Plant Archives Vol. 26, No. 2, 2026 pp. 2053-2062 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.no.2.282

IN VITRO ROOT INDUCTION IN SOYBEAN (*Glycine max* (L.) MERR.) CV. MAUS-158

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(Date of Receiving-11-07-2026; Date of Revision-19-09-2026; Date of Acceptance-29-09-2026)

ABSTRACT

This study establishes a reliable and efficient protocol for production of *in vitro* root induction potential from different explants of soybean (*Glycine max* (L.) Merr.) cv. MAUS-158. The Leaf, hypocotyl, root, cotyledon and callus explants were cultured on Murashige and Skoog (MS) medium supplemented with á-naphthaleneacetic acid (NAA) (0.25 and 0.6 mg/l) and indole-3-butyric acid (IBA) (0.3, 0.6, 1.2, 1.8 and 2.5 mg/l). The response of the explants was assessed based on root induction percentage, time of root initiation and root development during the culture period. Significant variation in rooting response was observed among the different explants. Root and callus explants exhibited the highest root induction response (83.33%), followed by hypocotyl (76.66%), cotyledon (75.00%) and leaf (61.66%) explants. The variation in rooting response indicated that explant type and auxin combination differentially influenced adventitious root formation. The best hormonal concentrations (NAA + IBA) for root initiation in various explants were 0.6+1.8 mg/l for leaf, 0.25+1.2 mg/l for Hypocotyl, 0.6+1.2 mg/l for root and cotyledon explants, and 0.25+2.5 mg/l for callus. Root and callus tissues showed comparatively greater competence for root induction, whereas leaf explants exhibited a lower rooting response under the tested conditions. The study demonstrated that the rooting potential of soybean explants varied considerably with tissue type, highlighting the importance of explant selection and auxin composition for efficient *in vitro* rhizogenesis. The optimized conditions identified in the present study provide a useful foundation for establishing *in vitro* root cultures of soybean cv. MAUS-158 and may facilitate subsequent applications in root biology and hairy-root induction studies.

***Key words*:** Adventitious root induction, auxins, explants, *Glycine max*, *in vitro*, IBA, NAA, soybean.

Introduction

Soybean (*Glycine max* (L.) Merr.) is an important leguminous crop cultivated worldwide for its high-quality protein and edible oil content, with major applications in food, feed, and industry (Hymowitz, 1970; Kereszt *et al*., 2007). The availability of its fully sequenced genome has further supported functional genomics, gene mapping and crop improvement research (Schmutz *et al*., 2010). Plant tissue culture provides an important platform for studying plant development and applying biotechnological approaches. Based on cellular totipotency, plant tissues can be cultured under controlled *in vitro* conditions to induce organogenesis and regeneration (Murashige and Skoog, 1962; Bhojwani and Razdan, 1996). However, soybean is relatively recalcitrant to *in vitro* regeneration, with morphogenic responses influenced by genotype, explant type, physiological status and culture conditions (Parrott *et al*., 1989; Bailey *et al*., 1993; Santarem *et al*., 1998). Thus, optimization of culture conditions for specific genotypes and explants is necessary for consistent regeneration responses.

Root induction is an important aspect of *in vitro* culture and provides a useful system for the studying root development and physiology (De Klerk *et al*., 1999).

Adventitious root formation is influenced by explant characteristics and plant growth regulators, particularly auxins such as indole-3-butyric acid (IBA) and á-naphthaleneacetic acid (NAA) (Hartmann *et al*., 2017; De Klerk *et al*., 1999; Ludwig-Müller, 2000). In soybean, rooting response varies with auxin type and concentration, and both IBA and NAA have been reported to influence adventitious root formation in hypocotyl explants (Liu *et al*., 1998).

The rooting capacity of explants differs according to their developmental stage, endogenous hormone levels and morphogenic competence (Hartmann *et al*., 2011; George *et al*., 2008). Therefore, comparative evaluation of leaf, hypocotyl, cotyledon, root and callus explants can identify tissues with greater competence for *in vitro* root formation. An efficient root induction system is valuable for root developmental studies and for establishing root cultures for physiological, biochemical and biotechnological applications (George *et al*., 2008; Ludwig-Müller, 2000). Although *in vitro* regeneration has been studied in soybean, explant-specific responses may vary with genotype and culture conditions (Parrott *et al*., 1989; Bailey *et al*., 1993). Hence, identifying suitable explant and auxin combinations is important for developing an efficient cultivars and explant specific rooting system.

In this context, soybean cv. MAUS-158 provides a relevant genotype for evaluating explant-specific rooting responses under controlled *in vitro* conditions. Therefore, the present study was undertaken to evaluate the *in vitro* root induction potential of leaf, hypocotyl, root, cotyledon and callus explants of soybean (*Glycine max* (L.) Merr.) cv. MAUS-158 under different combinations of NAA and IBA, with the aim of identifying the explant exhibiting superior rooting response.

Materials and Methods

Plant material

Soybean (*Glycine max* (L.) Merr.) cv. MAUS-158 was used as the plant material for the *in vitro* root induction study. Seeds were obtained from the Soybean Research Station, VNMKV, Parbhani. The seeds were thoroughly washed under running tap water and surface sterilized under aseptic conditions by treatment with 70% (v/v) ethanol for 30 seconds, followed by three rinses with sterile distilled water. The seeds were subsequently treated with 0.1% (w/v) Bavistin for 5 minutes and rinsed three times with sterile distilled water, followed by surface sterilization with 0.1% (w/v) mercuric chloride ($HgCl_2$) for 3 minutes and three final rinses with sterile distilled water. The sterilized seeds were inoculated on half-strength Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with 1.5% (w/v) sucrose and solidified with 0.8% agar. The medium pH was adjusted to 5.8 before autoclaving. Cultures were maintained at 25 ± 1°C under a 16 h light/8 h dark photoperiod. Different explants, including leaf, hypocotyl, cotyledon, root and callus, were excised from healthy *in vitro*-grown seedlings and used for evaluating their root induction potential under different NAA and IBA combinations.

Preparation of Explants for Root Induction

Healthy *in vitro*-grown soybean seedlings were used as the source of explants for the root induction study. Different explants, including leaf, hypocotyl, cotyledon and root tissues, were aseptically excised from the *in vitro*-grown seedlings at the appropriate developmental stage. The explants were cut into small, uniform pieces using sterile instruments to facilitate proper placement and contact with the culture medium. Leaf-derived callus was also used as an explant and was cut into small pieces before inoculation. The prepared leaf, hypocotyl, cotyledon, root, and callus explants were transferred to the respective root-induction media containing different combinations of NAA and IBA and evaluated for their rooting response under controlled *in vitro* conditions.

Plant Growth Regulators

The plant growth regulators á-naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA) were used for root induction. The required quantities of each regulator were accurately weighed and dissolved in a small volume of suitable solvent, then adjusted to the final volume with sterile distilled water. Stock solutions were prepared at a concentration of 10 mg/ml and filter-sterilized using membrane filters under aseptic conditions. The prepared stock solutions were stored at 4°C and used for preparing the required concentrations of NAA and IBA in the root induction media.

Procedure for Preparation of MS Culture Medium

MS culture medium was prepared under aseptic conditions in a laminar airflow cabinet fitted with a HEPA filter (0.22 µm). The working surface and hands were disinfected with 70% (v/v) ethanol, and glassware and instruments were exposed to UV light for approximately 20 minutes before use. For preparation of 1l MS basal medium, 50 ml of Stock A (20X) and 5 ml each of Stocks B, C and D (200X) were added to distilled water and mixed thoroughly. Sucrose (3%, w/v) was added and dissolved, and the volume was initially made up to approximately 900 ml. The pH was adjusted to 5.8 ± 0.2 using 0.1 N NaOH or 0.1 N HCl, followed by adjustment of the final volume to 1 l.

Agar-agar (0.8%, w/v) was added as a solidifying agent and dissolved by gentle heating. The medium was sterilized by autoclaving at 121°C and 15 psi for 20 minutes. After cooling, the required concentrations of NAA and IBA were added aseptically from their respective stock solutions. Approximately 25 ml of medium was dispensed into each culture bottle, which was capped, labeled and sealed with parafilm. The prepared media were stored at 25°C until further use.

Surface Sterilization and Inoculation of Seeds

All aseptic operations were performed in a laminar airflow cabinet. Soybean seeds were soaked in water for 10 minutes and decoted, then rinsed with sterile distilled water. The seeds were surface sterilized with 0.1% carbendazim for 5 minutes, 0.1% $HgCl_2$ for 3 minutes, and 70% ethanol for 90 seconds, with sterile water washes after each treatment (Abbasi *et al*., 2016). Finally, the seeds were rinsed three times with sterile distilled water and dried on sterile filter paper. The sterilized seeds were inoculated onto half-strength MS medium containing 3% sucrose and 0.8% agar. Cultures were incubated at 25±2°C under a 16 h light/8 h dark photoperiod. Seedlings obtained after 7-14 days were used as explant sources (Abbasi *et al*., 2016; Murashige & Skoog, 1962).

Preparation of Callus Explant:

After 10-14 days of germination, leaf explants were excised aseptically from *in vitro* grown seedlings. The leaf tissues were fragmented into small pieces (approximately 1 cm) using a sterilized scalpel and forceps. The fragmented leaf tissues were inoculated on cultured medium supplemented with an appropriate concentration of 2,4-dichlorophenoxyacetic acid (2,4-D). The cultures were maintained under controlled conditions, and callus formation was observed within two weeks of incubation (Abbasi *et al*., 2016).

Root Induction from Various Explants:

Root Induction from Leaf Explant:

Leaf explants obtained from *in vitro*-grown seedlings of *Glycine max* (L.) were excised under aseptic conditions and cut into small segments by removing the midrib and sides of the leaf using a sterile scalpel and forceps. The explants were carefully inoculated on MS basal medium with the abaxial surface in contact with the medium supplemented with appropriate concentrations of auxins, such as indole-3-butyric acid (IBA) and α-naphthaleneacetic acid (NAA), to induce roots (Baque *et al*., 2010; Zhang *et al*., 2020). The culture medium was fortified with 3% (w/v) sucrose as a carbon source, and the pH was adjusted to 5.8 ± 0.2 using 0.1 N NaOH or 0.1 N HCl prior to sterilization. Agar (0.8% w/v) was added as a solidifying agent, and the medium was sterilized by autoclaving at 121°C and 15 psi for 20 minutes (George *et al*., 2008). After inoculation, the cultures were incubated at 25 ± 2°C under controlled environmental conditions with an appropriate photoperiod. The explants were regularly monitored for contamination and response, and root initiation was generally observed within 1-3 weeks depending on the concentration of growth regulators and physiological condition of the explants (Bhojwani & Razdan, 2004). The induced roots were allowed to grow further before being used for subsequent experimental studies.

Table 1: Plant Growth Hormones used for callus induction and root induction treatments.

Sr. No.	Growth Regulator	Stock Concentration
1.	2,4 - D	**10 mg/ml**
2.	Indole-3-butyric acid (IBA)	
3.	Naphthaleneacetic acid (NAA)	

Root Induction from Hypocotyl Explant:

Hypocotyl explants were obtained from *in vitro*-grown seedlings under aseptic conditions. The hypocotyls were carefully excised and cut into small segments using a sterile scalpel and forceps. The cut surfaces were made slightly slanted to increase the surface area for better interaction with the culture medium. The explants were inoculated on MS basal medium supplemented with appropriate concentrations of auxins, such as indole-3-butyric acid (IBA) and α-naphthaleneacetic acid (NAA), to induce root formation (Chou *et al*., 2010). After inoculation, the culture bottles were capped, sealed and incubated at 25 ± 2°C under controlled environmental conditions with an appropriate photoperiod. The explants were regularly monitored to check for contamination and response to root initiation, which was generally observed within 1-3 weeks depending on the concentration of growth regulators and the physiological condition of the explants. The induced roots were allowed to grow on culture medium.

Root Induction from Cotyledon Explant:

Both mature and immature cotyledons were used as explants for root induction studies. Mature cotyledons were excised from *in vitro*-grown seedlings under aseptic conditions. A small wound was created at the proximal end, i.e., the point of attachment to the hypocotyl, to enhance root induction by stimulating cellular activity. Immature cotyledon explants were obtained from immature soybean pods. The seeds were surface sterilized, and the seed coat was carefully removed under

Table 2: *In vitro* root induction efficiency of Leaf explants in response to various growth regulators (IBA+NAA).

Treat-ment No.	Medium MS+NAA (mg/l) +IBA (mg/l)	No. of explants (20 per replication)	No. of Root Induced Explants			Total No. of Root Induced Explants	Rate of Root Induction (%)	Root Induced Explants Mean ± S.E.	Root Length (Cm)	Callus Response
			R1	R2	R3					
T1	0.25 + 0.3	60	12	14	11	37	61.66	12.333±0.882	12.67 ± 1.15^{b}	Low
T2	0.25 + 0.6	60	15	10	08	33	55.00	11.00±2.082	12.33 ± 0.58^{b}	Low
T3	0.25 + 1.2	60	13	11	13	37	61.66	12.333±0.667	10.67 ± 2.08^{c}	Low
T4	0.6 + 1.2	60	09	13	12	34	56.66	11.333±1.202	12.67 ± 2.52^{b}	Low
T5	**0.6 + 1.8**	**60**	**12**	**14**	**15**	**41**	**68.33**	**13.667±0.882**	**17.00 ± 1.00^{a}**	**Low**
T6	0.6 + 2.5	60	10	08	14	32	53.33	10.667±1.764	9.00 ± 1.00^{c}	Low
T7	0.6 + 0.6	60	14	11	12	37	61.66	12.333±0.882	13.33 ± 1.53^{b}	Low
IBA (mg/l)										
T8	0.6	60	14	12	12	38	63.33	12.667±0.667	10.33 ± 1.53^{c}	Low
T9	1.8	60	12	10	14	36	60.00	12.000±1.155	7.67 ± 0.58^{d}	Low
T10	**2.5**	**60**	**15**	**14**	**11**	**40**	**66.67**	**13.333±1.202**	**12.00 ± 1.00^{b}**	**Low**
T11	0.0 (Control)	60	00	00	00	00	00.00	0.000±0.000	0.000±0.000	-
							C.D.(5%)	**3.4247**		
							C.D.(1%)	**4.6061**		
							SE(m)	**1.1634**		
							SE(d)	**1.6453**		
							C.V. (%)	**18.22**		

Note: Treatment means were separated by DMRT at $p \leq 0.05$ and means followed with same letters are not significantly different by DMRT.

aseptic conditions. The cotyledons were then separated, and the embryo was removed. The isolated cotyledons were inoculated onto MS medium for further *in vitro* culture and evaluation of root induction (Lee *et al.*, 2021).

Root Induction from Root Explant:

Roots obtained from *in vitro*-grown soybean seedlings were used as explants to evaluate their root induction potential. The roots were carefully removed from the culture medium and excised from the stem. They were then gently washed with distilled water to remove any adhering medium and cut into small segments. These root segments were inoculated onto the rooting medium using sterile forceps. All procedures were carried out under aseptic conditions inside a laminar airflow cabinet to prevent contamination. The inoculated culture bottles were capped, sealed and incubated at 25 ± 2°C under controlled environmental conditions with an appropriate photoperiod. The explants were observed regularly to assess contamination and their response to root initiation, which was typically noted within 1-3 weeks, depending on the concentration of growth regulators and the physiological condition of the explants. The newly formed roots were then allowed to continue growing on the culture medium (Sujatha & Ranjitha Kumari, 2012).

Root Induction from Callus Explant:

Callus derived from soybean leaf explants was used as the source material for root induction. The callus was carefully removed from the culture bottles under aseptic conditions, and any adhering MS medium was gently removed. The cleaned callus tissue was then inoculated onto rooting medium supplemented with appropriate concentrations of auxins, such as indole-3-butyric acid (IBA) and α-naphthaleneacetic acid (NAA), to induce root formation (Sukweenadhi *et al.*, 2019). The culture bottles were carefully capped and incubated in the culture room under controlled conditions to facilitate root induction from the callus. The culture bottles were observed regularly to assess the callus response to root induction.

Results and Discussion

Effect of Explant Type on Root Induction in Soybean

Root induction was successfully achieved from leaf, hypocotyl, cotyledon, root, and callus explants of soybean (*Glycine max* L.) cv. MAUS-158 using MS medium supplemented with different concentrations of IBA and NAA. Root initiation was observed within 10–18 days of inoculation, although callus and cotyledon explants generally required a longer period of 14-21 days. Among the tested explants, root and callus showed the highest overall root induction response (83.33%), followed by hypocotyl (76.66%), cotyledon (75.00%), and leaf (61.66%) (Table 4). The variation in rooting response may be attributed to differences in the physiological condition, endogenous auxin levels, and regenerative capacity of the explants. Similar differences in root

Table 3: *In vitro* root induction efficiency of Hypocotyl explants in response to various growth regulators (IBA+NAA).

Treat-ment No.	Medium MS+NAA (mg/l) +IBA (mg/l)	No. of explants (20 per replication)	No. of Root Induced Explants			Total No. of Root Induced Explants	Rate of Root Induction (%)	Root Induced Explants Mean ± S.E.	Root Length (Cm)	Callus Response
			R1	R2	R3					
T1	0.25 + 0.3	60	15	14	16	45	75.00	15.000±0.577^{a}	11.00 ± 1.00^{b}	Moderate
T2	0.25 + 0.6	60	16	17	16	49	81.67	16.333±0.333^{a}	15.67 ± 0.58^{b}	Moderate
T3	**0.25 + 1.2**	**60**	**17**	**18**	**15**	**50**	**83.33**	**16.667±0.882^{a}**	**18.33 ± 1.15^{a}**	**Moderate**
T4	0.6 + 1.2	60	14	17	15	46	76.67	15.333±0.882^{a}	19.00 ± 1.00^{a}	Moderate
T5	0.6 + 1.8	60	16	14	15	45	75.00	15.000±0.577^{a}	15.33 ± 0.58^{b}	Moderate
T6	0.6 + 2.5	60	17	13	18	48	80.00	16.000±1.528^{a}	14.33 ± 1.53^{c}	Moderate
T7	0.6 + 0.6	60	18	16	15	49	81.67	16.333±0.882^{a}	18.67 ± 1.53^{a}	Moderate
IBA (mg/l)										
T8	0.6	60	16	12	13	41	68.33	13.667±1.202^{b}	6.33 ± 1.15^{d}	Moderate
T9	**1.8**	**60**	**18**	**15**	**14**	**47**	**78.33**	**15.667±1.202^{a}**	**11.33 ± 1.15^{b}**	**Moderate**
T10	2.5	60	17	14	15	46	76.67	15.333±0.882^{a}	**9.33 ± 1.53^{c}**	Moderate
T11	0.0 (Control)	60	00	00	00	00	00.00	0.000±0.000^{c}	0.000±0.000^{c}	-
							C.D.(5%)	**2.679**		
							C.D.(1%)	**3.6032**		
							SE(m)	**0.9101**		
							SE(d)	**1.2871**		
							C.V. (%)	**11.16**		

Note: Treatment means were separated by DMRT at $p \leq 0.05$ and means followed with same letters are not significantly different by DMRT.

induction potential among explant types have been reported in soybean and other leguminous crops (Baque *et al.*, 2010; Chou *et al.*, 2010; Sujatha & Ranjitha Kumari, 2012).

Effect of Auxin Concentration on Root Induction

The concentration and combination of IBA and NAA markedly influenced root induction in different soybean explants. The response varied with explant type, indicating that an appropriate auxin balance was essential for efficient root formation. Root explants showed a high rooting response even at lower auxin concentrations, whereas callus and cotyledon explants generally required longer incubation for root initiation. In the present study, higher rooting responses were obtained with suitable combinations of IBA and NAA, demonstrating the synergistic role of these auxins in adventitious root formation. Auxins are known to stimulate cell division, cell differentiation, and root primordium development, although excessive concentrations may inhibit root elongation or promote callus formation. Similar auxin-dependent responses have been reported during *in vitro* root induction in soybean and other plant species (Baque *et al.*, 2010; Zhang *et al.*, 2020; Sukweenadhi *et al.*, 2019).

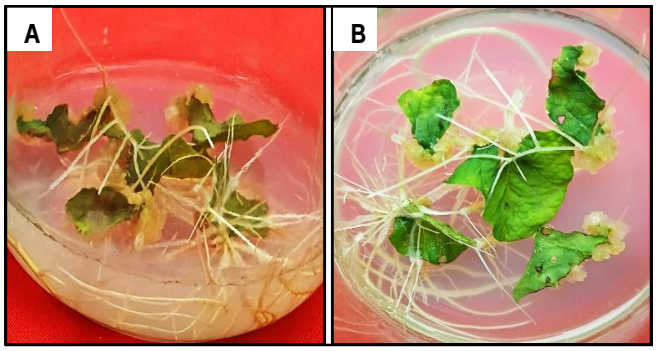


Fig. 1. Root induction in soybean leaf explants under optimum growth regulator treatments: (A) T5 - 0.6 mg L^{-1} NAA + 1.8 mg L^{-1} IBA; (B) T10 - 2.5 mg L^{-1} IBA.

Effect of Growth Regulator on Root Induction from Leaf Explants

The root induction response of *in vitro*-grown soybean leaf explants was evaluated using IBA alone and IBA in combination with NAA (Table 2; Fig. 1). Root initiation was first observed after approximately 15 days of culture, followed by progressive root elongation. Leaf explants showed comparatively delayed root initiation than the other explants evaluated.

Among the treatments, T5 (0.6 mg L^{-1} NAA + 1.8 mg L^{-1} IBA) recorded the highest root induction (68.33%), with a mean of 13.667 ± 0.882 rooted explants and a maximum root length of 18 cm. This was followed by T10 (2.5 mg L^{-1} IBA) with 66.67% and T8 (0.6 mg L^{-1} IBA) with 63.33% root induction. T1 (0.25 mg L^{-1} NAA + 0.3 mg L^{-1} IBA), T3 (0.25 mg L^{-1} NAA + 1.2 mg L^{-1}

Table 4: *In vitro* root induction efficiency of Root explants in response to various growth regulators (IBA+NAA).

Treat-ment No.	Medium MS+NAA (mg/l) +IBA (mg/l)	No. of explants (20 per replication)	No. of Root Induced Explants			Total No. of Root Induced Explants	Rate of Root Induction (%)	Root Induced Explants Mean ± S.E.	Root Length (Cm)	Callus Response
			R1	R2	R3					
T1	0.25 + 0.3	60	19	17	16	52	86.67	17.333±0.882[a]	20.67 ± 1.53[a]	Moderate
T2	0.25 + 0.6	60	16	17	16	49	81.66	16.333±0.333[a]	16.33 ± 1.53[b]	High
T3	0.25 + 1.2	60	17	18	16	51	85.00	17.000±0.577[a]	13.67 ± 1.53[c]	Moderate
T4	**0.6 + 1.2**	**60**	**18**	**17**	**19**	**54**	**90.00**	**18.000±0.577 [a]**	**22.33 ± 1.53 [a]**	**Low**
T5	0.6 + 1.8	60	16	18	16	50	83.33	16.667±0.667[a]	19.33 ± 1.53[a]	Moderate
T6	0.6 + 2.5	60	18	15	13	46	76.67	15.333±1.453[b]	17.00 ± 2.00[b]	Moderate
T7	0.6 + 0.6	60	19	17	14	50	83.33	16.667±1.453[a]	23.00 ± 2.00[a]	Moderate
IBA (mg/l)										
T8	0.6	60	16	14	17	47	78.33	15.667±0.882[a]	13.67 ± 1.53[c]	Moderate
T9	1.8	60	18	15	14	47	78.33	15.667±1.202[a]	13.00 ± 2.00[c]	High
T10	**2.5**	**60**	**17**	**17.**	**15**	**49**	**81.66**	**16.333±0.667 [a]**	**20.67 ± 1.53 [a]**	**Moderate**
T11	0.0 (Control)	60	00	00	00	00	00.00	0.000±0.000[c]	0.000±0.000[c]	-
							C.D.(5%)	**2.6461**		
							C.D.(1%)	**3.559**		
							SE(m)	**0.8989**		
							SE(d)	**1.2713**		
							C.V. (%)	**10.38**		

Note: Treatment means were separated by DMRT at $p \leq 0.05$ and means followed with same letters are not significantly different by DMRT.

IBA), and T7 (0.6 mg L^{-1} NAA + 0.6 mg L^{-1} IBA) each recorded 61.66%. T9 (1.8 mg L^{-1} IBA), T4 (0.6 mg L^{-1} NAA + 1.2 mg L^{-1} IBA), and T2 (0.25 mg L^{-1} NAA + 0.6 mg L^{-1} IBA) recorded 60.00%, 56.66%, and 55.00%, respectively. The lowest response among auxin treatments was observed in T6 (0.6 mg L^{-1} NAA + 2.5 mg L^{-1} IBA), with 53.33% root induction (Table 2).

The root induction was not observed in the control T11 (MS medium without plant growth regulators), confirming the importance of auxins for root induction. All auxin-supplemented treatments induced rooting with low callus formation. Overall, T5 (0.6 mg L^{-1} NAA + 1.8 mg L^{-1} IBA) was the most effective treatment, producing both the highest rooting percentage and maximum root length.

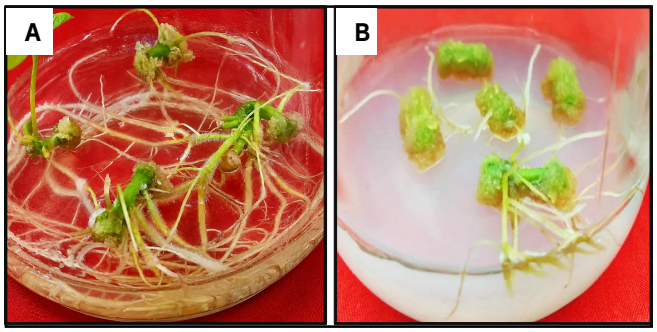


Fig. 2: Root induction in soybean Hypocotyl explants under optimum growth regulator treatments: (A) T3 - 0.25 mg L^{-1} NAA + 1.2 mg L^{-1} IBA; (B) T9 – 1.8 mg L^{-1} IBA.

The present findings are in agreement with Baque *et al.*, (2010), who reported that 1.0 mg L^{-1} IBA was effective for adventitious root induction from leaf explants of *Morinda citrifolia*. They also observed that increasing IBA and NAA concentrations promoted callus formation, while red light enhanced adventitious root production, indicating the importance of auxin concentration and light quality in root induction.

Effect of Growth Regulator on Root Induction from Hypocotyl Explants

The rooting response of soybean hypocotyl explants was evaluated using different concentrations and combinations of IBA and NAA (Table 3). Root initiation was observed after approximately 12 days, followed by gradual root elongation. Hypocotyls showed a comparatively higher rooting response than leaf explants (Fig. 2).

The treatment T3 (0.25 mg L^{-1} NAA + 1.2 mg L^{-1} IBA) recorded the highest rooting (83.33%), with 50 rooted explants, a mean of 16.667 ± 0.882, and a maximum root length of 19 cm. The T2 and T7 each recorded 81.67%, followed by T6 (80.00%) and T9 (78.33%). Treatment T4 and T10 recorded 76.67%, while T1 and T5 recorded 75.00%. The lowest response was observed in T8 (0.6 mg L^{-1} IBA), with 68.33% rooting, a mean of 13.667 ± 1.202, and a maximum root length of 5 cm (Table 4; Fig. 2). The roots were not observed in the PGR-free

Table 5: *In vitro* root induction efficiency of Cotyledon explants in response to various growth regulators (IBA+NAA).

Treat-ment No.	Medium MS+NAA (mg/l) +IBA (mg/l)	No. of explants (20 per replication)	No. of Root Induced Explants			Total No. of Root Induced Explants	Rate of Root Induction (%)	Root Induced Explants Mean ± S.E.	Root Length (Cm)	Callus Response
			R1	R2	R3					
T1	0.25 + 0.3	60	10	11	09	30	50.00	10.000±0.577[c]	12.67 ± 1.53	Moderate
T2	0.25 + 0.6	60	09	08	10	27	45.00	9.000±0.577[c]	10.00 ± 1.00	Moderate
T3	0.25 + 1.2	60	17	18	16	51	85.00	17.000±0.577[a]	15.67 ± 0.58	Low
T4	0.6 + 1.2	60	18	17	19	54	90.00	18.000±0.577[a]	13.33 ± 0.58	Moderate
T5	0.6 + 1.8	60	16	18	16	50	83.33	16.667±0.667[a]	12.33 ± 1.53	Moderate
T6	0.6 + 2.5	60	18	15	13	46	76.67	15.333±1.453[a]	11.00 ± 1.00	Low
T7	0.6 + 0.6	60	19	17	14	50	83.33	16.667±1.453[a]	20.00 ± 1.00	Moderate
IBA (mg/l)										
T8	0.6	60	16	14	17	47	78.33	15.667±0.882[a]	16.33 ± 1.15	Moderate
T9	1.8	60	18	15	14	47	78.33	15.667±1.202[a]	15.33 ± 2.08	Low
T10	2.5	60	17	17	15	49	81.67	16.333±0.667[a]	12.67 ± 1.53	Moderate
T11	0.0 (Control)	60	00	00	00	00	00.00	0.000±0.000[d]	0.000±0.000	-
							C.D.(5%)	**2.6129**		
							C.D.(1%)	**3.5142**		
							SE(m)	**0.8876**		
							SE(d)	**1.2553**		
							C.V. (%)	**11.25**		

Note: Treatment means were separated by DMRT at $p \leq 0.05$ and means followed with same letters are not significantly different by DMRT.

control (T11), whereas all auxin treatments induced rooting, confirming the importance of exogenous auxins. Moderate callus formation was observed along with rooting. Overall, T3 (0.25 mg L^{-1} NAA + 1.2 mg L^{-1} IBA) was identified as the optimum treatment, producing the highest rooting percentage and maximum root length.

The results of the present investigation are supported by the findings of Chou *et al.*, (2010), who reported that auxins such as IAA, IBA, and NAA effectively promoted adventitious root formation in soybean hypocotyls. They concluded that auxin-regulated peroxidase activity plays an important role in root induction and development.

Effect of Growth Regulator on Root Induction from Root Explants

Root explants were cultured on MS medium supplemented with different concentrations and combinations of IBA and NAA (Table 4; Fig. 3). Root initiation occurred rapidly, approximately 10 days after inoculation, followed by progressive elongation, indicating high responsiveness of root explants.

The treatment T4 (0.6 mg L^{-1} NAA + 1.2 mg L^{-1} IBA) showed the highest response, with 90.00% root induction, 54 rooted explants, a mean of 18.00 ± 0.577, and a maximum root length of 22 cm, with low callus formation. The treatment T1 (0.25 + 0.3 mg L^{-1}) recorded 86.67%, T3 (0.25 + 1.2 mg L^{-1}) 85.00%, T5 and T7 83.33% each, T2 and T10 81.66% each, and T8 and T9 78.33% each. The lowest response was observed in T6 (0.6 mg L^{-1} NAA + 2.5 mg L^{-1} IBA), with 76.67% rooting and a mean of 15.333 ± 1.453. Moderate callusing occurred in most treatments, with higher callus formation in T2 and T9. In control MS medium alone (T11), no any root induction was observed confirming the requirement of exogenous auxins for root induction. Thus, T4 was identified as the optimum treatment due to its highest rooting percentage, maximum root length, and low callus formation. The findings agree with Ho *et al.*, (2019), who reported that adventitious root development from leaf and root explants of *Polygonum multiflorum* varied with explant type and genotype.

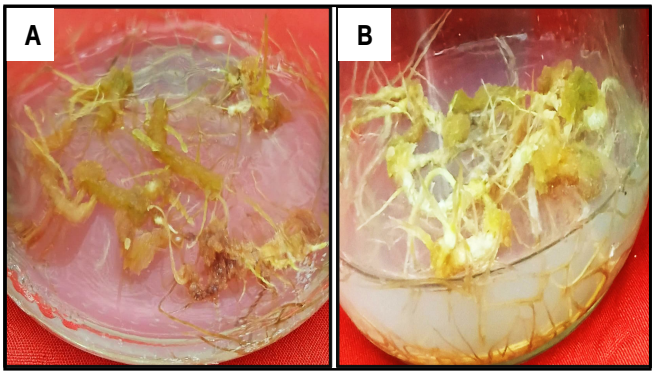


Fig. 3: Root induction in soybean Root explants under optimum growth regulator treatments: (A) T4 - 0.6 mg L^{-1} NAA + 1.2 mg L^{-1} IBA; (B) T10 - 2.5 mg L^{-1} IBA.

Table 6: *In vitro* root induction efficiency of Callus explants in response to various growth regulators (IBA+NAA).

Treat-ment No.	Medium MS+NAA (mg/l) +IBA (mg/l)	No. of explants (20 per replication)	No. of Root Induced Explants			Total No. of Root Induced Explants	Rate of Root Induction (%)	Root Induced Explants Mean ± S.E.	Root Length (Cm)	Callus Response
			R1	R2	R3					
T1	0.25 + 0.6	30	08	07	09	24	80.00	8.000±0.577[a]	26.00 ± 1.00	High
T2	0.25 + 2.5	30	09	08	10	27	90.00	9.000±0.577[a]	31.00 ± 1.73	High
T3	0.25 + 1.2	30	09	07	07	23	76.67	7.667±0.667[a]	17.67 ± 1.53	High
T4	0.6 + 1.2	30	10	07	09	26	86.67	8.667±0.882[a]	31.67 ± 0.58	High
T5	0.6 + 1.8	30	07	09	10	26	86.67	8.667±0.882[a]	14.33 ± 1.53	High
							C.D.(5%)	**2.0579**		
							C.D.(1%)	**2.8803**		
							SE(m)	**0.6667**		
							SE(d)	**0.9428**		
							C.V. (%)	**16.5**		

Note: Treatment means were separated by DMRT at $p \leq 0.05$ and means followed with same letters are not significantly different by DMRT.

Effect of Growth Regulator on Root Induction from Cotyledon Explants

Cotyledon explants were cultured on MS medium containing different IBA and NAA treatments (Table 5; Fig. 4). Root initiation occurred after approximately 18 days. The treatment T4 (0.6 mg L^{-1} NAA + 1.2 mg L^{-1} IBA) showed the highest rooting (90.00%), with 54 rooted explants, a mean of 18.00 ± 0.577, and 13 cm root length. The treatment T3 (0.25 + 1.2 mg L^{-1}) recorded 85.00% rooting with 51 rooted explants and a mean of 17.000 ± 0.577. T5 (0.6 + 1.8 mg L^{-1}) and T7 (0.6 + 0.6 mg L^{-1}) each recorded 83.33%, with T7 producing 19 cm roots. The treatment T10 (2.5 mg L^{-1} IBA) recorded 81.67%, T8 (0.6 mg L^{-1} IBA) and T9 (1.8 mg L^{-1} IBA) each 78.33%, and T6 (0.6 + 2.5 mg L^{-1}) 76.67%. The treatment T1 (0.25 + 0.3 mg L^{-1}) and T2 (0.25 + 0.6 mg L^{-1}) showed lower rooting of 50.00% and 45.00%, respectively. Low callusing occurred in T3, T6, and T9, while moderate callusing occurred in the remaining treatments. The T4 was best for rooting percentage, whereas T7 was best for root length.

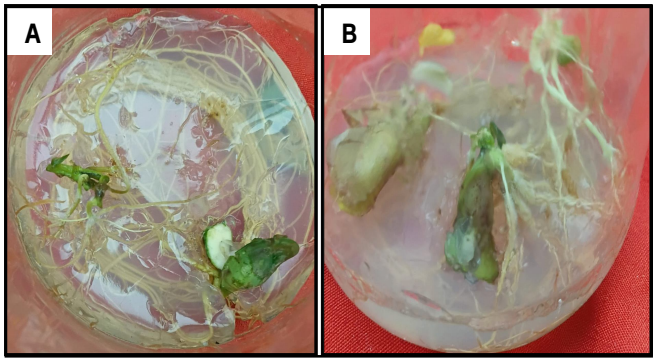


Fig. 4: Root induction in soybean Cotyledon explants under optimum growth regulator treatments: (A) T4 - 0.6 mg L^{-1} NAA + 1.2 mg L^{-1} IBA; (B) T10 - 2.5 mg L^{-1} IBA.

The findings are supported by Lee *et al.*, (2021), who established an *in vitro* adventitious root culture system from cotyledon explants of *Clitoria ternatea*. They reported the highest root induction (95.24%) with 2.50 mg L^{-1} NAA, while 1.50 mg L^{-1} NAA produced the maximum number of roots and 2.50 mg L^{-1} 4-Cl-IAA resulted in the longest roots. Their study also demonstrated the potential of *in vitro* roots for producing bioactive compounds, emphasizing the importance of auxin optimization for efficient root induction and root culture.

Effect of growth regulator on root induction from Callus explants

Callus explants were cultured on MS medium supplemented with different IBA and NAA treatments (Table 6; Fig. 5). Root initiation was observed after approximately 10 days, followed by gradual root elongation. The treatment T2 (0.25 mg L^{-1} NAA + 2.5 mg L^{-1} IBA) recorded the highest rooting (90.00%), with 27 rooted calli, a mean of 9.00 ± 0.577, and 30 cm root length. The treatment T4 (0.6 mg L^{-1} NAA + 1.2 mg L^{-1} IBA) and T5 (0.6 mg L^{-1} NAA + 1.8 mg L^{-1} IBA) each recorded 86.67% rooting, with a mean of 8.667 ± 0.882;

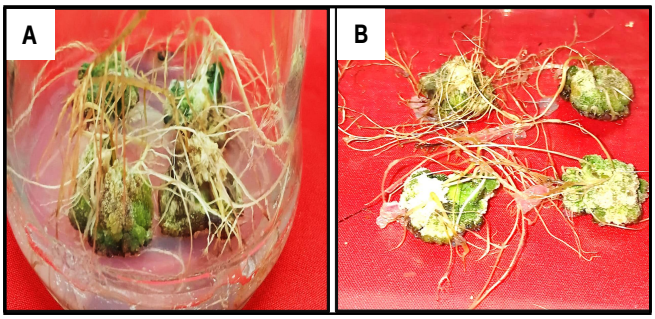


Fig. 5: Root induction in soybean Callus explants under optimum growth regulator treatments: (A) T2 - 0.6 mg L^{-1} NAA + 1.2 mg L^{-1} IBA; (B) T4 - 0.6 + 1.2

T4 produced the longest roots (32 cm). The treatment T1 (0.25 mg L^{-1} NAA + 0.6 mg L^{-1} IBA) recorded 80.00% rooting, with a mean of 8.00 ± 0.577 and 25 cm roots, whereas T3 (0.25 mg L^{-1} NAA + 1.2 mg L^{-1} IBA) showed the lowest response (76.67%), with a mean of 7.667 ± 0.667 and 16 cm roots.

High callus formation was observed in all auxin treatments, while no root formation occurred in the PGR-free control (T6), confirming the importance of exogenous auxins. Thus, T2 was the most effective treatment for rooting percentage, whereas T4 was superior for root elongation.

The findings are supported by Fazal *et al*., (2014), who reported that NAA concentration influenced adventitious root growth and biomass accumulation from leaf-derived callus of *Prunella vulgaris*. They found that 1.0 mg L^{-1} NAA promoted maximum biomass, while 0.5 mg L^{-1} NAA enhanced phenolic and flavonoid production, demonstrating the role of auxin concentration in regulating root growth and secondary metabolite production.

Conclusion

The present investigation demonstrated that both explant type and auxin concentration significantly influenced *in vitro* root induction in soybean (*Glycine max* L.) cv. MAUS-158. Root induction was successfully achieved from all explants tested, namely leaf, hypocotyl, root, cotyledon, and callus, on MS medium supplemented with IBA and NAA. Among the explants, root and callus exhibited the highest overall rooting response, followed by hypocotyl, cotyledon, and leaf explants. The combination of IBA and NAA was generally more effective than IBA alone, indicating a synergistic effect of auxins on root formation. The optimum treatments varied among explants: T5 (0.6 mg L^{-1} NAA + 1.8 mg L^{-1} IBA) for leaf, T3 (0.25 mg L^{-1} NAA + 1.2 mg L^{-1} IBA) for hypocotyl, T4 (0.6 mg L^{-1} NAA + 1.2 mg L^{-1} IBA) for root and cotyledon, and T2 (0.25 mg L^{-1} NAA + 2.5 mg L^{-1} IBA) for callus explants. The exogenous hormones are essential for root induction and without hormone no root induction was observed. Overall, root and callus explants proved to be the most suitable explant sources for efficient in vitro root culture establishment in soybean.

Acknowledgements

The authors gratefully acknowledge the Vilasrao Deshmukh College of Agricultural Biotechnology, Latur, and the CM Fund sponsored laboratory, Department of Plant Biotechnology for providing the essential laboratory facilities and institutional support required to conduct this study. The authors also extend their sincere thanks to the faculty members and technical staff of the Department of Plant Biotechnology for their valuable assistance and support throughout the experimental work and data analysis.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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