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Effect of sucrose and abscisic acid on potato (*Solanum tuberosum*) microtuberisation and dormancy

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ABSTRACT

Microtuberisation serves as a vital approach for the rapid propagation and long-term conservation of potato (*Solanum tuberosum* L.) germplasm. The present study was carried out during 2023–24 at Sardarkrushinagar Dantiwada Agriculture University, Dantiwada, Gujarat to evaluate the interactive effects of abscisic acid (ABA), sucrose concentrations, and genotype on *in vitro* microtuber formation across four potato cultivars, i.e. Kufri Badshah, Kufri Surya, Lady Rosetta, and Kufri Nilkanth. Nodal explants were cultured in Murashige and Skoog (MS) liquid medium supplemented with ABA (0.0, 0.1, 0.5, and 1.0 mg/L) and sucrose (8% and 10%) to assess their impact on key microtuberisation parameters. Results demonstrated that increased sucrose concentration significantly enhanced higher number of microtubers and microtuber weight, outperforming all treatments involving ABA. The combination of ABA with sucrose did not yield synergistic effects, indicating sucrose as the principal driver of microtuber development. Among the genotype, Kufri Badshah exhibited the highest microtuberisation efficiency, followed by Kufri Surya, Lady Rosetta, and Kufri Nilkanth. While ABA did not directly improve microtuber induction or growth metrics, it played a critical role in dormancy extension and prolongation of storage viability, an essential factor for transportation and germplasm preservation. Differential cultivar responses to ABA, highlighting the need for conservation strategies suited to each genotype. Overall, this study showed that sucrose is a key factor for microtuber formation, while ABA is crucial for dormancy regulation, providing useful information to improve microtuber production and long-term storage of potato germplasm.

Keywords: Abscisic acid, Dormancy, Germplasm conservation, *In vitro*, Microtuber, Microtuber storage, Potato cultivars

Potato (*Solanum tuberosum* L.) is the fourth most important food crop globally, playing a vital role in food security and agricultural economies. The production of pathogen-free seed potatoes and the rapid multiplication of elite cultivars are essential for sustainable potato cultivation. In this context, *in vitro* microtuberisation has emerged as a cornerstone technique. Microtubers, produced under controlled laboratory conditions, are small, dormant tubers that can be used for both planting and long-term germplasm conservation (Ahloowalia 1998).

Microtuberisation, the *in vitro* formation of miniature tubers, is widely employed in potato germplasm conservation, breeding programmes, and the production of pathogen-free planting material. This process is influenced by several factors, including the culture medium composition, hormonal treatments, cultivar genotype, and the type of medium (solid or liquid). Among these, sucrose plays a critical role as both a carbon source essential for energy production and biosynthesis, and as an osmotic agent and signaling molecule that activates pathways driving tuberisation (Xu *et al.* 1998a). It has been shown to upregulate genes involved in starch biosynthesis and tuber-specific metabolic processes, showing its key role in microtuber development (Fernie and Willmitzer 2001). High concentrations of sucrose promote tuber initiation by enhancing the expression of tuberisation-related genes and increasing starch accumulation (Sarkar and Naik 1998, Xu *et al.* 1998a). In this context, investigating the combined roles of sucrose and abscisic acid (ABA) in liquid media is particularly relevant for optimising microtuber production protocols.

Abscisic acid (ABA), a plant hormone known for its role in stress responses and dormancy regulation, has been extensively studied for its involvement in potato

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microtuberisation. While ABA enhances tuber quality by promoting dormancy and reducing sprouting, it is generally less effective as a sole inducer of tuber formation (Kumar *et al.* 2007). However, ABA plays a crucial regulatory role in tuber initiation and development, particularly under stress conditions such as water deficit and osmotic stress. It influences microtuber formation by modulating cellular and metabolic pathways and by acting as a signaling molecule that interacts with other phytohormones, such as gibberellins and cytokinins, to maintain the hormonal balance required for tuber induction. Studies have shown that elevated ABA levels during *in vitro* culture can stimulate starch accumulation, suppress apical dominance, and induce the expression of tuber-specific genes (Simko *et al.* 1997, Xu *et al.* 1998a). The interplay between sucrose and ABA in liquid media can optimise both the yield and quality of microtubers. However, the effectiveness of these treatments can vary significantly across cultivars due to genetic differences in hormonal sensitivity and carbohydrate metabolism (Estrada *et al.* 1986, Sarkar and Naik 1998).

In addition to its limited role in microtuber induction, abscisic acid (ABA) is essential for the induction and maintenance of tuber dormancy. Dormancy is a critical physiological stage that prevents premature sprouting during storage, ensuring tuber viability under unfavourable conditions. ABA plays a central role in initiating and sustaining dormancy by inhibiting cell division and elongation, thereby suppressing sprouting (Shuttle 2004). The onset and duration of dormancy are regulated by the dynamic balance between ABA biosynthesis and catabolism, which is influenced by environmental conditions, culture media composition, and genetic variability among potato cultivars (Destefano-Beltran *et al.* 2006). The effectiveness of ABA in promoting dormancy and contributing to microtuber quality is closely linked to the composition of the *in vitro* culture medium. Factors such as sucrose concentration, nitrogen sources, and the presence of other plant growth regulators can interact synergistically with ABA, affecting its performance (Mendes *et al.* 2020). Additionally, significant genotypic variability influences cultivar-specific responses to ABA, highlighting the necessity for customised culture protocols (Kumlay and Ercisli 2015). This study investigated the effects of ABA and sucrose concentration on microtuber formation in liquid media across four potato cultivars.

The objectives of this study were to evaluate the effects of abscisic acid (ABA) and sucrose on microtuber formation in liquid culture media. By examining these treatments across four potato cultivars (Kufri Badshah, Kufri Surya, Lady Rosetta, and Kufri Nilkanth), the study aimed to assess genotype-specific responses in terms of microtuber number, size, and weight. A key objective is to investigate the role of ABA not only in microtuber development but also in the induction and extension of dormancy, which is critical for long-term *in vitro* storage and germplasm conservation. Through this approach, the study seeks to optimise liquid media-based microtuberisation protocols for large-scale production of pathogen-free seed potatoes.

MATERIALS AND METHODS

Plant material: The present study was carried out during 2023–24 at Sardarkrushinagar Dantiwada Agriculture University, Dantiwada (24º19' N, 72º19' E; at an elevation of 154.42 m amsl), Gujarat. Four potato cultivars i.e. Kufri Badshah, Kufri Surya, Lady Rosetta and Kufri Nilkanth were selected for this study due to their commercial importance, diverse agronomic traits and varying responses to *in vitro* microtuberisation protocols (Shukla *et al.* 2023).

All potato cultivars were collected from Potato Research Station, Sardarkrushinagar Dantiwada Agriculture University, Deesa, Gujarat. The explants used for the experiments consisted of single-node stem cuttings derived from *in vitro* plantlets maintained in a growth chamber.

In vitro *microtuberisation*: Nodal segments were cultured on semi-solid Murashige and Skoog (MS, Hi media, India, Murashige and Skoog 1962) basal medium with vitamins containing 1.0 mg/L 6-Benzylaminopurine (BAP) and 3% sucrose and 0.8 g agar for shoot growth and development. Six-week-old *in vitro* shoots with 5–6 internodes were used for microtuberisation and sub-cultured on the MS basal medium with vitamins containing 8% sucrose and 0.8 g agar (Shukla *et al.* 2023). The *p*H of the medium was adjusted to 5.8 prior to autoclaving at 12°C and 118 kPa for 20 min. All culture vessels were kept in the growth chamber at 23 ± 2°C under a 16 h photoperiod at a light intensity of 40 µmol/m^2/s.

Evaluation of ABA on microtuberisation: The impact of ABA and sucrose concentration was assessed to optimise *in vitro* microtuberisation using explants with 5–6 internodes. Shoots were multiplied as previously described, and explants were transferred to media supplemented with ABA at concentrations of 0, 0.1, 0.5, and 1.0 mg/L, along with sucrose (8% and 10%). The experiment utilised 125 mL culture flasks, each containing 50 mL of medium per treatment and four shoots were cultured in each flask.

Liquid cultures were kept on a rotary shaker at 100 rpm, first under light for 3 days and then in the dark, under the same growth conditions as described earlier. After six weeks of incubation, the total number of microtubers was recorded. Microtubers larger than 0.5 cm were collected in autoclaved glass jars and stored in a refrigerator at 4°C in darkness. Microtuber germination was evaluated under *in vitro* and greenhouse conditions after 60 days storage. Microtubers were placed on MS basal medium (MS salts with vitamins containing 3% sucrose and 0.8 g agar) and kept under the same growth conditions as previously described. Under greenhouse conditions, microtubers were placed in a tray filled with a soil and coco peat mixture (25:75). Initially, the tray was kept in darkness for a week, and later, it was maintained at 25°C with a 16 h photoperiod. For all experiments, the number of microtuber/shoot and the average weight of microtuber (g) were recorded.

Data collection and analysis: All *in vitro* and greenhouse experiments on microtuber germination were arranged in

a completely randomised design (CRD). Each experiment consisted of five replicates and was repeated three times (n = 15). Data were analysed using one-way ANOVA for individual factor effects and three-factor ANOVA to assess the interaction effects of sucrose, ABA, and genotype. When the ANOVA indicated significance, mean comparisons were performed using Duncan's Multiple Range Test (DMRT). All statistical analyses were conducted using GRAPES software (Chen *et al.* 2008). Results are presented as means ± standard error, and different letters in the tables indicate significant differences at p= 0.05.

RESULTS AND DISCUSSION

Effect of different concentration of ABA and sucrose on microtuber development: *In vitro* cultures were successfully established for four potato cultivars (Kufri Surya, Kufri Badshah, Lady Rosetta, and Kufri Nilkanth) using a standard growth medium for shoot multiplication. Shoots grown in a liquid medium containing varying concentrations of abscisic acid (ABA: 0.1, 0.5, and 1.0 mg/L) and sucrose (8% and 10%) exhibited normal growth and development, comparable to those cultured on a semi-solid medium (Fig. 1 A, B, C). The number of microtubers/shoot was significantly influenced by the concentrations of ABA and sucrose across all four cultivars (Table 1). Kufri Badshah consistently produced the highest number of microtubers under all treatment combinations, with a maximum of 6.2 ± 0.37 microtubers/shoot observed at 80 g/L sucrose without ABA (Fig. 1 D). In contrast, Kufri Nilkanth exhibited the lowest microtuber formation, particularly under higher ABA concentrations and 100 g/L sucrose, with a minimum of 1.2 ± 0.2 microtubers/shoot.

Overall, microtuber production was significantly higher at 80 g/L sucrose compared to 100 g/L sucrose across all ABA concentrations for most cultivars. In the control, the number of microtubers was significantly higher than in ABA-treated cultures. Increasing ABA concentrations to 0.5 and 1.0 mg/L led to a gradual reduction in microtuber production across all cultivars. Furthermore, 80 g/L sucrose consistently supported greater microtuber formation than 100 g/L sucrose at all ABA concentrations.

Within cultivars, Kufri Surya and Lady Rosetta exhibited moderate reductions in microtuber number with increasing ABA, while Kufri Nilkanth was the most sensitive to ABA treatment, particularly at 0.5 and 1.0 mg/L combined with 100 g/L sucrose. Notably, the combination of 0.5 mg/L ABA and 80 g/L sucrose appeared more favourable for sustaining microtuber formation, especially in Kufri Badshah.

The interaction between cultivar, sucrose, and ABA concentration was observed non-significant for number of

Table 1 Effect of different ABA and sucrose concentrations on number of microtubers/flask in four potato cultivars

Cultivar name	Sucrose (g/L)	No. of microtubers/flask				
		ABA (mg/L)				Cultivar (Mean)
		0.0	0.1	0.5	1.0	
Kufri Surya	80	4.6 ± 0.50	2.2 ± 0.37	4.2 ± 0.58	3.0 ± 0.70	3.000^{b}
	100	3.6 ± 0.50	1.4 ± 0.24	3.2 ± 0.2	1.8 ± 0.37	
Kufri Badshah	80	6.2 ± 0.37	3.0 ± 0.31	5.4 ± 0.50	4.8 ± 0.37	4.325^{a}
	100	5.0 ± 0.70	2.8 ± 0.37	3.8 ± 0.37	3.6 ± 0.50	
Lady Rosetta	80	3.6 ± 0.67	2.6 ± 0.4	3.2 ± 0.37	3.4 ± 0.50	3.050^{b}
	100	3.2 ± 0.37	2.6 ± 0.24	3.0 ± 0.31	2.8 ± 0.37	
Kufri Nilkanth	80	3 ± 0.70	1.6 ± 0.24	3.0 ± 0.70	3.0 ± 0.44	2.350^{c}
	100	3.0 ± 0.44	1.2 ± 0.2	2.0 ± 0.31	2.0 ± 0.44	
ABA Conc. (Mean)		4.025^{a}	2.175^{c}	3.475^{b}	3.050^{b}	
Sucrose Conc. (Mean)	80					
	100					

Source of variation	Significance level	Source of variation	Significance level
Cultivar	p >0.001	Cultivar × ABA Conc.	NS
ABA Conc.	p >0.001	Cultivar × Sucrose Conc.	NS
Sucrose Conc.	p >0.001	ABA Conc. × Sucrose Conc.	NS
SEM±	0.952	Cultivar × ABA Conc. × Sucrose Conc.	NS
CV	30.666		

The cultivars were cultured in liquid Murashige and Skoog (MS) basal medium with vitamins supplemented with 1.0 mg/L 6-benzylaminopurine (BAP) and 3% sucrose. Cultures were maintained under dark conditions, and observations were recorded after 6 weeks. Values are SEM±. Means followed by the same lowercase letter in a column are not significantly different at $p\geq 0.05$ according to LSD test.

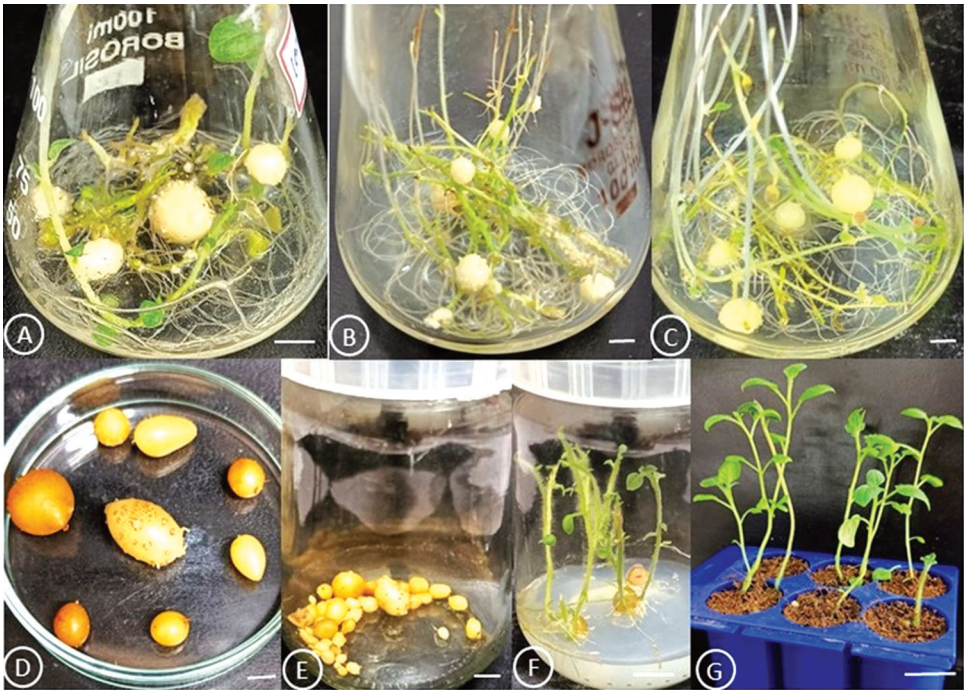

Fig. 1 Microtubers development in liquid MS basal medium containing 1.0 mg/L 6-Benzylaminopurine (BAP) with 8% sucrose (A); with 0.5 mg/L Abscisic acid (ABA) and 8% sucrose (B); and with 0.5 mg/L ABA and 10% sucrose (C); Microtubers harvested after 6 weeks of culture (D); and stored at 4°C for 8 weeks (E); Microtubers germination observed under *in vitro* (F) and greenhouse (G) conditions after 6 weeks.

microtubers. For instance, while Kufri Badshah maintained high microtuber numbers across both sucrose levels, Kufri Nilkanth showed a sharp decline in response to ABA at higher sucrose (100 g/L). This indicated that the effect of ABA was cultivar-specific and strongly modulated by sucrose concentration. The number of microtubers/flask was strongly influenced by both ABA and sucrose levels ($p< 0.001$), whereas the interaction effects among cultivar × ABA × sucrose concentrations were non-significant.

The weight of microtubers was significantly affected by different concentrations of abscisic acid (ABA) and sucrose level across the four potato cultivars studied (Table 2). Among the cultivars, Kufri Badshah consistently produced the heaviest microtubers under all treatment conditions, reaching a maximum weight of 0.66 ± 0.01 g/microtuber at 80 g/L sucrose without ABA. In contrast, Lady Rosetta and Kufri Nilkanth exhibited comparatively lower microtuber weights across treatments.

Overall, microtuber weight was higher under control conditions (0 mg/L ABA) compared to ABA-treated cultures. Increasing ABA concentrations to 0.5 and 1.0 mg/L generally resulted in a significant reduction in microtuber weight across all cultivars. Additionally, 80 g/L sucrose supported higher microtuber weights than 100 g/L sucrose at each ABA concentration.

Within cultivars, Kufri Surya and Lady Rosetta displayed notable decreases in microtuber weight with increasing ABA concentration. Kufri Nilkanth exhibited moderate sensitivity, particularly at 1.0 mg/L ABA combined with 100 g/L sucrose where the lowest microtuber weights were recorded (0.19 ± 0.01 g). The combination of 0.5 mg/L ABA and 80 g/L sucrose helped maintain relatively higher microtuber weights compared to 1.0 mg/L ABA, especially in Kufri Badshah and Kufri Surya.

The interaction between ABA and sucrose was more pronounced for microtuber weight than number of microtubers. While 80 g/L sucrose mitigated the negative effect of ABA across cultivars, 100 g/L sucrose enhanced ABA-induced reductions, especially in Kufri Nilkanth. This suggests that sucrose concentration modifies sensitivity to ABA, influencing both tuber initiation and biomass accumulation. The interaction effects of cultivar × ABA and ABA × sucrose were also significant ($p<0.001$), indicating that specific genotype-hormone-carbon source combinations play a crucial role in microtuber growth and development.

Overall, microtuber number and weight were both higher in control treatments compared to ABA-treated cultures. Increasing ABA concentrations (0.5 and 1.0 mg/L) led to a gradual decline in both parameters across all cultivars. Similarly, 80 g/L sucrose consistently promoted greater microtuber formation and higher weights than 100 g/L sucrose at all ABA concentrations. Among cultivars, Kufri Surya and Lady Rosetta showed moderate reductions in both number and weight with increasing ABA, while Kufri Nilkanth was the most sensitive, particularly at 1.0 mg/L ABA with 100 g/L sucrose. Notably, the combination of 0.5 mg/L ABA and 80 g/L sucrose was more favourable than 1.0 mg/L ABA in sustaining microtuber production and weight, especially in Kufri Badshah.

Effect of different concentration of abscisic acid and sucrose on microtuber storage and germination: All microtubers germinated under both *in vitro* and greenhouse conditions after two months' storage (Fig. 1E, F, G), however, germination was delayed in the ABA treatments under both conditions. Microtuber germination was significantly influenced by abscisic acid (ABA) treatment and growth conditions (*in vitro* and greenhouse) across the four potato cultivars (Table 3). In general, microtubers developed on medium supplemented with 0.5 mg/L ABA showed delayed germination compared to the control (0 mg/L ABA) under both *in vitro* and greenhouse conditions.

Across all cultivars, germination was faster *in vitro* than in

Table 2 Effect of different ABA and sucrose concentrations on microtuber weight in four potato cultivars

Cultivar Name	Sucrose (g/L)	Microtuber weight (g)				
		ABA (mg/L)				Cultivar (Mean)
		0.0	0.1	0.5	1.0	
Kufri Surya	80	0.56 ± 0.01	0.11 ± 0.01	0.44 ± 0.01	0.27 ± 0.01	0.33^{b}
	100	0.49 ± 0.01	0.13 ± 0.01	0.38 ± 0.01	0.23 ± 0.01	
Kufri Badshah	80	0.66 ± 0.01	0.17 ± 0.01	0.52 ± 0.01	0.35 ± 0.01	0.40^{a}
	100	0.58 ± 0.01	0.20 ± 0.01	0.45 ± 0.02	0.29 ± 0.01	
Lady Rosetta	80	0.51 ± 0.01	0.12 ± 0.01	0.39 ± 0.01	0.25 ± 0.01	0.31^{c}
	100	0.46 ± 0.01	0.17 ± 0.01	0.36 ± 0.01	0.19 ± 0.01	
Kufri Nilkanth	80	0.54 ± 0.01	0.15 ± 0.01	0.42 ± 0.01	0.20 ± 0.01	0.32^{bc}
	100	0.50 ± 0.01	0.16 ± 0.01	0.39 ± 0.01	0.19 ± 0.01	
ABA Conc. (Mean)		0.54^{a}	0.15^{d}	0.42^{b}	0.25^{c}	
Sucrose Conc. (Mean)	80					
	100					

Source of variation	Significance level	Source of variation	Significance level
Cultivar	p> 0.001	Cultivar × ABA Conc.	p >0.001
ABA Conc.	p> 0.001	Cultivar × Sucrose Conc.	NS
Sucrose Conc.	p> 0.001	ABA Conc. × Sucrose Conc.	p >0.001
SEM±	0.001	Cultivar × ABA Conc. × Sucrose Conc.	NS
CV	7.98		

The cultivars were cultured in liquid Murashige and Skoog (MS) basal medium with vitamins supplemented with 1.0 mg/L 6-benzylaminopurine (BAP) and 3% sucrose. Cultures were maintained under dark conditions, and observations were recorded after 6 weeks. Values are SEM±. Means followed by the same lowercase letter in a column are not significantly different at $p \geq 0.05$ according to LSD test.

Table 3 Effect of abscisic acid on microtuber germination (days) of four potato cultivars after 8 weeks of storage under *in vitro* and greenhouse conditions

Cultivar Name	Microtuber germination (days)			
	In vitro		Greenhouse	
	ABA (0)	ABA (0.5 mg/L)	ABA (0)	ABA (0.5 mg/L)
Kufri Surya	6.6 ± 0.50^{b}	11.4 ± 0.50^{b}	9.4 ± 0.50^{c}	16 ± 0.70^{b}
Kufri Badshah	8.2 ± 0.37^{a}	15.0 ± 0.70^{a}	13 ± 0.54^{a}	18.2 ± 0.58^{a}
Lady Rosetta	7.4 ± 0.50^{ab}	12.2 ± 0.86^{b}	11.6 ± 0.50^{ab}	15.2 ± 0.37^{b}
Kufri Nilkanth	7.0 ± 0.70^{a}	13.2 ± 1.01^{b}	11.4 ± 0.50^{b}	15.6 ± 0.50^{b}

Values represent means ± SEM, and different letters within a column represent significant differences between the treatments based on the DMRT test (p=0.05).

the greenhouse. Kufri Surya exhibited the fastest germination under control conditions, with an average of 6.6 ± 0.50 days *in vitro* and 9.4 ± 0.50 days in the greenhouse (Table 3). In contrast, Kufri Badshah had the slowest germination, taking 8.2 ± 0.37 days *in vitro* and 13 ± 0.54 days in the greenhouse under control conditions. ABA treatment at 0.5 mg/L significantly delayed germination times, with Kufri Badshah showing the longest delay, reaching 15.0 ± 0.70 days *in vitro* and 18.2 ± 0.58 days in the greenhouse.

Among the cultivars, Lady Rosetta and Kufri Nilkanth demonstrated intermediate germination responses, with ABA-treated microtubers germinating between 12.2–13.2 days *in vitro* and 15.2–15.6 days in the greenhouse. Overall, ABA treatment delayed microtuber germination significantly, and the delay was more pronounced under greenhouse conditions compared to *in vitro* conditions across all cultivars.

The interaction between cultivar, ABA treatment, and growth condition was also evident. For example, while Kufri Surya germinated rapidly in both conditions, Kufri Badshah exhibited the strongest ABA induced delay, particularly under greenhouse conditions. This indicates that ABA effects on dormancy release are cultivar-dependent and modulated by the growth environment.

One significant advancement in potato production is the development of tissue culture techniques for generating microtubers- small, *in vitro* produced tubers that serve as disease-free planting material (Donnelly *et al.* 2003). As demonstrated by Estrada *et al.* (1986), microtuber production has become a valuable tool for germplasm conservation and the rapid multiplication of elite potato cultivars (Gopal *et al.* 2004, Uchendu *et al.* 2016, Muthoni *et al.* 2019). These technologies allow researchers to preserve genetic diversity and produce high-quality plantlets under controlled conditions. In recent years, Efforts to achieve sustainable agriculture have further promoted the integration of microtuber technology, aiming to increase yields and improve crop quality. The integration of microtuber production into potato cultivation systems offers numerous advantages, including greater uniformity in crop production, reduced reliance on field-grown seed tubers, and improved disease resistance. Furthermore, *in vitro* techniques enable the rapid multiplication of potato cultivars with desirable traits, ensuring a consistent supply of high-quality planting material capable of withstanding both biotic and abiotic stresses (Mohamed and Girgis 2023).

Sucrose played a central role in enhancing microtuber formation, with each cultivar performing best when sucrose was the sole supplement in the medium. As a primary carbon source, sucrose supplies the energy and structural substrates necessary for cellular processes, while also functioning as an osmotic agent that trigger physiological responses that promote tuberisation (Xu *et al.* 1998b). Moreover, sucrose acts as a signaling molecule, promoting the expression of genes involved in starch biosynthesis, storage protein accumulation, and tuber-specific metabolic pathways (Fernie and Willmitzer 2001). The increase in osmotic pressure induced by sucrose is particularly important for promoting tuber swelling and differentiation in liquid culture systems. The highest microtuber yield observed in the cultivar Kufri Badshah suggests that this genotype is especially efficient in utilising sucrose for tuberisation. Such genotype-specific responses may stem from variations in carbohydrate metabolism and differential sensitivity of tuberisation-related genes to sucrose signaling, as reported in earlier studies (Thieme 1992, Ahloowalia 1998, Sarkar and Naik 1998).

The results of this study highlighted the potential of abscisic acid (ABA) supplementation in optimising microtuber production and extending microtuber dormancy without negatively affecting microtuber quality or germination rate. Although ABA treatment (particularly at 0.5 mg/L) slightly reduced the number and weight of microtubers compared to control conditions, the reductions were not significant enough to compromise microtuber viability or practical use. Similar findings have been reported where ABA contributed to improved tuber quality without severe inhibition of tuber formation (Xu *et al.* 1998a, Emaraa *et al.* 2017).

Abscisic acid (ABA), a hormone well known for its roles in stress response and dormancy regulation, demonstrated limited effectiveness in promoting microtuber formation under *in vitro* conditions when compared to sucrose. Its influence on reducing sprouting and enhancing tuber dormancy (Kumar *et al.* 2007) indicated that ABA contributes more to improving microtuber quality and storability than to increasing yield. Furthermore, the combination of ABA with sucrose did not result in higher microtuber yields than sucrose alone, implying that although ABA may support carbohydrate-induced tuberisation, it cannot initiate the process on its own. These findings are consistent with previous reports which highlight ABA's role in enhancing tuber quality traits, rather than directly activating the developmental pathways responsible for microtuber formation (Rook *et al.* 2006, Sonnewald and Sonnewald 2014).

Dormancy is a critical physiological phase in potato tubers, essential for maintaining their viability during storage. All microtubers germinated successfully under both *in vitro* and greenhouse conditions (Fig. 1) following two months of storage at 4ºC. While ABA did not significantly affect microtuber size or number, it plays a crucial role in regulating dormancy, extending storage duration, and enhancing the overall utility of *in vitro* germplasm conservation.

Germination studies revealed that ABA-treated microtubers germinated significantly later than untreated ones, both *in vitro* and under greenhouse conditions. However, germination percentage remained unaffected, suggesting that ABA primarily acts to extend dormancy rather than inhibit viability, consistent with earlier studies (Coleman *et al.* 2001, Donnelly *et al.* 2003, Suttle 2004, Destefano-Beltran *et al.* 2006). Extended dormancy is particularly beneficial for microtuber storage, as it reduces premature sprouting and allows more flexibility in planting schedules (Thieme 1992, Di *et al.* 2024). Abscisic acid (ABA) plays a pivotal role in extending dormancy in potato microtubers, thereby maintaining their viability during storage (Suttle and Hultstrand 1994). In this study, microtubers treated with ABA exhibited prolonged dormancy compared to untreated ones, consistent with previous reports. ABA achieves this by suppressing sprouting and metabolic activity, ensuring that microtubers remain viable for extended periods. This dormancy-regulating function makes ABA a valuable component in germplasm conservation protocols, as it reduces the need for frequent subculturing, lowers the risk of germplasm loss during long-term *in vitro* storage, and conserves resources while preserving genetic integrity. Additionally, the reduced metabolic activity in ABA-treated microtubers enhanced their storage potential, supporting the long-term maintenance of germplasm collections (Mendes *et al.* 2020).

Furthermore, the beneficial impact of moderately high sucrose concentration (80 g/L) on both microtuber number and weight supports previous observations that moderately high sucrose levels promote osmotic conditions favourable for tuber initiation (Khuri and Moorby 1995). Cultivar-specific responses to ABA, notably the better performance

of Kufri Badshah, suggest genetic variability that could be utilised for breeding programmes aiming to improve microtuber storage and sprouting characteristics. ABA at 0.5 mg/L represents an effective tool to extend the dormancy of potato microtubers while maintaining their quality and germination potential, providing valuable implications for large-scale seed potato production systems (Suttle and Hultstrand 1994, Gopal *et al.* 2004).

This study underscored the critical role of sucrose in promoting microtuber formation and highlights the potential of ABA as a supplementary agent to enhance tuber quality and extend dormancy. The observed variability in cultivar responses emphasises the need for genotype-specific optimisation of *in vitro* culture conditions. Future research should explore the combined effects of sucrose and ABA across a range of concentrations to identify possible synergistic interactions. In addition, transcriptomic and metabolomic analyses could uncover the molecular pathways responsible for the differential responses among cultivars. By extending dormancy and reducing metabolic activity, ABA not only improves storage efficiency but also reduces the risks of germplasm loss or genetic drift. These advantages are particularly valuable for the long-term preservation of potato germplasm, which are crucial for breeding programmes and global food security.

This study evaluated the effects of abscisic acid (ABA), with and without sucrose, on microtuber formation in liquid media across four potato cultivars. Sucrose alone, at 8% and 10% concentrations, produced the highest numbers, sizes, and weights of microtubers. Treatments combining ABA with sucrose did not significantly enhance microtuberisation and were generally less effective than sucrose alone. Among all treatments, 8% sucrose was the most effective across cultivars, confirming its role as the primary inducer of *in vitro* microtuber development. Although ABA did not directly increase microtuber yield, its role in inducing dormancy and extending storage supports its use in germplasm conservation. These findings highlighted the importance of integrating ABA's dormancy-regulating function with optimised sucrose concentrations and cultivar-specific protocols to improve the efficiency and sustainability of microtuber production system.

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