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Research Article

In vitro organogenesis in Mothbean [*Vigna aconitifolia* (Jacq.)

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Abstract
Five varieties of mothbean viz., GMO 1, GMO 2, CZM 2, Jwala and RMO 40 were investigated for in vitro organogenesis. Significant differences were observed among the varieties, explants, medium combinations and their two and three way interactions for the days taken for green colouration of callus, per cent green colouration and number of shoots per callus piece except for variety × explant, explant × medium and variety × explant × medium interactions in case of days to green colouration of callus and explant × medium for number of shoots per callus piece. Among the six media tested, MS + 1.0 mg l^{-1} IAA + 3.0 mg l^{-1} K took significantly less number of days to green colouration of callus (21.462), showed the highest percentage of green colouration (60.086) and also produced maximum number of shoots per callus piece (5.556). Of the different varieties, GMO 2 was found to be the most responsive variety for regeneration of plants. The callus derived from leaf explants was found superior to the hypocotyl derived callus in plant regeneration. Medium. MS + 1.0 mg l^{-1} IAA + 3.0 mg l^{-1} K was recognized as the most efficient medium for plant regeneration from established callus cultures of mothbean. The differences among the varieties and medium combinations were significant for per cent rooting. The medium factor contributed maximally towards the total variation followed by variety. On the other hand, the contribution of variety × medium was the least towards the total variation. Of the different varieties, GMO2 was found to be the most responsive variety and the MS medium supplemented with 1.0 mg l^{-1} NAA was the best medium for root regeneration.

Key words:
Mothbean, in vitro, organogenesis

Introduction

Mothbean [*Vigna aconitifolia* (Jacq.) Marechal] (2n = 22) belongs to fabaceae family is an important food grain legume crop that is cultivated over the entire world. It is regarded as a quality pulse crop in India for its excellent protein quality, high digestibility and freedom from flatulent effects associated with other pulses. The crop is unable to fulfill increasing demand because of low yield potential, which is thought to be result of biotic and abiotic factors as well as low genetic variability.

One of the major steps in any yield improvement program is the creation of a broad based gene pool. Interspecific hybridization has proved impossible due to cross-incompatibility and hybrid sterility. To date, a reproducible and reliable transformation system that would enable genes of interest to be inserted into mothbean lines is not available in existing genotypes. Consequently, genetic transformation combined with traditional breeding methods may prove helpful in improving both the quality and yield of mothbean.

Plant tissue culture offer new strategies for improvement of agricultural crops. Plant cell culture has provided a new and an exciting option for obtaining increased genetic variability relatively rapid and without much sophisticated technology (Larkin and Scowcroft, 1981). Henceforth, an efforts were made for organogenesis from callus cultures in mothbean.

Material and Methods

Experimental materials:The experimental materials include five genotypes of mothbean *viz*., GMO 1, GMO 2, CZM 2, Jwala and RMO 40. The most commonly grown varieties in a stressed ecosystem were used for the investigation. The seeds harvested from pureline were grown aseptically and hypocotyl and leaf were used as explants.

Regeneration of plants from established callus cultures: The healthy looking and fast growing callus masses derived from hypocotyl and leaf explants sources were selected for plant regeneration. The MS medium supplemented with K, IAA and their selective combinations were tried for regeneration. The equal quantity of healthy callus mass was employed to each test tube. The cultures were inoculated at 26±2ºC under light the photoperiod in the culture room was maintained at 16/8 hours light/dark cycle. The observation like days taken for green colouration of callus, per cent green colouration and number of shoots per callus piece were recorded periodically.

Rooting and establishment of regenerated plants: The regenerated shoots were separated from the callus masses. Old roots, if any, remnants of callus and old leaves were removed. The shoots of approximately 3 to 4 cm of height were then transferred to rooting medium (plate 4). The MS medium supplemented with different concentrations of NAA was tested for rooting of shoots. The observations like days taken for root initiation, number of roots per shoots and per cent rooting were recorded.

For establishment, plantlets with 3-4 leaves and having 3-4 well developed roots were transferred to 20 × 10 cm polythene bags containing a specific potting mixture. The potting mixture consisted farm yard manure (FYM), soil and sand 1:2:1 ratio.

Observations recorded: The observations recorded from the different experiments are as under.
Per cent green colouration of callus:From the piece of callus, the regenerated shoots measuring more than 0.5 cm length were counted and recorded after one month of culture.
Number of shoots per callus piece: The numbers of shoots were counted from each callus piece.
Per cent rooting: Number of roots recorded out of total number of shoots cultured for rooting were counted after 15 days of inoculation and expressed as percentage.

The statistical analysis for the research data was carried out as per the procedure of factorial completely randomized design according to Steel and Torrie (1960). The data in percentages were transformed using arcsin transformation prior to analysis.

Results and Discussion

The callus cultures required for this experiment were initially maintained for 3-4 subcultures on MS medium supplemented with 1.0 mg l^{-1} 2, 4-D.

Per cent green colouration of callus: Out of different regeneration media tried, MS + 1.0 mg l^{-1} IAA + 3.0 mg l^{-1} K recorded significantly highest percentage of green colouration of callus (60.086). On the other hand, the lowest percentage (32.835) was observed for MS basal medium (Table 1). Among the five varieties, the callus of GMO 2 showed the maximum percentage of green colouration (52.254) as against (45.017) per cent recorded for RMO 40 calli (Table 2). The leaf derived calli gave higher percentage of green colouration (49.666) as compare to the hypocotyl calli (44.433) (Table 3).

In case of variety × explant interaction (Table 4), the highest mean percentage of greening (55.718) was recorded by the calli derived from the leaf explant of variety GMO 2. The hypocotyl derive calli of Jwala recorded the lowest percentage of green colouration (41.369).

The results of variety × medium interaction (Table 5) showed that the variety GMO 2 recorded the highest percentage (72.643) of green colouration of callus on both MS + 1.0 mg l^{-1} IAA + 3.0 mg l^{-1} K. The lowest percentage of green colouration (30.690) was recorded by the calli of variety CZM 2 on MS basal medium which was at par with GMO 1 (32.404) on the same medium.

The results of explant × medium interaction (Table 6) showed that callus derived from the leaf explants recorded the highest percentage of green colouration (64.928) on MS +1.0 mg l^{-1} IAA + 3.0 mg l^{-1} K. The hypocotyl callus recorded the lowest percentage (31.744) of green colouration on MS basal medium.

The data on variety × explant × medium interactions (Table 7) revealed that the callus derived from leaf explants of variety GMO 2 recorded the highest percentage of green colouration (73.110) on MS + 1.0 mg l^{-1} IAA + 3.0 mg l^{-1} K. However, it was at par with the per cent green colouration and of varieties CZM 2 (72.880) and Jwala (71.954) of the same medium. The leaf callus of the same variety also showed (72.426) per cent green colouration on MS + 1.0 mg l^{-1} IAA + 5.0 mg l^{-1} K. The callus derived from hypocotyl explants (28.122) of variety CZM 2 recorded the lowest percentage of green colouration on MS basal medium which was at par with the varieties GMO 1(31.313) and GMO 2 (31.550) on the same medium.

Number of shoots per callus piece: Irrespective of varieties and explants used, MS + 1.0 mg l^{-1} IAA + 3.0 mg l^{-1} K recorded significantly the highest number of shoots (5.556) per callus piece, while MS basal medium produced the lowest number of shoots (2.408) per callus piece (Table 8). Out of five varieties tested, variety GMO 2 produced significantly the highest number of shoots (4.747) per callus piece irrespective of media and explants used (Table 9). The results given in Table 10 indicated that across all the media and varieties tested, the callus induced from leaf explants yielded significantly higher number of shoots (4.301) per callus piece than the callus from hypocotyl explants (3.901).

The variety × explant interaction as presented in Table 11 showed that the callus derived from leaf explants of variety GMO 2 produced the maximum number of shoots (5.333). The callus derived from the hypocotyl explants of variety CZM 2produced minimum number of shoots (3.703) per callus piece. The results are in contrast with the findings of Bhargava and Chandra (1983). They obtained shoot regeneration through hypocotyl explants and effective role of K alone or in combination with IAA in induction of shoots from hypocotyl explants of mothbean.

The variety × medium interaction effects (Table 12) revealed that the highest number of shoots (6.740) was produced by the callus GMO 2 on MS + 1.0 mg l^{-1} IAA + 3.0 mg l^{-1} K followed by the number of shoots (6.140) produced on MS + 1.0 mg l^{-1} IAA + 5.0 mg l^{-1} K of the same variety. The minimum number of shoots (2.170) was noticed on the MS basal medium for the variety GMO 1. Bhargava and Chandra (1989) showed that K-IAA combinations were more effective than BAP-IAA in inducing shoot regeneration from leaf explants of mothbean.

The explant × medium interaction effect for the number of shoots per callus piece was found non-significant. However, the callus derived from leaf explants produced the highest number of shoots (5.708) on MS + 1.0 mg l^{-1} IAA + 3.0 mg l^{-1} K and least number of shoots (2.320) on MS basal medium of the same explant.(Table 13). On the other hand, the hypocotyl explants produced maximum number of shoots (5.404) on MS + 1.0 mg l^{-1} IAA + 3.0 mg l^{-1} K while the lowest number of shoots (2.496) produced on MS basal medium. These are in accordance with the results obtained by Eapen *et al.* (1986). They obtained optimum shoot regeneration of leaf and cotyledon explants on MS supplemented with the K and IAA. The report of Jain and Chopra (1988) also showed maximum shoots buds per leaf explant on MS + 1.0 mg l^{-1} IAA + 5.0 mg l^{-1} K.

The results of variety × explant × medium interaction are presented in Table 14. Significantly the highest number of shoots per callus piece (7.100) were recorded by the callus derived from leaf explants of variety GMO 2 on MS medium containing 1.0 mg l^{-1} IAA and 3.0 mg l^{-1} K which was at par with the shoots produced (6.680) on MS + 1.0 mg l^{-1} IAA + 5.0 mg l^{-1} K. Hypocotyl callus of the same variety i.e. GMO 2 also produced the highest number of shoots (6.380) on MS + 1.0 mg l^{-1} IAA + 3.0 mg l^{-1} K. Thus callus derived from hypocotyl and leaf explants of variety GMO 2 was found to be equal effective in terms of shoot production on MS + 1.0 mg l^{-1} IAA + 3.0 mg l^{-1} K. Jain and Chopra (1988) reported genotypic differences with leaf derive callus of 169 germplasm lines of *Vigna aconitifolia.* Bhargava and Chandra (1989) obtained differential response of genotypes to different combinations of hormones from callus of leaf explants of mothbean. Bhargava and Chandra (1983) observed distinct differences in regeneration response among different cultivars.

Rooting and establishment of regenerated plants: As more number of shoots of variety GMO 2 was available, rooting experiments were first performed on these shoots. The differentiated shoots of about 3 to 4 cm height were separated aseptically for transfer on root induction medium. The MS medium supplemented with different concentrations of NAA was used for the rooting experiment.

Per cent rooting: The comparison of mean of different varieties (Table 16) showed that variety GMO 2 recorded significantly the highest per cent (67.752) rooting, whereas, CZM 2 recorded the lowest (41.640). Such a genotype differences were observed by Bhargava and Chandra (1983) for rooting of regenerated shoots of mothbean.

Among the different medium combinations used (Table 15), MS medium supplemented with 1.0 mg l^{-1} NAA recorded the highest percentage (67.065) rooting which was at par with the per cent (64.195) rooting recorded by MS medium supplemented with 0.50 mg l^{-1} NAA. The MS medium having 0.10 mg l^{-1} NAA recorded significantly the lowest per cent (32.188) rooting. The importance of medium has also been shown by numerous workers (Eapen *et al.*, 1986; Bhargava and Chandra, 1989 and Choudhary *et al.*, 2009) to obtain good rooting in *in vitro* regenerated shoots of mothbean.

The variety × medium interaction effects revealed that the highest percentage (76.048) of rooting was recorded by variety GMO 2 on MS medium supplemented with 1.0 mg l^{-1} NAA and the lowest per cent (26.804) rooting was recorded by variety CZM 2 on MS + 0.10 mg l^{-1} NAA (Table 17).

For the establishment of plants, the rooted plantlets were transplanted in the polythene bags containing a potting mixture (sand + soil + FYM in 1:2:1 ratio). These plants were the kept under the high humidity. Higher per cent of the plants established in the polythene bags. Good performance of this mixture may be attributed to its ability to retain optimum moisture and to provide good aeration and nutrients. Afterwards, these plants were planted in the field conditions.

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Table 1. The influence of different media on per cent green colouration of callus.

Media (MS + mg l^{-1})	Mean
MS basal	29.076 (32.835)
MS + 1.0 IAA	44.836 (42.283)
MS + 1.0 IAA + 1.0 K	50.510 (45.606)
MS + 1.0 IAA + 2.0 K	56.812 (49.243)
MS + 1.0 IAA + 3.0 K	72.820 (60.086)
MS + 1.0 IAA + 5.0 K	61.366 (52.244)

S.Em = 0.401 C.D. = 1.111 C.V. % = 6.022

Figures in parenthesis are percentage values

Table 2. The influence of different varieties on per cent green colouration of callus.

Varieties	GMO 1	GMO 2	CZM 2	Jwala	RMO 40
Mean	51.100 (45.857)	60.369 (52.254)	49.579 (45.267)	52.184 (46.854)	49.620 (45.017)

S.Em = 0.366 C.D. = 1.014 C.V. % = 6.022

Figures in parenthesis are percentage values

Table 3. The influence of various explants on per cent green colouration of callus.

Explants	Hypocotyl	Leaf
Mean	48.457 (44.433)	56.683 (49.666)

S.Em = 0.231 C.D. = 0.641 C.V. % = 6.022

Figures in parenthesis are percentage values

Table 4. The influence of variety × explant interaction effect on colouration of callus (%).

Explants	Varieties				
	GMO 1	GMO 2	CZM 2	Jwala	RMO 40
Hypocotyl	51.757 (46.220)	55.127 (48.790)	45.390 (42.462)	43.337 (41.359)	46.677 (43.332)
Leaf	50.443 (45.494)	65.610 (55.718)	53.767 (48.072)	61.030 (52.349)	52.563 (46.701)

S.Em = 0.517 C.D. = 0.641 C.V. % = 6.022

Figures in parenthesis are percentage values

Table 5. The influence of variety × medium interaction effect on per cent green colouration of callus.

Media (MS + mg l^{-1})	GMO 1	GMO 2	CZM 2	Jwala	RMO 40
MS basal	28.360 (32.404)	30.550 (33.874)	25.770 (30.690)	30.540 (33.787)	29.980 (33.421)
MS + 1.0 IAA	45.060 (42.427)	46.600 (43.302)	45.160 (42.465)	45.330 (42.550)	42.030 (40.672)
MS + 1.0 IAA + 1.0 K	55.600 (48.517)	53.050 (47.034)	36.190 (37.191)	58.270 (50.339)	49.440 (44.949)
MS + 1.0 IAA + 2.0 K	56.740 (49.162)	64.730 (53.949)	55.550 (48.458)	49.850 (45.192)	57.190 (49.432)
MS + 1.0 IAA + 3.0 K	62.320 (52.440)	90.350 (72.643)	75.880 (62.343)	73.130 (60.577)	62.420 (52.490)
MS + 1.0 IAA + 5.0 K	58.520 (50.193)	76.750 (62.724)	58.920 (50.430)	55.980 (48.739)	56.660 (49.136)

S.Em = 0.896 C.D. = 2.483 C.V. % = 6.022

Figures in parenthesis are percentage values

Table 6. The influence of explant × medium interaction effect on per cent green colouration of callus.

Media (MS + mg l^{-1})	Hypocotyl	Leaf
MS basal	27.364 (31.744)	30.788 (33.926)
MS + 1.0 IAA	40.652 (39.861)	49.020 (44.705)
MS + 1.0 IAA + 1.0 K	47.308 (43.723)	53.712 (47.489)
MS + 1.0 IAA + 2.0 K	52.896 (46.942)	60.728 (51.545)
MS + 1.0 IAA + 3.0 K	65.940 (55.239)	79.700 (64.928)
MS + 1.0 IAA + 5.0 K	56.584 (49.086)	66.148 (55.402)

S.Em = 0.567 C.D. = 1.571 C.V. % = 6.022

Figures in parenthesis are percentage values

Table 7. The influence of variety × explant × medium interaction effect on per cent green colouration of callus.

Media (MS + mg l-1)	Explants									
	Hypocotyl					Leaf				
	Varieties					Varieties				
	GMO 1	GMO 2	CZM 2	Jwala	RMO40	GMO 1	GMO 2	CZM 2	Jwala	RMO 40
MS basal	26.640 (31.313)	27.020 (31.550)	21.840 (28.122)	27.900 (32.155)	33.420 (35.580)	30.080 (33.495)	34.440 (36.198)	29.700 (33.258)	33.180 (35.418)	26.540 (31.261)
MS + 1.0 IAA	45.100 (42.451)	42.240 (40.781)	39.620 (39.257)	36.540 (37.462)	39.760 (39.363)	45.020 (42.403)	50.960 (45.822)	50.700 (45.673)	54.120 (47.638)	44.300 (41.991)
MS + 1.0 IAA + 1.0 K	60.600 (51.419)	49.580 (45.030)	41.420 (40.307)	42.840 (41.146)	42.100 (40.714)	50.600 (45.615)	56.520 (49.038)	30.960 (34.075)	73.700 (59.531)	56.780 (49.184)
MS + 1.0 IAA + 2.0 K	56.840 (49.220)	58.520 (50.184)	50.680 (45.661)	45.120 (42.465)	53.320 (47.179)	56.640 (49.104)	70.940 (57.713)	60.420 (51.304)	54.580 (47.919)	61.060 (51.685)
MS + 1.0 IAA + 3.0 K	60.900 (51.595)	90.080 (72.175)	61.260 (51.805)	56.620 (49.080)	60.840 (51.539)	63.740 (53.285)	90.620 (73.110)	90.500 (72.880)	89.640 (71.954)	65.000 (53.440)
MS + 1.0 IAA + 5.0 K	60.460 (51.324)	63.320 (53.021)	57.520 (49.619)	51.000 (45.844)	50.620 (45.624)	56.580 (49.062)	90.180 (72.426)	60.320 (51.240)	60.960 (51.633)	62.700 (52.647)

S.Em = 1.267 C.D. = 3.512 C.V. % = 6.022

Table 8. The influence of different media on number of shoots per callus piece.

Media (MS + mg l^{-1})	Mean
MS basal	2.408
MS + 1.0 IAA	2.830
MS + 1.0 IAA + 1.0 K	3.714
MS + 1.0 IAA + 2.0 K	4.736
MS + 1.0 IAA + 3.0 K	5.556
MS + 1.0 IAA + 5.0 K	5.364

S.Em = 6.725 C.D. = 0.186 C.V. % = 11.594

Table 9. The influence of different varieties on number of shoots per callus piece.

Varieties	GMO 1	GMO 2	CZM 2	Jwala	RMO 40
Mean	3.894	4.747	4.203	4.085	3.759

S.Em = 6.139 C.D. = 0.170 C.V. % = 11.594

Table 10. The influence of various explants on number of shoots per callus piece

Explants	Hypocotyl	Leaf
Mean	3.901	4.301

S.Em = 3.882 C.D. = 0.108 C.V. % = 11.594

Table 11. The influence of variety × explant interaction effect on number of shoots per callus piece.

Explants	Varieties				
	GMO 1	GMO 2	CZM 2	Jwala	RMO 40
Hypocotyl	3.940	4.160	3.703	3.783	3.920
Leaf	3.847	5.333	4.703	4.387	3.597

S.Em = 8.681 C.D. = 0.108 C.V. % = 11.594

Table 12. The influence of variety × medium interaction effect on number of shoots per callus piece.

Media (MS + mg l^{-1})	GMO 1	GMO 2	CZM 2	Jwala	RMO 40
MS basal	2.170	2.620	2.380	2.560	2.310
MS + 1.0 IAA	2.570	3.060	2.740	3.030	2.750
MS + 1.0 IAA + 1.0 K	3.150	4.560	3.990	3.500	3.370
MS + 1.0 IAA + 2.0 K	4.570	5.360	4.780	4.810	4.160
MS + 1.0 IAA + 3.0 K	5.110	6.740	5.890	5.130	4.910
MS + 1.0 IAA + 5.0 K	4.710	6.140	5.440	5.480	5.050

S.Em = 0.150 C.D. = 0.417 C.V. % = 11.594

Table 13. The influence of explant × medium interaction effect on number of shoots per callus piece.

Media (MS + mg l^{-1})	Hypocotyl	Leaf
MS basal	2.496	2.320
MS + 1.0 IAA	2.652	3.008
MS + 1.0 IAA + 1.0 K	3.444	3.984
MS + 1.0 IAA + 2.0 K	4.292	5.180
MS + 1.0 IAA + 3.0 K	5.404	5.708
MS + 1.0 IAA + 5.0 K	5.120	5.608

S.Em = 9.510 C.D. = NS C.V. % = 11.594

Table 14. The influence of variety × explant × medium interaction effect on number of shoots per callus piece.

Media (MS + mg l^{-1})	Explants									
	Hypocotyl					Leaf				
	Varieties					Varieties				
	GMO 1	GMO 2	CZM 2	Jwala	RMO 40	GMO 1	GMO 2	CZM 2	Jwala	RMO 40
MS basal	2.480	2.560	2.420	2.600	2.420	1.860	2.680	2.340	2.520	2.200
MS + 1.0 IAA	2.540	2.480	2.860	2.480	2.900	2.600	3.640	2.620	3.580	2.600
MS + 1.0 IAA + 1.0 K	3.480	3.640	3.560	3.220	3.320	2.020	5.480	4.420	3.780	3.420
MS + 1.0 IAA + 2.0 K	4.820	4.300	3.600	4.360	4.380	4.320	6.420	5.960	5.260	3.940
MS + 1.0 IAA + 3.0 K	5.440	6.380	5.300	4.700	5.200	4.780	7.100	6.480	5.560	4.620
MS + 1.0 IAA + 5.0 K	4.880	5.600	4.480	5.340	5.300	4.540	6.680	6.400	5.620	4.800

S.Em = 0.213 C.D. = 0.589

Table 15. The influence of different media on per cent rooting.

Media (MS + mg l^{-1})	Mean
MS + 0.10 NAA	32.188 (34.405)
MS + 0.25 NAA	59.880 (52.127)
MS + 0.50 NAA	78.404 (64.195)
MS + 1.00 NAA	82.760 (67.065)

S.Em = 1.077 C.D. = 3.031 C.V. % = 9.890

Figures in parenthesis are percentage values

Table 16. The influence of different varieties on per cent rooting.

Varieties	GMO 1	GMO 2	CZM 2	Jwala	RMO 40
Mean	64.65 (55.29)	82.64 (67.75)	44.22 (41.64)	73.27 (61.27)	51.76 (46.27)

S.Em = 1.204 C.D. = 3.388 C.V. % = 9.890

Figures in parenthesis are percentage values

Table 17. The influence of variety × medium interaction effect on per cent rooting.

Media (MS + mg l^{-1})	GMO 1	GMO 2	CZM 2	Jwala	RMO 40
MS + 0.10 NAA	23.800 (29.345)	56.840 (49.218)	19.920 (26.804)	32.000 (34.555)	28.380 (32.103)
MS + 0.25 NAA	61.160 (51.842)	89.200 (71.942)	29.240 (32.958)	81.180 (65.343)	38.620 (38.551)
MS + 0.50 NAA	84.280 (68.115)	91.180 (73.710)	60.180 (51.147)	90.200 (73.057)	66.180 (54.858)
MS + 1.00 NAA	89.360 (71.894)	93.340 (76.048)	67.540 (55.650)	89.700 (72.155)	73.860 (59.578)

S.Em = 2.408 C.D. = 6.777 C.V. % = 9.890

Figures in parenthesis are percentage values.