control group showed no roots (figure 2). These results demonstrate that IBA considerably lengthens roots (figure 3). According to Frick and Strader (2018), auxins—in particular, indole-3-butyric acid, or IBA—have a significant impact on root differentiation and improvement. The current study uses IBA for root development in the Asterix and Bartina variety. The results are primarily compared with those of Stefancic *et al.* (2005), who used the 'GiSelA 5' dwarfing cherry rootstock to investigate the effects of two applied auxins (IAA and IBA) on the development of roots and shoots in leafy cuttings. The findings showed that IBA stimulated early root growth more effectively than IAA. Compared to Farhatullah and Sayeed (2007), who discovered that their longest roots (3.7 cm) developed on MS medium with 0.248 mg L−1 GA3, the results are superior. The cultivar Spunta generated the most roots (10.40 per explant) on media containing IBA, according to a separate study by Al-Taleb *et al.* (2011). The findings of (Zaman *et al.,* 2001), who examined the impact of four distinct treatments and three distinct auxins on potato meristem culture, provide additional support for the study. Four distinct concentrations of IBA were available for the treatments: 0, 0.1, 0.5, and 1.0 mg/L. The findings showed that the size of the roots was significantly influenced by the different auxins. Notably, 1 mg/L IBA had the highest root count (23.7), followed by 1 mg/L NAA (20.1). According to the current investigation, Asterix and Bartina generated the most roots and the longest roots at 1 mg/l of IBA.

## CONCLUSION

The study found that Plant Growth Regulators (PGRs) improved growth parameters in both potato varieties, Asterix and Bartina. Asterix performed very well in terms of shoot development and root initiation. The combination of BAP and IAA was most valuable in raising number and length of shoots and total number of leaves in both varieties. Furthermore, IBA was observed to promote early root growth. The ideal concentration of 4.5mg/l of BAP and 1mg/l of IAA to promote shoot proliferation and 1 mg/l of Indole Butyric Acid (IBA) to promote root initiation can be used to develop effective and inexpensive protocols for in vitro potato propagation. This can provide useful insights for future study and food production.

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| Sr. No. | Author's name | Contribution | Signature |
|---|---|---|---|
| 1. | Iqra Shafique | Conducted the research and wrote the manuscript | |
| 2. | Khalid Mehmood | Conceptualized and supervised the research | |
| 3. | Sabir Hussain Shah | Supervised and assisted the research work | |
| 4. | Ruqia Mehmood | Analyzed the data statistically | |
| 5. | Zuhair Hasnain | Reviewed the manuscript | |