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Effect of plant growth regulators and explants on organogenesis of soybean (*Glycine max* L.)

Vahid Mehrizadeh and Ebrahim Dorani*

Department of Plant Breeding and Biotechnology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran.
*Corresponding author; dorani@tabrizu.ac.ir

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Abstract

Objective: The soybean plant is one of the most important crops, and it is known as a rich source of vegetable oil and protein in the world. Despite its advantages, soybean production faces different challenges and requires genetic improvement. Soybean transformation provides an attractive advancement for soybean breeding programs, allowing the production of novel and genetically diverse plant materials. Therefore, developing an efficient plant regeneration protocol is necessary for the transformation programs. In this study, a simple, efficient, and repeatable protocol was developed for *in vitro* organogenesis of the Saman soybean cultivar.

Methods: The soybean seeds were cultured on different strengths of MS medium (full and half-strength MS) in combination with different concentrations of BAP/Kin (0, 0.5, 1, 1.5, 2, 2.5, and 3 mg/L). After germination, cotyledon, hypocotyl, and primary leaf explants were cultured on the shoot induction media with different concentrations of BAP/Kin (0, 1, 1.5, and 2 mg/L) in combination with IBA/NAA (0, 0.1, and 0.5 mg/L) to evaluate their organogenesis potential. In the next step, for shoot elongation, all regenerated buds were transferred to two media (MS and ½ MS) in combination with BAP (0, 0.1, 0.2, and 0.4 mg/L) and GA_3 (0, 0.1, 0.5, and 1 mg/L). At the final stage, for root induction, the elongated shoots were cut and cultured in different strengths of the MS medium (full and half-strength MS) in combination with different concentrations of IAA, IBA, and NAA (0, 0.1, 0.5, 1, 1.5, and 2 mg/L). All experiments were conducted as a factorial arrangement based on a completely randomized design.

Results: The results showed that the highest percentage of seed germination (97.18%) was obtained in full-strength MS medium supplemented with 2 mg/L BAP. The maximum number of shoot induction per explant (7.1 shoots) was observed in the medium containing 1.5 mg/L BAP and 0.1 mg/L IAA from the cotyledon explants. The maximum rate of shoot elongation (8.5 cm) was achieved in the half-strength MS medium containing 0.5 mg/L GA_3 and 0.2 mg/L BAP. Also, the maximum number of roots was produced in the half-strength of MS medium supplemented with 1.5 mg/L IBA. Finally, the rooted plantlets after acclimatization were transferred into the greenhouse for flowering and pod maturation.

Conclusion: The results of this study can be used for gene transfer and genetic engineering research for the Saman cultivar and other soybean cultivars.

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Introduction

Soybean (*Glycine max* L.) is one of the most important legumes and is known as a rich source of vegetable protein and oil in the world. Among the oilseed crops, soybean has wide utilization, and the products of soybean have an important role in the human diet. In addition, it has particular importance in animal nutrition (Sehaole and Mangena 2024). The world production of soybeans has increased because of the increase in demand for soybean grain derivatives (Wang *et al*. 2022). The soybean plant is cultivated as a valuable crop in most parts of the world, but its production has some challenges because it is sensitive to environmental stresses, especially drought, salinity, weeds, and pests (Vargas-Almendra *et al*. 2024). The improvement for protein and oil quality, and resistance to pests, diseases, herbicides, and stresses are some of the main objectives of soybean breeding programs (Mangena 2021).

Classical breeding methods of soybean are limited due to its self-pollination and low genetic diversity among its cultivars (Begum *et al.* 2019). Biotechnology techniques, including genetic engineering and tissue culture, can be effective in improving such plants (Raza *et al.* 2019). Tissue culture and plant regeneration are necessary for the production of transgenic plants, and also, these techniques create new possibilities for improving the soybean quality. In this regard, developing an efficient plant regeneration protocol is the first step in transformation programs (Long *et al.* 2022). Soybean is known as a recalcitrant plant for *in vitro* regeneration (Rehman *et al.* 2025). Various factors, such as genotype, explant, culture medium, plant growth regulators (PGR), and environmental conditions, affect the regeneration of soybean (Tiwari *et al.* 2023).

Genotype, along with PGR, plays a key role in the regeneration of various plants, especially legumes (Pratap *et al.* 2018). The soybean regeneration extremely depends on the genotype, so the preparation of a suitable protocol for the regeneration of specific genotypes is necessary (Xu *et al.* 2022; Zhong *et al.* 2024). Explant is one of the effective factors in soybean regeneration. Also, it seems that the selection of a suitable explant can overcome some of the problems associated with

soybean regeneration (Arun *et al.* 2016). Various parts of the soybean have been used as explants for regeneration, including shoot meristems (Liu *et al*. 2004), cotyledons (Zhong *et al.* 2024), half-seeds (Sojková *et al.* 2016), hypocotyls (Raza *et al.* 2017), and embryo axes (Joyner *et al.* 2010).

PGRs are one of the effective factors in the plant tissue culture and play an important role in the regeneration of plants (Mangena 2021). The most useful PGRs are auxins, cytokinins, and gibberellins, as they affect cell growth, tissue development, and plant regeneration (Li *et al*. 2017). Different studies showed that the proportion of cytokinin to auxin determines the rate of regeneration in dicotyledonous plants (Lee *et al*. 2020; Muzika *et al*. 2024). Generally, auxins stimulate cell elongation and plant growth, and cytokinins trigger cell division, and when combined, they can promote shoot formation (Paes de Melo *et al*. 2020). Both BAP and TDZ have been reported to be effective cytokines for shoot regeneration in some soybean cultivars (Raza *et al*. 2017). Also, the combination of BAP and NAA has been shown to improve shoot regeneration and the number of shoots from soybean explants (Rehman *et al*. 2025). On the other hand, some studies reported the positive influence of pretreatment of soybean seeds with cytokinins such as BAP or TDZ on the regeneration of shoots (Shan *et al*. 2005; Raza *et al*. 2017; Mangena 2021).

In this study, the effect of different concentrations of auxin and cytokinin on *in vitro* germination of seeds, organogenesis, shoot elongation, and rooting of elongated shoots in soybean (*Glycine max* L.) was investigated.

Materials and Methods

Plant material

The seeds of the Saman cultivar of soybean (*Glycine max* L.) were provided by the Seed and Plant Improvement Institute, Karaj, Iran. Saman cultivar is one of the most important and economical soybean varieties in Iran.

In vitro seed germination and explant preparation

In this study, the soybean seeds were surface-sterilized with the chlorine gas. For this purpose, the seeds were incubated for 24 hours inside a desiccator with a glass containing 100 ml of bleach (5% v/v) and 4 ml of hydrochloric acid (37%). After 24 hours of incubation, the seeds were taken out and rinsed three times with sterile distilled water (Figure 1A).

The sterilized seeds of soybean were cultured on the MS and half-strength MS media (Murashige and Skoog 1962). All culture media contained 3% sucrose and 0.6% agar, supplemented with different concentrations of BAP (0, 0.5, 1, 1.5, 2, 2.5, and 3 mg/L) or Kin (0, 0.5, 1, 1.5, 2, 2.5, and

3 mg/L). Nine seeds were cultured in the culture bottles, and six bottles were used for each treatment as replications. In all experiments, the cultures were maintained in the growth chamber at 25±2 °C and 16/8 h light/dark photoperiod. In this study, three different explants of soybean (cotyledon, hypocotyl, and primary leaf) were used for organogenesis. The cotyledon and hypocotyl explants were cut from 6-day-old soybean seedlings, and the primary leaf explants were cut from 12-day-old germinated plantlets.

Shoot induction

Different combinations of PGR, including Kin (0, 1, 1.5, and 2 mg/L), BAP (0, 1, 1.5, and 2 mg/L), NAA (0, 0.1, and 0.5 mg/L) and IAA (0, 0.1, and 0.5 mg/L) were used for organogenesis of Saman cultivar and their effects on the hypocotyl, cotyledon, and primary leaf explants were investigated. Also, in this experiment, MS medium salts along with B5 vitamins (Gamborg *et al.* 1968) were used as the culture medium. The hypocotyl explant was put horizontally on the culture medium, and the cotyledon and primary leaf explants were put on the dorsal surface. All cultures were transferred to a growth chamber and after two weeks, the explants were transferred to the fresh medium. After six weeks, the number of regenerated shoots per explant was determined for all treatments.

Shoot elongation

For shoot elongation, all regenerated buds of soybean were transferred to the shoot elongation media. To achieve this goal, two media (MS and ½ MS) in combination with different concentrations of BAP (0, 0.1, 0.2, and 0.4 mg/L) and GA_3 (0, 0.1, 0.5, and 1 mg/L) were used and after four weeks (two subcultures), the shoot lengths were measured with a ruler.

Rooting and acclimatization

In this experiment, to investigate the rooting in the Saman soybean cultivar, the elongated shoots were isolated and transferred into rooting media. For this aim, two media (MS and ½ MS) were used in combination with different concentrations of IAA, IBA, and NAA (0, 0.1, 0.5, 1, 1.5, and 2 mg/L) with 20 g/L sucrose and 6 g/L agar. To acclimatize the *in vitro*-rooted plantlets of soybean were transferred into plastic cups, containing sterile peat moss and perlite. Then, the cups were covered with plastic bags, and the ½ Hoagland solution was used for irrigating the plantlets. After one week, the plastic bags were removed gradually, allowing the plantlets to acclimatize to the new environment. After two weeks, the acclimatized plantlets were transferred into large pots and kept under controlled conditions until flowering and maturity of the pods.

Experimental design and statistical analysis

For the shoot regeneration, an experiment was conducted with two factors (combination of PGR and types of explants) based on a completely randomized design (CRD) with three replications per each treatment. Also, germination, shoot elongation, and rooting experiments were conducted with two factors (as mentioned in the previous sections) based on a CRD with four or six replications. After the test of assumptions, the data were analyzed using MSTATC (version 6) and SPSS (version 21) statistical software. The data means were compared by Tukey's honestly significant difference test at 0.05 probability level.

Results

The effect of different cytokinins on seed germination and seedling growth

Analysis of variance of the data showed that the PGR, strengths of MS medium, and their interaction had a significant effect on seed germination (Table 1). Comparisons of the means for the combination of PGR and different strengths of MS medium showed that the lowest germination (13.88%) was observed in the ½ MS medium without PGR, and the highest germination (97.18%) was observed in the MS medium supplemented with 2 mg/L BAP (Table 2).

Based on our observation, after three days of culture, germination of soybean seeds began in all treatments. Seedlings grown in the media containing BAP had large cotyledons with small and thick hypocotyls compared to Kin. On the other hand, seedlings obtained from the media without PGR (control) had small cotyledons and thin hypocotyls. Also, among the different media, the large cotyledons and thicker hypocotyls were observed on the seedlings grown in full-strength MS medium as compared to seedlings from the ½ MS medium. In total, the highest percentage of germinated seeds with rapid growth, enlarged cotyledons, green chlorophyll, healthy, and thick hypocotyls was observed in full MS medium supplemented with 2 mg/L BAP (Figure 1 B-C).

Table 1. Analysis of variance of the effect of plant growth regulators and different strengths of MS medium on germination percentage of soybean seeds.

S.O.V	df	Mean squares
Plant growth regulators	12	5768.52**
Strength of MS medium	1	12296.314**
Plant growth regulators × Strength of MS medium	12	145.87**
Error	130	2.33
C.V. (%)		34.1

**: Significant at $p \leq 0.01$.

Table 2. The germination percentage of soybean seeds as affected by the combination of the different plant growth regulators and strengths of MS medium after six days.

BAP (mg/L)	Kin (mg/L)	Germination (%)	
		Half-strength MS medium	Full-strength MS medium
0	0	13.88	19.44
0.5	0	30.55	41.65
1	0	47.21	63.86
1.5	0	55.53	77.75
2	0	66.62	**97.18**
2.5	0	75.00	86.10
3	0	80.52	83.30
0	0.5	19.44	27.80
0	1	25.00	36.10
0	1.5	33.33	50.00
0	2	44.43	61.10
0	2.5	50.00	75.00
0	3	52.70	63.90
HSD5%		3.303	

HSD: Tukey's honestly significant difference test.

The effect of explants and plant growth regulators on organogenesis

Based on our observation, after 10 days, all explants had enlarged and swollen with the signs of callus induction. Also, the callus induction was observed mostly at the hypocotyl explants in the media containing NAA. After three weeks, the signs of regeneration were observed in the explants. After six weeks, all regenerated shoots were recorded.

The results of the analysis of the data showed that the effects of PGR, explants, and their interaction were significant, at 1% probability level, on shoot induction of the Saman cultivar (Table 3). Comparison of the means for the combination of PGR and types of explants showed a significant difference among treatments, and there was direct regeneration in most treatments (Table 4).

It was found that the presence of an auxin, along with cytokinin, compared to cytokinin alone, plays a significant role in the organogenesis of soybean. The combination of BAP with IAA was found to be the best combination for soybean regeneration. Also, there was a significant difference between types of explants in the ability of regeneration, and in all treatments, the cotyledon explants had the highest regeneration efficiency among explants. For example, the highest number of shoot regeneration per explant for the hypocotyl and primary leaf explants were 3.6 and 5.0 in medium, respectively, supplemented with 2 mg/L BAP and 0.1 mg/L IAA, while the highest number of shoot

regeneration per explant for cotyledon (7.1) was achieved in the medium supplemented with 1.5 mg/L BAP and 0.1 mg/L IAA (Figure 1 D-E).

Table 3. Analysis of variance of the effect of plant growth regulators and explants on organogenesis of soybean.

S.O.V	df	Mean squares
Plant growth regulators	22	16.78**
Explants	2	841.30**
Plant growth regulators × Explants	44	0.68**
Error	138	0.02
C.V. (%)		19.4

**: Significant at $p \leq 0.01$.

Table 4. Comparisons of means of the combination of different plant growth regulators and explants on soybean organogenesis after six weeks.

BAP (mg/L)	Kin (mg/L)	NAA (mg/L)	IAA (mg/L)	Number of shoots per explant		
				Hypocotyl	Cotyledon	Primary leaf
0	0	0	0	0.00	0.00	0.00
1	0	0	0	0.00	0.60	0.00
1.5	0	0	0	0.00	2.76	0.60
2	0	0	0	0.60	2.00	0.93
0	1	0	0	0.00	0.60	0.00
0	1.5	0	0	0.00	1.43	0.00
0	2	0	0	0.00	1.00	0.60
1.5	0	0.1	0	1.76	2.76	2.30
2	0	0.1	0	2.30	3.10	2.76
1.5	0	0.5	0	1.30	1.96	1.44
2	0	0.5	0	1.76	2.40	1.76
0	1.5	0.1	0	1.00	2.53	1.76
0	2	0.1	0	1.53	2.76	2.30
0	1.5	0.5	0	0.60	1.76	1.43
0	2	0.5	0	1.43	2.30	1.53
1.5	**0**	**0**	**0.1**	3.00	**7.10**	4.50
2	0	0	0.1	3.60	5.60	5.00
1.5	0	0	0.5	3.10	4.86	4.00
2	0	0	0.5	2.76	3.76	3.33
0	1.5	0	0.1	2.30	3.60	2.76
0	2	0	0.1	2.76	4.33	3.33
0	1.5	0	0.5	1.00	2.76	1.53
0	2	0	0.5	1.76	3.00	2.53
HSD5%					0.489	

HSD: Tukey's honestly significant difference test.

The effect of media and plant growth regulators on shoot elongation

The regenerated explants were transferred to the shoot elongation media, and after two weeks, the length of the shoots was recorded. Analysis of variance of the data showed that the PGR, different strengths of MS medium, and their interaction had a significant effect on shoot elongation at the 1% probability level (Table 5). Mean comparisons of the combination of PGR with different strengths of MS medium (Table 6) showed that the increasing concentration of BAP and GA_3 caused shoot elongation in both media. Moreover, the culture medium also played an important role in shoot elongation, and the length of the shoots in the ½ MS medium was higher than that of the MS medium. In total, the highest shoot length (8.5 cm) was achieved in the ½ MS medium, supplemented with 0.5 mg/L GA_3 and 0.2 mg/L BAP (Figure 1 F-G). Also, the lowest shoot length was observed in the ½ MS medium without PGR.

The effect of auxin and medium on the rooting of the shoots

Analysis of the data showed that the different types and concentrations of auxins, different strengths of MS medium, and their interaction had a significant effect on rooting traits of the soybean explants (Table 7). Comparison of the means for combined effects of the two factors (Table 8) showed that the maximum number (6.5) and length (11.2 cm) of roots were obtained in the ½ MS medium, supplemented with 1.5 mg/L IBA, and the minimum number and length of roots were produced in the media without auxins (Figure 1 H-I).

Different auxins enhanced and supported the rooting of the regenerated shoots, because the lowest number and length of roots were produced in the media without auxin. Also, among the types of auxins tested, IBA was the most effective auxin as compared with other auxins for rooting of the shoots. So that, the maximum number and length of roots were obtained from IBA and the minimum number and length of roots was produced by NAA. Also, the culture media were effective in the rooting of shoots. So, the ½ MS medium was more effective than the MS medium in the rooting of the Saman cultivar. In total, the maximum number and length of roots were produced in the ½ MS media, supplemented with 1.5 mg/L IBA. Also, the rooted plantlets were maintained in the growth chamber for acclimatization. After acclimatization (Figure 1 J-K), the plantlets were transferred to the greenhouse for flowering and pod maturation (Figure 1 L).

Discussion

The results of this study showed that the use of full MS medium with 2 mg/L BAP is necessary for germination of soybean seed and preparation of suitable explants for regeneration. Similar to our

findings, it has been reported that the use of cytokinins in the seed germination of soybean stimulates regeneration (Raza *et al.* 2017). Also, the reduction in the germination efficiency was found in the ½ MS medium, compared with the full MS medium. This phenomenon indicates that the nutritional requirement affected the germination efficiency of soybean. Furthermore, media containing BAP as compared with media containing Kin affected the germination efficiency of soybeans. Similar to our findings, Mangena *et al*. (2021) and Shan *et al*. (2005) suggested that the treatment of soybean seeds with PGR such as TDZ or BAP enhanced the germination and regeneration efficiency.

Table 5. Analysis of variance of the effect of plant growth regulators and different strengths of MS medium on shoot elongation.

S.O.V	**df**	**Mean squares**
Plant growth regulators	15	10.84**
Strength of MS medium	1	121.48**
Plant growth regulators × Strength of MS medium	15	3.67**
Error	96	0.17
C.V. (%)		23.5

**: Significant at $p \leq 0.01$

Table 6. Mean comparisons of the interaction effect of plant growth regulators and different strengths of MS medium on the shoot elongation after two weeks.

BAP (mg/L)	**GA_3 (mg/L)**	**Length of shoot (cm)**	
		½ MS medium	**Full MS medium**
0	0	0.0	0.5
0	0.1	3.3	1.9
0	0.5	4.5	2.8
0	1	5.7	3.7
0.1	0	4.3	2.3
0.1	0.1	4.2	2.8
0.1	0.5	5.3	3.2
0.1	1	5.7	3.6
0.2	0	5.4	3.1
0.2	0.1	6.0	3.5
0.2	**0.5**	**8.5**	4.3
0.2	1	7.0	4.7
0.4	0	5.0	3.6
0.4	0.1	4.6	4.5
0.4	0.5	5.5	5.1
0.4	1	5.0	5.6
HSD5%		1.14	

HSD: Tukey's honestly significant difference test.

Table 7. Analysis of variance of root traits as affected by auxin types and different strengths of MS medium.

S.O.V	df	Mean squares	
		Number of roots	Root length
Auxins	15	10.44**	50.48**
Strength of MS medium	1	31.0**	30.71**
Auxins × Strength of MS medium	15	0.84*	2.72**
Error	96	0.47	0.17
C.V.(%)		16.5	22.9

*, **: Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Table 8. Mean comparisons of the interaction effect of different auxin types and strengths of MS medium on root traits after two weeks.

NAA (mg/L)	IAA (mg/L)	IBA (mg/L)	Number of roots		Root length (cm)	
			½ MS medium	Full MS medium	½ MS medium	Full MS medium
0	0	0	1.25	0.50	1.2 l	0.5 m
0.1	0	0	1.75	1.00	2.4 k	1.1 l
0.5	0	0	2.25	1.50	3.2 j	2.6 k
1	0	0	2.75	2.25	4.9 g	5.6 f
1.5	0	0	3.75	1.75	7.1 d	4.2 i
2	0	0	3.00	1.50	4.1 i	3.3 j
0	0.1	0	2.25	1.25	4.3 hi	3.6 j
0	0.5	0	2.75	1.75	5.4 f	4.7 gh
0	1	0	3.00	2.25	9.5 b	7.5 d
0	1.5	0	3.50	2.75	6.1 e	6.3 e
0	2	0	4.75	3.25	4.8 g	4.2 i
0	0	0.1	2.75	2.25	6.2 e	4.7 gh
0	0	0.5	3.5	3.00	7.1 d	6.2 e
0	0	1	4.75	4.50	8.1 c	9.4 b
0	**0**	**1.5**	**6.50**	3.75	**11.2 a**	8.1 c
0	0	2	3.75	3.00	6.1 e	4.5 ghi
HSD5%			1.897		1.141	

HSD: Tukey's honestly significant difference test.

Figure 1. Soybean organogenesis; (A) Sterilization of the soybean seeds, (B) Culture of the seeds of soybean on the culture mediums, (C) 6-days old seedlings, (D) Culture of the explants on the shoot induction media, (E) Shoot induction, (F-G) Shoot elongation, (H-I) Rooting of the elongated shoots, (J-K) Acclimatization of the regenerated plants under controlled conditions, (L) Regenerated plants with seed pods.

In vitro soybean regeneration is affected by various factors, including genotypes, PGR, explants, etc. (Sehaole and Mangena 2024). Among these, PGRs play an important role in the regeneration of explants during tissue culture (Long *et al.* 2022). The results of our study confirmed that the regeneration potential of the cotyledon explant was higher than that of the other explants (primary leaf and hypocotyl). Therefore, the cotyledon explant was the most suitable explant for the regeneration of the Saman cultivar. Tiwari *et al.* (2023) also reported that the highest rate of soybean regeneration was obtained from the cotyledon explant. More investigation showed that among the different treatments of PGR, the combination of 1.5 mg/L BAP with 0.1 mg/L IAA was determined as the best PGR combination for regeneration of the Saman cultivar using different explants. It was also found that the BAP was more effective than Kin in the soybean regeneration. Rehman *et al.* (2025) also reported that the maximum shoot induction of the AARI soybean cultivar was observed on the MS media containing 2.0 mg/L BAP and 0.1 mg/L NAA. Also, according to Begum *et al.*

(2019), the highest rate of shoot induction was produced in the media supplemented with 1.5 mg/L BAP in the BARI-5 cultivar of soybean. In another study, the highest efficiency of regeneration was observed at a concentration of 2.0 mg/L BAP in the LS677 cultivar of soybean (Mangena *et al.* 2015). However, some reports, such as Khan *et al.* (2024), indicated that the regeneration of cotyledon explants was achieved only by the combination of BAP with Kin.

Based on our findings, the lowest shoot elongation was observed on the PGR-free media. Therefore, for elongation of regenerated shoots, GA_3 and BAP were important PGRs for the regulation of plant growth. Also, our results showed that the optimal concentration of a combination of GA_3 and BAP (0.5 mg/L GA_3 and 0.2 mg/L BAP) with half MS medium improved the shoot length in the Saman cultivar. According to Pasternak and Steinmacher (2024), the GA_3 hormone promoted the stem elongation, IAA mainly promoted cell elongation, and BAP promoted cell division. In another study, Li *et al.* (2017) indicated that the combination of 1.00 mg/L GA_3 and 0.1 mg/L IAA resulted in the highest shoot elongation of 34% and 26% for Jack Purple and Tianlong-1 soybean cultivars, respectively. Also, Arun *et al*. (2016) reported that the highest rate of shoot elongation was 90% in the MS medium when 1.45 µM GA_3 and 49.42 µM spermine were added into the shoot elongation medium. While Mangena *et al.* (2015) used the MS medium without PGR for the elongation of the regenerated shoots of the soybean cultivar LS 677.

Finally, our results showed that the presence of auxins is necessary for the rooting of the elongated shoots. So, the ½ MS medium was determined as the most suitable medium, and IBA as the most effective auxin, for the rooting of shoots. The highest root number and length were observed in the ½ MS medium supplemented with 1.5 mg/L IBA. It was also found that IBA was more effective than IAA and NAA in rooting of the elongated shoots. Raza *et al.* (2017) also used the half-strength B5 medium, supplemented with 1.0 mg/L IBA for rooting of the elongated shoots of nine soybean cultivars. In addition, Ma and Wu (2008) reported the usage of the MSB5 medium containing 0.5 mg/L IBA for rooting of the elongated shoots in four cultivars of soybean. While, Rehman *et al.* (2025) reported the highest root induction of shoots of the soybean cultivar AARI from the combination of 0.5 mg/L IBA and 1.2 mg/L IAA.

Conclusion

Based on our results, chlorine gas effectively sterilized the soybean seeds. Suitable explants for soybean regeneration seeds can be obtained with cytokinins that are necessary for germination. Among different kinds of cytokinins, BAP was more effective than Kin in the germination of soybean seeds. The maximum germination was obtained in the full MS medium with 2 mg/L BAP. Cotyledon

explant was the most suitable explant, and the combination of BAP with IAA was as the best hormone combination for the regeneration of the Saman cultivar. The maximum number of regenerated shoots was in the medium containing 1.5 mg/L BAP with 0.1 mg/L IAA from the cotyledon explant. The most effective PGR for the shoot elongation was obtained from the combination of GA_3 and BAP. The highest shoot elongation was observed in the ½ MS medium containing 0.5 mg/L GA_3 and 0.2 mg/L BAP. Finally, the suitable medium and auxin for rooting of shoots of Saman cultivar were obtained from the ½ MS medium with IBA. The results, the maximum root number and length were produced in the ½ MS medium with 1.5 mg/L IBA.

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Ethical Considerations

The authors avoided data fabrication and falsification.

Conflict of Interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

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