

AN EFFICIENT AND REPRODUCIBLE TISSUE CULTURE PROCEDURE FOR CALLUS INDUCTION AND MULTIPLE SHOOTS REGENERATION IN GROUNDNUT (*Arachis hypogaea* L.)

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ABSTRACT

This study was carried out to optimize *in vitro* regeneration system of groundnut through tissue culture technology that can provide good environment in terms of food security. The two varieties of groundnut (Golden and BARI 2001) were used and an effective *in vitro* regeneration system was established for selecting the best responsive variety to tissue culture using embryo as an explant sources. MS media having 5.5 mg/l BAP and 1.5 mg/l NAA was found to be best by producing the highest callus induction frequencies (86 & 78%) in Golden and BARI 2001, respectively. Similarly, the maximum multiple shoots/plant (9 & 6) with optimum length of shoots (4.8 & 4.1 cm) was obtained with the application of 4 mg/l BAP along with 1 mg/l NAA and 1.1 mg/l TDZ in Golden and BARI 2001, respectively. In case of roots initiation, the highest root frequency (90 & 76%) having optimum roots/shoots (8.3 & 6.2) and substantial root length (8.1 & 7.0 cm) was obtained in Golden followed by BARI 2001 variety on MS media having 1 mg/l phytohormone IBA. Results showed that callus and multiple shoot induction and *in vitro* rooting, varied with different hormonal combinations in the media and the genotype that might be due to genetic variations between the varieties. This study delivers a base line for efficient *in vitro* propagation and genetic transformation in elite groundnut genotypes for desirable characteristics.

Key words: Callus induction, Embryo slices, Genetic transformation, Multiple shoots, Plant growth regulators

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INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is the most dominant oilseed crop and is ranked 3rd among oil producing crops in the world (Upadhyaya *et al.*, 2003). Groundnut is mainly cultivated throughout the world with specific regions like tropical, sub-tropical, and temperate areas (Cuc *et al.*, 2008). Plant breeding of groundnut is very difficult, time consuming and requires highly skilled labors to perform emasculation, crossing and selection due to their self-pollination and narrow genetic base behaviors (Pasupuleti *et al.*, 2013; Moretzsohn *et al.*, 2005). Gene transformation limits all these hurdles and introduces important genes of good agronomic characteristics (Tiwari *et al.*, 2008; Lemaux, 2008). But an efficient regeneration method is prerequisite for genetic transformation approach. So, the establishment of an adaptive tissue culture system for groundnut would be very useful for improved production with good seed quality (Sharma and Anjaiah, 2000).

Successful plants regeneration system relies on many factors such as plants growth regulators, composition of media, genotype, explants, photoperiod,

temperature and the environment (Ishag *et al.*, 2009; Shah *et al.*, 2013; Shah *et al.*, 2014 a-b). BAP along with NAA promoted callus induction; while TDZ and BAP were reported for initiation of multiple shoot cultured explants (Hutchinson *et al.*, 1994). In the past groundnut *in vitro* regeneration was conducted by using the whole immature cotyledon (Robinson *et al.*, 2011, Palanivel *et al.*, 2002), cotyledonry nodes and mature embryos (Lacroix *et al.*, 2003), mature dry seeds (Baker *et al.*, 1995), leaflets (Chengalrayan *et al.*, 2001; Tiwari and Tuli, 2009), hypocotyls (Venkatachalam *et al.*, 1997), mature epicotyl (Shan *et al.*, 2009). However, it is difficult to obtain more planting material in short time due to the larger size of these explants.

In the past decades, tissue culture technique has long been applied to achieve somaclonal variation by plant breeders (Kaeppler *et al.*, 2000). Selection of plant species through somoclonal variations that are used to create genetic variability among crop plants and the struggles are being done to improve crop yield, oil contents, and cultivars development of *Arachis hypogaea* that have resistance against various diseases (Robinson *et al.*, 2011). The plant breeders have been struggling for a long time to improve groundnut yield, quality and to

develop resistant varieties for pest, drought, fungus, cold and salt but unfortunately, limited success has been achieved by conventional breeding (Banerjee *et al.*, 2007). While genetic transformation has become a popular tool for transferring desirable genes into crops without any barrier in less time as compared to conventional plant breeding (Taji *et al.*, 2002). But an efficient tissue culture protocol is pre-requisite for successful genetic transformation. Therefore, we planned this study to optimize an efficient and reproducible tissue culture procedure for callus induction, multiple shoot regeneration and *in vitro* root induction in groundnut.

MATERIALS AND METHODS

Plant material, disinfection and explants preparation:

The seeds of two groundnut cultivars namely Golden and BARI 2001 were taken from Barani Agricultural Research Institute (BARI), Chakwal, Pakistan. Seventy percent ethanol was used for surface sterilization of mature and healthy seeds. Surface sterilization was done for two minutes with 70% ethanol and then for fifteen minutes with 6% sodium hypochlorite (NaOCl) along with continuous shaking. Then their seeds were washed with double distilled water until the removal of traces of ethanol and NaOCl. These seeds were put in double distilled water for one and half hour to facilitate zygotic embryo excision. Embryos slice; as a source of explants was removed aseptically from their seeds by ripping out the seed coat. Through bilateral cutting of these seeds, very thoroughly radicle and plumule were excised and then their embryos were cut into several pieces.

Plant growth regulators and culture conditions: This research study was conducted at NIGAB, NARC, Islamabad. Murashige and Skoog (1962) basal media with vitamin at pH 5.8 fortified with 30 g/l sucrose and 3 g/l gum powder was used in all the cultures. Various levels of cytokinin (BAP, KIN) and auxins (NAA, TDZ, IAA) on MS medium were used to obtain callus and multiple shoots regeneration (Tables 1 and 2). *In vitro* root induction was achieved with the application of post autoclaved IBA in MS media.

Effect of growth regulators on callus and multiple shoot induction: MS basal media having various levels of BAP, IAA and NAA was used for callus induction (Table 1). Then explants were placed in contact with the media under tissue paper to protect them from high light intensity at 28 °C. The calli were induced and explants were expanded five times in their original size after ten days. Subsequently, the explants were sub cultured for more ten days on the same media for further regeneration. The regenerated calli continued to proliferate and then sub-culturing on multiple shoot induction media (MSM), having hormonal combinations of BAP, KIN and TDZ (Table 2) for 10-15 days under sixteen hours'

photoperiod having fluorescence light intensity of 50 $\mu\text{molm}^{-2}\text{s}^{-1}$ and 65-70% relative humidity at 25 ± 2 °C (Shah *et al.*, 2015; Shah *et al.*, 2020).

In vitro rooting and plantlet acclimatization:

Individual shoots (2-5 cm in length) were aseptically cut from multiple shoots and were put on growth regulator free MS fortified by IBA for 2-3 weeks and repeated three times (Table 3). Data was recorded after four weeks for rooted plants, No. of roots/explant and root length. Then these were transferred to hydroponics condition in the glass house containing Yoshida solution (Yoshida *et al.*, 1976) for further development of roots for 10 days. For acclimatization maintenance of plantlets in a combination of manure and sand (1:3) at a constant temperature of 30 °C in a glass house for fourteen days after profuse root elongation (about 3 cm) with secondary roots.

Statistical analysis: The whole experimental trials were arranged using completely randomized design. The significant difference was noticed by using ANOVA technique at $P \leq 0.05$ and Duncan's multiple range test was applied to check the significant differences among means. For this purpose, statistics software namely The Statistix v. 8.1 was used (Analytical Software, 2005).

RESULTS

Morphogenetic response of explants on callus induction media:

In order to obtain callus cultures, embryo slices were placed horizontally in contact with the surface of callus induction media (CIM) having various combination of PGRs (Table 4) (Figure a). After 10 days of culturing, explants expanded five times in their original size (Figure b) and turned either brown and compact (Golden) (Figure c) or yellow and friable (BARI 2001) (Figure d). In the first experiment, response of explants was checked by placing embryo slices on MS media supplemented only with different levels of BAP (Table 4), where the callus induction frequency (CIF) was no more than 19% & 13% in Golden and BARI 2001, respectively. In order to enhance callus induction frequency different levels of PGRs (BAP + IAA and BAP + NAA) on MS media were used (Table 4). Optimum CIF (86% & 78 %) was obtained on CIM 13 in these two varieties (Table 4). No any calli formed in control. Furthermore, at the higher levels of BAP and NAA (CIM 10) callus inhibition was seen in both varieties.

BAP in combination with TDZ and KIN promotes multiple shoot induction:

From the results it has been confirmed that regeneration of multiple shoots attained when healthy callus was transferred on multiple shoot regeneration media (MSM) with reduced levels of hormones. BAP alone as well as in combination with TDZ and KIN was used to facilitate the multiple shoot

induction. The brownish and dead parts of calli were removed with sharp, sterilized scalpel and sub-cultured on MSM media that have PGRs (Table 5) for 15 days. The calli swelled radially from the excised portion and prolonged the apical meristem five times more than the calli placed on simple MS media (devoid of hormone). After 15 days of explants on MSM, approximately 10-20 buds per explant were formed. In control experiment (hormone-free medium) no bud formation occurred. The results showed significant variation in number of multiple shoots/ plant between the varieties (Table 5). The explants on MSM1-MSM5 were unable to induce maximum multiple shoots in both the varieties (Table 5). MSM9 facilitated multiple shoot induction (4.6 & 4.1) with considerable shoot length (4.8 & 4.3 cm) and number of leaves/shoot (4.4 & 4.1) (Table 6) as compared to MS media devoid of hormone and MSM1-MSM5 in Golden and BARI 2001. The most effective combination of these PGRs was observed on MSM13 media producing highest number of multiple shoots/ plant (9.0 and 6.6) with highest shoot length (6.0 & 5.1 cm) and maximum number of leaves/shoot (6.1 & 5.3) in Golden and BARI 2001, respectively as shown in Table 5 and Table 6. The MSM10 & MSM15 having higher concentrations of hormones inhibited the multiple shoot formation, average shoot length and leaves/explant forming the calli on the lower surface of shoots in both the varieties (Figure f). After the excision of older shoots, new shoots were being produced on the clump by placing on fresh MSM. The buds and shoots produced on older clumps were sub cultured on shoot elongation media

(SEM) (data not shown) after every two weeks and transferred on MSM for multiple shoots regeneration.

Root induction needs no PGR application: On average of 2-4 cm elongated shoots were detached of multiple shoots bunch formed from callus that has been shifted to RIM. Substantial rooting system was seen on MS media fortified with IBA concentrations (autoclaved and filter sterilized). After 2-3 weeks, regenerated shoots produced roots via callus induction in both varieties (Figure g). The shoots on RIM1- RIM5 did not promote rooting (Table 7). The Highest rooting efficiency was achieved in Golden variety on RIM8, where 90% of the regenerated shoots produced roots when cultured medium exhibiting maximum root length (8.1 cm) and maximum number of roots/plants (8.3) (Table 7). In variety BARI 2001 there were a minute response to rooted plants (76%) with smaller root length (7.0 cm) and reduced number (6.3) of roots/plant compared to Golden (Table 7). Roots on RIM8 were healthy and normal in appearance, whereas the use of IBA (>1.5 mg/L) lead to inhibit rooting system calli formation on the basal portion of plantlets resulting in the thickness and shortening of roots (Figure h). then these plants were shifted to hydroponic condition having yoshida solution for the elongation of roots. After 2 weeks transferring of explants on hydroponics system, significant root development occurred in both the varieties. The plantlets were effectively adapted in glasshouse for a week and then shifted in pots containing 1:3 manure and sand with 85 % survival rate. These plants appeared uniform, healthy, and morphologically com-parable to the donor plants (Figure. i).

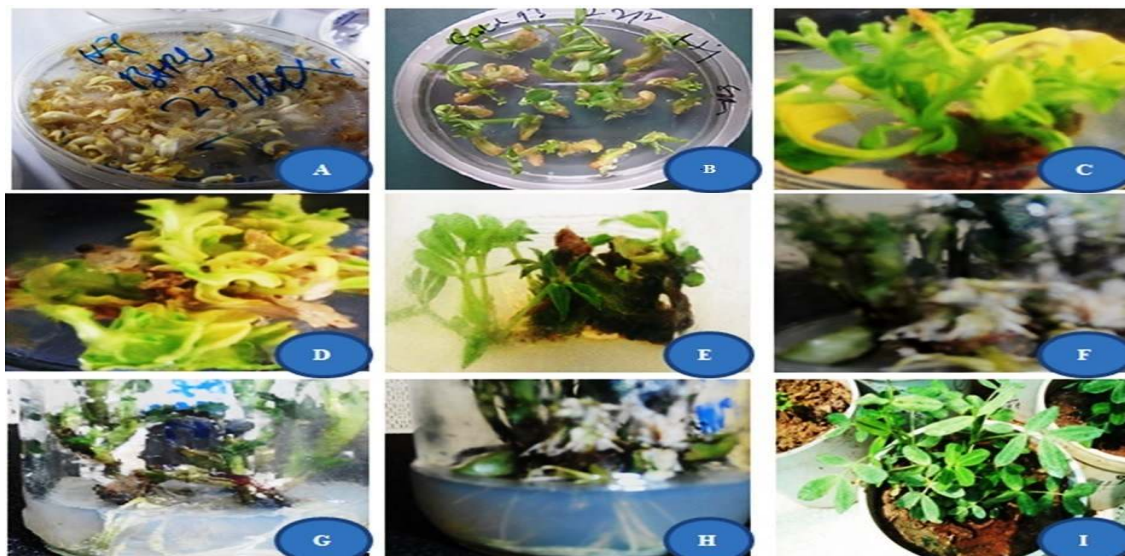


Figure 1. Steps involved in *in vitro* regeneration of groundnut (a) Embryo slices used as explants (b) Enlarged explants on Callus induction media (c) Yellow and friable texture of callus in BARI 2001 (d) Brown and compact texture of callus in Golden with multiple shoots (e) Multiple shoot regeneration from callus in BARI 2001 (f) Multiple shoot inhibition on higher levels of hormones g) Explants with roots on root induction media h) Root inhibition on higher levels of IBA (i) Plants in pot.

Table 1. Callus induction media used for callus proliferation in groundnut.

Media	Composition
CIM1	MS + BAP (1.0 mg/l)
CIM2	MS + BAP 2.0 mg/l
CIM3	MS + BAP 3.0 mg/l
CIM4	MS + BAP 4.0 mg/l
CIM5	MS + BAP 5.0 mg/l
CIM6	MS + IAA (2.0 mg/l) + BAP (3.5 mg/l)
CIM7	MS + IAA (2.5 mg/l) + BAP (4.5 mg/l)
CIM8	MS + IAA (3.0 mg/l) + BAP (5.5 mg/l)
CIM9	MS + IAA (3.5 mg/l) + BAP (6.5 mg/l)
CIM10	MS + IAA (4.0 mg/l) + BAP (7.5 mg/l)
CIM11	MS + NAA (0.5 mg/l) + BAP (3.5 mg/l)
CIM12	MS + NAA (1.0 mg/l) + BAP (4.5 mg/l)
CIM13	MS + NAA (1.5 mg/l) + BAP (5.5 mg/l)
CIM14	MS + NAA (2.0 mg/l) + BAP (6.5 mg/l)
CIM15	MS + NAA (2.5 mg/l) + BAP (7.5 mg/l)

MS: 4.3 g/l MS basal salts and vitamins (Murashige and Skoog, 1962), NAA: 1-naphthaleneacetic acid, IAA: Indole-3-acetic acid, BAP: 6-benzylaminopurine

Table 2. Multiple shoot induction media used for multiple shoot formation in groundnut.

Media	Composition
MSM1	MS + BAP (4.0 mg/l)
MSM2	MS + BAP (4.5 mg/l)
MSM3	MS + BAP (5.0 mg/l)
MSM4	MS + BAP (5.5 mg/l)
MSM5	MS + BAP (6.0 mg/l)
MSM6	MS + BAP (4.0 mg/l) + Kin (0.5 mg/l)
MSM7	MS + BAP (4.5 mg/l) + Kin (1.0 mg/l)
MSM8	MS + BAP (5.0 mg/l) + Kin (1.5 mg/l)
MSM9	MS + BAP (5.5 mg/l) + Kin (2.0 mg/l)
MSM10	MS + BAP (6.0 mg/l) + Kin (2.5 mg/l)
MSM11	MS + BAP (4.0 mg/l) + TDZ (0.5 mg/l)
MSM12	MS + BAP (4.5 mg/l) + TDZ (1.0 mg/l)
MSM13	MS + BAP (5.0 mg/l) + TDZ (1.5 mg/l)
MSM14	MS + BAP (5.5 mg/l) + TDZ (2.0 mg/l)
MSM15	MS + BAP (6.0 mg/l) + TDZ (2.5 mg/l)

Kin: Kinetin, TDZ: Thidiazuron

Table 3. Root induction media used for root formation in groundnut.

Media	Composition
RIM1	MS + IBA (0.5 mg/l) (IBA used after autoclaving)
RIM2	MS + IBA (1.0 mg/l) (IBA used after autoclaving)
RIM3	MS + IBA (1.5 mg/l) (IBA used after autoclaving)
RIM4	MS + IBA (2.0 mg/l) (IBA used after autoclaving)
RIM5	MS + IBA (2.5 mg/l) (IBA used after autoclaving)
RIM6	MS + IBA (0.5 mg/l) (IBA used after filter sterilization)
RIM7	MS + IBA (1.0 mg/l) (IBA used after filter sterilization)
RIM8	MS + IBA (1.5 mg/l) (IBA used after filter sterilization)
RIM9	MS + IBA (2.0 mg/l) (IBA used after filter sterilization)
RIM10	MS + IBA (2.5 mg/l) (IBA used after filter sterilization)

IBA: Indole-3-butyric acid

Table 4. Assessment of various combinations of PGRs on callus induction in groundnut.

Callus induction media	GOLDEN		BARI 2001	
	No. of explants responded (%)	Callus induction (%)	No. of explants responded (%)	Callus induction (%)
CIM1	13.3 ± 1.5	6.3 ± 1.5	10.6 ± 2.5	5.3 ± 1.5
CIM2	23.5 ± 3.0	10.3 ± 1.7	17.3 ± 2.8	8.0 ± 2.2
CIM3	35.6 ± 3.7	13.5 ± 2.3	26.2 ± 3.9	10.3 ± 2.1
CIM4	45.3 ± 3.9	19.7 ± 3.4	33.3 ± 3.9	13.2 ± 2.3
CIM5	38.1 ± 4.1	15.5 ± 3.1	26.6 ± 3.1	9.0 ± 1.8
CIM6	45.6 ± 4.3	40.6 ± 3.8	40.5 ± 4.0	33.3 ± 3.2
CIM7	57.3 ± 5.3	51.3 ± 4.0	49.9 ± 4.4	38.5 ± 4.1
CIM8	68.1 ± 5.6	58.1 ± 4.2	53.6 ± 4.3	41.7 ± 4.4
CIM9	61.0 ± 5.2	43.7 ± 3.9	56.4 ± 5.2	39.3 ± 4.5
CIM10	50.4 ± 4.8	39.5 ± 4.3	45.2 ± 4.2	30.2 ± 4.3
CIM11	58.8 ± 4.9	45.3 ± 4.7	46.7 ± 4.7	38.8 ± 3.2
CIM12	74.7 ± 5.3	66.3 ± 4.3	70.7 ± 5.1	61.3 ± 4.4
CIM13	92.2 ± 5.5	86.6 ± 5.5	88.3 ± 5.3	78.3 ± 4.8
CIM14	86.2 ± 5.1	70.4 ± 4.9	75.4 ± 5.0	60.7 ± 4.3
CIM15	73.7 ± 4.8	61.3 ± 4.7	61.7 ± 4.4	49.5 ± 4.9

The best results have been shown by bold letters. The values of standard deviation (n = 3) have been given after ± symbol.

Table 5. Assessment of various combinations of PGRs on multiple shoot formation in groundnut.

Multiple shoot induction media	GOLDEN		BARI 2001	
	No. of explants responded (%)	No. of shoots/ explants	No. of explants responded (%)	No. of shoots/ explants
MSM1	44.7 ± 3.7	1.4 ± 0.5	40.4 ± 3.3	1.3 ± 0.5
MSM2	50.5 ± 3.9	1.9 ± 0.6	48.6 ± 3.7	1.9 ± 0.7
MSM3	71.3 ± 5.5	2.7 ± 0.7	64.6 ± 5.3	2.5 ± 0.9
MSM4	65.3 ± 5.3	3.4 ± 0.4	60.3 ± 3.1	3.1 ± 0.8
MSM5	59.6 ± 4.2	3.1 ± 0.3	49.7 ± 4.2	1.9 ± 0.4
MSM6	51.4 ± 3.1	2.1 ± 0.7	45.6 ± 4.1	1.5 ± 0.6
MSM7	63.7 ± 4.4	3.3 ± 0.8	56.3 ± 4.3	3.0 ± 0.8
MSM8	65.2 ± 5.2	4.6 ± 0.6	69.5 ± 4.3	4.1 ± 0.6
MSM9	80.1 ± 5.8	4.1 ± 0.3	62.7 ± 4.7	3.6 ± 0.5
MSM10	71.6 ± 5.8	3.7 ± 0.3	59.4 ± 4.0	2.4 ± 0.4
MSM11	59.9 ± 4.2	3.2 ± 0.6	53.3 ± 4.3	3.3 ± 0.8
MSM12	70.5 ± 5.5	5.0 ± 0.7	58.8 ± 5.0	3.7 ± 0.9
MSM13	88.3 ± 5.1	9.2 ± 1.1	74.2 ± 5.3	7.1 ± 1.2
MSM14	83.7 ± 5.9	6.3 ± 1.1	68.3 ± 5.6	5.2 ± 1.1
MSM15	71.5 ± 5.5	3.7 ± 0.5	56.5 ± 5.1	3.2 ± 0.9

The best results have been shown by bold letters. The values of standard deviation (n = 3) have been given after ± symbol.

Table 6. Assessment of various combinations of PGRs on number of leaves per explant and average height of shoots (cm) in groundnut.

Multiple shoot induction media	GOLDEN		BARI 2001	
	No. of leaves per explants	Average height of shoots (cm)	No. of leaves per explants	Average height of shoots (cm)
MSM1	3.3 ± 0.8	2.1 ± 0.8	3.1 ± 0.7	2.2 ± 0.4
MSM2	3.5 ± 0.8	2.5 ± 0.9	3.6 ± 0.5	2.2 ± 0.4
MSM3	3.9 ± 0.3	3.8 ± 0.6	3.8 ± 0.8	3.4 ± 0.9
MSM4	4.2 ± 0.3	3.6 ± 0.7	4.0 ± 1.1	3.1 ± 0.8
MSM5	3.3 ± 0.9	3.1 ± 0.7	3.1 ± 0.6	2.9 ± 0.8
MSM6	3.4 ± 0.3	2.5 ± 0.8	3.3 ± 0.9	2.1 ± 0.5
MSM7	3.8 ± 0.8	3.0 ± 0.7	3.9 ± 0.9	2.8 ± 0.8
MSM8	4.4 ± 0.8	4.8 ± 0.6	4.1 ± 0.7	4.3 ± 0.6
MSM9	4.2 ± 0.7	4.3 ± 0.6	3.8 ± 0.8	3.9 ± 1.1

MSM10	3.3 ± 0.3	3.0 ± 0.9	3.3 ± 0.9	3.5 ± 0.9
MSM11	3.0 ± 0.4	2.8 ± 0.4	3.1 ± 0.7	2.8 ± 0.4
MSM12	3.3 ± 0.3	3.1 ± 0.3	3.4 ± 0.5	2.8 ± 0.6
MSM13	6.1 ± 1.3	6.0 ± 1.1	5.3 ± 1.2	5.1 ± 0.9
MSM14	4.2 ± 0.9	3.9 ± 0.7	3.3 ± 0.3	3.3 ± 0.9
MSM15	3.7 ± 0.5	3.3 ± 0.3	3.5 ± 0.3	2.9 ± 0.4

The best results have been shown by bold letters. The values of standard deviation (n = 3) have been given after ± symbol.

Table 7. Assessment of various combinations of IBA on number of roots per shoot and average root length (cm) in groundnut.

Root induction media	GOLDEN			BARI 2001		
	No. of shoots producing roots (%)	No. of roots per shoot	Root length (cm)	No. of shoots producing roots (%)	No. of roots per shoot	Root length (cm)
RIM1	15.5 ± 2.3	0.0 ± 0.0	0.0 ± 0.0	11.3 ± 2.3	0.0 ± 0.0	0.0 ± 0.0
RIM2	23.3 ± 3.0	0.8 ± 0.3	2.0 ± 0.4	17.5 ± 2.0	0.6 ± 0.3	1.7 ± 0.4
RIM3	35.4 ± 4.7	3.1 ± 0.5	2.8 ± 0.7	26.8 ± 3.3	2.3 ± 0.7	2.0 ± 0.6
RIM4	46.7 ± 5.1	1.3 ± 0.3	2.1 ± 0.6	38.4 ± 3.3	1.3 ± 0.5	1.6 ± 0.6
RIM5	39.3 ± 4.1	0.8 ± 0.3	1.7 ± 0.3	27.3 ± 2.9	0.6 ± 0.3	1.3 ± 0.3
RIM6	50.4 ± 3.8	4.4 ± 0.7	2.0 ± 0.6	42.6 ± 4.6	2.4 ± 0.8	1.3 ± 0.3
RIM7	58.8 ± 3.0	6.3 ± 1.1	5.8 ± 0.8	50.7 ± 5.1	4.2 ± 1.0	3.6 ± 0.8
RIM8	90.5 ± 5.8	8.3 ± 1.4	8.1 ± 1.5	76.3 ± 5.8	6.3 ± 1.3	7.0 ± 1.1
RIM9	79.6 ± 5.5	4.8 ± 0.8	6.3 ± 1.3	70.8 ± 4.8	3.4 ± 0.9	4.3 ± 0.7
RIM10	76.3 ± 4.6	3.2 ± 0.3	4.7 ± 0.8	66.6 ± 4.4	2.1 ± 0.3	2.7 ± 0.8

The best results have been shown by bold letters. The values of standard deviation (n = 3) have been given after ± symbol.

For *in vitro* root regeneration individual shoots were detached from the group of multiple shoots and moved to MS media having IBA (0.5-2.5 mg/l) for 2 weeks. It was evaluated from this study that there is no need of phytohormones for root formation (Asylin-Ozudogru *et al.*, 2005). About 2-5 cm shoots were shifted to root induction media and later on after 3-4 weeks, root formation started in both the varieties. It is already reported that various doses of IBA, optimum root induction was seen at 1.5 mg/l IBA (Perveen *et al.*, 2011), giving the highest root induction frequency with maximum root length in both the varieties. At 1.5 mg/l IBA healthy roots were appeared on the base of shoots while, at higher levels of IBA (> 1.5 mg/l) roots become thick and shorten due to the formation of callus, resulting in inhibition of roots formation. After abundant formation of roots, these plantlets were shifted to hydroponics culture in Yoshida solution (Yoshida *et al.* 1976) for 1-2 weeks and after every 4 days fresh Yoshida solution was added. When significant root elongation with lateral roots developed, then these plants were acclimatized in plastic bags. After some time (2 weeks) these acclimatized plants were again transferred to soil pots to maintain and grow in new environmental condition. Similar results were also reported by Venkatachalam *et al.* (1997); Banerjee *et al.* (2007); Verma *et al.* (2009) by using mixture of hormones i.e. IBA, NAA and KIN for root induction in groundnut. Present technique provides a reliable and high frequency yielding process for obtaining morphologically normal peanut plants in a short time.

Conclusion: The present study provides a reliable and high frequency yielding process for obtaining morphologically normal peanut plants in a short time. The best hormonal combination was BAP along with NAA resulting in the highest callus induction frequency. The highest CIF was noted with 5.5 mg/l BAP and 1.5 mg/l NAA in Golden, compared to other combinations. BAP in combination with TDZ and KIN promoted multiple shoot induction. MS media having 5.5 mg/l BAP and 1.5 mg/l NAA was found to be best by producing the highest callus induction frequencies (86 & 78%) in Golden and BARI 2001, respectively. Similarly, the maximum multiple shoots/ plant (9 & 6) with optimum length of shoots (4.8 & 4.1 cm) was obtained with the application of 4 mg/l BAP along with 1 mg/l NAA and 1.1 mg/l TDZ in Golden and BARI 2001, respectively. Roots on RIM8 [MS + IBA (1.5 mg/l)] were healthy and normal in appearance, whereas the use of IBA (>1.5 mg/L) led to inhibit the rooting system.

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