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Effects of Abscisic Acid (ABA) and Benzylaminopurine (BAP) Derived Minitubers on Germination and Yield of Two Potato Cultivars (*Solanum tuberosum* L.)

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ABSTRACT

Potato, the world's fourth most important food crop, is primarily vegetatively propagated, making the production of high-quality, virus-free seed tubers essential. This experiment was conducted in two stages at the University of Jiroft during the years 2022–2023. In the first stage, the plantlets were subjected to sprinkler (foliar) application of growth regulators to the mother plants. The treatments were applied in two sequential steps: stolon induction and tuberization, using Abscisic Acid (ABA), Benzylaminopurine (BAP), and the combined treatment BAP + ABA, under controlled conditions. In the second stage, the tubers produced from the first stage were evaluated for germination characteristics, seedling establishment, and subsequent performance under field conditions. The measured traits included the number of sprouts on minitubers, sprout length, days to seedling emergence, seedling emergence percentage, number of stems per plant, morphological characteristics, and yield and yield components. Results showed that use of BAP during the tuberization stage increased sprout length by 41% and 52% in cv. Santé and cv. Colomba, respectively, compared with the control plants. Moreover, the shortest days to seedling emergence were observed in this treatment compared with the control. The highest seedling emergence percentage was recorded with ABA application during the stolon induction stage, showing an average increase of 40% over the control. Additionally, cv. Colomba exhibited a 10% higher seedling emergence than cv. Santé. In cv. Santé, the combined treatment BAP + ABA during the tuberization stage increased the number of sprouts by 50% compared with the control. In contrast, the greatest number of sprouts on tubers in cv. Colomba was obtained with BAP applied during the tuberization stage, with an increase of 33% over the control. In both cultivars, plants treated with BAP + ABA during the tuberization stage produced the highest number of tubers per plant.

Keywords: Hairy roots, Bioreactor systems, Secondary metabolites, Elicitors

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Introduction

Potato (*Solanum tuberosum* L.) with an annual production of 400 million tons and an average yield of 40 tons per hectare, is recognized as the fourth most important food crop in the world (Anonymous, 2023). Potato is predominantly propagated vegetatively through tubers. Minitubers are small tubers potatoes produced from micropropagated plantlets under in vitro conditions, which are subsequently planted at high densities in greenhouses using culture media or pots containing various substrates. Minituber size ranges from 5 to 25 mm, and their weight varies between 0.1 and 10 g (Struik, 2007). The production of Minitubers from in vitro plantlets allows for more rapid multiplication of seed tubers, better planning, and a reduction in the number of field generations (Rentzsch et al., 2012). Minitubers generally exhibit a longer dormancy period compared to conventional potato tubers, which can be critical for field establishment. For seed tuber producers, rapid and uniform tuber sprouting is a prerequisite for proper establishment of healthy plants. Sprout growth can be stimulated by various chemical, hormonal, and physical treatments. Depending on the timing of application, these substances can directly terminate tuber dormancy and induce sprout growth (Suttle, 2008). Cytokinins and gibberellins are known to break dormancy and initiate sprout growth (Hartmann et al., 2011). Struik (2007) reported that application of synthetic cytokinin terminates tuber dormancy and initiates active sprout growth. Hartmann et al., (2011) found that benzylaminopurine application induced dormancy break in tubers but did not affect sprout growth. application of plant growth regulators is widely used to modify sprouting characteristics and break tuber dormancy (Kaya et al., 2023). Plant Growth Regulators (PGRs) are chemical compounds that regulate a wide range of processes in plants (Ahmadi Lahijani et al., 2018). Among various hormones, abscisic acid (ABA) and cytokinins (CK) play fundamental roles not only in regulating senescence-related processes but also in establishing source-sink strength and modulating dormancy initiation and release (Bednarek et al., 2021). There is evidence regarding the effect of cytokinins on enhancing tuberization (Rossouw, 2008). Cytokinins are recognized as inducers of cell division (Suttle, 1995). Low concentrations of cytokinins have been shown to be more effective in promoting sprout growth in potato tubers compared to higher concentrations (Rossouw, 2008). Cytokinins are more effective than gibberellins in shortening the dormancy period of sprouts; however, they stimulate sprout growth to a lesser extent than gibberellins (Rossouw, 2008). Abscisic acid plays a role in the initiation and maintenance of tuber dormancy (Blauer et al., 2013). Although ABA content in tubers declines during storage, no consistent correlation has been observed between ABA levels and sprouting behavior across different potato cultivars (Biemelt et al., 2000). As a plant growth inhibitor, abscisic acid positively influences the onset and induction of tuberization (Farran et al., 2006). ABA promotes the mobilization of carbohydrates stored in stems and leaves, facilitating their translocation and accumulation in reproductive organs such as seeds and storage organs, thereby accelerating the process of carbohydrate deposition in these tissues (Mahajan et al., 2024). Sprouting can be stimulated by the application of chemical agents either before or after harvest (Dutta et al., 2024). Once tuber dormancy is broken, the sprouts on the seed tuber begin to grow. Upon sprouting initiation, tubers transform into a source organ that supports the growth and development of sprouts, accompanied by structural and metabolic changes as well as alterations in gene expression patterns (Davies, 2010). This sprout growth is dependent on the supply of energy, nutrients, and other essential resources provided by the mother tuber (Struik, 2007). Within each tuber, sprouts compete for available resources, particularly when the mother tuber is small, as is the case with minitubers. The growth of potato plants derived from Minitubers under field conditions is largely influenced by the sprouting pattern established during tuber storage (Suttle et al., 2012). Numerous studies have investigated the effects of chemical treatments on dormancy break and subsequent sprout characteristics (Suttle et al., 2012). Furthermore, there is little knowledge concerning the impact of maternal plant foliar application on the growth traits of the resulting tubers (Suttle et al., 2012). The objective of this study was to evaluate the effects of Abscisic Acid (ABA) and Benzylaminopurine (BAP) applications during stolon induction and tuberization stages on the subsequent germination characteristics and field performance of Colomba and Sante potato cultivars.

Materials and Methods

This experiment was conducted in two stages at the University of Jiroft during the years 2022–2023. Plantlets of two Potato cultivars (Solanum tuberousum L. cvs. Sante and Colomba) were used in this study.The first experiment was arranged as a factorial experiment in a completely randomized design (CRD). The second experiment was also factorial, but arranged in a randomized complete block design (RCBD). In the first stage, the plantlets were subjected to sprinkler (foliar) application of growth regulators to the mother plants.

First Experiment: Micropropagation and Maternal Plant Treatment

Using in vitro tissue culture techniques, micropropagation was performed, and uniform plantlets were produced at Partikan Bazar Gostar Company. Murashige and Skoog (MS) basal medium was employed for this purpose (Suttle et al., 2012). The MS culture medium was prepared based on micro and macronutrient salts and vitamins, without hormones, supplemented with 30 g/L sucrose, and pH was adjusted to approximately 5.8. The plantlets were transplanted into cubic containers (50 × 50 × 50 cm) filled with a 1:1 (v/v) mixture of perlite and cocopeat. Cultivation was carried out in a representative greenhouse in Anbarabad in early January 2022. The photoperiod was set at 12/12 h (light/dark), day and night temperatures were maintained at 27 ± 20 C and 20 ± 2 C, respectively, and relative humidity was adjusted to 40 ± 5% (Bhaskara, 2017). Hoagland nutrient solution was applied weekly to supply plant nutritional requirements (Bhaskara, 2017). During the growth period, hilling was performed twice, at 30 and 50 day after planting, to cover the lower stem nodes. No pests or diseases were observed throughout the experimental period. The experiment was arranged as a factorial based on a completely randomized design with three replications. Each replicate consisted of 12 plantlets (four plantlets per container). The first factor included two potato) Colomba and Sante) , and the second factor comprised seven hormonal treatments. Tubers were harvested manually 120 days after planting. Following harvest, Minitubers were washed with water, air-dried for 24 hours, and subsequently stored in darkness at 5 ± 1 C with a relative humidity of 85 ± 5%. After 15 weeks of storage, tubers were removed from darkness and exposed to artificial light at ambient temperature for two weeks prior to planting. The presence of at least one sprout with a minimum length of five millimeters in at least 80% of tubers was considered as the criterion for sprouting and termination of tuber dormancy. The number of activated sprouts and sprout length were recorded.

Second Experiment: Field Evaluation

Tubers obtained from the first experimental phase were planted in research farm in Anbarabad County, located at 57 58' 30" E longitude, 28 47' 43" N latitude, and an altitude of 601 meters above sea level, in late October 2023. This study was arranged as a factorial experiment based on a randomized complete block design with three replications. The first factor consisted of two potato cultivars (Colomba and Sante), and the second factor comprised tubers derived from the seven hormonal treatments applied in the first experiment. uniform tubers (15–25 mm in diameter) were planted in plots of 2.3 m^2 in three lines with a distance of 75 cm, a distance between the lines of 20 cm, and a depth of 8 cm. Fertilizer requirements were determined based on soil test results. Nitrogen was applied at 300 kg ha^{-1} from urea source, half at planting and the remainder as topdressing four weeks after emergence and prior to hilling. Phosphorus was applied at 90 kg ha^{-1} as diammonium phosphate ($(NH_4)_2HPO_4$) at planting, and potassium was applied at 100 kg ha^{-1} as potassium nitrate at planting. The experimental field had been fallowed for two years prior to planting. Selected physicochemical properties of the soil are presented in Table 1. For hormonal treatments, both plant growth regulators, BAP (6-Benzylaminopurine, Sigma) and ABA (2-cis, 4-trans-Abscisic acid, Sigma), were applied at a concentration of 50 µM via foliar spraying. Tween® (Riedel-de Haen) at a concentration of 0.5% (v/v) was used as a surfactant. Control plants were sprayed with distilled water containing 0.5% (v/v) Tween®. To ensure adequate solution uptake, foliar application was carried out until complete wetting of the plants was achieved. Hormone spraying was performed at the end of the light period under indirect sunlight to prevent rapid photodegradation. Plant growth regulator treatments were applied at seven levels and at two growth stages, including: BAP at early stolonization, ABA at early stolonization, BAP + ABA at early stolonization, BAP at early tuberization, ABA at early tuberization, BAP + ABA at early tuberization, and control (sprayed with distilled water).

Table 1. Physicochemical characteristics of the soil in depth of 0- 30 cm.

Texture	Available Potassium (mg.kg-1)	Available Phosphorus (mg.kg-1)	Total nitrogen (%)	Organic carbon (%)	Electrical conductivity (EC) (dS.m^{-1})	pH
Clay loam	130	10	0.1	1.2	1.11	7.21

Irrigation was carried out using a drip irrigation system. Weed control was performed through a single application of the herbicide metribuzin (sencor) (70% WP) at a rate of 1 kg ha^{-1} before weed seed germination, supplemented by two rounds of hand weeding. No pests or diseases were observed during the experimental period. Day to emergence were calculated as the number of days from planting to the emergence of at least 50% of seedlings. Emerged plants relative to the total number of planted tubers per treatment were considered as the percentage of germinated and established seedlings. At physiological maturity, plants were manually harvested from a 1 m^2 area within each plot, excluding border rows, for yield assessment. Three plants per replication were selected to measure leaf area, shoot dry weight, and tuber-related traits. Green leaf area was determined using a leaf area meter (Li-3100 area meter, LI-COR, Lincoln, NE). For dry weight determination, shoot samples were oven-dried at 70 °C for 24 hours until constant weight was achieved. Tuber number per plant, tuber yield, and average tuber weight were recorded. Average tuber weight per plant was calculated as the ratio of total tuber weight per plant to the number of tubers per plant.

Statistical analysis

After confirming normality, data were analyzed using SAS software (9.1). Means were compared using the Least Significant Difference (LSD) test at the 5% probability level.

Results and Discussion

Sprout Number per Minituber

Analysis of variance revealed that sprout number per minituber was significantly affected ($P \leq 0.01$) interaction cultivar at plant growth regulator (Table 2). The response of cultivars to growth regulator application varied. In both Sante and Colomba cultivars, Minitubers derived from BAP + ABA application at tuberization and stolonization stages did not show statistically significant differences in sprout number; however, both treatments produced significantly more sprouts per minituber compared to control plants. In the Sante cultivar, application of ABA or BAP alone at either stolonization or tuberization stages did not significantly differ from each other (Table 3). In contrast, BAP treatment at the tuberization stage significantly increased sprout number per minituber in Colomba compared to the control, with the highest sprout number (4.6 sprouts per minituber) recorded in this cultivar under this treatment, although no such positive effect was observed in Sante (Table 3). Therefore, it can be concluded that BAP + ABA application effectively promotes sprout production on minitubers through dormancy release and stimulation of cell division. However, cultivar differences in response to growth regulator applications were evident. These findings are consistent with those reported by Kumar et al., (2007) and Mahajan et al., (2024).

Sprout Length

According to the analysis of variance, the effect of cultivar was significant at the 5% probability level, and the effect of growth regulators was significant at the 1% probability level on sprout length (Table 2). Colomba cultivar produced longer sprouts compared to Sante cultivar. The highest sprout length in Sante (8 mm) and Colomba (8.8 mm) was recorded under BAP treatment at the stolonization stage (Table 3). BAP application at the tuberization stage increased sprout length in both Sante and Colomba cultivars by 41% and 52%, respectively, compared to control plants (Table 3). The stimulation of sprout elongation by BAP application is likely due to the induction of cell division and subsequent growth. Newly formed cells probably act as novel sinks for carbohydrates, thereby enhancing sprout growth. The differential responses observed between cultivars may be attributed to differences in morphology, endogenous physiological mechanisms, and varying sensitivity to plant growth regulators. These findings are consistent with those reported by Kuluev et al., (2016), Bhaskara (2017), and Kumar et al., (2011). Recent findings have highlighted the complex interplay of phytohormones such as Abscisic Acid (ABA) and cytokinins like Benzylaminopurine (BAP) in regulating critical stages of potato development, from dormancy release to tuber initiation (Saidi and Hajibarat., 2021).

Day to Emergence

Analysis of variance revealed that day to emergence was significantly affected only by the application of plant growth regulators at the 1% probability level (Table 2). BAP application at both tuberization and stolonization stages, on average, reduced the number of days to emergence in both cultivars compared to ABA treatment (Table 3). No statistically significant differences were observed between BAP and BAP + ABA treatments at either stolonization or tuberization stages and the control plants in both Sante and Colomba cultivars (Table 3). BAP application at the stolonization stage resulted in seedling emergence at 7 and 8 days after planting in Colomba and Sante cultivars, respectively, which was on average five day earlier in Colomba and four day earlier in Sante compared to control plants. BAP application removes callose deposits from blocked plasmodesmata, allowing assimilates to move toward dormant meristematic cells, thereby stimulating growth and accelerating seedling emergence under field conditions. Li et al., (2019) reported that the application of growth-promoting hormones enhances the translocation of photosynthetic assimilates to meristematic cells, thereby increasing the rate of seedling emergence. Cytokinins and gibberellins may facilitate the removal of callose deposits from blocked plasmodesmata, permitting photosynthates to reach dormant meristematic cells and stimulating the initiation of their growth (Reinoso et al., 2011). Sensitivity to applied plant hormones depends on processes such as uptake, metabolism, and perception of received signals (Salimi et al., 2010). Postharvest management strategies, including the exogenous application of ABA, have been shown to significantly enhance the germination rate of mini-tuber seeds, with studies reporting rates as high as 97.33% under optimal conditions (Zhu et al., 2023).

Table 2. Analysis of variance for effect of application of abscisic acid and benzylaminopurine on number of sprouts, length of sprouts and day to emergence of two potato cultivar in second experiment

		MS		
S.O. V	df	Number of sprouts per Minitubers	Length of sprouts	Day to emergence
Replication (Block)	2	0.48	0.38	0.11
Cultivar (C)	1	9.5**	3.85*	2.91^{ns}
Plant growth regulator (PGR)	6	4.02**	27.61**	30.03**
Cultivar (C) × Plant growth regulator (PGR)	6	2.2^{**}	0.75^{ns}	1.34^{ns}
Error	26	0.63	0.42	0.77
C.V. (%)		18.5	11.9	7.7

Table 3. Mean comparison of effect of application of abscisic acid and benzylaminopurine on number of sprouts, length of sprouts and day to emergence of two potato cultivars in first experiment

Plant growth regulator	Number of sprouts per Minitubers		Length of sprouts		Day to emergence	
	Sante	Colomba	Sante	Colomba	Sante	Colomba
Control	2.2^{b}	3.1^{b}	4.1^{c}	4.3^{d}	13^{a}	13^{ab}
BAP S	2.5^{bc}	2.8^{b}	8^{a}	8.8^{ab}	8^{c}	7^{c}
ABA S	2.7^{bc}	2.1^{c}	3.5^{d}	4.3^{d}	14^{a}	14^{a}
BAP + ABA S	4^{b}	4.4^{a}	6^{b}	7^{bc}	13^{a}	14^{a}
BAP T	1.5^{c}	4.6^{a}	8.2^{a}	9.5^{a}	9^{c}	9^{cb}
ABA T	1.6^{c}	2.3^{c}	4^{c}	4^{d}	12^{b}	11^{b}
BAP + ABA T	4.1^{a}	4.5^{a}	6.3^{b}	6^{cd}	11^{b}	11^{b}

In each row, mean followed by similar letters are not significantly different ($p > 0.05$) using LSD test.

Seedling Emergence Percentage

Based on the analysis of variance, the effects of cultivar and plant growth regulators on seedling emergence percentage were significant at the 1% probability level (Table 4). According to the mean comparison results, the highest seedling emergence percentage in both Sante and Colomba cultivars was observed in the ABA treatment applied at the stolonization stage, on average, it was 30% higher in the Columba cultivar and 20% higher in the Sante cultivar than in the control plants. (Table 5). Additionally, seedling emergence percentage in the Colomba cultivar was 10% higher than in Sante (Table 5). Mean comparisons also showed no statistically significant differences among ABA, BAP, and BAP + ABA treatments applied at the tuberization stage (Table 5). In this study, tubers harvested from plants treated with ABA exhibited a higher seedling emergence percentage compared to other treatments. Abscisic acid, by inducing a longer dormancy period during storage, aligned bud break more closely with the planting time, thereby preventing damage, breakage, and decay of sprouts on seed tubers during storage. Moreover, it increased sprout length after planting, contributing to improved seedling emergence. Bednarek et al., (2006) reported that ABA induces dormancy in seeds, preventing precocious germination under stress conditions and protecting both seeds and sprouts (Tanaka et al., 2006). Furthermore, ABA stimulates the translocation of assimilates toward tubers, increasing their starch and carbohydrate content (Travaglia et al., 2007). Consequently, these tubers, with higher energy reserves, support greater seedling emergence percentage, higher stem and plant number, and improved plant establishment. Minitubers with longer sprout length (up to 8 mm) exhibit shorter emergence times when planted (Dutta et al., 2024).

Number of Plants

The results of the analysis of variance showed that the effects of cultivar and plant growth regulators on the number of plants per square meter were significant at the 1% probability level (Table 4). Mean comparison results indicated that the ABA treatment at the stolonization stage in the Colomba cultivar produced the highest number of plants, with 7.2 plants per square meter, representing a 37% increase over the control plants (Table 5). In the Sante cultivar, the highest number of plants was observed with ABA application at the tuberization stage, which was 41% higher than the control. Moreover, the number of plants in the Colomba cultivar was 10% higher than in Sante (Table 5). Based on the findings of this study, it can be reported that the application of abscisic acid results in the formation of thicker and more robust sprouts. These vigorous sprouts, due to reduced damage and breakage, contribute to an increased number of plants. Suttle et al., (2012) reported that, unlike gibberellin-which promotes excessive sprout growth and increases the risk of damage and breakage-abscisic acid induces the development of shorter, thicker, and sturdier sprouts, thereby enhancing plant establishment in the field. Abscisic acid application reduced sprout fragility and susceptibility to damage, increased plant number, and decreased the number of days to sprout emergence. Gibberellin application significantly increased sprout length but led to greater brittleness and vulnerability, especially at planting time, thereby reducing the quality of micro-tubers. In contrast, sprouts resulting from ABA application at both the stolonization and tuberization stages exhibited reduced sprout length on tubers, increased plant number per square meter, and improved seedling establishment (Davies, 2010).

Number of Stems

Analysis of variance showed that the number of stems per plant was significantly affected by cultivar, plant growth regulators, and their interaction at the 1% probability level (Table 3). According to the mean comparison results, the Colomba cultivar produced an average of 35% and 15% more stems per plant than Sante at the stolonization and tuberization stages, respectively. In both Sante and Colomba cultivars, the application of BAP + ABA at the tuberization stage resulted in an average of 3.1 and 4.1 stems per plant, respectively, corresponding to 29% and 15% more stems compared to the control plants (Table 5). Thus, it can be stated that micro-tubers with a higher number of sprouts produce more stems in the subsequent generation. Haverkort et al., (2012) observed a significant linear relationship between the number of sprouts on seed tubers and the number of stems, stolons, tubers, and also between sprout number and tuber yield. They attributed differences in sprout number per seed tuber primarily to variations in pre-sprouting conditions or cultivar differences. Soaking tubers in a 20 ppm benzyladenine solution broke apical dominance and increased the number of sprouts per tuber (Mingo-Castel et al., 1976). Haverkort et al., (2012) further reported that the number of potato stems increased with a higher number of sprouts on seed tubers.

Table 4. Analysis of variance for effect of application of abscisic acid and benzylaminopurine on Emergence Percentage, Plant Per and Stem Per Plant of two potato cultivars in second experiment

		MS		
S.O. V	Df	Emergence Percentage	Plant Per m^2	Stem Per Plant
Replication (Block)	2	825**	5.05**	0.40^{ns}
Cultivar (C)	1	1362.5**	8.95**	8.13**
Plant growth regulator (PGR)	6	1066.3**	5.31**	6.03**
Cultivar (C) × Plant growth regulator (PGR)	6	113.2^{ns}	0.72^{ns}	1.75**
Error	26	111.5	0.50	0.30
C.V. (%)		15.5	12.8	16.5

Table 5. Mean comparison of effect of application of abscisic acid and benzylaminopurine on Emergence Percentage, Per Plant and Stem Per Plant of two potato cultivars in first experiment

Plant growth regulator	Emergence Percentage		Plant Per m^2		Stem Per Plant	
	Sante	Colomba	Sante	Colomba	Sante	Colomba
Control	70.1^{b}	70.2^{b}	3.8^{c}	4.5^{c}	2.5^{ab}	3.5^{a}
BAP S	85.5^{ab}	88.2^{ab}	4.8^{b}	6.2^{ab}	1.8^{ab}	1.8^{b}
ABA S	89.9^{ab}	100^{a}	6^{ab}	7.2^{a}	1.8^{ab}	2.2^{b}
BAP + ABA S	86.2^{ab}	91.2^{ab}	6.1^{ab}	6.5^{ab}	2.7^{ab}	4^{a}
BAP T	70.5^{b}	70.2^{b}	4.2^{bc}	5^{b}	1.3^{b}	2^{b}
ABA T	85.1^{ab}	84.1^{ab}	6.4^{a}	6.1^{ab}	1.4^{b}	2.5^{a}
BAP + ABA T	73.2^{b}	75.7^{b}	4.3^{ab}	5.5^{bc}	3.5^{a}	4.1^{a}

In each row, mean followed by similar letters are not significantly different ($p > 0.05$) using LSD test.

Leaf Area

Based on the analysis of variance, leaf area was significantly affected by cultivar and plant growth regulators at the 1% probability level, and by their interaction at the 5% probability level (Table 6). Mean comparison results showed that the highest leaf area per plant was observed in the Colomba cultivar with the application of BAP + ABA at the tuberization stage, reaching 1200 cm^2 per plant-39% higher than the control plants. In contrast, for the Sante cultivar, the highest leaf area was recorded with ABA application at the tuberization stage, with 998 cm^2 per plant, representing a 38% increase over the control (Table 7). In this study, the leaf area of the Colomba cultivar was generally greater than that of Sante. This difference between cultivars in response to plant growth regulator application may be attributed to variations in plant morphology, such as leaf area as a surface for hormone absorption, or to differences in the mechanisms of uptake and internal metabolism. These findings are consistent with the results of other researchers (Knowles and Knowles, 2006; Kaya et al., 2023; Kumar et al., 2007). Application of BAP + ABA at tuber initiation stage increased soluble carbohydrate content of both cultivars (Ahmadi Lahijani et al., 2018). Yadav et al., (1997) also reported that application of BAP stimulated accumulation of soluble sugar, proline and amino acids in Cicer plants.

Shoot Dry Weight

Analysis of variance showed that shoot dry weight was significantly affected by plant growth regulators and their interaction with cultivar at the 1% probability level (Table 6). Mean comparison results indicated that the highest shoot dry weight in Colomba and Sante cultivars, with values of 2.31 and 2.28 g per plant, respectively, was obtained from the BAP + ABA treatment applied at the tuberization stage. This represented an average increase of 15% in Colomba and 3% in Sante compared to the control plants. Furthermore, shoot dry weight at the tuberization stage was 2% higher in Colomba than in Sante (Table 7). Based on these findings, it can be concluded that the application of plant growth regulators enhances shoot dry weight by improving seedling establishment in the field, increasing canopy cover, and boosting the number of stems and plants per square meter. The differential response observed between potato cultivars following growth regulator application may be attributed to morphological factors and differences in internal uptake and metabolic mechanisms. These results are consistent with the findings of other researchers (Mahajan et al., 2024; Kaya et al., 2023; Viola et al., 2007). Roosta et al., (2015) reported that plant dry weight, diameter of tubers and tuber yield of potato plants were increased by application of BAP in vitro.

Table 6. Analysis of variance for effect of application of abscisic acid and benzylaminopurine on Leaf area and shoot dry weight of two potato cultivars in second experiment

		MS	
S.O.V	Df	Leaf area	Shoot dry weight
Replication (Block)	2	55854^{ns}	38.20**
Cultivar (C)	1	50489**	15.21^{ns}
Plant growth regulator (PGR)	6	48998**	174.98**
C × PGR	6	20047*	92.40**
Error	26	21995	11.20
C.V. (%)		19.2	16.1

Table 7. Mean comparison of effect of application of abscisic acid and benzylaminopurine on Leaf area and shoot dry weight of two potato cultivars in first experiment

Plant growth regulator	Leaf area (Cm2 Plant $^{-1}$)		Shoot dry weight (g Plant^{-1})	
	Sante	Colomba	Sante	Colomba
Control	611^{d}	735^{bc}	2.11^{a}	1.98^{b}
BAP S	720^{c}	549^{d}	1.54^{bc}	1.58^{bc}
ABA S	415^{e}	389^{c}	1.11^{c}	0.92^{c}
BAP + ABA S	552^{ce}	521^{d}	1.01^{c}	1.55^{bc}
BAP T	819^{b}	845^{b}	1.91^{ab}	1.61^{bc}
ABA T	998^{a}	862^{b}	1.75^{b}	1.97^{b}
BAP + ABA T	982^{a}	1002^{a}	2.28^{a}	2.31^{a}

In each row, mean followed by similar letters are not significantly different ($p > 0.05$) using LSD test.

Number of Tubers

Analysis of variance revealed that the effect of cultivar on tuber number was significant at the 1% probability level, while the effect of plant growth regulators was significant at the 5% probability level (Table 8). Mean comparison results showed that, in both Sante and Colomba cultivars, the BAP + ABA treatment applied at the tuberization stage produced the highest number of tubers per plant, with averages of 6 and 7 tubers, respectively. This represented increases of 37% in Sante and 22% in Colomba compared to the control plants (Table 9). Overall, the Colomba cultivar produced an average of 15% more tubers per plant than Sante (Table 9). Application of BAP + ABA at the tuberization stage increased the number of sprouts on mother tubers, stem number, and tuber number per plant in both potato cultivars. These findings suggest that a higher number of sprouts on the mother tuber leads to increased tuber production in the subsequent generation. Reinoso et al., (2011) reported that an increase in stem number per plant was associated with higher tuber number but a decrease in average tuber weight. The application of growth regulators accelerates seedling emergence, increases stem and tuber number per plant, and reduces average tuber weight in potato (Bednarek et al., 2021). Following dormancy break, sprout growth depends on the mother tuber for the supply of energy, nutrients, and other compounds required for development (Haverkort et al., 2012). The genotypic-specific response to PGRs, as seen in our cvs. Santé and Colomba, has also been documented in other recent studies. For instance, García-García et al., (2019) reported differential responses to BAP and ABA between the 'Atlantic' and 'Alpha' potato varieties during in vitro tuberization, indicating that endogenous hormonal balances play a decisive role.

Average Tuber Weight

Analysis of variance revealed significant effects of cultivar, plant growth regulators, and their interaction on average tuber weight at the 1% probability level. Mean comparison results showed that the Sante cultivar produced an average single tuber weight of 66.7 g, which was 25% higher and 16.5 g heavier per tuber than the Colomba cultivar. In the Sante cultivar, the application of BAP at the stolonization stage resulted in the highest average tuber weight (66.7 g per tuber), representing an 18% increase over the control (Table 9). In the Colomba cultivar, plants treated with BAP + ABA at the stolonization stage produced the highest average tuber weight (50.2 g per tuber), which was 29% higher than the control plants (Table 9). It appears that the application of BAP and BAP + ABA at the stolonization stage stimulates cell division in tubers, leading to heavier tubers with higher carbohydrate content, thereby increasing tuber yield (Bhaskara, 2017). Furthermore, some researchers have reported that cytokinin activates starch biosynthesis enzymes during the onset of tuberization, resulting in starch accumulation and enhanced sink capacity of developing potato tubers (Mingo-Castel et al., 1976). Cytokinin application to mother plants stimulates cell division in tubers, producing more vigorous tubers with greater carbohydrate reserves. These tubers provide better support for sprouts, leading to the development of more and stronger sprouts, which in turn results in more robust plants. This contributes to improved plant establishment and increased tuber yield (Kaya et al., 2023). Xu et al., (1998) found that ABA/GA ratio has a determining role in the initiation of tuberization in potato. Liu and Xie (2001) reported that different cytokinin concentrations increased minituber size and weight, and a linear relationship was observed between these two traits.

Tuber Yield

Analysis of variance showed that tuber yield was significantly affected by plant growth regulators at the 5% probability level (Table 8). Mean comparison results indicated that the highest tuber yield was observed in the Colomba cultivar (2282 g/m^2) and the Sante cultivar (2120 g/m^2) under BAP + ABA and BAP treatments, respectively, both applied at the stolonization stage. These represented increases of 36% and 33% compared to the control plants, respectively. At the stolonization stage, the Colomba cultivar produced the highest tuber yield, which was 7% greater than that of the Sante cultivar (Table 9). The findings of this study demonstrate that the application of BAP + ABA and BAP at the stolonization stage enhances tuber yield in potato plants by increasing the number of sprouts per minituber, subsequently increasing stem and plant number, and ultimately improving tuber number and weight. These results are consistent with those reported by Dalla Rizza, Vilaro, and Izquierdo (2019); Pospisilova and Batkova (2004); and Li et al., (2007), who stated that growth regulators such as BAP + ABA and BAP increase final potato yield by enhancing yield components (e.g., tuber weight, tuber number, etc.). Application of BAP, ABA, or their combination at tuber initiation improved key physiological traits-enhancing photosynthesis and chlorophyll via higher soluble carbohydrate accumulation-and significantly increased tuber yield components (yield per plant, mean tuber weight, and tuber number), with a stronger response in Agria Fontane cultivar (Ahmadi Lahijani et al., 2018). The positive effect of BAP application observed in our study aligns with recent findings by Thinakaran et al., (2025), who demonstrated that BAP, particularly when combined with optimal sucrose concentrations, is a highly significant factor for improving in vitro tuberization and tuber weight in potato cultivars.

Harvest Index

Based on the analysis of variance, the effect of plant growth regulators on harvest index was significant at the 1% probability level, and the interaction effect of cultivar and growth regulators was significant at the 5% probability level (Table 8). Mean comparison results showed that in the Sante cultivar, application of BAP + ABA at the stolonization stage resulted in a harvest index of 80%, which was on average 35% higher than that of the control plants. In the Colomba cultivar, ABA treatment at the stolonization stage also produced a harvest index of 80%, representing an 11% increase compared to the control (Table 9). Based on these findings, it can be concluded that the positive effects of the combined application of BAP + ABA on the number of stems per plant, yield, yield components, and ultimately harvest index are attributed to the synergistic effects of these growth regulators when applied together. These results are consistent with the findings of Dutta et al., (2024), Suttle (1995), and Travaglia et al., (2007).

Table 8. Analysis of variance for effect of application of abscisic acid and benzylaminopurine on Tuber per plant, Mean tuber weight, Tuber yield and Harvest index of two potato cultivars in second experiment

		MS			
S.O. V	Df	Tuber per plant	Mean tuber weight	Tuber yield	Harvest index
Replication (Block)	2	3.01	55.01	15251	0.002
Cultivar (C)	1	18.75**	808.5**	32217^{ns}	0.006^{ns}
Plant growth regulator (PGR)	6	4.5*	620.3**	74187*	0.059**
Cultivar (C) × Plant growth regulator (PGR)	6	1.5^{ns}	152.7**	11995.75^{ns}	0.015*
Error	26	0.85	75.20	105521	0.002
C.V. (%)		15.3	19.2	17.4	8.5

Table 9. Mean comparison of effect of application of abscisic acid and benzylaminopurine on Tuber per plant, Mean tuber weight, Tuber yield and Harvest index of two potato cultivars in first experiment

Plant growth regulator	Tuber per plant		Mean tuber weight (g)		Tuber yield (g m-^{2})		Harvest index (%)	
	Sante	Colomba	Sante	Colomba	Sante	Colomba	Sante	Colomba
Control	3.8^{b}	5.5^{ab}	55.2^{b}	35.5^{ab}	1420^{bc}	1459^{bc}	52^{c}	71^{b}
BAP S	4.0^{b}	5.6^{ab}	66.7^{a}	42.2^{ab}	2120^{a}	1890^{ab}	73^{a}	75^{ab}
ABA S	3.4^{c}	4.4^{b}	46.3^{bc}	47.8^{a}	1151^{c}	1325^{c}	67^{ab}	80^{a}
BAP + ABA S	5.8^{a}	5.7^{ab}	42.8^{bc}	50.2^{a}	2120^{ab}	2282^{a}	80^{a}	72^{b}
BAP T	3.7^{b}	6.5^{ab}	45.2^{bc}	34.4^{ab}	1052^{c}	1620^{bc}	55^{bc}	61^{c}
ABA T	5.1^{ab}	6.3^{ab}	39.2^{bc}	25.5^{b}	1400^{bc}	1282^{c}	60^{b}	50^{d}
BAP + ABA T	6.0^{a}	7.0^{a}	30.5^{c}	25.2^{b}	1350^{bc}	1310^{c}	54^{c}	60^{c}

In each row, mean followed by similar letters are not significantly different ($p > 0.05$) using LSD test.

Conclusion

The results demonstrated that the application of BAP and ABA can effectively improve the sprouting process on seed tubers and enhance plant establishment in the field. Overall, BAP appears to increase the number and length of sprouts, by reducing seedling emergence time, while ABA reduces sprout length on tubers and improves the percentage and uniformity of seedling emergence and establishment under field conditions. Since sprout growth largely depends on the energy supply from the mother tuber, both BAP and ABA seem to enhance the physiological sink strength of the tubers. Stronger tubers provide greater energy reserves to support sprout development during subsequent growth stages in the field. These effects are cultivar-dependent, and further research is recommended to better understand the advantages and limitations of using each growth regulator to improve tuber sprouting, plant establishment, and minituber production.

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