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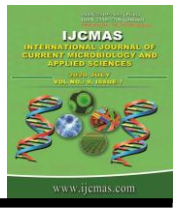

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Indirect Organogenesis for Regeneration in Brinjal (*Solanum melongena L.*) Var. KKM 1

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A B S T R A C T

Brinjal is an important nutritional vegetable crop of tropical and subtropical regions. It is highly responsive to various tissue culture techniques. The aim of this study was to develop an efficient protocol to standardize the concentration of sterilants for seed and explants sterilization for *in vitro* germination and regeneration respectively. The explants used are cotyledonary leaf, stem and hypocotyl of *in vitro* grown brinjal var.KKM1. For sterilization of seeds and explants, 5% sodium hypochlorite treatment for 3 minutes is the most effective sterilant concentration against the microbiological contamination. In subsequence, the regeneration protocol was optimised for the brinjal var.KKM1. From the *in vitro* grown seedlings, different explants such as hypocotyl, cotyledenary leaf and stem were taken for callus induction. Highest number of callus was obtained in MS medium containing 2 mgl^{-1} NAA and 0.5 mgl^{-1} BAP. Among the explants used, cotyledonary leaf and stem were highly responsive to callus induction. Further the callus was proliferated in the same media and was used for regeneration by using different plant growth hormones. Regeneration of shoots from callus was more efficient in MS media containing 2.0 mgl^{-1} BAP, 1.0 mgl^{-1} Kn and 0.5 mgl^{-1} NAA. The successful rooting was recorded on ½ MS media supplemented with 0.5 mgl^{-1} IBA. This *in vitro* regeneration process might be useful for producing disease free plants especially little leaf of brinjal, the most common infection in the brinjal var.KKM1.

Introduction

Brinjal is a widely adaptive and highly productive vegetable crop of tropical and subtropical regions. It is one of the important horticultural plants of the Solanaceae family. It has significant medicinal values (Khan, 1979). It has higher calorie, iron, phosphorous and riboflavin content. There are different varieties of brinjal according to colour and

shape. One such variety KKM1 was developed through single plant selection from a local type collected from Kulathur area and released by Department of Horticulture, Agriculture College and Research Institute, Killikulam during the year 1995. The plants are compact bearing small-sized white coloured egg-shaped fruits in the cluster of 2-4 with green calyx. The potential yield is 36 tonnes/ha. This variety has got a local preference in Tirunelveli district of Tamilnadu.

Brinjal is affected by several biotic stresses and Phytoplasma associated disease is one of the most significant diseases that causes yield loss of up to 40 per cent (Mitra, 1993 and Rao *et al.,* 2010). In India, little leaf disease of brinjal was first reported by Thomas and Krishnaswami (1939). The infected plants are characterised by small, soft, thin and malformed leaves showing yellow discolouration. The plants are generally stunted in growth with short internodes and auxiliary buds proliferation leading to phyllody symptoms (Rao *et al.,* 2010). The brinjal variety, KKM1 is severely affected by the Phytoplasma little leaf disease and hence to address this problem, the present study was undertaken to produce disease free planting materials using tissue culture methods.

The common problem of contamination in tissue culture by various microorganisms such as bacteria and fungi affects the callus proliferation and regeneration. Hence effective explants sterilization method (Dodds and Roberts, 1985; Sen *et al.,* 2013) is needed to produce disease free seedlings. Many sterilants cause toxicity to plant tissues and hence optimisation of the concentration of sterilization chemicals and duration of exposure of explants to sterilants are to be deduced for minimum explants injury to achieve better survival (CPRI 1992). The sterilization protocol of brinjal seeds for *in vitro* seed germination and the explants of *in vitro* seedlings viz, cotyledonary leaf, hypocotyl and stem for tissue culture is to be formulated for elimination of microbial contaminants by using different surface sterilizing agents such as sodium hypochlorite, mercuric chloride and ethanol.

The efficacy of *in vitro* organogenesis of brinjal highly depends on the type of explants used and complemented by a combination of plant growth regulators (Magioli and Mansur, 2005). Hence, the regeneration efficiency of brinjal var.KKM 1 is studied using different explants viz., cotyledonary leaf, hypocotyl and stem obtained from the *in vitro* germinated seedlings. With this prelude, the present study necessitates the development of an efficient sterilization protocol for the explants of brinjal var.KKM1. Also regeneration protocol optimisation from various explants of *in vitro* grown brinjal seedlings is a pre requisite for producing disease free plantlets for crop improvement.

Materials and Methods

Sterilization of seeds and explants

Seeds of brinjal variety KKM1 were obtained from the Department of Horticulture, Agriculture College and Research Institute, Killikulam. The seeds were washed in running tap water for 15 minutes. The non-viable floating seeds and debris were discarded. The washed seeds were subjected to sterilization using various sterilizing agents such as 5% sodium hypochlorite for 3min and 5min, 0.1% mercuric chloride for 2min and 3min and 70% ethanol for 10sec and 30sec under aseptic condition in laminar airflow chamber. Then the seeds were washed using sterile distilled water for 4 to 5 times and air dried by spreading on sterilized filter paper in a petridish. The sterilized seeds of ten to twelve numbers were inoculated in 100 ml

conical flasks containing 30 ml agar solidified MS medium free of hormones and the seeds were allowed to germinate. The per cent germination of seeds was recorded after 7 days of inoculation. Similarly, the explants viz., cotyledonary leaf, hypocotyl and stem from *in vitro* germinated seedlings were sterilized following the above mentioned sterilization treatments for callus induction and contamination percentage was recorded in all explants.

Callus induction

The explants such as cotyledonary leaf, stem and hypocotyls were dissected out from 10 to 12 days old *in vitro* germinated seedlings and inoculated in MS medium supplemented with different combination of plant growth hormones viz., 2, 4-D at 2.0 and 3.0 mgl^{-1}, BAP at 0.5, 1.0, 1.5 and 2.0$mgl^{-}1$, IAA at 0.5, 1.0, 1.5 and 2.0mgl^{-1} and NAA at 0.5, 1.0, 1.5 and 2.0mgl^{-1} concentration. The inoculated explants were kept in dark condition with 26°C temperature for callus initiation. The calli formed were subcultured for every three weeks in the same media prepared freshly for callus proliferation.

In vitro regeneration

The friable and embryogenic calli is selected from the proliferated callus and was inoculated in MS medium supplemented with various combination of hormones viz., BAP at 2.0 and 3.0 mgl^{-1}, NAA at 0.5 mgl^{-1} and kinetin at 1.0 mgl^{-1} concentration for shoot initiation.

The inoculated callus cultures were kept in culture conditions of 2000 lux light intensity, 16/8 hrs photoperiod and 26°C temperature. The regenerated shoots were transferred to rooting medium containing MS basal along with the hormone IBA at two different concentrations of 0.5 and 1.0 mgl^{-1}.

Hardening

The plantlets after sufficient development of root system, were taken carefully from the culture tubes and the agar was washed in running tap water. The plantlets were transferred to small plastic pots containing sterilized sand, soil and FYM in 1:2:1 ratio and kept inside the polyhouse for two to three weeks for acclimatization.

Data recording and analysis

The experiments were conducted in Completely Randomized Design. The analysis of variance for different characters was performed and means were compared by the Duncan's Multiple Range Test (DMRT).

Results and Discussion

Sterilization of seeds and explants

The chemical sterilants viz., sodium hypochlorite, mercuric chloride and ethanol were used at various concentrations and exposure time to determine the most efficient procedure for sterilization of seeds of brinjal var.KKM1 for *in vitro* germination and the explants viz., cotyledonary leaf, stem and hypocotyl from *in vitro* grown seedlings for callus formation (Table 1).

The germination of brinjal var.KKM1 seeds initiated within three to four days of incubation. The maximum germination percentage was recorded as 91.7% using 5% sodium hypochlorite for 3 minutes exposure and contamination percentage was 5.1 (Table 1; Fig 1c). The use of sodium hypochlorite for effective surface sterilization of explants from different plant sources has been widely reported (Durosomo *et al.,* 2014; Anoop Badoni *et al.,* 2010; Maina *et al.,* 2010). The explants such as cotyledonary leaf, stem and hypocotyl for callus induction showed lesser

contamination percentage of 3.86%, 5.2% and 6.0% respectively by using 5% sodium hypochlorite for 3 min exposure time compared to the exposure period of 5 min and other sterilizing agents (Fig 1d).

Treatment with 70% ethanol was not promising since the seed germination was 16.23% even though contamination was 15.33% and response of explants was not evident. The explants such as cotyledonary leaf, stem and hypocotyl used for callus formation also showed higher contamination percentage using 70% ethanol (Table.1;Fig 1e,1f). Ethanol is phytotoxic and a strong sterilizing agent with good bacteriostatic activity. For these reasons, the explants should be exposed to a lower concentration of ethanol for a short period of time. But the use of reduced concentration of ethanol does not completely destroy bacteria and hence to improve effectiveness in sterilization procedure, ethanol is generally used prior to treatment with other sterilization compounds.

Mercuric chloride at 0.1% concentration for exposure time of 3 minutes recorded 73.33% germination and 7.10 % contamination (Table.1; Fig 1a). The contamination percentage of cotyledonary leaf, stem and hypocotyls were recorded as 8.86%, 8.4% and 8.4% respectively, when 0.1% $HgCl_2$ was used as sterilant for 2 minutes exposure (Fig 1b), whereas it was 5.1%, 7.3% and 6.2% respectively for 3 minutes exposure (Table.1). There are many reports of surface sterilization of explants in plant tissue culture using 0.1% $HgCl_2$ (Sarkar *et al.,* 2006; Alim *et al.,* 2014). The exposure of explants to $HgCl_2$ may have negative effects on the survival rate of explants and $HgCl_2$ treatments for longer period leads to browning and death of explants (Danso *et al.,* 2011).

The sterilization treatments used in the seeds of Brinjal KKM1 variety showed better results with 91.7 per cent germination by sodium hypochlorite at 5 % concentration for the duration of 3 min followed by mercuric chloride at 0.1% concentration for 3 min. Also surface sterilization of explants viz., cotyledonary leaf, hypocotyl and stem showed lesser contamination percentage by using 5 % sodium hypochloride for 3 minutes. Hence, this concentration of sterilant was the most effective for seeds and explants sterilization.

Callus induction

Callusing of brinjal KKM1 variety could be successfully induced using explants from *in vitro* germinated seedlings. The cotyledonary leaf, stem and hypocotyl were cultured on MS media with BAP, NAA, 2,4-D and IAA in 14 different combinations. Among these, MS media with hormonal combination of 2 mgl^{-1}NAA and 0.5 mgl^{-1}BAP promoted callus induction of cotyledonary leaf explant in 6.33 days (Fig 2a) followed by MS media with 1 mgl^{-1}NAA and 1 mgl^{-1}BAP in 6.66 days and MS media with 0.5 mgl^{-1}IAA and 2 mgl^{-1}BAP in 7.0 days.. Media with other hormonal combinations resulted in slow progress of callus induction. The maximum per cent response for callus induction is 56.66% in MS media with hormones 2 mgl^{-1}IAA and 0.5 mgl^{-1} BAP. MS media with 3 mgl^{-1} 2,4-D and 1 mgl^{-1} BAP and MS basal with 0.5 mgl^{-1}IAA and 2 mgl^{-1} BAP resulted in 55% callus induction using cotyledonary leaf explants (Table 2). The profuse callus proliferation was observed from cotyledon explants in MS medium containing 2 mgl^{-1}BAP or 2.5 mgl^{-1}BAP (Mohinder Kaur *et al.,* 2011) and in MS with 3 mgl^{-1}2, 4-D and 0.05 mgl^{-1} BAP (Alim *et al.,* 2014).

The hormonal combination of 2 mgl^{-1} NAA and 0.5 mgl^{-1}BAP in MS basal media required 9.33 days (Fig 2b) for callus induction from stem explants followed by 1 mgl^{-1}NAA and 1

mgl^{-1} BAP in 9.66 days and 0.5 mgl^{-1}IAA and 2 mgl^{-1}BAP in 10.33 days. Also a significant callus induction percentage of 55% was recorded in the same hormonal combination of 2mgl^{-1} NAA and 0.5mgl^{-1} BAP in MS basal media (Table 2). Ray *et al.,* (2010) reported that the highest amount of callus was produced in MS medium containing 2.0 mgl^{-1} BAP and 0.5 mgl^{-1}NAA using the stem explant.

The hormonal combination of 3 mgl^{-1} 2,4-D and 0.5 mgl^{-1}BAP used for hypocotyl explants required 12.66 days (Fig 2c) for callus induction followed by 0.5 mgl^{-1}NAA and 2 mgl^{-1}BAP in 13 days and 3 mgl^{-1} 2,4-D in 13.33 days (Table 2). The callus induction percentage was 53.33% in the hormonal combination of 2 mgl^{-1} NAA and 0.5 mgl^{-1}BAP and 49.66% in MS media with 2 mgl^{-1}BAP and 0.5 mgl^{-1}IAA. Among the three explants, cotyledonary leaf was more responsive to callus induction and proliferation compared to stem and hypocotyl explants (Fig 2d, 2e, 2f).

In vitro regeneration

Significant regeneration of shoot was achieved from cotyledonary leaf, stem and hypocotyl explants on MS media with 2 mgl^{-1} BAP, 1 mgl^{-1} kinetin and 0.5 mgl^{-1} NAA (Fig 2g, 2h, 2i). Number of days taken to shoot regeneration by the explants obtained from cotyledonary leaf and stem was 38.33 and 39.33 days respectively. whereas the hypocotyl explants took 47.66 days for shoot regeneration. Number of shoots derived per callus of cotyledonary leaf is 3 per callus clump and shoot length was 3.93 cm in MS media with 2 mgl^{-1} BAP, 1 mgl^{-1} kinetin and 0.5 mgl^{-1}NAA (Table 3; Fig 3a). Earlier reports suggest that the high frequency organogenesis of shoots was achieved from calli of cotyledonary leaf in MS supplemented with1.0 mgl^{-1} BAP and 1.0 mgl^{-1} Kn (Sarker *et al.,* 2006) and 2.0 mgl^{-1} BAP and 0.3 mgl^{-1} IAA (Baradhan *et al.,* 2012). Average number of shoots from callus of hypocotyl explants was 0.6 per callus clump when MS basal medium containing 2.5 mgl^{-1} BAP and 0.5 mgl^{-1} IAA was used (Baradhan *et al.,* 2012).

Half strength MS medium supplemented with 0.5 mgl^{-1} IBA and 1.0 mgl^{-1} IBA are more effective for root induction from the shoots derived from calli of all the three explants, cotyledonary leaf, hypocotyl and stem. The number of days taken for root formation from *in vitro* shoots was ranging between 26 to 29.33. The maximum number of roots recorded in ½ MS medium supplemented with 1.0 mgl^{-1} IBA was 2.66 to 3 per shoot and root length was 2.56 cm (Table 4; Fig 3b). MS basal medium was reported to be effective for root induction (Taha *et al.,* 2002). Jadhav *et al.,* (2014) and Zayova *et al.,* (2012) noticed that the brinjal regenerated shoots successfully formed roots on MS hormone-free medium and also in medium containing 0.1 mgl^{-1} IBA when *in vitro* shoots from calli of the explants, cotyledon and hypocotyl were used for root formation.

The plantlets after sufficient development of root system, were taken carefully from the culture tubes and the agar was washed in running tap water. The plantlets were transferred to small plastic pots containing sterilized sand, soil and FYM in 1:2:1 ratio and kept inside the polyhouse for two to three weeks for acclimatization (Fig 3c). After three weeks the plants were transferred to small earthern pot and kept inside the glasshouse (Fig 3d).

In conclusion the brinjal var.KKM1, is highly susceptible to Phytoplasma infection and hence to eliminate it by tissue culture techniques, standardization of a protocol for regeneration is attempted.

Table.1 Effect of different chemicals on *in vitro* germination of brinjal var. KKM1 seeds and sterilization of explants

S.No	Treatment	Sterilant	Concentration (%)	Duration	Seeds		Cotyledonary leaf	Stem	Hypocotyl
					Germination % *	Contamination % *	Contamination % *		
1	T1	NaOCl	5	3 min	91.70	5.10	3.86	5.20	6.00
2	T2	NaOCl	5	5 min	50.40	6.73	6.23	8.30	7.30
3	T3	$HgCl_2$	0.1	2 min	41.70	6.06	8.86	8.40	8.40
4	T4	$HgCl_2$	0.1	3 min	73.33	7.10	5.10	7.30	6.20
5	T5	Ethanol	70	10 sec	8.16	8.40	22.3	26.53	36.00
6	T6	Ethanol	70	30 sec	16.23	15.33	25.7	29.36	38.50
CD value (0.0.5)					1.137	0.139	0.178	0.840	1.036

*Mean of three replications
The treatment means are compared using Duncan Multiple Range Test (DMRT).
The mean followed by common letter(s) are not significantly different (p=0.05).

Table.2 Effect of different hormonal combinations on callus induction from cotyledonary leaf, stem and hypocotyls of brinjal variety KKM1

Hormonal combination mgl^{-1}				Cotyledonary leaf		Stem		Hypocotyl	
2,4 D	BAP	IAA	NAA	Days to callus induction *	callus induction % *	Days to callus induction *	callus induction % *	Days to callus induction *	callus induction % *
2	-	-	-	12.00^{bcd}	25.00^{e}	13.66^{bcd}	25.00^{def}	15.66^{bcde}	18.33^{d}
3	-	-	-	13.00^{abc}	29.33^{cde}	13.00^{cd}	28.33^{cd}	13.33^{fgh}	45.00^{a}
2	0.5	-	-	12.66^{bc}	28.33^{de}	12.00^{de}	33.33^{bc}	16.66^{abc}	30.00^{bc}
2	1	-	-	14.33^{a}	35.00^{bcd}	15.00^{ab}	35.00^{b}	18.00^{a}	23.33^{bcd}
3	0.5	-	-	12.33^{bcd}	35.00^{bcd}	14.00^{bc}	21.66^{ef}	12.66^{h}	46.66^{a}
3	1	-	-	9.33^{e}	55.00^{a}	14.33^{abc}	20.00^{f}	14.33^{efg}	26.66^{bcd}
-	2	0.5	-	7.00^{f}	55.00^{a}	10.33^{ef}	55.00^{a}	13.33^{fgh}	49.66^{a}
-	1	1	-	9.00^{e}	53.33^{a}	13.00^{cd}	26.66^{de}	15.00^{de}	25.00^{bcd}
-	1.5	1.5	-	11.00^{d}	40.00^{b}	15.33^{ab}	21.66^{ef}	16.00^{bcd}	20.00^{cd}
-	0.5	2	-	9.00^{e}	56.66^{a}	13.66^{bcd}	26.66^{de}	17.00^{ab}	33.33^{b}
-	2	-	0.5	11.66^{cd}	36.66^{bc}	16.00^{a}	20.00^{f}	13.00^{gh}	45.00^{a}
-	1	-	1	6.66^{f}	53.33^{a}	9.66^{f}	55.00^{a}	16.00^{bcd}	26.66^{bcd}
-	1.5	-	1.5	13.33^{ab}	36.66^{bc}	14.33^{abc}	21.66^{ef}	15.33^{cde}	20.00^{cd}
-	0.5	-	2	6.33^{f}	53.33^{a}	9.33^{f}	55.00^{a}	14.66^{def}	53.33^{a}
CD value (0.05)				1.652	7.864	1.712	6.451	1.526	10.079

*Mean of three replications
The treatment means are compared using Duncan Multiple Range Test (DMRT).
The mean followed by common letter(s) are not significantly different (p=0.05)

Table.3 Effect of different hormonal combinations on *in vitro* shoot regeneration of the calli derived from cotyledonary leaf,stem and hypocotyls of brinjal variety KKM1

Calli	Harmonal combinations (mg/l)			Days required for shoot regeneration*	Number of shoots per explants*	Shoot length(cm)*
	BAP	NAA	kinetin			
Calli from cotyledonary leaf	3	-	-	42.33^{d}	1.00^{d}	3.06^{e}
	3	0.5	-	45.33^{c}	1.33^{cd}	3.26^{cd}
	2	-	1	42.66^{d}	1.33^{cd}	3.26^{cd}
	2	0.5	1	38.33^{e}	3.00^{a}	3.93^{a}
Calli from stem	3	-	-	45.66^{c}	1.66^{cd}	3.10^{e}
	3	0.5	-	47.00^{b}	1.66^{cd}	3.33^{c}
	2	-	1	47.33^{ab}	1.00^{d}	3.13^{de}
	2	0.5	1	39.33^{e}	2.66^{ab}	4.03^{a}
Calli from hypocotyl	3	-	-	48.33^{a}	1.66^{cd}	3.56^{b}
	3	0.5	-	48.33^{a}	1.33^{cd}	3.00^{e}
	2	-	1	48.33^{a}	1.66^{cd}	3.30^{c}
	2	0.5	1	47.66^{ab}	2.00^{bc}	4.00^{a}
CD value (0.05)				1.051	0.932	0.159

*Mean of three replications
The treatment means are compared using Duncan Multiple Range Test (DMRT).
The mean value followed by common letter(s) are not significantly different (p=0.05).

Table.4 Effect of different hormonal combinations on *in vitro* root formation from the shoots derived from calli of cotyledonary leaf, stem and hypocotyls of brinjal variety KKM1

Shoot	Hormonal combinations*	Days required for root regeneration*	Number of roots per plant*	Root length(cm)*
Shoots derived from calli of cotyledonary leaf explants	IBA 0.5 mg/l	26.00^{b}	1.33^{b}	2.26^{b}
	IBA 1.0 mg/l	27.66^{ab}	3.00^{a}	2.16^{bc}
Shoots derived from calli of stem explants	IBA 0.5 mg/l	28.33^{a}	1.33^{b}	2.13^{c}
	IBA 1.0 mg/l	29.33^{a}	2.66^{a}	2.56^{a}
Shoots derived fromcalli of hypocotyl explants	IBA 0.5 mg/l	27.33^{ab}	1.33^{b}	2.20^{bc}
	IBA 1.0 mg/l	28.66^{a}	2.66^{a}	2.53^{a}
CD value (0.05)		2.011	0.938	0.119

*Mean of three replications
The treatment means are compared using Duncan Multiple Range Test (DMRT).
The mean followed by common letter(s) are not significantly different (p=0.05).

Fig.1 *In vitro* seed germination of brinjal variety KKM1 seeds using different sterilants

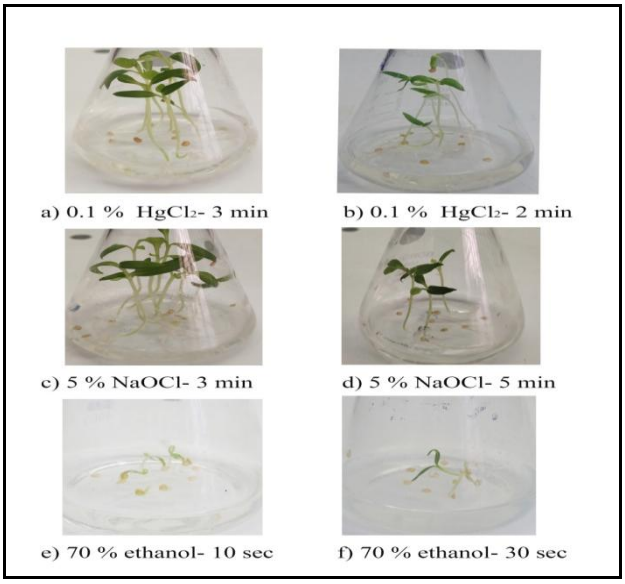
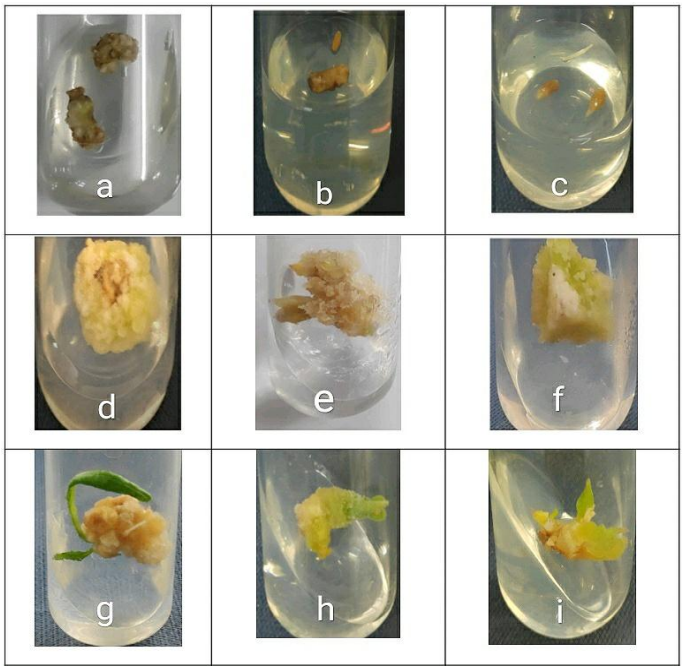
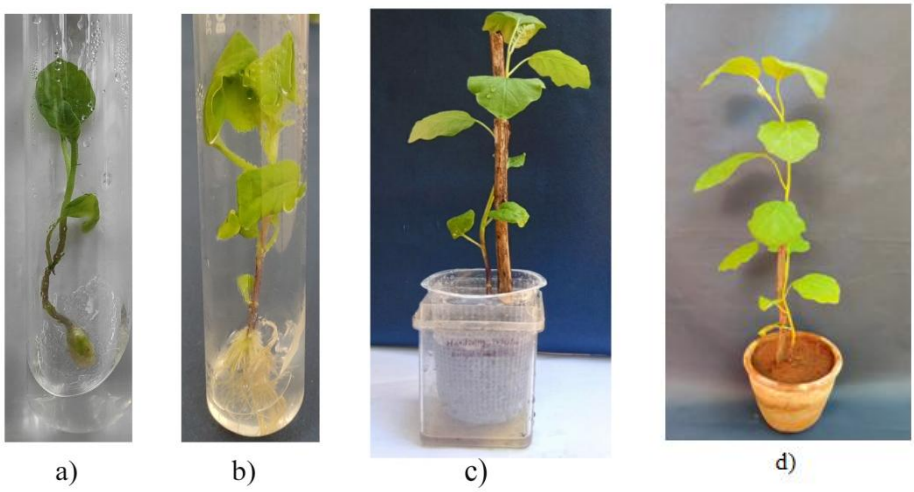


Fig.2 Callus induction and regeneration of cotyledonary leaf, stem and hypocotyls explants



a. Callus induction of cotyledonary leaf explant in 6.33 days using MS + 2 mg/l NAA and 0.5 mg/l BAP; b. Callus induction of stem explant in 9.33 days using MS + 2 mg/l NAA and 0.5 mg/l BAP; c. Callus induction of hypocotyl explants in 12.66 days using MS + 3mg/l 2,4-D and 0.5 mg/l BAP; d. Callus proliferation from cotyledonary leaf explants after 20 days **(**MS + 2 mg/l NAA and 0.5 mg/l BAP) **;** e. Callus proliferation from stem explants after 20 days **(**MS + 2 mg/l NAA and 0.5 mg/l BAP) **;** f. Callus proliferation from hypocotyls explants after 20 days (**MS +** 3mg/l 2,4-D and 0.5 mg/l BAP); g. shoot regeneration from cotyledonary leaf explants after 38.33 days using MS + 2 mg/l BAP, 1 mg/l kinetin and 0.5 mg/l NAA; h. shoot regeneration from stem explants after 39.33 days using MS + 2 mg/l BAP, 1 mg/l kinetin and 0.5 mg/l NAA; i. shoot regeneration from hypocotyls explants after 47.66 days using MS + 2 mg/l BAP, 1 mg/l kinetin and 0.5 mg/l NAA

Fig.3 *In vitro* formation of shoots and roots and hardening of brinjal var.KKM1 from cotyledonary leaf explants



Shooting of callus from cotyledonary leaf explants using MS + 2 mg/l BAP, 1 mg/l kinetin and 0.5 mg/l NAA, b. Rooting of shoots derived from the callus of cotyledonary leaf explants using ½MS + 1.0mg/l IBA, c. Hardening of *in vitro* developed plantles of brinjal variety KKM1, d. *In vitro* regenerated plants transferred to pot.

Surface sterilization with sodium hypochlorite at 5% concentration for 3 min exposure is efficient in eliminating contamination in all the three explants taken from *in vitro* germinated seedlings. The callus induction and proliferation was prominent on MS medium containing 2.0mgl^{-1} NAA and 0.5 mgl^{-1} BAP from cotyledonary leaf. The shoot regeneration through callus from cotyledonary leaf and stem was efficient in MS media with 2 mgl^{-1} BAP, 1 mgl^{-1}kinetin and 0.5 mgl^{-1} NAA. Half strength MS medium supplemented with 0.5 and 1.0 mgl^{-1} IBA was more effective for root induction. To conclude, this sterilization protocol for explants of brinjal var.KKM1 and regeneration protocol will be highly useful to produce *in vitro* brinjal plants for resistance to little leaf of brinjal caused by Phytoplasma.

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