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RESEARCH ARTICLE

Effects of different growth hormones on *Solanum tuberosum* L. callus induction and regeneration

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Abstract

This study was carried out to develop a protocol for callus induction, regeneration, shoot and root induction in two potato cultivars (Riviera and Almonda) that can be further used to develop genetically modified plants. Callus was induced from leaf and internodal explants on Murashige and Skoog's medium (MS) supplemented with different combinations of hormones. The highest percentage of callus induction was obtained from the internodal explants of Riviera cultivar was 100% in Callus Induction Media I (CIM I) supplemented with 2 mg/L Benzyl Adenine (BA), 0.2 mg/L α-Naphthalene Acetic Acid (NAA), 2 mg/L 2,4-Dichlorophenoxy acetic acid (2,4-D), 0.2 mg/L Kinetin (Kin) and 0.2 mg/L gibberellic acid (GA3). After one-month calli were shifted to Shoot Induction Media I and II (SIM I and SIM II), the highest percentage for shoot induction was 33.3% with the leaf explants of Riviera cultivar on SIM II supplemented with 2 mg/L BA, 0.2 mg/L NAA, 0.5 mg/L Kin and 0.2 mg/L GA3. The root induction percentage was 100% for both cultivars. Regenerated potato plants had normal leaf shapes with uniform morphology. These findings provide valuable insights for developing tissue culture protocols for potato regeneration, which are essential for genetic engineering and potato breeding programs.

Keywords

Callus induction; growth hormones; *Solanum tuberosum* L.; tissue culture

Introduction

Solanum tuberosum L. (Potato) holds global significance as a valuable non-cereal food crop, ranking fourth in acreage and production worldwide (1-5). It is recognized for its nutritional richness, and potatoes are a vital source of starch, protein, vitamins and dietary fiber, contributing to immune system health, blood pressure regulation and improved brain function. Beyond nutrition, potatoes play a crucial role in the livelihoods of millions globally (6-11). In Iraq, potatoes have two growing seasons-spring (December to mid-February) and autumn (late August to mid-September) (12). Key potato varieties in Iraq include Riviera, Almonda, Nicola, Agria, JIP 1600-1, Dimont, Arezona, Naema, Sevara, Porin and Alpada (13). However, traditional breeding is often limited by potato genotypes and lengthy breeding cycles; biotechnology emerges as a solution for challenges faced by potato farmers, with a focus on plant regeneration from cell and tissue culture (14-22).

Callus induction, shoot regeneration and root induction are fundamental steps in potato tissue culture and are essential for the successful regeneration of genetically engineered plants.

Researchers have made significant progress in potato callus induction and plant regeneration by optimizing concentrations of plant growth regulators (23-24). Successful *in vitro* plant regeneration from various potato explants, including leaves, stems, tuber discs, and unripe zygotic embryos, depends on factors like genotype, culture medium composition, and growth regulatory concentrations (25-30). By studying the effects of different hormone combinations, optimal conditions for each stage of regeneration can be developed. These insights are essential for facilitating the production of genetically engineered potato plants with desirable traits (31). Plant growth regulators used for callus induction are Auxin and cytokinin (32-33).

In this study, for callus induction, BA, NAA, 2, 4-D, Kin and GA3 have been used; for shoot induction, BA, NAA, GA3, and Kin, and root induction, IBA has been used in two cultivars, Riviera and Almonda.

The present study aimed to achieve the optimal composition of plant hormones to obtain regeneration potato plants with the best formulation. Additionally, it is essential to identify the best composition of media and hormones that can be used as a protocol in the genetic engineering of potatoes.

Materials and Methods

The study was carried out in the laboratory of the Plant Biotechnology Department at Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq.

Plants Materials

The Potato cultivars Nederland (Riviera and Almonda) used in this study were obtained from Nahar Al Awrad Company / Abo-graib / Baghdad / Iraq.

Explants and surface sterilization

Potato cultivars were soaked in gibberellin (10 mg/L) to break the dormancy and obtain tuber sprouts. Tuber sprouts were then washed for an hour under tap water.

Tuber sprouts of the potato cultivars were sterilized with 10% sodium hypochlorite (NaOCl) and then treated with 70% ethanol for 1 min. In the final step, tuber sprouts were rinsed 2-3 times with sterilized double distilled water. Tuber sprouts were shifted on MS media (pH 5.7) and seedlings were obtained after growth at 24 ˚C under light and dark (16:8h) in a growth chamber.

Callus Induction Media (CIM)

Leaves and internodal (1-1.5 cm) explants were used after 4 weeks and cultured in two combinations of Callus Induction Media (CIM) I and II. For both CIM I and II, MS media containing basal media were complemented with 20 g/L sucrose, 7.5 g/L agar, GA3 (0.2 mg/L), BAP (2 mg/L), and NAA (0.2 mg/L) (pH: 5.7). CIM I, also contained 2,4-D (2 mg/L) and Kin (0.2 mg/L). The pH was adjusted (5.7). After one month, the average fresh callus weight was determined.

Shoot Induction Media SIM

After 4 weeks, the callus shifted to Shoot Induction Media (SIM) I, II and III and was kept in the growth chamber as in the previous condition. Every 4 weeks, the callus was weighted and subcultured. For all SIM I, II and III MS media containing basal media complemented with 20 g/L sucrose, 7.5 g/L Agar, GA3 (0.2 mg/L), BAP (2 mg/L) and NAA (0.2 mg/L). SIM II also contains Kin (0.5 mg/L). The pH for all was adjusted to (5.7). After two months, the average length was determined.

Root Induction Media RIM

After shoot regeneration (3 months), plantlets were transferred to RIM, which contained basal media complemented with 20 g/L sucrose, 7.5 g/L Agar, IBA (2 mg/L), and sulcide (0.1 mg/L) with pH (5.7).

Data Analysis

Data on callus induction, callus morphology, callus fresh weight, %regeneration, number of shoots/callus clump, shoot length, number of nodes/shoot and number of leaves/shoots were taken.

Results and Discussion

Callus Induction

The results showed callus induction from both leaf and internodal explants from both potato cultivars, Riviera and Almonda. The result revealed that the highest callus formation for leaves was 96.3% for the Almonda cultivar, while for Riviera, 93.3% on CIM I (Table 1, Fig 1B), which included MS media with 2 mg/L BA, 0.2 mg/L NAA, 2 mg/L 2,4-D, 0.2 mg/L Kinetin and 0.2 mg/L Gerbilline. While, for CIM II (Table 1), which includes MS media with 2 mg/L BA, 0.2 mg/L NAA, and 0.2 mg/L Gerbilline was recorded (77.0% and 70.0%) for callus formation for Riviera and Almonda, respectively.

The results also showed that the highest percentage of callus formation for internodes was 100% in the Riviera cultivar, while for Almonda was 93.2%, both for CIM I (Table 2). At the same time, callus formation was (95.0% and 77.4%) both for CIM II for Riviera and Almonda, respectively.

Callus induction media and varieties affect callus fresh weight and callus morphology. Table 1, Fig 1A and Fig 4 illustrate how cultivars differ in their response to media formulations regarding fresh weight and callus induction percentage derived from leaf explant. CIM I and CIM II had a significant effect on fresh weight among cultivars. The absence of significant differences among columns with similar letters indicates that these cultivars demonstrate similar responses to media composition in both cultivars. The results showed that the highest average callus weight of the Riviera variety was 0.184g, while Almonda was 0.153g both for CIM I and the highest weight using CIM II was 0.034g and 0.011g for Riviera and Almonda, respectively. These findings agreed with

Table 1. Effect of different combinations of plant growth regulators on callus induction from *in vitro* leaf explants of potato cultivars after four weeks in culture.

Potato cultivar	Culture media	Callus induction (%)	Average of fresh callus weight (g)	Callus morphology
Almonda	CIM I	96.3 a	0.153 a	Friable
	CIM II	70.0 b	0.011 b	friable
Columns with the same letter are not significantly different, using Duncan's Multiple Range Test at 5% level".				
Riviera	CIM I	93.3 a	0.184 a	Compact
	CIM II	77.0 b	0.034 b	Compact
Columns with the same letter are not significantly different, using Duncan's Multiple Range Test at 5% level				
Contents of the CIM I= 2 mg/L BA+ 0.2 mg/L NAA+ 2 mg/L 2,4-D + 0.2 mg/L Kin + 0.2 mg/L GA_3				
Contents of the CIM II= 2 mg/L BA+ 0.2 mg/L NAA+ 0.2 mg/L GA_3				

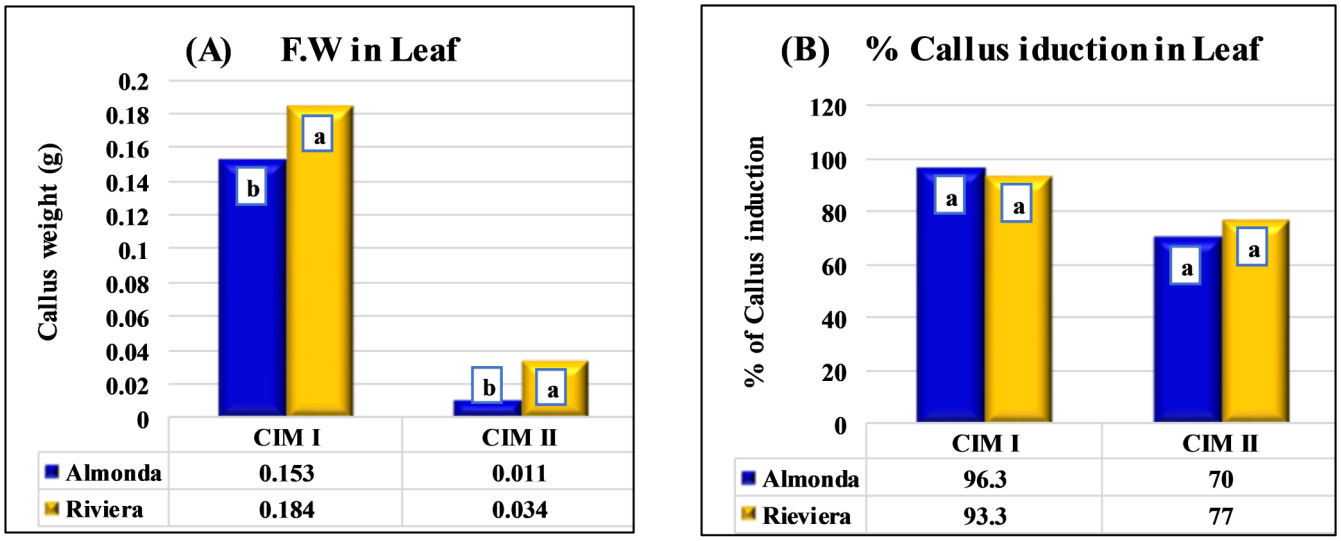


Fig. 1. Comparison between cultivars in the effect of media on fresh weight from leaves and the callus induction percentage. A: fresh weight in leaf for both cultivars. B: Callus induction percentage in leaf for both cultivars. Columns with the same letter are not significantly different, using Duncan's Multiple Range Test at a 5% level".

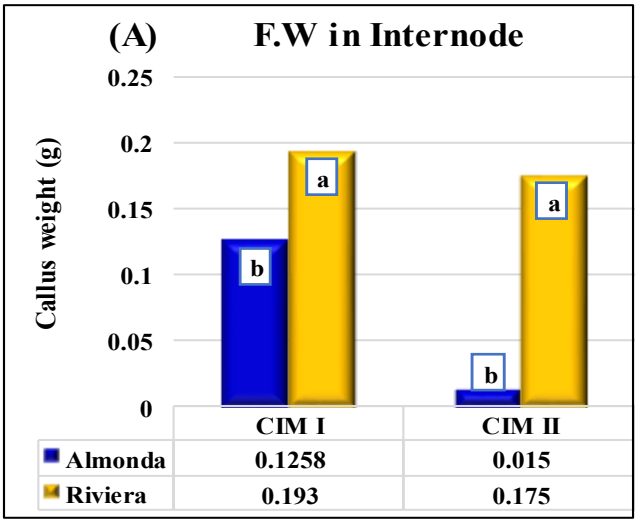
previous studies (34), which noted that the cultivars had no significant effect on callus induction, but they had a significant effect on callus mass.

The results showed that the combination of 2,4-D and Kin with BAP and NAA had a significant effect on callus induction and, on average, fresh callus weight, suggesting that the 2,4-D and Kin with BAP and NAA improved callus induction. While with the internodes of Riviera, there was a non-significant effect on callus induction (Table 2 and Fig. 2B). These results agreed with Kumar et al (35), which revealed that the highest callus induction was on MS medium supplement with 2,4-D and Kin.

The results (Table 2, Fig 2A) also revealed that CIM I and CIM II had a significant effect on fresh weight in both cultivars. Also, there was a significant effect of Cultivars in callus induction in CIM II, while there were no significant differences among cultivars in CIM I. Fig 2A illustrates that the highest average weight of callus for internodes was 0.193g in the Riviera cultivar and for Almonda 0.1258g, both for CIM I. While for CIM II, it was 0.175g and 0.015g for Riviera and Almonda, respectively. These findings agreed with previous studies (36), which revealed that the internodal explant had a significant effect on callus weight.

Table 2: Effect of different combinations of plant growth regulators on callus induction from *in vitro* **internode** explants of potato cultivars after four weeks in culture.

Potato cultivar	Culture media	Callus induction (%)	Average of fresh callus weight (g)	Callus morphology
Almonda	CIM I	93.2 a	0.1258 a	Friable
	CIM II	77.4 b	0.015 b	Compact
Columns with the same letter are not significantly different, using Duncan's Multiple Range Test at 5% level				
Riviera	CIM I	100 a	0.193 a	Friable
	CIM II	95.0 a	0.175 b	Compact
Columns with the same letter are not significantly different, using Duncan's Multiple Range Test at 5% level				
Contents of the CIM I= 2 mg/L BA+ 02 mg/L NAA+ 2 mg/L 2,4-D + 0.2 mg/L Kin + 0.2 mg/L GA_3				
Contents of the CIM II= 2 mg/L BA+ 0.2 mg/L NAA+ 0.2 mg/L GA_3				



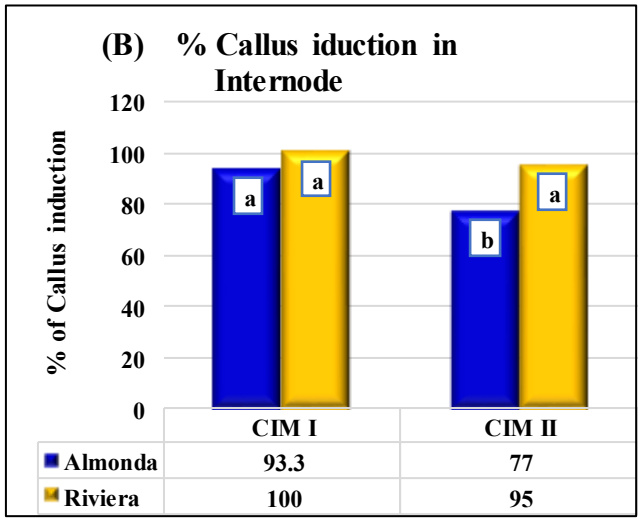


Fig. 2. Comparison between cultivars in the effect of media on fresh weight from internode and the callus induction percentage. A: fresh weight in internodal for both cultivars. B: Callus induction percentage in internodal for both cultivars. Columns with the same letter are not significantly different, using Duncan's Multiple Range Test at a 5% level.

The data regarding callus morphology showed different colors and textures; most calli were yellowish white, yellowish green, and yellow (Fig. 4) (Table 1 and 2). Half of the calli texture was compact, and half was friable.

In the current study, it was observed that the auxin (2, 4-D) and cytokinin (Kin), along with BA and NAA, were the most effective for callus induction. These findings agreed with Abu Zeid, et al (37), Metwali et al. (38), Bakhsh (39), and Kumar et al. (35), who noted that 2,4-D and Kin are the best dose compared to other combinations to enhance callus induction from potato. Also, the results are in agreement with Turhan (40), who noted that Kin and NAA gave the best percentage for callus induction.

The results also showed that the 2,4-D with BA gave effective callus induction, which is consistent with findings from other studies (31,41).

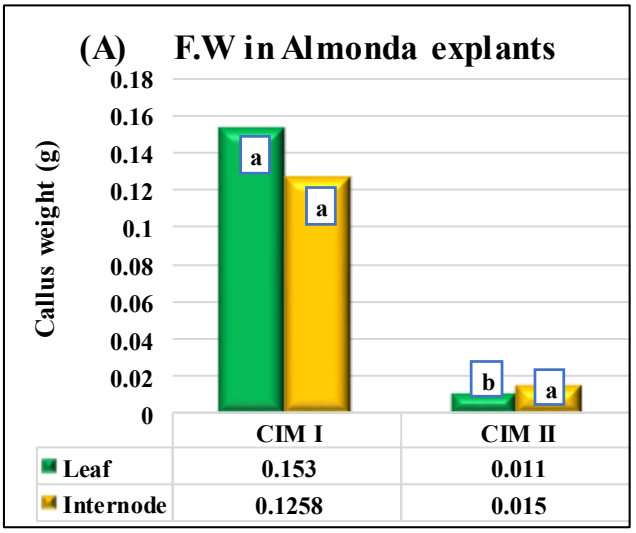
GA_3 promotes development and elongation in plants according to Abdul (42), who reported that GA_3 promotes cell division which leads to elongation.

The results (Fig. 3 and 4) also showed there was a significant effect on the explant source in the Almonda cultivar in CIM I, while in CIM II, there was no significant effect. Haque et al (43) and Dhital et al (44) noted that the callus induction from leaf explants had a significant effect than internode explants. Riviera Cultivar showed no significant effect of explant source in CIM I; however, CIM II had a significant impact on leaf and internode. Their results were agreed by Huda et al (36), who noted that internodes gave higher callus weight in comparison with leaf explants.

These findings were consistent with previous studies (45,41,31), which noted that there was a different response among growth media regulations due to genetic variation and physiological differences.

Regeneration

Table 3 shows the effect of both SIM I and SIM II on shoot induction (%), average number of shoots/explant and average length (cm) from leaf explant of both Cultivars. The results revealed that the Riviera cultivar was superior in shoot forming to Almonda, who gave 0% shoot induction. The shoot induction percentage from Riviera leaves was 17.95% and 33.33% on SIM I and SIM II,



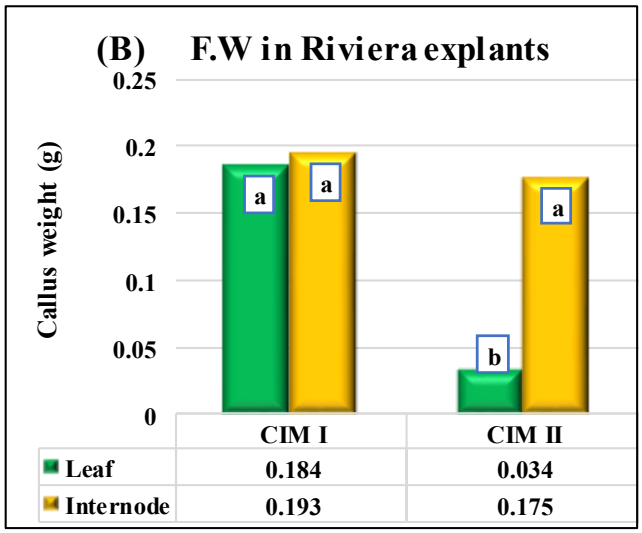


Fig. 3. Comparison between effects explants sources (leaf and internode) on the callus fresh weight. A: fresh wight in Almonda explants. B: fresh wight in Almonda explants. Columns with the same letter are not significantly different, using Duncan's Multiple Range Test at a 5% level.

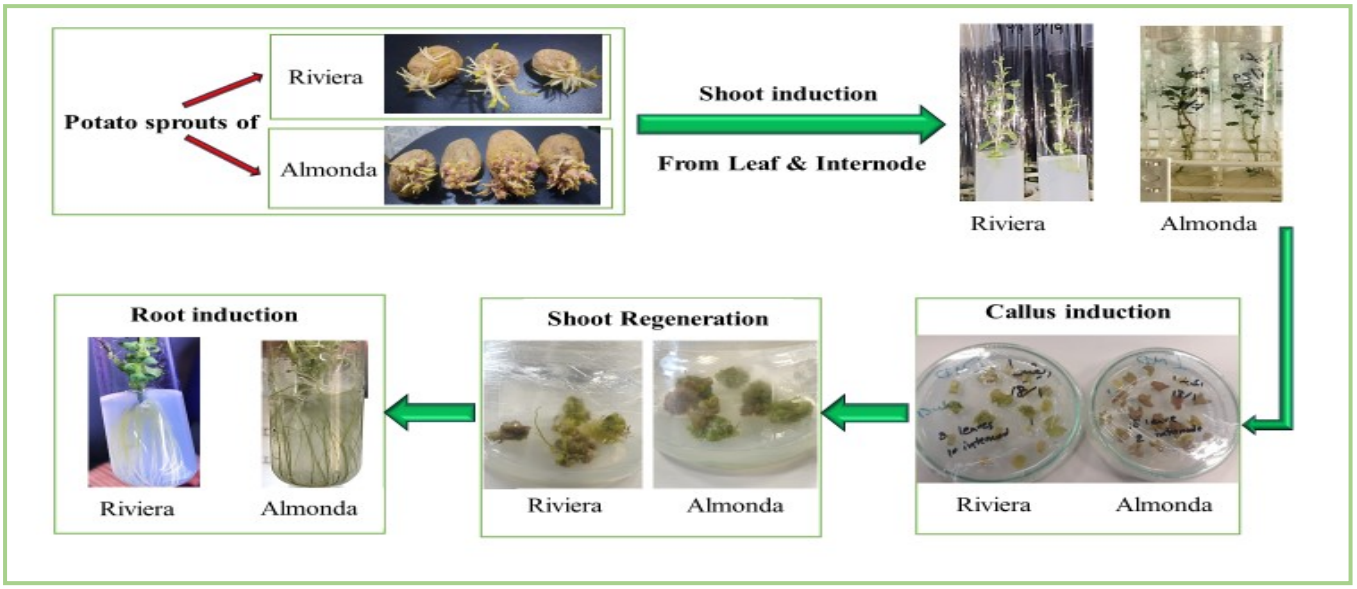


Fig 4. Steps for callus induction, shoot, and root regeneration.

Table 3: Effect of different combinations of plant growth regulators on shoot organogenesis from callus induced from leaf explants of potato cultivars.

Potato cultivar	Culture media	Shoots induction (%)	Average number of shoots/ explant	Average length (cm.)
Almonda	SIM I	0 a	0 a	0 a
	SIM II	0 a	0 a	0 a
Columns with the same letter are not significantly different, using Duncan's Multiple Range Test at 5% level				
Riviera	SIM I	17.9 b	1.1 b	7.1 a
	SIM II	33.3 a	9.4 a	6.2 a
Columns with the same letter are not significantly different, using Duncan's Multiple Range Test at 5% level				
Contents of the SIM I= 2 mg/L BA+ 0.2 mg/L NAA+ 0.2 mg/L GA_3				
Contents of the SIM II= 2 mg/L BA+ 0.2 mg/L NAA+ 0.2 mg/L GA_3+ 0.5 mg/L Kin				

respectively. The results demonstrate a significant effect in shoot induction, Average number of shoots/explants and Average length between SIM I and SIM II for the Riviera cultivar. The shoot induction in SIM I 17.9b while in SIM II 33.3a. The average number of shoots per explant was 1.1b and 9.4a in SIM I and SIM II, respectively. The average length was 7.0 cm in SIM I and 6.0 cm in SIM II.

Table 4 shows the effect of both SIM I and SIM II on shoot induction (%), average number of shoots/explants, and average length (cm) from the internode explant of both Cultivars. The results showed that there was a significant effect in shoot induction, Average number of shoots/explants and Average length between SIM I and SIM II for the Riviera cultivar, while for Almonda, there was not any significant effect. The shoot induction percentage of Riviera internodes was 12% and 16.6%, while Almonda gave 5.2% and 1.8% on SIM I and SIM II, respectively.

The average number of shoots per explant was 2.3b and 3.5a in SIM I and SIM II, respectively, for Riviera and in Almonda was 1.0a for both SIM I and SIM II. The average length in Riviera was 8.2 cm in SIM I and 6.3 cm in SIM II. While in Almonda it was 5.4 and 5.6 for SIM I and SIM II, respectively.

Table 4: Effect of different combinations of plant growth regulators on shoot organogenesis from callus induced from internode explants of potato cultivars.

Potato cultivar	Culture media	Shoots induction (%)	Average number of shoots/explants	Average length (cm.)
Almonda	SIM I	5.2 a	1.0 a	5.4 a
	SIM II	1.8 a	1.0 a	5.6 a
Columns with the same letter are not significantly different, using Duncan's Multiple Range Test at 5% level				
Riviera	SIM I	12.0 b	2.3 b	8.2 a
	SIM II	16.6 a	3.5 a	6.3 b
Columns with the same letter are not significantly different, using Duncan's Multiple Range Test at 5% level				
Contents of the SIM I= 2 mg/L BA+ 0.2 mg/L NAA+ 0.2 mg/L GA_3				
Contents of the SIM II= 2 mg/L BA+ 0.2 mg/L NAA+ 0.2 mg/L GA_3+ 0.5 mg/L Kin				

SIM II gave higher shoot induction, this may be due to the KIN, which has an effect along with other growth regulators in inducing shoot organogenesis.

Different media combinations gave an understanding of using different plant growth regulators in each media. SIM I media is supplemented with BA, NAA, and GA_3, while SIM II includes an additional hormone, Kin, along with BA, NAA and GA_3. The observed variations in shoot induction and growth characterized between SIM I and SIM II are mainly a result of differences in hormonal content. These results were demonstrated by Shibli et al (46) and Priyadarshani and Batra (47) who noted that there was a significant reduction in both leaf and internode length and shoot organogenesis by adding BA and Kin in MS media. Also, Pereira and Fortes (48) and Priyadarshani and Batra (47) noted that GA_3 has a significant effect on micropropagation in Potatoes.

The Results in Table 5 and Fig 4 showed the effect of Indole-3-Butyric Acid on rooting of potato cultivars after four weeks in culture. The table showed root induction (%), the average number of roots/explants and the average length (cm) originating from leaves and internodes from both cultivars. The leaves of the Almonda cultivar did not give any root induction (0%), while the internodes gave a 100% root induction percentage with an average number of roots/explants of about 30, with 7.1 as the average length. The leaves and internodes of Riviera cultivars gave a 100% root induction percentage with an average number of roots/explants of about 37 and 35 for leaves and internodes, respectively. The average length is about 5.1cm for leaves and 4.9cm for internodes. These results agree with other studies (22,24,49), which noted that the best results were obtained by using basil MS medium with IBA.

Conclusion

This study aimed to develop a protocol for callus induction and shoot and root regeneration of *Solanum tuberosum* L. in two cultivars (Almonda and Riviera) using different plant growth regulators. Overall, this study successfully provides valuable insights for developing a protocol for potato regeneration by tissue culture technique, which is good for genetic engineering and breeding programs for potatoes.

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Authors' contributions

AW and DM conceptualized the idea and planned and designed the research work. DM carried out all experiments. AN supervised research activity. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

The author did not use AI technology to write this article.

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Table 5. Effect 2 mg/L of IBA on rooting of potato cultivar after four weeks in culture.

Potato cultivar	Origin of explant	Root induction (%)	Average number of roots/ explants	Average length (cm.)
Almonda	Leaf	0 b	0 b	0 b
	Internode	100 a	30 a	7.1 a
Columns with the same letter are not significantly different, using Duncan's Multiple Range Test at 5% level				
Riviera	Leaf	100 a	37 a	5.1 a
	Internode	100 a	35 a	4.9 a
Columns with the same letter are not significantly different, using Duncan's Multiple Range Test at 5% level				

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