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Standardization of indirect regeneration protocol in Lady Rosetta cultivar of potato (*Solanum tuberosum*)

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ABSTRACT

The present experiment was conducted during 2021–22 at School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab to standardize indirect somatic embryogenesis for rapid callus induction, plantlet regeneration and shoot proliferation in the popular potato (*Solanum tuberosum* L.) cv. Lady Rosetta. Nodal and internodal regions were used as explants for the process under sterilized laminar air flow cabinet conditions. The experiment was laid out in a completely randomized design (CRD) with three replications. About 9.78% callus induction were noticed in the explants cultured on Murashige and Skoog (MS) media supplemented with growth regulators i.e 4.0 mg/L 2, 4-Dichlorophenoxy acetic acid (2,4-D) and 0.5 mg/L Kinetin (Kin) for induction of callus and regeneration of shoot tissues from callus. Simultaneously, 48.9% plantlet regeneration from calli was obtained in the MS media with 4 mg/L Benzyl Amino Purine (BAP) and 1 mg/L Kin. We observed better callus induction at 4.0 mg/L 2,4-D and 0.5 mg/L Kin, shoot induction at 4.0 mg/L BAP and 1.0 mg/L Kin and root induction in half-strength MS media. The regenerated shoots were efficiently grown on rooting media, later transferred for hardening and acclimatized under field conditions. The present study serves as an efficient protocol to conduct tissue culture experiment using potato explants for calli induction, shoot regeneration and rooting in cv. Lady Rosetta. These findings will serve as a significant finding for regenerating potato plants from calli for genetic transformation/genome editing studies.

Keywords: Callus, Hardening, Internode, Potato, Tissue culture

Potato (*Solanum tuberosum* L.), a short-day annual crop belonging to the Solanaceae family, is nutritionally enriched with vitamins, proteins, carbohydrate (starch) and minerals i.e. phosphorus, potassium and magnesium (Subrahmanyeswari *et al.* 2024). Potato has high demand in the food production in fresh as well as processing markets (Dolnicar *et al.* 2021). Considering the importance of potato in research and development as a vegetable crop, the United Nations considered 2008 as an "International Year of the Potato" (Lutaladio and Castaldi 2009). Globally, the second-largest producer of potatoes is India contributing towards agricultural economy (Singh and Dutt 2024).

Although potato is a highly nutritional crop, it is susceptible to bacterial and fungal infections that hinders the crop productivity halting the production and marketing sectors (Ashu *et al.* 2018). The plant propagation in potato occurs through sexual and asexual methods through true potato seed and tubers, respectively, but these have less multiplication rate (Gu *et al.* 2024). To overcome this difficulty, tissue culture approaches serve as a substitute for vegetative mass multiplication of plants in a limited space excluding season-independent constraints and genetic or morphological alteration in plant species (Jain 2001, Char *et al.* 2023).

The optimization parameters include the use of growth regulators viz. Benzyl Adenine (BAP), Gibberellic acid (GA_3), Kin, Zeatin, 2,4-D, Chlormequat chloride, α-naphthalene acetic acid (NAA) and Chlorocholine chloride, which are vital for the *in vitro* direct potato regeneration resulting in the formation of a large number of shoots and roots (Mohapatra and Batra 2017, Subrahmanyeswari *et al.* 2024). Tissue culture approach in an optimized manner is widely used for the propagation of potato plants (Walia *et al.* 2021, Moletsane *et al.* 2022, Sharde *et al.* 2024, Yousef *et al.* 2024, Massa *et al.* 2024). The Lady Rosetta is the processing potato variety which possesses high dry matter and low reducing sugars.

The significance of this study is to obtain the standardized protocol for generation of callus, root and shoot tissues so that these tissues can be utilized for tissue-specific genome editing and omics studies. Hence, the objective

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of the research was the development of indirect somatic embryogenesis protocol that will be considered good for growth and multiplication in future genetic transformation/ genome editing experiments.

MATERIALS AND METHODS

The present experiment was conducted during 2021–22 at School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab. The explants obtained from the seed tubers of Lady Rossetta with IC number (CIP-2003-0001) were sprouted and maintained in 3 kg pots filled with autoclaved soil under controlled conditions in a plant growth chamber. After collection of explants, they were washed with teepol and mercuric chloride for surface sterilization of the plant material. The explants were then excised into nodes and internodes from the potato cv. Lady Rosetta under sterilized conditions in laminar air flow cabinet. Till date, tissue culture has been done in potato cultivar Kufri Jyoti (Rawat *et al.* 2017), Kufri Sindhuri (Sharma *et al.* 2023) and Gudiene and Belete potato varieties (Hajare *et al.* 2021). No work has been done on the potato cv. Lady Rosetta justifying the novelty of the research. Moreover, this variety has high demand in the food processing industry equipped with low sugar and starch content with longer shelf life. Collected explants were treated with 1% carbendazim^{TM} and 0.1% mercuric chloride for 10 and 8 min followed by 2–3 washings with autoclaved distilled water.

Culturing of explants was done on MS media grown with growth regulators i.e 2, 4-D, Benzyl amino purine (BAP) and Kinetin (Kin) at various concentrations. Kin and 2, 4-D were optimized for the induction of callus and subsequently, Kin and BAP were used for callus regeneration. Explants (from nodes and internodes) were cultured on callus inducing media with constant Kin concentration of 0.5 mg/L and consecutively, increasing concentration of 2,4-D (0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/L). Similarly, the selected calli were placed on regeneration media having increasing concentrations of BAP (0.0, 0.5, 1.0, 2.0, 2.5, 3.0, 4.0 and 5.0 mg/L) with constant concentration of Kin i.e. 0.5 mg/L and 1.0 mg/L. Control media were also provided both for the induction and regeneration (having no hormone). Later, the root induction was also optimized on half- and full-strength MS media. The germinated plants were transferred to moist cotton for 2–3 days and then transferred to the cocopeat and soil mixture for further growth analysis in glass house. All the data obtained was subjected to completely randomized design (CRD) having three replication with statistical analysis using SAS software (Littell *et al.* 1996). The average means were compared through standard error values and critical difference at 5% value (CD @5%).

RESULTS AND DISCUSSION

Effect of 2,4-D *on callus induction*: 2,4-D is an effective auxin for the stimulation of cell growth and division for callus induction. It is the preferable growth regulator for induction of callus in potato (El-Sherbini *et al.* 2024). To finalize the optimum concentration of 2, 4-D for callus induction in cv. Lady Rosetta, various concentrations ranging from 0.0, 1.0, 2.0, 3.0, 4.0, 5.0 mg/L was used with a constant Kin concentration of 0.5 mg/L. The callus induction data was recorded in triplicates. Callus induction was not found in the control MS media without any hormones. The details of the interaction of 2, 4-D and Kin on callus induction is shown in Table 1 and Fig. 1.

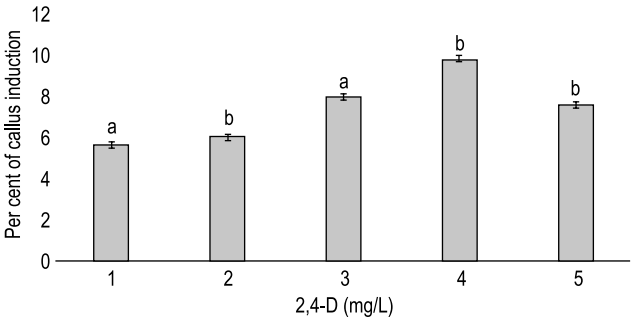

Fig. 1 Per cent callus induction on variable concentrations of 2,4-D (mg/L) at 1.0, 2.0, 3.0, 4.0, 5.0 and constant 0.5 mg/L Kin concentration.
Note: The graph represents the mean, standard deviation and Duncan's multiple range test for comparison of treatment of means in three replications completely randomized design (CRD). a and b, Statistically significant difference at $P<0.05$.

The 2,4-D at 4.0 mg/L was the most efficient concentration for the induction of 9.78% calli with a compact texture. Similarly, Mohamed *et al.* (2024) reported the compact, nodular brown, yellow or green calli during the culturing of stem nodal segments of potato and the highest calli fresh weights of 2.22 ± 0.85 and 1.25 ± 0.64 gram were obtained in Lady rosetta and Chara potato cultivars respectively. Further, time duration for callus induction at this particular concentration was less in comparison with other concentrations. Thus, 4.0 mg/L 2, 4-D and 0.5 mg/L Kin was found optimum in potato cv. Lady Rosetta. Shallal (2024) also used 2,4-D at varying concentration and found that 2, 2.5, and 3 mg/L of 2,4-D were the most suitable concentrations for inducing high amount of callus

Table 1 Callus induction on MS media supplemented with different 2,4-D concentrations

2, 4-D (mg/L)	Per cent callus induction	Time duration (days)	Callus texture	Degree of callus formation
0	0	0	--	--
1	5.62	16.85	Compact	+
2	6.03	16.64	Loose	++
3	7.95	11.0	Loose Watery	++
4	9.78	7.81	Compact	+++
5	7.56	9.23	Loose	++

--, No callus; +, Poor callus; ++, Moderate callus; +++, Massive callus.

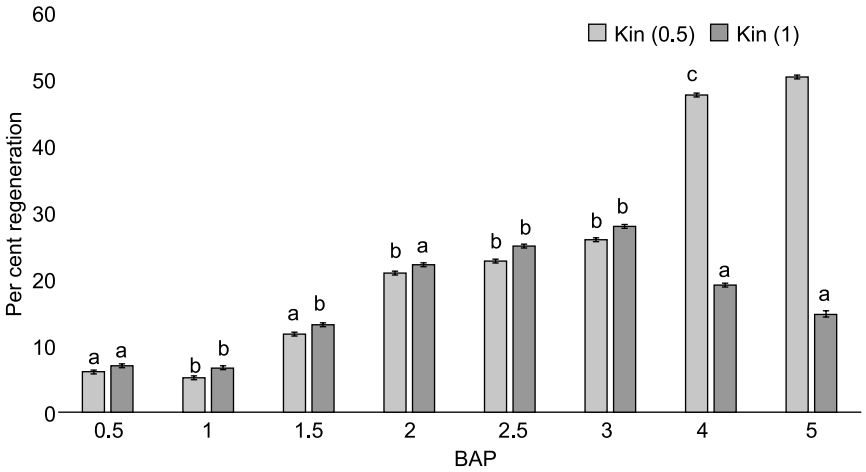

Fig. 2 Per cent regeneration of callus tissue on supplementation of different concentrations of BAP (mg/L) with constant concentrations of 0.5 and 1.0 (mg/L) Kinetin.
Note: Regeneration in MS media supplemented with various concentrations of BAP (mg/L) at of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0 concentration with constant Kin at 0.5 and 1.0 (mg/L) concentrations. DMRT analysis of the data showed the mean ± standard error of three independent replications. a, b and c, Statistically significant difference at $P<0.05$.

in potato cultivar Arizona. Mohamed *et al.* (2024) studied the combined effect of thidiazuron (TDZ) and 2,4-D at 1 and 5 ppm (combined and/or individually) on stem nodal segments in two potato cultivars i.e. Lady Rosetta and Chara that cause the induction of callus formation.

Effect of BAP on callus regeneration: BAP is a cytokinin which is well known for cell division, bud formation, shoot multiplication and proliferation. The variable concentrations of BAP (0.5, 1.0, 2.0, 2.5, 3.0, 4.0 and 5.0 mg/L) at two constant Kin concentrations viz. 0.5 mg/L and 1.0 mg/L were optimized to observe callus regeneration. Callus regeneration was not seen in the control MS media. The detailed description of the regeneration of calli using BAP concentrations was shown in Table 2 and Fig. 2.

The highest callus regeneration efficiency for plantlet regeneration of about 50.43% within approximately 15 days was observed on BAP (5 mg/L) and Kin (0.5 mg/L) media. We observe that subsequent increase in the BAP concentration from 0.5–5 mg/L led to the enhancement in the per centage plantlet regeneration. Similarly, MS media supplemented with BAP approximately 4.0 mg/L showed highest frequency of shoot regeneration (Salehi *et al.* 2014, Yasmin *et al.* 2022).

Simultaneously, the supplementation of MS media with 5.0 mg/L BAP and 1.0 mg/L kinetin showed the highest percentage (50.83%) of plantlet regeneration. Although BAP concentration of 5.0 mg/L enhanced the percentage of calli regeneration, we observed browning effect at this concentration at both 0.5 and 1.0 Kin (mg/L) which is not desirable, hence, subsequent BAP addition was not done. Hence, MS medium supplemented with 4.0 mg/L BAP and 1 mg/L Kin was used for plantlet regeneration. Similarly, it has also been reported that the enhancement in BAP concentration from 1–3 mg/L also increase the percentage of calli in Arizona potato cultivar (Shallal 2024). Sharde *et al.* (2024) found that 3.0 mg/L BAP showed better *in vitro* competence in sprout cultures of potato cultivars. Babadjanova *et al.* (2024) found that the culturing of the internodal stem explants in MS media with 1.5 mg/L BAP and 1 mg/L NAA resulted in 92% and 100% callus formation rates in the Desiree and Sarnav potato variety, respectively. The occurrence of higher BAP concentration also resulted in the development of the brown dilated calli around the base

Table 2 Percentage of calli regeneration at different days

BAP (m/L)	Days for regeneration	Percentage of calli regenerated	Callus texture	Callus colour	Degree of callus formation	Days for regeneration	Percentage of calli regenerated	Callus texture	Callus colour	Degree of callus formation
	BAP and 0.5 Kinetin (mg/L)					BAP and 1.0 Kinetin (mg/L)				
0	--	--	--	--	--	--	--	--	--	--
0.5	46.83	6	Loose	G	+	7	45.83	Loose	G	+
1	47.7	5.16	Compact	G	+	6.83	45.9	Compact	G	+
1.5	44.8	11.81	Compact	YG	++	13.16	43.16	Compact	YG	++
2	42.48	20.9	Loose Watery	YG	++	22.16	38.9	Loose Watery	YG	++
2.5	35.16	22.83	Loose	YG	++	25	34.33	Loose	YG	++
3.0	26.16	26	Loose	YG	++	28	25.9	Loose	YG	++
4.0	21.23	47.81	Compact	W	+++	19.16	48.9	Compact	W	+++
5.0	16.48	50.43	Brownish Compact	W	+++	14.83	50.83	Brownish Compact	W	+++

--, No callus; +, Poor callus; ++, Moderate callus; +++, Massive callus; Y, Yellowish; G, Greenish; YG, Yellow greenish.

of the explant near the cut surface (Zhang *et al.* 2005). The enrichment of MS media with BAP and Kinetin showed positive outcomes in stimulating shoot formation in potato (Sivakumar *et al.* 2024). The combination of BAP and Kin result in the significant increase in the number of microtubers formed during *in vitro* propagation in potato (Daurov *et al.* 2024). BAP concentration at 0.0 and 2 mg/L is effective for rapid shoot proliferation in Agria potato cultivar (Motallebi-Azar *et al.* 2011).

Overall, BAP at 4.0 mg/L and Kin at 1 mg/L concentration with compact white callus may be recommended for plantlet regeneration in potato cv. Lady Rosetta.

Multiple shoot and root regeneration: MS media fortified with growth regulators i.e 4.0 mg/L 2,4-D, 4.0 mg/L BAP and 1.0 mg/L Kin was used for further studies. The green callus at 0.5 and 1.0 mg/L BAP was visible after transferring to regeneration media. The gradual stages of plantlet regeneration on callus induction media and regeneration media were shown in Fig. 3. Subsequently, root initiation was observed from the regenerated shoot in the green callus in the regeneration media. Multiple well-developed shoots were formed on the standardized regeneration media. Shirin *et al.* (2007) also employed the use of MS media fortified with BAP, NAA and Kin which proved to be efficient for shoot regeneration. The combination of 2-4, D and BAP in MS media is a widely accepted media for efficient shoot multiplication in potato (Ijaz *et al.* 2017, Liaqat 2022).

Observations on root growth on full- and half-strength MS media showed that half-strength MS media produced maximum root length (5.63–7.5 cm) within ~9 days. However, full- strength MS media showed maximum root length (3.8–7 cm) in ~12 days. The detailed description of the root growth analysis was shown in Table 3. Half-strength MS media was preferrable for better shoot proliferation in potato (Kazemiani *et al.* 2012, Mollika *et al.* 2024, Behera *et al.* 2024). The use of half-strength MS media showed both root formation and shoot multiplication simultaneously in *Typhonium flagelliforme* (Rezali *et al.* 2017). Dewir *et al.* (2020) reported that the reduction of MS media salts to half-strength resulted in the stimulation of root initiation within 10 days followed by 100% rooting at three-week duration in sweet potato. They obtained 96% survival rate during transfer to *ex vitro* conditions without any morphological alterations or disease symptoms. Further, quarter-strength MS followed by half-strength MS medium also showed maximum number of roots and root length during *in vitro* propagation in *Spathiphyllum* and *Eucalyptus grandis* × *E. nitens* hybrid respectively (Mokotedi *et al.* 2000, Donmez *et al.* 2022).

Table 3 Root grown on full- and half-strength MS media

Media	Observations			
Half-strength MS media	Number of days	7.0	8.3	9.3
	Root length (cm)	5.3–6.9	5.5–7.0	5.63–7.5
Full-strength MS media	Number of days	9.03	10.53	12.05
	Root length (cm)	3.4–6.5	3.5–6.72	3.8–7

The use of internodes for indirect regeneration showed the highest frequency of multiple shoot regeneration (Mollika *et al.* 2024). The optimization factors have profound effect on the development of numbers of lateral shoots and node, root number, length of shoots, and percentage of

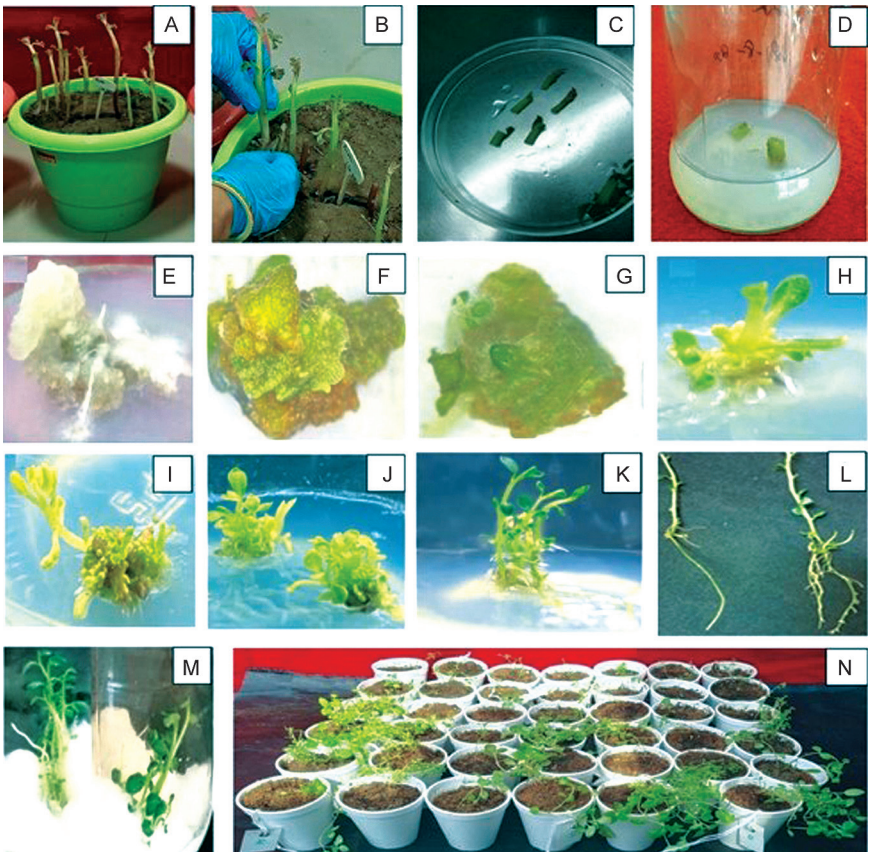

Fig. 3 Gradual stages of indirect somatic embryogenesis of potato cv. Lady Rosetta (A) Explants of LR potato plants grown under controlled conditions; (B) Sampling; (C) Excision of nodes and internodes from the explant; (D) Explants placed on the MS media supplemented with 2, 4-D and Kin; (E) callus formed after 14 days; (F, G) Green callus formed after transferring to regeneration MS media supplemented with BAP and Kin; (H, I and J) Initiation of shoots from green calli; (K) Multiple developing shoots formed with leaves on regeneration medium; (L) Plantlets with well- developed shoots and roots; (M) Hardening of rooted plants in glass jars on moist cotton and (N) Plants having root and shoot system growing under greenhouse conditions.

callus induction (Kazemiani *et al.* 2012). Therefore, there is an urgency for the development of micropropagation system for the propagation of micro propagules to surpass the food insecurity and permit the commercial cultivation (Behera *et al.* 2024).

The acclimatization of the rooted plants was done on the moist cotton followed by hardening in thermocol jars filled with autoclaved cocopeat: perlite: vermiculite in 3:1:1 ratio in the glass house. The hardening procedure using moist cotton in tissue culture acclimatization experiments was also preferrable during hardening of banana plants cv. Grand Naine (Manchanda *et al.* 2018) and *Tylophora indica* (an important medicinal plant) (Kaur *et al.* 2011). Cocopeat as potting medium showed higher growth survival percentage as compared to other media such as vermicompost and saw-dust during micro-propagation of apple rootstocks (Modgil *et al.* 2008). One sixty-two plants were transferred to glass-house after hardening and all the plants survived. Around 4–6 plants showed yellowing of leaves and that also showed slow growth when kept under sunlight.

The best results were obtained for callus induction at 4.0 mg/L 2, 4-D and 0.5 mg/L Kin, shoot induction at 4.0 mg/L BAP and 1 mg/L Kin and induction of roots in half-strength MS media in the potato cv. Lady Rosetta. The major advantages of opting for tissue culture method are the generation of large number of clones/plants from single seed or explants, space utilization and reduction of the chances of plant diseases under aseptic conditions (Jain 2001). Keeping in view the importance of potato cultivation and market, a large gap exists between the supply and demand of potato production in India. Therefore, optimization of plant tissue culture procedure is primary requirement to ensure the production of large-scale planting materials. These optimization parameters would surely minimize the chances of risk associated with use of toxic insecticides and fungicides.

This protocol may be recommended for commercial application for raising disease-free and micro-propagated potato plants with high regeneration competence. The successful propagation of potato plants at commercial scale occurs through shoot multiplication and proliferation system under *in vitro* conditions. We report here the potential conditions for producing multiple shoots through indirect means in potato cv. Lady Rosetta in MS media which could be used for genetic transformation/genome editing experiments in future.

The standardization of indirect somatic embryogenesis for rapid callus induction, plantlet regeneration and shoot proliferation in the popular potato cv. Lady Rosetta was performed. The optimizing parameters include the supplementation of Murashige and Skoog (MS) media with growth regulators i.e 2,4-Dichlorophenoxy acetic acid (2,4-D), Benzyl Amino Purine (BAP) and Kinetin (Kin) for callus induction and shoot regeneration from callus. The systematic protocol of *in vitro* to *in vivo* conditions developed an efficient tissue culture protocol in cv. Lady Rosetta. These findings could be exploited for genetic transformation/genome editing studies.

REFERENCES

Ashu, Bishnoi D K, Bhatia J K, Baskaur and Sheoran O P. 2018. Production and marketing challenges of potato cultivation in Haryana, India. *International Journal of Current Microbiology and Applied Sciences* **7**(3): 1349 –55

Babadjanova F I, Ubaydullaeva K A, Asrorov A M, Rakhmanov B K, Abdullaev A N, Bolkiev A A, Abdullaev S A, Eshmurzaev J B and Buriev Z T. 2024. Effects of plant growth regulators on callogenesis and embryogenesis in sarnav and desiree potato (*Solanum tuberosum* L.) varieties. *Plant Science Today* **11**(1): 215–22.

Behera S, Chauhan V B, Monalisa K, Meher R K, Kar S K, Pati K, Bansode V V, Nedunchezhiyan M, Verma A K, Naik P K and Naik S K. 2024. *In vitro* plant regeneration, genetic fidelity, biochemical analysis and anticancer activity of anthocyanin-rich purple flesh sweet potato var. 'Bhu Krishna'. *South African Journal of Botany* **166**: 332–43.

Char M, Subrahmanyeswari T, Bhattacharyya S and Gantait S. 2023. Meta-Topolin-induced *in vitro* propagation, field evaluation, flow cytometry and molecular marker-based genetic stability assessment of potato cv. Badami alu. *Plant Cell, Tissue and Organ Culture* **155**(3): 809–26.

Daurov D, Daurova A, Sapakhova Z, Kanat R, Akhmetzhanova D, Abilda Z, Toishimanov M, Raissova N, Otynshiyev M, Zhambakin K and Shamekova M. 2024. The impact of the growth regulators and cultivation conditions of temporary immersion systems (tiss) on the morphological characteristics of potato explants and microtubers. *Agronomy* **14**(8): 1782.

Dewir Y H, Aldubai A A, Kher M M, Alsadon A A, El-Hendawy S and Al-Suhaibani N A. Optimization of media formulation for axillary shoot multiplication of the red-peeled sweet potato (*Ipomoea batatas* [L.] Lam.) 'Abees'. 2020. *Chilean Journal of Agricultural Research* **80**(1): 3–10.

Dolnicar P. 2021. Importance of potato as a crop and practical approaches to potato breeding. *Solanum tuberosum*: *Methods in Molecular Biology*, Vol. 2354. Dobnik D, Gruden K, Ramsak Z and Coll A (Eds). Humana, New York. https://doi.org/10.1007/978-1-0716-1609-3_1

Donmez D, Erol M H, Bicen B, Simsek O and Kacar Y A. 2022. The effects of different strength of MS Media on *in vitro* propagation and rooting of *Spathiphyllum*. *Anadolu Tarım Bilimleri Dergisi* **37**(3): 583–92.

El-Sherbini S S, El-Hadary M H and Abdel-Rahman M M. 2024. Impact of PEG combined with 2, 4-Dichlorophenoxyacetic acid on improving vitality and friability of the induced callus in spunta and cara potato cultivars. *Delta Journal of Science* **48**(1): 1–2.

Gu J, Struik P C, Evers J B, Lertngim N, Lin R and Driever S M. 2024. Quantifying differences in plant architectural development between hybrid potato (*Solanum tuberosum*) plants grown from two types of propagules. *Annals of Botany* **133**(2): 365–78.

Hajare S T, Chauhan N M and Kassa G. 2021. Effect of growth regulators on *in vitro* micropropagation of potato (*Solanum tuberosum* L.) Gudiene and Belete varieties from Ethiopia. *The Scientific World Journal* **2021**(1): 5928769.

Ijaz S, Shah E and ul Haq I. 2017. Evaluating the effect of photomixotrophic conditions for expedite and efficient *in vitro* tuberization system on the morphological, biochemical,

anatomical and physiological characteristics of microtubers. *International Journal of Horticultural Science* **7**.

Jain S M. 2001. Tissue culture-derived variation in crop. *Euphytica* **118**: 153–66.

Kaur H, Anand M and Goyal D. 2011. Establishment of an efficient protocol for micropropagation of stem explants of *Tylophora indica*, an important medicinal plant. *African Journal of Biotechnology* **10**(36): 6928–32.

Kazemiani S, Motallebi-azar A, Mohaddes N, Kiomarsy F, Yarmohammadi F and Etedali F. 2012. Effect of different concentrations of sucrose and bap on shoot proliferation on MS strength basal media in potato cv. Agria. *Biology and Environment: Proceedings of the Royal Irish Academy* **3**(1): 63–72.

Liaqat A. 2022. Optimization of lectin gene transformation protocol in potato cultivar GN2 by using GUS as a reporter gene. Ph.D Thesis, Capital University.

Littell R C, Milliken G A, Stroup W A and Wolfinger R D. 1996. SAS system for mixed models. SAS Institute, Cary, North Carolina.

Lutaladio N and Castaldi L. 2009. Potato: The hidden treasure. *Journal of Food Composition and Analysis* **22**(6): 491–93.

Manchanda P, Gill M I S, Megha S and Gosal S S. 2018. Production of superelite planting material through *in vitro* culturing in banana. *Biotechnologies of Crop Improvement* **1**: 93–129.

Massa R, Al-Biski F and Al-Maarri K. 2024. Clonal selection for salinity tolerance in some potato (*Solanum tuberosum*) varieties by callus culture. *Potato Research* **67**(2): 647–61.

Modgil M, Sharma T and Thakur M. 2008. Commercially feasible protocol for rooting and acclimatization of micropropagated apple rootstocks. *(In) International Symposium on Biotechnology of Fruit Species: BIOTECHFRUIT* pp. 209–14.

Mohamed H A, Tawfik M M, Hussein E A and Ibrahim A H. 2024. Morphogenic responses of two potato cultivars explants to sucrose, photoperiods and growth regulators. *Alfarama Journal of Basic and Applied Sciences* **5**(2): 151–62.

Mohapatra P P and Batra V K. 2017. Tissue culture of potato (*Solanum tuberosum* L.): A review. *International Journal of Current Microbiology and Applied Sciences* **6**(4): 489–95.

Mokotedi M O, Watt M P, Pammenter N W and Blakeway F C. 2000. *In vitro* rooting and subsequent survival of two clones of a cold-tolerant *Eucalyptus grandis* × *E. nitens* hybrid. *HortScience* **35**(6): 1163–65.

Moletsane M S, Kimurto P K and Oyoo M E. 2022. Effect of presprouting plant growth regulators and natural materials on dormancy, growth, and yield of potatoes (*Solanum tuberosum* L.). *Advances in Agriculture* **2022**(1): 9932606.

Mollika S R, Islam T, Hoque M I and Sarker R H. 2024. Indirect *in vitro* regeneration in four varieties of potato (*Solanum tuberosum* L.) from internodal segments and leaf explants. *Plant Tissue Culture and Biotechnology* **34**(1): 83–92.

Motallebi-Azar A, Kazemiani S, Kiumarsie F and Mohaddes N. 2011. Shoot proliferation from node explants of potato (*Solanum tuberosum* cv. Agria). II. Effect of different concentrations of NH4NO3, hydrolyzed casein and BAP. *Romanian Biotechnological Letters* **16**(3): 6181–86.

Rezali N I, Sidik N J, Saleh A, Osman N I and Adam N A. 2017. The effects of different strength of MS media in solid and liquid media on in vitro growth of *Typhonium flagelliforme*. *Asian Pacific Journal of Tropical Biomedicine* **7**(2): 151–56.

Rawat T S, Krishnaprasad BT and Anil VS. 2017. Direct and indirect regeneration of potato cultivar Kufri jyoti. *IOSR Journal of Biotechnology and Biochemistry* **3**: 31–34.

Salehi M, Hosseini B and Jabbarzadeh Z. 2014. High–frequency *in vitro* plantlet regeneration from apical bud as a novel explant of *Carum copticum* L. *Asian Pacific Journal of Tropical Biomedicine* **4**: S424–S428.

Shallal H H. 2024. Effect of BAP along with 2,4-D on the induction of shoots formation on potato cultivar arizona *in vitro*. *University of Thi-Qar Journal of agricultural research* **13**(1): 201–11.

Sharde R, Tripathi M K, Bhatt D, Tiwari S, Sharma M, Tomar Y S and Tripathi N. 2024. Influence of plant growth regulators on *in vitro* morphogenesis in sprout culture of potato (*Solanum tuberosum* L.). *Potato Research* **67**(2): 399–420.

Sharma M, Sharma M, Salgotra R K, Sharma M and Singh A K. 2023. Standardization of protocol for *in vitro* tuberization in potato (*Solanum tuberosum*) cultivar Kufri Sindhuri. The Indian *Journal of Agricultural Sciences* 93(1): 84–88.

Shirin F, Hossain M, Kabir M F, Roy M and Sarker S R. 2007. Callus induction and plant regeneration from internodal and leaf explants of four potato (*Solanum tuberosum* L.) cultivars. *World Journal of Agricultural Sciences* **3**(1): 1–6.

Singh B and Dutt S. 2024. Seventy five years of potato in India. *Indian Farming* **74**(5): 3–8.

Singh B, Raigond P, Dutt S and Kumar M. 2020. Potatoes for food and nutritional security. Potato: Nutrition and food security, pp.1–12.

Sivakumar P, Sasikala K, Prabha T, Selvaraj K V, Karunakaran V, Selvamurugan M, Shree G K and Kiruba M. 2024. Microtuber Induction and Plant Regeneration of Potato (*Solanum tuberosum* L.): A Review. *International Journal of Plant and Soil Science* **36(3)**: 166–76.

Subrahmanyeswari T, Bandyopadhyay S and Gantait S. 2024. Direct regeneration-mediated *in vitro* propagation and microtuberization of potato: An appraisal of the past ten-year period. *South African Journal of Botany* **172**: 768–78.

Walia A, Anil B, Samnotra RK, Gaur A and Pankaj P. 2021. *In vitro* propagation studies for late blight resistant potato variety Kufri Girdhari. *Biological Forum – An International Journal* **13**(3): 375–80.

Yasmin S, Hasan J, Hossain S, Saha S and Khatun F. 2022. Auxin and cytokinin synergism in micropropagation for mass production of Aloe vera. *BioTechnologia (Pozn)* **103**(3): 301–10.

Yousef A N, Danial G H, Ibrahim D A and Barakat S E. 2024. Propagation and callus regeneration of potato (*Solanum tuberosum* L.) cultivar 'desiree' under salt stress conditions. *Science Journal of University of Zakho* **12**(3): 294–98.

Zhang Z, Zhou W and Li H. 2005. The role of GA, IAA and BAP in the regulation of *in vitro* shoot growth and microtuberization in potato. *Acta Physiologiae Plantarum* **27**: 363–69.