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RESEARCH ARTICLE

# Effects of plant growth regulators on callogenesis and embryogenesis in sarnav and desiree potato (*Solanum tuberosum* L.) varieties

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## Abstract

Somatic embryos play a pivotal role in the production of high-quality potatoes and seed breeding. This study focused on determining the concentrations of 1-naphthaleneacetic acid (NAA) and 6-benzyl amino purine (BAP) in the formation of callus tissue and callus induction. Our goal was to assess the efficiency of potato explants with the highest potential for somatic embryo production. To achieve this, we cultivated Sarnav and Desiree potato varieties under *in vitro* tissue culture conditions, utilizing the obtained tissue cultures for subsequent experiments. The MS nutrient media were enriched with NAA and BAP at ratios of 1.5: 1, 1: 1.5, and 1: 1 mg/L, along with NAA concentrations of 1.5, 1, or 2 mg/L. Somatic embryogenesis experiments were conducted using various MS nutrient media, enriched with BAP and $GA_3$ at concentrations of 1: 0.5, 0.4: 0.1, 0.5: 0.2, and 0.1: 0.1 mg/L of plant growth regulators. During the course of the study, diverse callus formations were observed in both leaf and internodal stem explants. Among the nutrient media, the M2 medium enriched with 1: 1.5 mg/L of NAA and BAP yielded the highest callus formation rates: 92% for the Desiree variety and 100% for the Sarnav variety, specifically in internodal stem explants. Notably, the index of embryo formation in leaf explants selected for somatic embryogenesis within the SE4 medium was 70% for the Sarnav variety and 65% for the Desiree variety. The inclusion of BAP and $GA_3$ at a ratio of 0.1: 0.1 mg/l in the SE4 nutrient medium resulted in somatic embryogenesis in 80% of calli for the Sarnav variety and 78% for the Desiree variety. These findings underscore the potential for regenerating plants through somatic embryogenesis in the Sarnav potato variety, a significant development with implications for genetic transformation studies involving this particular variety.

## Keywords

*Solanum tuberosum*; callus; 1-naphthaleneacetic acid (NAA); 6-benzyl amino purine (BAP); gibberellic acid ($GA_3$); auxin; cytokinin

## Introduction

Potato (*Solanum tuberosum* L.) holds a crucial position as one of the primary crops cultivated for nutritional purposes. With the global population on the rise, the demand for potato products is also increasing. As a result, it becomes paramount to explore alternative approaches to bolster crop

productivity. An avenue of great significance lies in the regeneration of plants from callus tissues through somatic embryogenesis, a technique employed for the development of novel biotechnological varieties. Employing tissue culture and genetic transformation methods for generating new germplasm has the potential to enhance potato quality, confer disease resistance, and improve various agronomic traits (1). Promising strides have already been taken in the realm of regenerating potato plants, as evidenced by positive outcomes achieved during research endeavours (2). Various plant tissues, such as leaves, stems, and others have been harnessed for regeneration through the mechanism of somatic embryogenesis in potato biotechnology. The successful formation of callus tissue hinges upon factors such as the specific plant organs utilized, the choice of plant growth regulators, and the influence of external environmental factors (3). Notably, plant growth regulators emerge as pivotal elements, exerting diverse effects on the growth of distinct tissues.

An essential aspect of the research involves pinpointing the optimal dosage of plant growth regulators incorporated into nutrient media to facilitate the formation of callus tissue from explants, ultimately leading to the acquisition of somatic embryos. Among the phytohormones governing plant growth and development, auxin stands out as a key player (4, 5, 6, 7), orchestrating vital processes in the expansion and maturation of various plant organs (8). Extensive investigations have delved into the critical factors underpinning the formation of callus tissues and the subsequent generation of regenerants through somatic embryogenesis in potatoes. The quantity of auxin and cytokinin in the nutrient medium emerges as a pivotal determinant of cellular activity, hinting at potential crosstalk between these two hormones (9). The successful implementation of somatic embryogenesis hinges upon a confluence of factors: the source of plant organs, the composition of the artificial nutrient medium, including the combination of growth regulators, and the influence exerted by external environmental conditions (10).

In contrast, somatic embryos are obtained in other plant types through the manipulation of plant growth regulators – cytokinin and auxins such as thidiazuron (TDZ) and 1-naphthaleneacetic acid (NAA) (1, 11). The selection of appropriate explants assumes paramount importance in the initiation of callus tissue formation and subsequent somatic embryogenesis (12). Throughout plant ontogenesis, the embryogenic potential exhibits a continuous decline contingent upon the species in question (13). However, the internode segment and leaf tissue have high embryogenic potential (14), making youthful tissues particularly proficient in generating embryos (10).

Somatic embryos play a crucial role in producing high-quality potatoes and seed breeding. In this study, we aimed to determine the optimal concentrations of 1-naphthaleneacetic acid (NAA) as an auxin and benzyl aminopurine (BAP) as a cytokinin for callus formation, along with BAP and gibberellic acid ($GA_3$) for somatic embryogenesis in the local Sarnav variety. The effects of these plant growth regulators on the local Sarnav variety were compared with those on the Desiree (*S. tuberosum* L) variety, which was used as a control.

## Materials and Methods

### *Plant material*

The research was carried out at the Laboratory of Transgenomics and Tissue Culture at the Center of Genomics and Bioinformatics. In this study, the Sarnav and Desiree potato varieties, which were cultivated *in vitro* in the laboratory, were employed. The process of somatic embryo development was observed using an NLCD-307B Digital Binocular Microscope with a monitor set at 100× magnification.

### Preparation of culture media

All the reagents used for the growth media were procured from PhytoTech Labs (USA). For the preparation of 1 L of callus induction medium, 4.31 g MS nutrient (Murashige & Skoog), 15-30 g sucrose, and varying ratios of NAA and BAP were included (Table 1). The pH of the culture medium was adjusted to 5.8 (Sartorius P 009, USA), and 8 g phyto agar was added.

**Table 1.** The chemical composition of callus induction and somatic embryogenesis media

| Culture media | MS reagent (g/L) | Sucrose (g/L) | Phyto-Agar (g/L) | NAA (mg/L) | BAP (mg/L) | Myo inositol (g/L) | $GA_3$ (mg/L) | pH |
|---|---|---|---|---|---|---|---|---|
| **The composition of callus induction culture media (M)** | | | | | | | | |
| M1 | 4.31 | 30 | 8 | 1.5 | 1 | – | – | 5.8 |
| M2 | 4.31 | 30 | 8 | 1 | 1.5 | – | – | 5.8 |
| M3 | 4.31 | 30 | 8 | 2 | 2 | – | – | 5.8 |
| M4 | 4.31 | 30 | 8 | 1.5 | – | – | – | 5.8 |
| M5 | 4.31 | 30 | 8 | 1 | – | – | – | 5.8 |
| M6 | 4.31 | 30 | 8 | 2 | – | – | – | 5.8 |
| M7 | 4.31 | 30 | 8 | – | – | – | – | 5.8 |
| **The composition of somatic embryogenesis (SE) media** | | | | | | | | |
| SE1 | 4.31 | 20 | 8 | – | 1 | 0.1 | 0.5 | 5.8 |
| SE2 | 4.31 | 20 | 8 | – | 0.4 | 0.1 | 0.1 | 5.8 |
| SE3 | 4.31 | 20 | 8 | – | 0.5 | 0.1 | 0.2 | 5.8 |
| SE4 | 4.31 | 20 | 8 | – | 0.1 | 0.1 | 0.1 | 5.8 |
| SE5 | 4.31 | 20 | 8 | – | – | 0.1 | – | 5.8 |

The preparation of culture medium for somatic embryogenesis followed the above-described procedure. The following amounts of chemicals were added per litre: sucrose (20 g), myoinositol (0.1 g). Various concentrations of BAP and $GA_3$ were taken for different culture media (Table 1).

The prepared media were sterilized for 20 minutes using an autoclave (Jeio Tech ST-65G, Korea) at a temperature of 121°C and under a pressure of 0.75 – 1.0 atmosphere.

### Statistical Analysis

The number of explants in each experiment made 100 samples. The statistical analysis of the data obtained in this work was performed in One-Way ANOVA.

### Growing condition

The explants were cultured on callus growth media (M1, M2, M3, M4, M5, M6, and M7) for a duration of two months. Subsequently, they were maintained on culture media for an additional month for somatic embryogenesis (SE1, SE2, SE3, and SE4). All stages of the experiment were conducted under sterile conditions within a specific laminar box (Heal Force HF safe-1200, China). The humidity level and temperature were maintained at 72-74% and 20-22 °C, respectively. The light cycle consisted of 16 hours of daylight followed by 8 hours of darkness, with a light intensity of 2000-3000 lux for culturing.

## Results and Discussion

The formation of calli from the cut surface of the explants was observed after 5-7 days *in vitro* for all hormone combinations. After eight weeks of growth in culture media, the data were analyzed to assess callus formation, and the percentage of explants that developed calli was calculated. In terms of the degree of development, the Sarnav variety produced more calli in the nutrient media M2, M3, M4, M5, and M6 (Fig. 1).

After two months of growth in culture media, the callus of the Desiree variety exhibited a friable structure with different colors: green-yellow (M1, M4, M6), green (M3), and brown-green (M2, M5). Similarly, the callus of the Sarnav variety also displayed various colors: light green

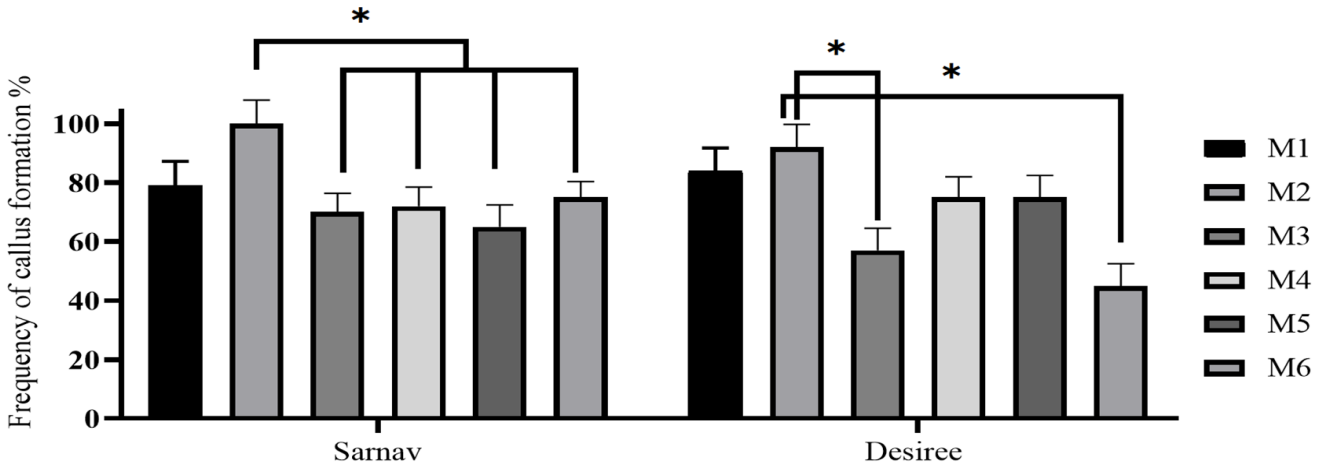


**Fig. 1.** Effect of different concentrations of NAA/BAP and NAA on callus proliferation of leaf explants of Sarnav and Desiree varieties. *P* value for Sarnav and Desiree varieties made 0.0001 and 0.01, respectively.

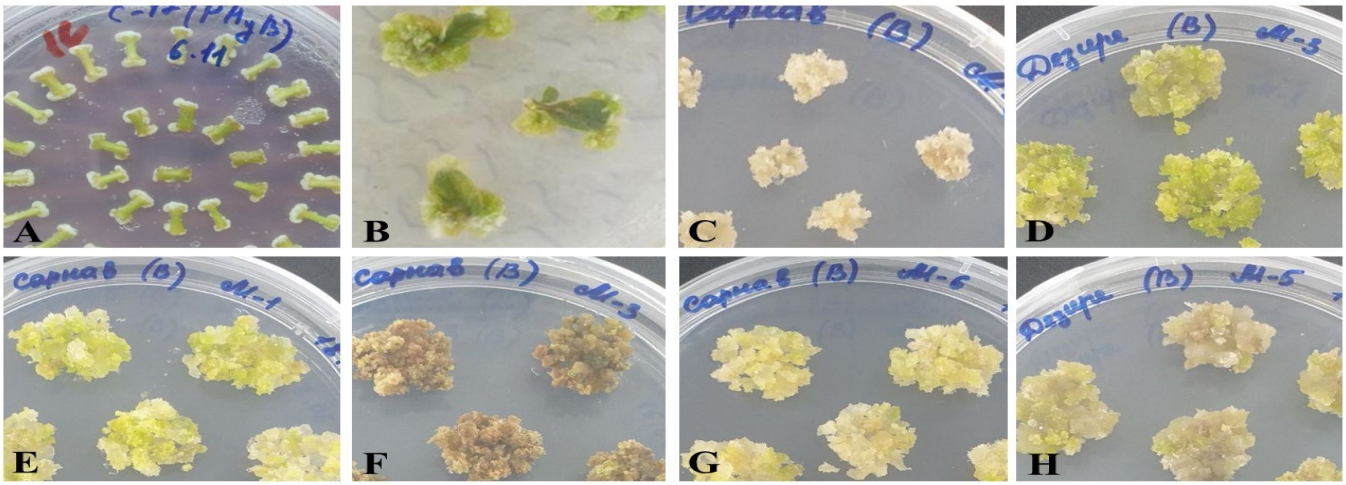


**Fig. 2. (A-H).** Effect of auxin and cytokinin ratio on callus proliferation in potato explants (internodal stems and leaves) grown *in vitro*

***A:** Yellowish-white callus formed from internodal stem explant in M2 (1/1.5mg/L) nutrient medium of Sarnav variety; **B:** Yellowish-white callus formed from leaf explant in M2 (1: 1.5 mg/L of NAA and BAP) nutrient medium of Sarnav variety; **C:** Yellowish-white callus after 20 days in M1 (1.5: 1 mg/L of NAA and BAP) nutrient medium of Sarnav variety: **D:** Green callus obtained from leaf explants of Desiree variety in M3 (2: 2 mg/L of NAA and BAP) nutrient medium; **E:** Light-green callus obtained from Sarnav variety in M1 (1.5: 1 mg/L of NAA and BAP) nutrient medium; **F:** Brown callus obtained from Sarnav variety in M3 (2: 2 mg/L of NAA and BAP) nutrient medium; **G:** Green-yellow callus of variety in M6 (only NAA 2 mg/L) nutrient medium; **H:** Brownish-green callus of Desiree variety in M5 (only NAA 1 mg/L) nutrient medium.

(M1, M2, M4, M5), brown (M3), and green-yellow (M6) (Fig. 2). The callus tissues of the leaf and internodal stem explants from both the Sarnav and Desiree varieties exhibited a friable structure.

Calli formation was initially observed in the M1, M2, and M3 culture media. The explants of both varieties, when grown in all growth media, exhibited compact, light green-coloured calli. Within a month, these calli transitioned to a friable appearance, undergoing a change from their initial light green colour in many of the growing cultures. In the Desiree variety, calli grown in M2 and M5 media showed a brown-yellow colour. The friable light green colour was evident in both M1, M4, and M6 media for both varieties. M7 media, which did not contain NAA and BAP, did not support the development of calli, highlighting the significance of these plant growth regulators (Table 2).

The results of this study indicate that embryogenic calli can be obtained, specifically when auxin is utilized. Kumlay & Ercisli (16) achieved the production of green friable callus within one week using various concentrations of auxin and cytokinin from potato varieties. The highest level, reaching 100%, was observed in calli obtained from leaf explants (M2, M3, M4, and M5), calculated based on the ratio of explants that produced calli to the total number of cultured explants. The formation of compact and friable calli has been associated with levels of chlorophyll and carotenoids. Increased iron (Fe) supply resulted in higher contents of chlorophyll and carotenoids in compact calli of the Iwa potato (*S. tuberosum*) cultivar. Compact calli were characterized by their green color, while friable calli exhibited a cream-colored, soft, and easily separable mass (17). These findings are consistent with the results obtained in this study.

The callogenesis results obtained from NAA alone revealed that the level of callus formation from leaf explants of both Sarnav and Desiree varieties in M4, M5, and M6 nutrient media ranged between 65% and 100%. Desiree exhibited a slightly lower frequency on M6. However, the level of callus formation for internodal stem explants ranged between 65% and 75% in the Sarnav variety when cultured in M4, M5, and M6 nutrient media. In the Desiree variety, these levels ranged from 45% to 75% (Fig. 3). Similar outcomes were observed in the study by Harun-Or-Rashid *et al.* (2), where they achieved positive results using potato explants such as stem internodes of Surjamukhi, Granola, Sheelbilati, Arun, and Sindurkouta genotypes by applying BAP at 5.0 mg/L + IAA at 2.0 mg/L + $GA_3$ at 1.0 mg/L.

### *Combined effects of NAA-BAP*

Cytokinin, a plant hormone, is widely used to induce callus formation in tissue culture. It can induce cell expansion via interactions with auxin and gibberellin. The results showed that the development of callus tissues in potato

**Table 2.** Effects of different concentrations of NAA/BAP and NAA on callus proliferation of leaf explants of Sarnav and Desiree varieties

| Media | Texture and color in one week | | Texture and color in two months | |
|---|---|---|---|---|
| | Sarnav | Desiree | Sarnav | Desiree |
| M1 | Compact light green | Compact light green | Friable light green | Friable green-yellow |
| M2 | Compact light green | Compact light green | Friable light green | Friable brown-green |
| M3 | Compact light green | Compact light green | Friable brown | Friable green |
| M4 | Compact light green | Compact light green | Friable light green | Friable green yellow |
| M5 | Compact light green | Compact light green | Friable light green | Friable brown-green |
| M6 | Compact light green | Compact light green | Friable green-yellow | Friable green-yellow |
| M7 | Callus was not formed | | | |

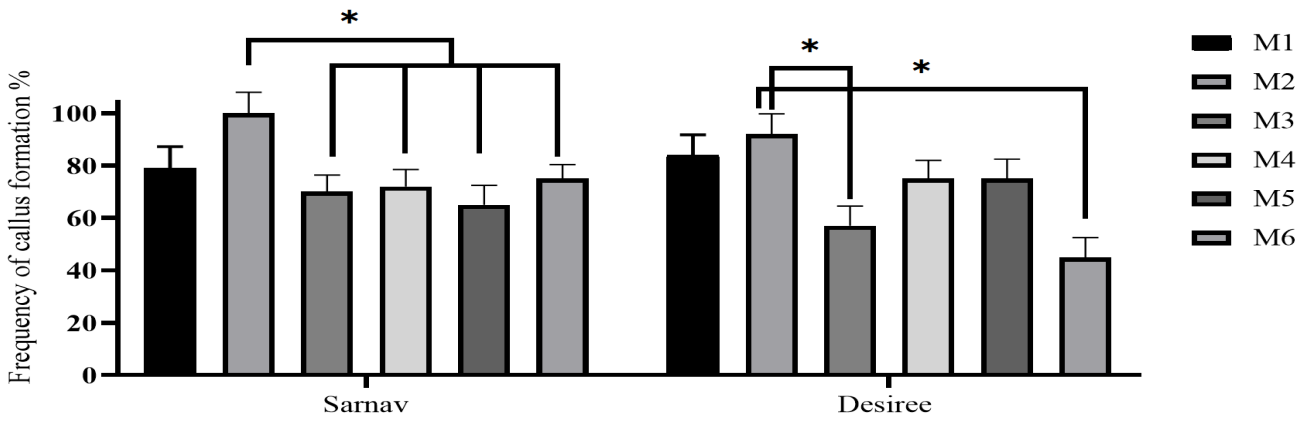


**Fig. 3.** Effects of NAA/BAP and different concentrations of NAA on the callus proliferation of internodal stem explants of Sarnav and Desiree

leaf and stem explants was influenced by the concentrations of plant growth regulators. The formation of callus tissues in the leaf explants of the Sarnav variety showed promising results in M2, M3, M4, and M5 nutrient media. The callus formation index from the Desiree variety leaf explants reached 100% in the M2 medium.

The highest rate of callogenesis in internodal stem explants was observed in the M2 nutrient medium, reaching 92% in the Desiree variety and 100% in the Sarnav variety, respectively. Yasmin *et al.* (14) reported higher callus formation from potato leaf explants than stem explants at 2.0 and 2.5 mg/L of BAP and NAA. Gudeva *et al.* (18) demonstrated enhanced callus formation in potato internodal stem explants using 2 mg/L NAA and 6 mg/L BAP. Klimek-Chodacka *et al.* (10) found that a combination of BAP (3 mg/L) and NAA (0.5 mg/L) resulted in maximum growth rate and biomass accumulation in *Nigella damascena*. Li *et al.* (19) achieved effective results using 2,4-D and kinetin at 1 mg/L and 0.5 mg/L for the leaf, hypocotyl, and stem of the *Bupleurum chinense*. Auxin and cytokinin are effective for callus tissue formation and somatic embryogenesis in most plants (20). Our work also demonstrates similar results with 1.0:1.5 mg/L ratio of these two plant growth regulators, which resulted in the highest level of callus formation in both the Sarnav and Desiree varieties. Additionally, MS medium containing 1.0 mg NAA was found to be efficient for the Sarnav variety.

## Explants effect

Callus formation was initiated in both genotypes' explants (leaf and internodal stem). However, the extent of formation varied depending on the plant organs used as explants. Leaf explants exhibit superior growth compared to stem explants, with callus formation ranging from 65% to 100% in both Desiree and Sarnav varieties. In a study conducted by Hanh *et al.* (11), focused on kiwi plant callus formation, different parts were used. Meristem explants demonstrated higher efficacy compared to leaves and stems. In other study, different genotypes and potato tissues, including internodal stems, leaves, and roots were studied (12).

## *Callus development*

Within a span of five to ten days of cultivation, both varieties began to exhibit signs of somatic embryogenesis development, including an increase in callus formation rate, the number of explants generating callus, and the emergence of callus tissue (Table 1), which was influenced by the concentration of plant growth regulators. Over this period, explants from both varieties underwent a transformation, losing their original characteristics across all media. Initially, compact and light-green callus tissues appeared, gradually transitioning into delicate and yellowish-white forms. The coloration of callus tissues during the initial formation phase is contingent on the specific plant organs used. Specifically, leaf explants produced light-green callus tissues, whereas stem explants yielded yellow-white calli (Table 3). After a duration of two months, all explants underwent a change in their initial appearance to acquire a fragile light-green or green-yellow hue. This change is attributed to the development of chloroplasts within callus cells, influenced by light exposure. The observed color and texture of callus tissue plays a pivotal role in sustaining the process of cell proliferation, thereby enhancing the likelihood of somatic embryo formation from these cells.

Calli usually have various colours. Somatic embryos mainly appear in friable tissues of pale green, yellow, light green, and green colours. This enhances the possibility of regenerating plants. The formation of brown colour is linked with the excessive amount of phenolics in tissues, impeding plant growth (21).

The shape and size of the explants underwent irreversible changes, followed by the appearance of small, irregular cellular masses around the cut edges, eventually developing into a callus. After a span of 20 days (for both the leaf and internodal stem), the callus displayed different transformation. In the case of leaf explants of the Sarnav variety, compact light green callus appeared in the M3 nutrient medium, but later turned brown after 2 months. This could be possibly due to the high content of auxin and cytokinins, which accelerated the cell division but also initiated the ageing process. Similarly, in the leaf explants of the Desiree variety, embryogenic cells initially exhibited a light green appearance, eventually transitioning to brown-green after 2 months in M2 and M5 nutrient media. Khalafalla *et al.* (13) documented the

**Table 3.** Effect of different concentrations of NAA/BAP and NAA on callus proliferation of internodal stem explants of Sarnav and Desiree

| Media | Texture and color after one week | | Texture and color after two months | |
|---|---|---|---|---|
| | Sarnav | Desiree | Sarnav | Desiree |
| M1 | Friable yellow white | Friable yellow white | Friable light green | Friable light green |
| M2 | Friable yellow white | Friable yellow white | Friable light green | Friable light green |
| M3 | Friable yellow white | Friable yellow white | Friable light green | Friable green yellow |
| M4 | Friable yellow white | Friable yellow white | Friable light green | Friable green |
| M5 | Friable yellow white | Friable yellow white | Friable green-yellow | Friable light green |
| M6 | Friable yellow white | Friable yellow white | Friable green-yellow | Friable green-yellow |
| M7 | Callus was not formed | | | |

formation of yellow, watery calli in 100% of explants within 7-17 days across different potato varieties, noting the highest rate of callus formation. Our finding align with those in other works (2, 14).

### *Somatic embryo induction and development*

Callus tissues (obtained from internodal stems and leaves) cultivated in M2 nutrient media, characterized by a high index of callus formation, were subjected to treatment with different concentrations of SE1 (1:0.5 mg/L of BAP and $GA_3$), SE2 (0.4:0.1 mg/L of BAP and $GA_3$), SE3 (0.5:0.2 mg/L of BAP and $GA_3$), SE4 (0.1:0.1 mg/L of BAP and $GA_3$), and MS control. Following this, the callus tissues were transferred to five distinct nutrient media. Within two weeks of this transfer, embryogenic cells became apparent. Noteworthy changes in the shape and color of the callus tissues of both Sarnav and Desiree potato varieties were observed. Embryogenic cells were identified by their rounded morphology (Fig. 4). The microscopic examination unveiled various stages during the observation of embryogenic cells. The onset of somatic embryogenesis within callus tissues was contingent upon the composition of the nutrient media and external environmental factors such as temperature, light intensity, and humidity, which were optimized in previous trials. During this process, cells underwent differentiation, culminating in the formation of spherical structures (Fig. 5). The molecular mechanisms underpinning somatic embryogenesis in plants remain incompletely elucidated. Notably, overexpression of BBM genes has been reported to enhance embryo formation from callus tissue in dicotyledonous plants (22).

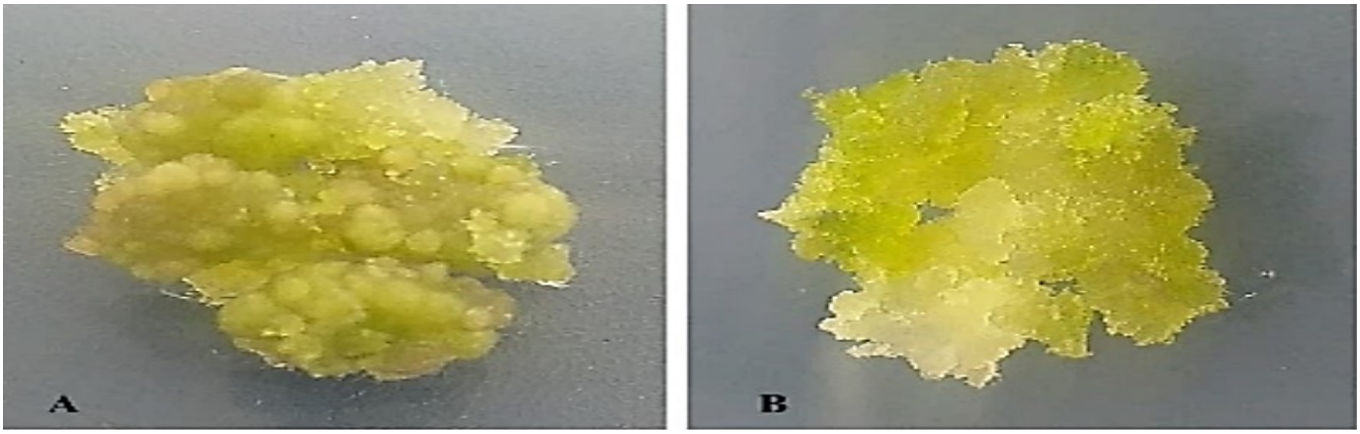


**Fig. 4. (A-B).** Sarnav potato callus tissue in an SE4 nutrient medium, (**A**) embryogenic callus tissue, (**B**) non-embryogenic callus tissue

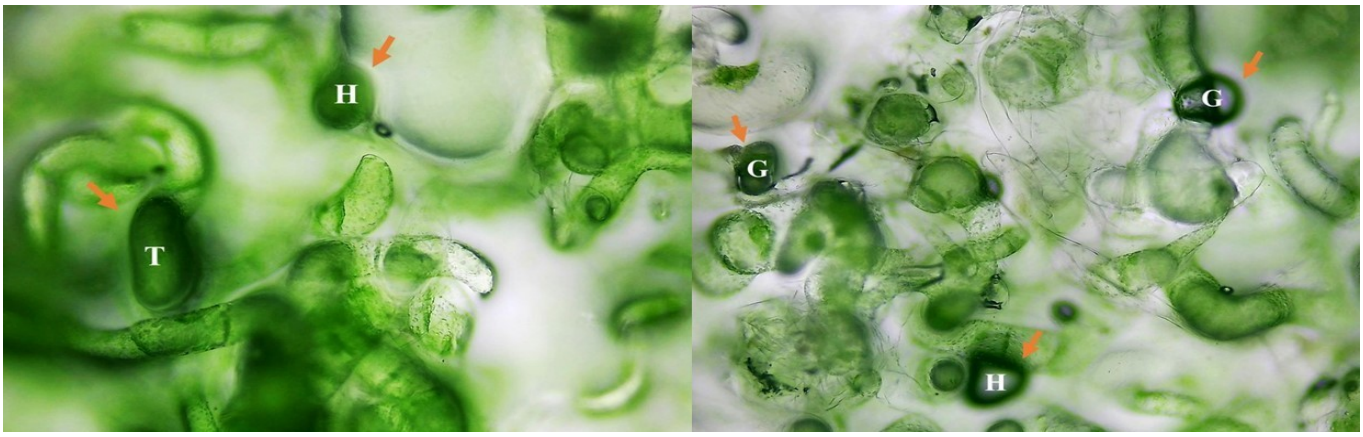


**Fig. 5.** Stages of development of the somatic embryo of the Sarnav potato variety in an SE4 nutrient medium, (G) globular stage embryo; (H)

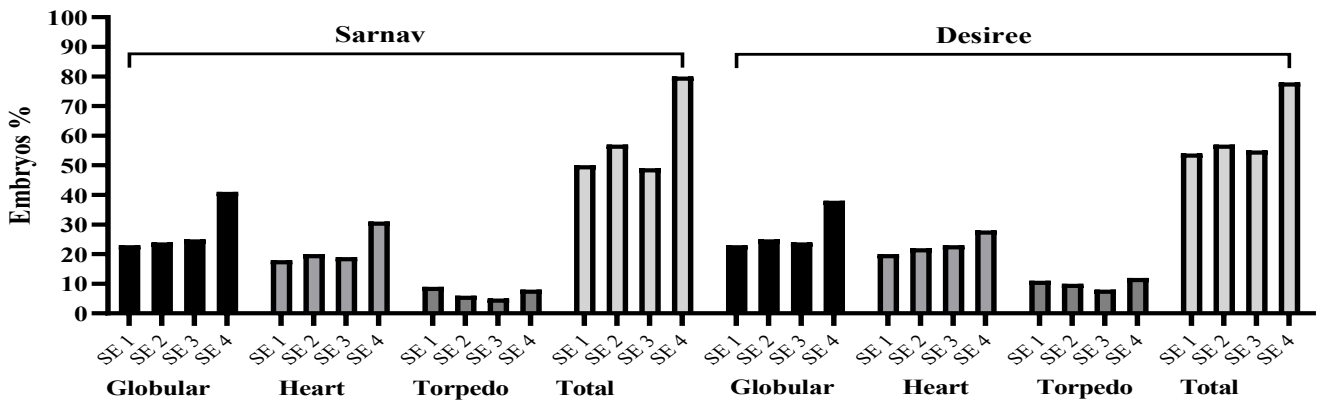


**Fig. 6.** Development of somatic embryos obtained from leaf explants in maturation medium

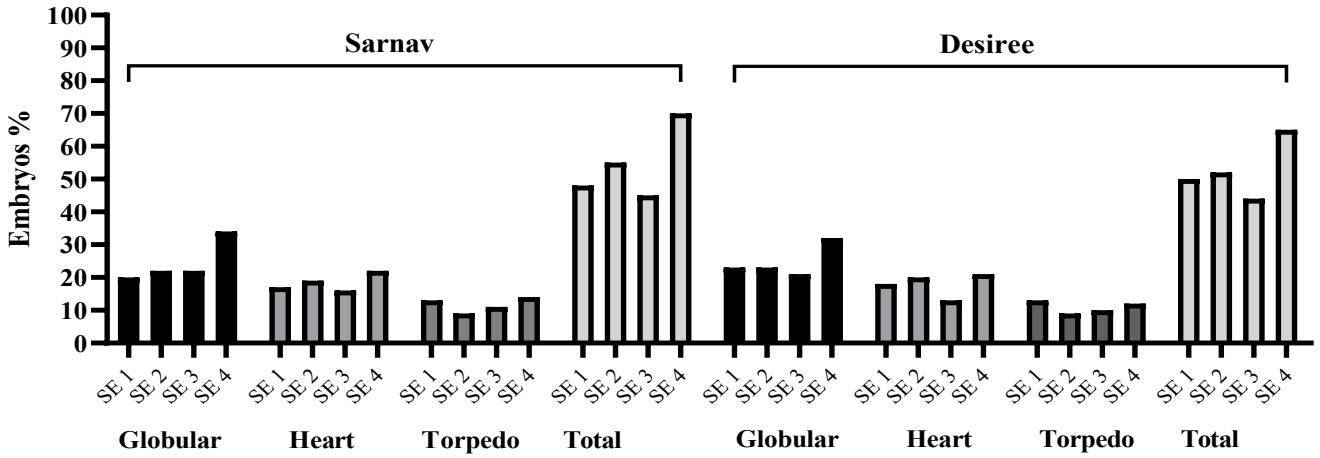


**Fig. 7.** Development of somatic embryos obtained from internodal stem explants in maturation medium

In this experiment, it was observed that the embryos of the globular, heart, and torpedo stages were formed from the explant parts of potato leaves and internodal stems. As shown in Fig. 6 and Fig. 7, internodal stem calli produced more somatic embryos (globular and heart-shaped) than leaf explants, indicating that internodal stem explants were more sensitive than leaf explants. In the research of Liang *et al.* (23) on *Scaevola sericea* plant leaf explants led to better embryo development than roots.

A higher incidence of globular and heart-shaped embryo formation was observed in both types of explants from the Sarnav and Desiree potato varieties when cultured in a nutrient medium containing 0.1 mg/L of BAP and 0.1 mg/L of $GA_3$. The pivotal role of auxin in plant development underscores the necessity of comprehensive investigation into its mechanisms in plant growth (24). Petrášek & Friml (25) underscore the importance of differential auxin distribution in various aspects of plant development. The formation of embryogenesis is influenced by endogenous auxin gradients and maxima, facilitated by cell-to-cell transport. Studies on *Arabidopsis thaliana* by Su and Zhang (26) revealed varying auxin levels in different embryonic stages, suggesting its regulatory role. Notably, auxin gradients crucially contribute to stem cell formation in embryonic callus, mediated through the regulation of PIN1, thus unravelling the mechanism of somatic embryogenesis.

The assessment of total embryo count and the quantification of embryos at globular, heart-, and torpedo-shaped stages were conducted separately for both leaf and stem explants of the Sarnav and Desiree varieties after 10 weeks of cultivation. The outcomes demonstrated the superior efficacy of the BAP/$GA_3$ ratio of 0.1:0.1 mg/L in the nutrient medium. The impact of varying cytokinin levels on the rate of somatic cell division has been explored in other research. In a similar study, somatic embryos were successfully induced in *Wedelia calendulacea* plant using a nutrient medium containing BAP and $GA_3$ in a 1.0:0.5 mg/L ratio (27). Likewise, a high rate of somatic embryo development was achieved in *Olea europaea* using a medium containing NAA and $GA_3$ in a 0.1:0.1 mg/L ratio (28).

## Conclusion

Callus induction was performed on two potato varieties using NAA: BAP and NAA-enriched variants of the MS nutrient media. Somatic embryogenesis was then carried out using BAP: $GA_3$-enriched medium. The M2 medium, containing 1 mg/L NAA and 1.5 mg/L BAP, exhibited the highest efficiency, with no significant differences observed in either variety or tissue type. Notably, the Sarnav variety exhibited similar callogenesis to the Desiree variety across various conditions. The SE4 culture medium, enriched with 0.1 mg/L BAP and 0.1 mg/L $GA_3$, yielded the highest rate of embryo formation in stem and leaf explants of both varieties. Furthermore, internodal stem explants exhibited slightly faster somatic embryos than leaf explants, making them a more suitable choice for somatic embryogenesis in these varieties.

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## Authors' contributions

FIB, KAU, ANA, AAB, SAA and JBE carried out experiments. FIB, KAU and ZTB designed the work. FIB, BKR and AMA drafted the manuscript. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest**: Authors do not have any conflict of interests to declare.

**Ethical issues** : None.

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