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Short communication

Effect of age and size of hypocotyl explant on *in vitro* shoot regeneration in eggplant

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ABSTRACT

In the present study, effect of size and age of hypocotyl explant on *in vitro* organogenetic responses was assessed in eggplant cv. Manjarigota. Size and age did not affect callus-initiation response, but showed marked influence on shoot regeneration response. Hypocotyl explants 1.5cm long showed highest shoot regeneration response (77.4%); either increase or decrease in size resulted in reduced response. Five to 15 day old hypocotyl explants showed direct shoot regeneration from cut ends, whereas 20-30 day old hypocotyl explants showed indirect shoot regeneration from callus produced on cut ends. Five day old explants were most responsive, with highest (91.23%) and thirty day old explants least responsive with reference to shoot regeneration response (20.85%). Shoot regeneration frequency decreased with increasing age, whereas shoot regeneration efficiency increased with increasing age of hypocotyl explants.

Key words: Eggplant, shoot regeneration, age and size, hypocotyl explants

Eggplant (S*olanum melongena* L.) is an important, popular and nutritious vegetable grown in Asia and other parts of the world. Ability of brinjal to respond well to tissue culture has allowed application of various, powerful biotechnology techniques in this crop for management of genetic resources and improvement (Magioli and Mansur, 2005). Several studies on morphogenetic potential in brinjal have been undertaken to demonstrate relative importance of explant type, genotype and growth regulators in *in vitro* regeneration response. Bhatia *et al* (2004) reported optimum age and size of explants to be important for achieving good regeneration in tomato.

Curuk *et al* (2002) reported that young hypocotyl explants showed rapid shoot regeneration. Cell aggregate size (total cell mass) is also important in determining organogenetic competence, cell division and further response of an explant (Compton, 2000). However, no effort has been made to assess and document the effect of age and size of explant on shoot regeneration response in eggplant. Manjarigota is a round, striped, tastier and preferred brinjal variety in India, and hypocotyl explant was seen to be highly responsive to regeneration (Prakash *et al*, 2008). Hence, we made an effort to document the effect of size and age of hypocotyl explants on *in vitro* morphogenetic response in brinjal cv. Manjarigota.

Breeder seed of eggplant cv. Manjarigota was obtained from Division of Vegetable Crops, IIHR, Bangalore during 2003-05 and 2008-10 experimental periods. Aseptic cultures of explants were raised as per the protocol of Prakash *et al* (2007). Hypocotyl segments (after removing apical meristem and the basal root stubs) were cultured on Shoot Regeneration Medium as per Murashige and Skoog (1992) [SRM: full strength MS major and minor salts, organics and vitamins, 3% sucrose (w/v), 0.8% agar (w/v), 2μM BAP and 0.05μM NAA]. pH of the medium was adjusted to 5.75±0.05 prior to autoclaving. Cultures were incubated at 25±1°C with 16/8h photoperiod under light intensity of 30-40 $\mu E m^{-2} s^{-1}$. To study the effect of size, hypocotyl explants of different sizes (short 0.5cm, medium 1.0cm, long 1.5cm and very long 2.0cm) were cultured on SRM in 20 replications in glass petri dishes twice over. To study the effect of age, hypocotyl explants of different ages (5, 10, 15, 20, 25 and 30 days after germination) were cultured on SRM in four replications in petri dishes and the experiment was repeated. Data were subjected to ANOVA to test significance of the observed results. Comparison between mean values of treatment was made by LSD to identify best treatments.

The best combination of cytokinin (BAP) and auxin

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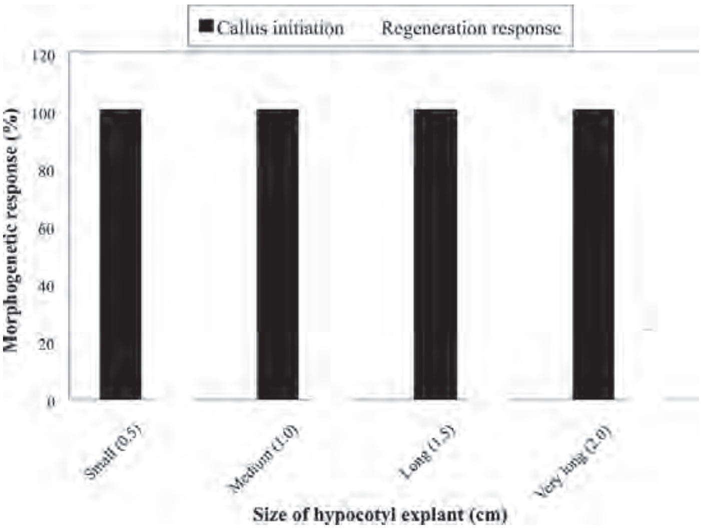

Fig 1. Effects of size of hypocotyl explants on organogenetic response in eggplant cv. Manjarigota (CD for regeneration response - 14.15; Significant at $P \leq 0.01$)

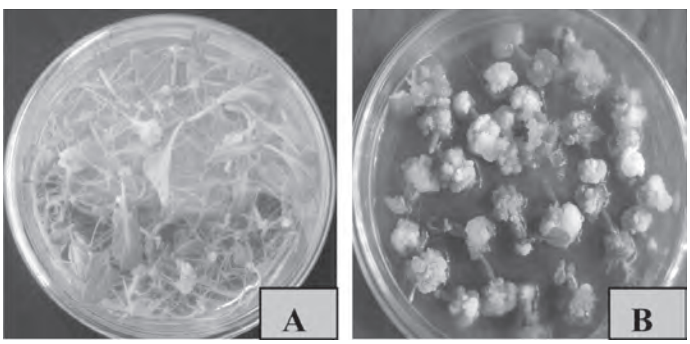

Fig 2. Organogentic response in (A) 5-day old (B) 30-day old hypocotyl explants of eggplant on shoot regeneration medium

(NAA) obtained from among the various concentrations tested (Prakash *et al*, 2008) was used for inducing shoots in hypocotyl explants. Size of hypocotyl explant did not affect callus initiation response, but, significantly affected shoot regeneration. Long-sized (1.5cm) hypocotyl explants were most responsive (77.4%) (Fig.1). Decrease or increase in size of the explant resulted in reduced regeneration response. Similarly, Frary and Earle (1996) reported reduction in shoot regeneration response with increasing or decreasing size of explants, whereas, 1 cm long hypocotyl explants were optimum for shoot regeneration in tomato (Bhatia *et al*, 2004). In our study, explants of all ages showed callus initiation response (100%) after 3-5 days of culture (data not shown). Hypocotyl explants 5-15 old days showed direct shoot regeneration from the cut end (Fig. 2-A) after 13-15 days of culture. These gave rise to shoots at one end, and adventitious roots at the other end, indicating polarity. Hypocotyl explants 20-30 days old gave rise to shoots from profuse and compact callus produced at the cut ends after 3-4 weeks of culture. Furthermore, age significantly affected both frequency (No. of explants showing regeneration response/No. of explants cultured) and efficiency (Average No. of shoots per explant) of shoot regeneration on the hypocotyl explant (Table 1). Explants five and 30 days old showed highest (91.23%) and lowest (20.85%) shoot regeneration response, respectively. Shoot regeneration frequency decreased with increase in age, whereas, efficiency increased with increase in age of hypocotyl explants. Thirty days old explants showed highest shoot regeneration efficiency (1.9/explant). This may be perhaps due to the ability of young, undifferentiated or recently differentiated cells of hypocotyl explants to redirect their developmental programme and, thereby, produce new shoots faster than do old explants (Curuk *et al*, 2002).

Tissues are known to lose organogenetic competence with increase in age (Pastori *et al*, 2001) and, in such cases, intermediate callus phase appears to be necessary for organogenesis. The intermediate callus phase may be responsible for the delayed response in older explants. Similarly, in previous studies on eggplant, callus-mediated shoot regeneration response appeared after 3-4 weeks of culture on 4-6 week old explant, irrespective of genotype, explant type and growth regulators (Magioli and Mansur, 2005). Murashige (1974) reported that ratio of the phytohormones in culture medium controls organogentic response of a tissue in a crop plant. However, in our laboratory, five-day-old hypocotyl explants showed shoot regeneration from cut ends without callus phase on all growth regulator combinations tested (Prakash *et al*, 2008). Therefore, under the present culture conditions, age of explant was found to be a critical factor that decided the mode of organogenesis *in vitro* and frequency of shoot regeneration in eggplant.

Table 1. Effect of age of hypocotyl explant on organogenetic response in eggplant cv. Manjarigota

Age of hypocotyl explant (Days old)	Regeneration frequency*(%)	No. of shoots/ explants
5	$91.23^{a} \pm 0.019$	$1.076^{a} \pm 0.033$
10	$73.75^{b} \pm 0.019$	$1.096^{ba} \pm 0.033$
15	$72.42^{bc} \pm 0.016$	$1.125^{b} \pm 0.024$
20	$60.69^{bc} \pm 0.016$	$1.380^{b} \pm 0.020$
25	$36.97^{d} \pm 0.022$	$1.610^{b} \pm 0.046$
30	$20.85^{d} \pm 0.016$	$1.900^{b} \pm 0.126$
SEM	3.58	0.062
CD at *P:0.01*	10.87	0.33

Values are mean ± SE of 15 replications; *Fractions were converted into percentage; percentage data was subjected to angular transformation before analysis

In conclusion, use of optimum-size explants appears to be necessary for obtaining good shoot regeneration. Young hypocotyl explants were rapid and hastened the process of shoot regeneration. This can save time and other inputs in future basic research and crop improvement studies in eggplant.

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