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RESEARCH ARTICLE

EFFECT OF GENOTYPE ON SHOOT REGENERATION FROM HYPOCOTYLS, COTYLEDONS AND LEAF EXPLANTS FROM SIX CULTIVARS OF EGGPLANT (*SOLANUM MELONGENA* L.)

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ABSTRACT

Interaction of genotype and explants with medium, for their in vitro plant regeneration via shoot induction in eggplant (Solanum melongena) to select an elite cultivar with high shoot regeneration frequency for establish a protocol for commercial use in growing tissue culture industries, has been studied. Hypocotyls , cotyledon and leaf explants of six commercially grown Indian cultivars, Pant Rituraj, Punjab Sadabahar, IVBL-9, IVBR-3, IVBR-1, BRSPS-14, which vary in shape, size and color were used in this study. A combination of BA 0.5 mg L^{-1} and Kinetin 0.5 was found to be optimum for shoot regeneration from hypocotyl and cotyledon explants while BA with IBA was found to be optimum for leaf explants. Genotype, explant and genotype- explant interaction had highly significant effects on shoot regeneration with genotype making use of maximum effect on shoot regeneration. IVBR-1 was found to be most responsive genotype for regeneration of shoots from hypocotyls and cotyledon among the genotype while Punjab Sadabahar was found to be most responsive for regeneration of shoots from leaf explants. Among the explants hypocotyls yielded the maximum number of adventitious shoots followed by cotyledon and leaf. Rooting of regenerated shoots was achieved in basal MS medium. Complete plantlets were transferred to soil after initial hardening for 2 weeks under 70-80% relative humidity.

INTRODUCTION

The eggplant, aubergine, or brinjal (*Solanum melongena* L) is a plant of the family *Solanaceae* (also known as the nightshades) and genus *Solanum*. It bears a fruit of the same name, commonly used as a vegetable in cooking. As a nightshade, it is closely related to the tomato and potato and is native to India and Sri Lanka (Tsao and Lo In, 2006), cultivated in countries such as Spain, France, Italy, Greece, North Africa and Asia mostly in India and China. It is a good source of vitamins and minerals (particularly iron) making its total nutritional value comparable with tomato (Kalloo 1993). In India, the eggplant fruit forms an integral part of the diet for the majority of the vegetarian population. Apart from being an important vegetable crop it has been used in traditional medicines (Khan 1979). *In vitro* propagation technique may contribute to the solution of several agronomic problems in this crop, and this fact was successfully achieve by Gleddie *et al* 1983, in tissue and cell culture and protoplast culture by Gleddie *et al* 1985. Organogenesis has been successfully achieved in cultivated and wild varieties and their hybrids. Fassuliotis 1975 was first to report regeneration in *S. sisymbriifolium lam* a wild species of eggplant. One of the most important factors affecting *in vitro* propagation of the eggplant is genotype and genotypic effect (Sharma and Rajam 1995, Alicchio *et al* 1982).Similar effect have been observed in other crops as in red peeper (Christopher & Rajam 1996) and in *Primula vulgaris* (Schween & Schwenkel 2003). The aim of the present study was to observe the effect of genotype of commercially grown Indian cultivar under *in vitro* condition for plant regeneration via organogenesis from hypocotyl, cotyledon and leaf explants of six cultivars of eggplant. Furthermore, attempts have been made to identify a particular genotype and explants which produces of large number of shoots.

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MATERIAL AND METHODS

Seeds of six eggplant cultivars- Pant Rituraj, Punjab Sadabahar, BRSPS-14, IVBR-1, IVBR-3, and IVBL-9 obtained from crop improvement division, Indian Institute of Vegetable Research, Varanasi, India. The seeds were washed with cetrimide solution and rinsed with sterile distilled water for three times in laminar flow. Further the seeds were surface sterilized with commercial bleach sodium hypochlorite (NaOCl) 5%v/v for 10 minutes followed by five times rinsing with sterile distilled water in laminar flow. The sterilized seed were then inoculated on ½ MS basal

(Murashige and Skoog 1962) medium in flask (Scot Duran, Germany) sealed with PARAFILM "M" (AMERICAN NATIONAL Can Chicago, USA) The medium was supplemented with 1.5% (w/v) sucrose and 0.8% (w/v) agar, pH 5.7, sealed flask were kept in dark at 26±1^{0}C temperature, 65 to 70 % humidity and 16/8 hour day/ night photoperiod till the emergence of radical later., the plates were kept in white fluorescent light.

Culture conditions

The flask were incubated at 26±1^{0}C temperature, 65 to 70 % humidity and 16/8 hour day/ night photoperiod. Light was provided at 3000 lux by cool-white fluorescent light.

Organogenesis

Ten days old seedlings from all six cultivars were taken for hypocotyls culture. Hypocotyls explants were excised from the region between sub apical and sub basal.. The cotyledons were cut at their both ends to make the wound at its margin. Two explants were taken from each seedling. Further they were cultured on BA 0.5 mg L^{-1} to 2 mg L^{-1} with kinetin 0.5 mg L^{-1} to 2 mg L^{-1} alone or in combination..Five explants were inoculated in each petriplate (15 mm X 90 mm Scot Duran, Germany) sealed with PARAFILM "M" (AMERICAN NATIONAL Can Chicago, USA). ..Leaf explants were taken from 40 days old seedling for regeneration of shoots under *in vitro* condition. The 10 mm width and 10 mm. length of explants were taken for shoot regeneration. Leaf explants were initially cultured on MS medium supplemented with Kin 0.5 mg L^{-1} to 3 mg L^{-1} and BAP 0.5 mg L^{-1} to 3 mg L^{-1} alone or in combination but these media combination did not yield satisfactorily shoot regeneration so, we further leaf explants was cultured on BA 0.5 mg L^{-1} to 3 mg L^{-1} and IBA 0.2 mg L^{-1} to 1 mg L^{-1} in alone or in combination.. Five explants were cultured on each petriplate. The petriplates were sealed with parafilm. Number of regenerated shoots was counted after 3 weeks.

Rooting of regenerated shoot

The regenerated adventitious shoots obtained after 3 weeks of culture on appropriate medium were transferred to basal MS medium for rooting. The data was analyzed for mean (average) for variables were compared through t-Test ($P < 0.05\%$) with standard error by using statistical package SPSS Version 10.1.

Hardening of rooted plantlet

When shoot attained 7-9 cm. height, they were taken out from test tube; the roots were washed with water to remove the traces of media. They were planted in disposable glass, containing autoclaved soil and sand (1:1) mixture and irrigated with tap water. The plants were covered with polythene bags and kept in culture room under *in vitro* condition, After 12 days polythene bags were removed for 4, 8 and 12 hours on each day subsequently, after that the bags were finally removed and plants were transferred to pots containing soil, sand and yard manure in polyhouse .under 70-80% humidity conditions.

RESULTS AND DISCUSSION

Shoot regeneration

Hypocotyls and cotyledons of all six cultivars were initially cultured on M S medium supplemented with BA or Kin or combined. There was an increase in number of adventitious shoots formed with same concentration of BA and Kin. The effect of Kin for shoot regeneration as well as for number of adventitious shoot was better to BA. The maximum number of adventitious shoots (7.6 per explants) obtained on medium containing BA 0.5 mg L^{-1} with Kin 0.5 mg L^{-1} from hypocotyl explants with cv.IVBL-9. While cotyledon explants yielded maximum number of adventitious shoots (6.9 shoots per explants) on medium BA 2.0 mg L^{-1} with Kin 2.0 mg L^{-1} from cv. IVBR-1.

Table 1. Media combinations (mg/ml) shown by alphabets in Figure No.6 to 9.

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
0	BA 1.0	BA 2.0	BA 3.0	IBA 0.2	IBA 0.5	IBA 1.0	BA 1.0+ IBA 0.2	BA 2.0+ IBA 0.2	BA 3.0+ IBA 0.2	BA 1.0+ IBA 0.5	BA 2.0+ IBA 0.5	BA 3.0+ IBA 0.5	BA 1.0+ IBA 1.0	BA 2.0+ IBA 1.0	BA 3.0+ IBA 1.0

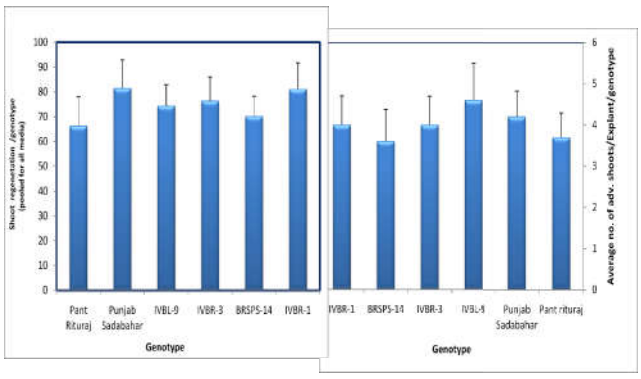
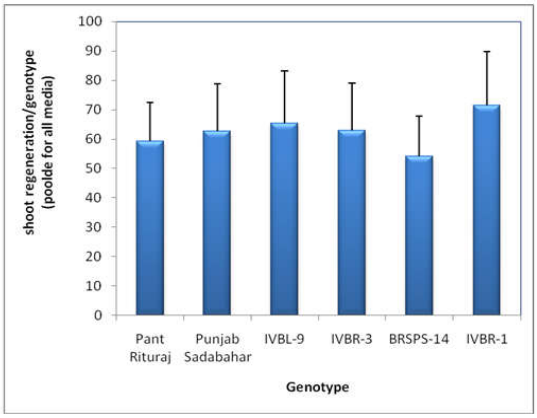


Fig 1 Shoot organogenesis from hypocotyl explants (Data from all media was pooled) vertical bars represent the mean ± SEM.

There was an increase in number of adventitious shoots formed with increasing BA concentration compare to Kin alone, while higher no. of adventitious shoots were obtained at increasing concentration of BA with Kin. Leaf explants were initially cultured on same combination used for hypocotyls and cotyledons explants but shoot regeneration could not achieved so we used MS medium with BA and IBA alone or in combination and obtained significant result. From leaf explants, highest no. of adventitious shoots (5.2 shoots per explants) obtained on medium MS supplemented with IBA 0.2 mg L^{-1} from cv. Punjab Sadabahar. Whereas in case of shoot regeneration from all three explants with all medium was obtained as cultivar IVBR-1 was found for better shoot regeneration from hypocotyls and cotyledons than other genotype. Explants hypocotyl responded better than cotyledons and leaf explants, and yielded more shoots in all



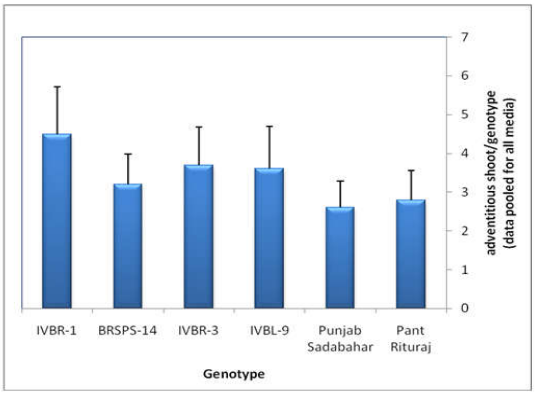


Fig 2. Shoot organogenesis from cotyledon explants (Data from all media was pooled) vertical bars represent the mean ±SEM

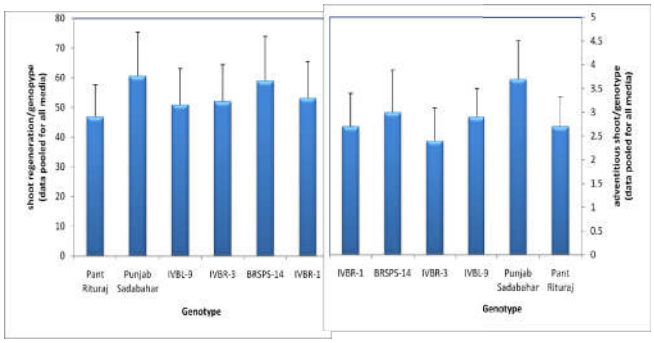


Fig 3 Shoot organogenesis from leaf explants (Data from all media was pooled) vertical bars represent the mean ±SEM.

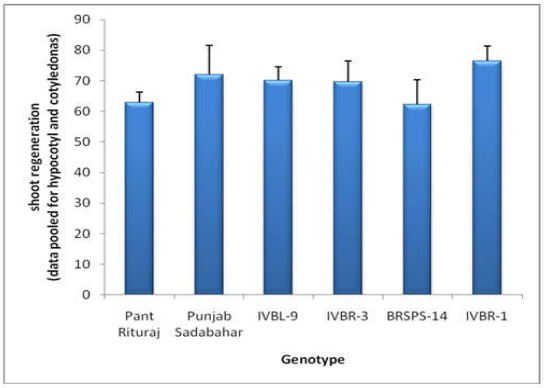


Fig 4. *In vitro* response for shoot regeneration from different genotype (data pooled for all media, hypocotyl s and cotyledons) vertical bars represent the mean ±SEM.

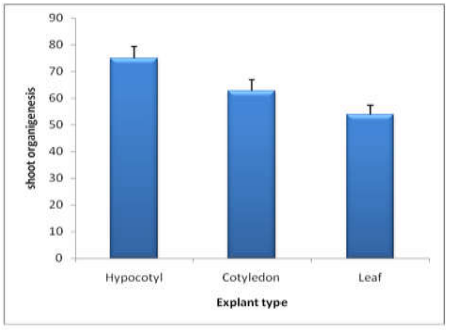


Fig .5 Comparison of shoot organogenesis among three different explants (data pooled for all media and genotype)

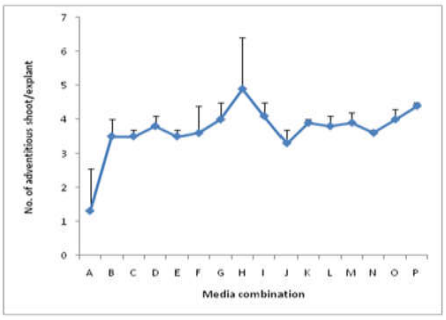


Fig 6. *In vitro* responses of media (data pooled for media, genotype, hypocotyl and cotyledonas) vertical bars represent the mean ±SEM.

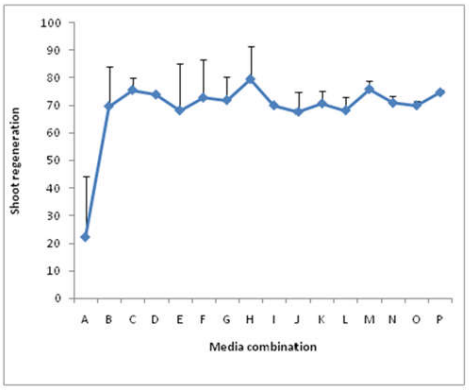


Fig 7. *In vitro* responses of media (data pooled for individual media with all genotype, hypocotyl and cotyledons) vertical bars represent the mean ±SEM.

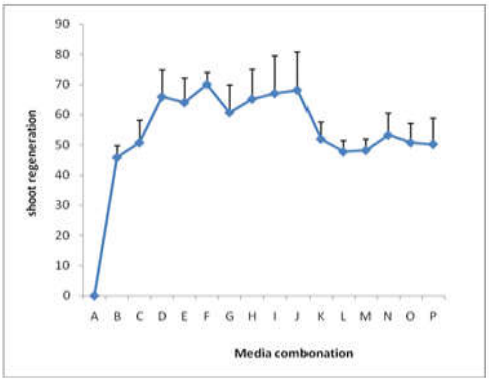


Fig 8. *In vitro* responses of media for shoot regeneration (data pooled for individual media with all genotype of leaf explants) vertical bars represent the mean ± SEM.

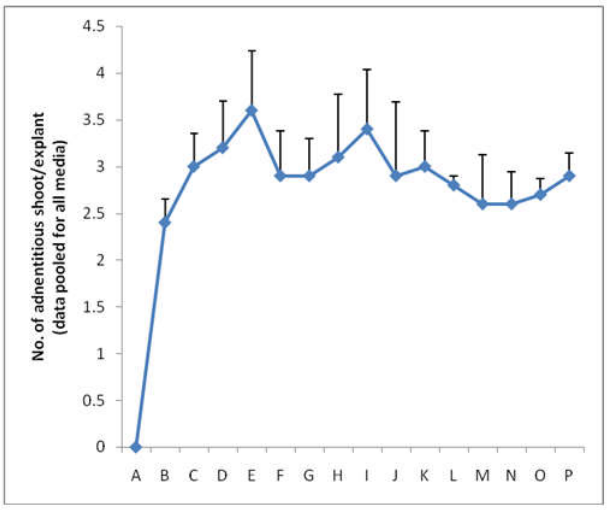


Fig 9. *In vitro* responses of media for no. of adventitious shoot per explant (data pooled for individual media with all genotype of leaf explants) vertical bars represent the mean ± SEM.

different media assessed, media, BA 0.5 mg L^{-1} and Kin 0.5 was finer for inducing shoots from hypocotyl and cotyledon explants from all genotype while IBA 0.5 mg L^{-1} was found most responsive for leaf explants from all genotype (Fig 10).

The adventitious shoots obtained from all three types of explants on all media could be rooted on initially MS basal medium and then on half strength MS basal medium and whole plants were obtained routinely. However (Sharma and Rajam, 1995) was unsuccessful to induce rooting of adventitious shoots regenerated on medium containing BA alone, while in our study, rooting was obtained with all media, whether BA alone or in combination with other phytohormones. This difference may occur due to different genotypes used in two studies. The earlier studies in eggplant reported for mostly for the organogenesis and somatic embryogenesis from hypocotyl (Matsuoka and Hinata 1979), from leaf explant (Gleddie *et al.*1983), from hypocotyl, cotyledonas and leaf (Sharma and Rajam, 1995), LS medium with 2,4-D was used by Allicho *et al*. (1982) for their study on plant regeneration from eggplant genotype,

Fig 10. Shoot regeneration and plantlet formation. (A) shoot regeneration from hypocotyls; (B) Shoot regeneration from cotyledons;(C) shoot regeneration from leaf; (D) rooting of shoots; (E) hardening of plantlet;(F) Regenerated plants in polyhouse.

genotypes analyzed on sixteen different media. This effect may be due to specific interaction between genotype, explants and medium at the same time. Among sixteen

whereas the present study was to focussed on *in vitro* whole plant regeneration from hypocotyl, cotyledonas and leaf explant via direct shoot regeneration and select the elite

genotye with explant and medium for further investigation and other purposes. The present study demonstrate the shoot regeneration from MS basal medium with hypocotyl explants (up to 48.6 %) with all genotype, this is in agreement with earlier report by (Matsuoka and Hinata 1979). Earlier IAA-cytokinin combinations have been used to induce adventitious shoots in eggplant from hypocotyl (Kamat and Rao 1978), hypocotyls, cotyledons and leaf (Sharma and Rajam 1995), leaf explants (Kowalozyk *et al*.1983), while combination of two cytokinin (BA and Kin) was not used before present study for shoot organogenesis from hypocotyl and cotyledon explants, however this combination could not regenerate shoots from leaf explants so we approached new different combination of IBA and BA and yielded shoot regeneration significantly from all six genotype. Kin with IAA combination have earlier been used for shoot regeneration from hypocotyl (Kamat and Rao 1978), while (Gleddie *et al*. 1985), used different concentration of Kin and Zeatin for shoot regeneration from leaf.

When the shoot regeneration response of individual genotypes (pooled for cotyledons and hypocotyls explants) was analyzed, it was seen that the maximum number of shoots was regenerated from IVBR-1 followed by Punjab Sadabahar and IVBL-9 while BRSPS-14, had poor shoot regeneration potential (Fig 4). Genotypic differences have earlier been reported for organogenesis in eggplant (Matsuoka and Hinata 1979; Alicchio et al. 1982; Sharma and Rajam 1995). It has also been observed in tomato (Tan et al. 1987), red peeper (Christopher and Rajam 1996), *Brassica* (Khehra and Mathias 1992), barley (Bregitzer 1992). Among the different explants, the greatest number of shoots (pooled for all genotypes) was obtained from hypocotyls explants, followed by cotyledons and leaves (Fig. 5), this finding is agreed with Sharma and Rajam (1995) but difference in shoot regeneration frequency and number of shoots may be due to different phytohormones and genotypes used in the two studies. However, Alicchio et al. (1982) earlier reported that callus derived from leaf and cotyledon explants had a higher regenerative potential than callus derived from hypocotyls. This may be due to the different basal media and phytohormones used in the two studies. Alicchio *et al.* (1982) used LS medium (Linsmaier and Skoog 1965) with 2,4-D while we have used MS medium with BA and Kin, BA and IBA alone or in combination. Variations in *in vitro* response have been known to occur due to the different basal media (Bregitzer, 1992) and phytohormones used (Wofford *et al.* 1992).Differences may occur due to the different genotypes used in related studies. Interestingly, a higher number of adventitious shoots was obtained from cotyledons rather than hypocotyls in our study in cultivar IVBR1 (Fig.1& 2), while more number of adventitious shoots was obtained from leaf rather than cotyledons in Punjab Sadabahar (Fig.2 & 3). Sharma and Rajam (1995) used BA-IAA combination with MS medium, reported higher number of adventitious shoots obtained from cotyledons rather than hypocotyls in one of cultivars, and this disparity in result, occurred due to selection of different genotypes and media in two studies. In our studies the genotype- explants interaction was found to be highly significant (Fig 4.) and implies that differences exist for the organogenic response of each explants of all the six cultivars studied. Significant interactions between the different components of variation, including *in vitro* morphogenesis have also been observed in soybean (Komatsuda *et al*. 1991, Amberger *et al*. 1992) and barley (Bregitzer 1992). These findings suggest that the IVBR-1 cultivar was the best for shoot regeneration from hypocotyls and cotyledons while cultivar Punjab Sadabahar was most suitable for shoot organogenesis from leaf explants. Data on the average number of shoots from three explants (hypocotyls, cotyledons and leaf of six genotype on sixteen media are presented through Fig 1 to 9. Variation was observed for Individual treatments of explants, genotype and medium significantly affected shoot regeneration in this study. Analysis of variance conducted on the six genotype individually (Fig 1 to 9) revealed that the explant was the primary source of variation for shoot regeneration in six genotype. Analysis of variance has been employed to determine different components of variation in tissue culture studies of red peeper (Christopher and Rajam 1996) *Solanum melongena* (Sharma and Rajam 1995), *Brassica* (Khehra and Mathias 1992), soybean (Komatsuda et al. 1991; Amberger et al. 1992) and barley (Bregitzer 1992). Genotypic differences for the number of shoots regenerated (pooled for sixteen media) was observed in this study (Figure 6 to 9, Table.1). All of the tested genotypes, cv. IVBR-1 regenerated highest number of shoots and cv. Pant Rituraj the least from hypocotyl and cotyledon explants while in case of regeneration of shoots from leaf explants, Punjab Sadabahar regenerated highest number of shoots and Pant Rituraj the least. Such genotypic difference for shoot regeneration has been reported for red peeper (Christopher and Rajam 1996) and for *Solanum melongena* (Sharma and Rajam 1995). Quantitative differences were also observed in the ability of the three explants to produce shoots (data pooled for sixteen media). Hypocotyl explants regenerated more shoots followed by cotyledon and leaf. (Fig 7). Genotype and explant effects on shoot regeneration were highly significant but their interaction had only a minor effect on shoot regeneration (Fig-8). Hypocotyl explants consistently regenerated shoots more than cotyledons and leaf explants across all the genotypes tested (Fig-9). However the degree of the differences among explants differed.

Evaluation of the sixteen media for hypocotyls, cotyledon and leaf, for shoot regeneration (pooled for all media and hypocotyls with cotyledons) revealed that IVBR-1 was most responsive while Punjab Sadabahar was found most responsive for leaf explants Fig.4 & Fig. 3 respectively. In present study, it was found that genotypic characters have strongly affected the shoot induction from all three explants for all eggplant cultivar under *in vitro* condition. Every cultivars has some different specific phenotypic characters so, it is obvious that regeneration of shoot from different cultivars are influenced by their genetic constitution, internal hormonal concentration etc. The developments in the field of biotechnological applications has taken place, the full potential is yet to be exploited for important of eggplant using this regeneration protocol. This success of *in vitro* shoot regeneration makes the use of appropriate genetic transformation program for (*Solanum melongena* L.) specially mentioned varieties of eggplant and direct regeneration may be positive for the stability of new genetic information in any transgenic plant.

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REFERENCES

Alicchio, R., Del Grosso, E., and Boschieri, E. (1982). Tissue culture and plant regeneration from different explants in six cultivars of *Solanum melongena*. *Experientia* 38: 449-450.

Amberger, L. A.,Palmer, R. G. and Shoemaker R.C. (1992). Analysis of culture-induced variation in soybean. *Crop Science* 32: 1103-8.

Arous, S., Boussaid, M. and Marrakchi, M. (2001). Plant regeneration from zygotic embryo hypocotyls of Tunician chili (*Capsicum annum* L.). *J. Appl. Hort.* 3 (1): 17-22.

Bregitzer, P. (1992). Plant regeneration and callus type in barley: effects of genotype and Culture medium. *Crop science* 32: 1108-12.

Christopher, T. and Rajam, M. V. (1996). Effect of genotype, explant and medium on *in vitro* regeneration of red pepper. *Plant Cell Tissue and Org. Cult.* 46: 245-250.

Fassuliotis, G. (1975). Regeneration of whole plants from isolated stem parenchyma cells of *Solanum sismbriifolium*. *J. Am. Soc. Hort. Sci* 100: 636-638.

Gleddie, S., Keller, W. and Setterfield, G. (1983). Somatic embryogenesis and plant regeneration from leaf explants and cell suspensions of *Solanum melongena* (eggplant). *Can. J. Bot.* 61: 656-666.

Gleddie, S., Keller, W. and Settlerfield, G. (1985) Plant regeneration from tissue, cell and protoplast cultures of several wild *Solanum* species. *J. Plant Physiol.* 119: 405-418.

Kallo, G. (1993). Eggplant (*Solanum melongena* L) Genetic improvement of Vegetable crops; *Pergamom Press Oxford* : 587-604.

Kamat, M. G. and Rao, N. A. (1978). Vegetative multiplication of eggplants *Solanum melongena* using tissue culture techniques. *Plant Sci. Lett.* 13: 57-65.

Khan, R. (1979). *Solanum melongena* and its ancestral forms In Hawkes, J., Lester, R, N. and Skelding, A. D. (Eds), The Biology and Taxonomy of Solanaceae; *Academic Press London* : 629-636.

Khehra, G. S. and Mathias, R.J. (1992). The interaction of genotype, explant and media on the regeneration of shoots from complex explants of *Brassica napus* L. *Journal of Experimental Botany* 43: 1413-18.

Komatsuda, T., Kaneko, K. and Oka, S. (1991). Genotype x sucrose interaction for somatic embryogenesis in soybean. *Crop Science* 31: 333-337.

Kowalozyk, T.P., Mackenzie, I.,A. and Cocking, E.C. (1983). Plant regeneration from organ explants and protoplasts of medicinal plant *Solanum khasianum* CB. Clarke var. Chatterjeeanum Sengupta (Syn. *Solanum viarum* Dunal). *Z. Pflanzenphysiol* 11: 55–68.

Matsuoka, H. and Hinata, K. (1979). NAA- Induced organogenesis and embryogenesis in hypocotyl callus of *Solanum melongena* L. *J. Exp. Bot*. 30: 363-370.

Modgil, M., Sharma, D. R., Bhardwaj, S. V. and Khosla K. (1994). *In vitro* propagation of apple (*Malus domestica* Borkh.) cv. Golden Delicious; *Indian J. Hort.* 51: 111-118.

Mukherjee, S. K., Rathnasbapathi, B. and Gupta, N. (1991). Low sugar and osmotic requirements for shoot regeneration from leaf pieces of *Solanum melongena* L. *Plant Cell Tissue and Organ Culture* 25: 12-16.

Murashige, T. and Skoog. F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol. Plant* 15: 473-497.

Rotino, G. L., Falavigna, A. and Restaino, F. (1987). Production of anther-derived plantlets of eggplant. *Capsicum Newsletter* 6: 89-90.

Schween, G. and Schwenkel, H. G. (2003). Effect of genotype on callus induction, shoot regeneration, and phenotypic stability of regenerated plants in the greenhouse of *Primula ssp*. *Plant Cell tissue and Organ Cultur* 72: 53-61.

Sharma, P. and Rajam, M. V. (1995). Genotype, explant and position effects on organogenesis and somatic embryogenesis in eggplant *(Solanum melongena* L.); *J. Exp. Bot.* 46: 135-141.

Tan, M. M. C., Colijin-Hooymans, C.M., Lindhout, W.H. and Kool, A.J. (1987). A comparison of shoot regeneration from protoplasts and leaf discs of different genotypes of the cultivated tomato. *Theoretical and Applied Genetics* 75: 105-8.

Tsao, Lo. (2006) "Vegetables: Types and Biology". Handbook of Food Science, Technology, and Engineering by Yiu H. Hui. *CRC Press* ISBN 1574445510.

Yadav, J. S. (1997). Polyamine-mediated regulation of somatic embryogenesis and lateral root differentiation in eggplant (*Solanum melongena*). Ph.D. Thesis. University of Delhi South Campus, New Delhi, India.

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