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BRIEF COMMUNICATION

High frequency *in vitro* regeneration of *Lathyrus sativus* L.

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Abstract

A simple and efficient protocol for high frequency plant regeneration of a grain legume grasspea (*Lathyrus sativus* L.) is described. Of different explant types tested epicotyl segments were most responsive. Murashige and Skoog's (1962) medium augmented with 17.76 μM 6-benzyladenine + 10.74 μM α-naphthaleneacetic acid showed the highest percentage of direct shoot regeneration. Among cultivars IC-120487 showed the highest regeneration frequency (80 %) with maximum shoot numbers (8.2 shoots per explant) and maximum average shoot length (4.1 cm). About 78 % of the regenerated shoots were rooted in half-strength MS medium containing 2.85 μM indole-3-acetic acid. After primary hardening the plantlets were established in soil with a survival rate of 75 %.

Additional key words: adventitious regeneration, epicotyl segment, grain legume, grasspea.

Grasspea (*Lathyrus sativus* L.) is an important grain legume crop in several tropical and sub-tropical countries and is well-adapted to adverse agricultural condition such as flooding, drought, salinity, low soil fertility and pathogen-infested soil. Unfortunately, its potential has not been fully utilized because of the presence of a neurotoxic amino acid, β-N–oxalyl-L-α,β-diamino-propionic acid (ODAP) (Sachdev *et al*. 1995).

Agrobacterium-mediated genetic transformation aimed at reduction of ODAP could bring forth a genetic improvement of this crop (Zambre *et al.* 2002). In this paper we report a high frequency direct-adventitious plant regeneration protocol using epicotyl segments of grasspea that could be useful for *Agrobacterium*-mediated genetic improvement of this hitherto under-exploited crop.

Five cultivars of grasspea (*Lathyrus sativus* L.) were used, of which seeds of four cultivars namely IC-120451, IC-120453, IC-120478, IC-120487 were obtained from the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. A local cultivar "Nayagarh local" was collected from the Pulses Research Station, Nayagarh, Orissa, India. Seeds were kept under running tap water for 30 min followed by 8-min treatment with 5 % (v/v) aqueous solution of *Teepol* (*Reckitt's Colman*, Kolkata, India) and rinsed 5 - 6 times with double-distilled water. Then the seeds were surface-sterilized with 0.1 % (m/v) aqueous solution of mercuric chloride for 5 min followed by five rinses in autoclaved double distilled water. The surface-sterilized seeds were inoculated in 300 cm^3 screw-capped jars (*Excel corporation*, Alleppey, Kerala, India) containing MS basal medium gelled with 0.8 % (m/v) agar (*Hi-media*, Mumbai, India). The pH of the medium was adjusted to 5.8 before autoclaving at 121 °C and 104 kPa for 20 min. The seeds were allowed to germinate at temperature of 25 ± 1 °C and photon flux density of 35 μmol m^{-2} s^{-1} provided by cool white fluorescent tubes (*Philips*, Bangalore, India) and 60 % relative humidity.

Five different explant types (cotyledon, hypocotyl, epicotyl, internode and leaf) harvested from 7-d-old axenic seedlings were used for plant regeneration. Segments of these explants from all the five cultivars,

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Abbreviations: BA - 6-benzyladenine; IAA - indole-3-acetic acid; IBA - indole-3-butyric acid; IPA - indole-3-propionic acid; MS medium - Murashige and Skoog's (1962) medium; NAA - α-naphthaleneacetic acid.

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after having removed the meristematic region, were inoculated in 300 cm^3 glass jars (4 explants per jar) containing Murashige and Skoog's (1962; MS) medium supplemented with growth regulators 2.22 - 22.19 μM 6-benzyladenine (BA) or 2.69 - 16.11 μM α-naphthalene-acetic acid (NAA) alone or in combinations. The pH of all the media was adjusted to 5.8 before gelling with 0.8 % agar. The original epicotyl segments were sub-cultured twice on shoot multiplication medium (MS + 17.76 μM BA + 10.74 μM NAA) after each harvest of the shoots. All the cultures were maintained under similar conditions as described earlier for seed germination. Well-developed shoots (3.5 - 4.0 cm) were excised and transferred to 30 cm^3 culture tubes (*Borosil*, Mumbai, India) containing half-strength MS medium gelled with 0.8 % (m/v) agar. The medium was augmented with 1.42 - 11.42 μM indole-3-acetic acid (IAA) or 1.23 - 9.8 μM indole-3-butyric acid (IBA), or 1.32 - 10.57 μM indole-3-propionic acid (IPA). After 5 - 7 d of root initiation the rooted shoots were transferred to auxin-free half-strength MS medium for further elongation of roots.

For shoot proliferation experiment each treatment consisted of 5 replicates (culture vessels) and the experimental unit was four explants per vessel. In the rooting experiment, each treatment consisted of 11 replicates (culture tubes) and one explant per experimental unit. Each experiment was repeated thrice at an interval of 4 days. Visual observations of cultures showing shoot differentiation and the number of shoots per explant, shoot length, root number and root length were recorded after 30 d.

Plantlets with well developed roots were removed from culture tubes and, after washing the roots in running tap water, they were transferred to plastic pots (7.5 cm diameter) containing autoclaved *Vermi-compost* (*Ranjan's Agrotech*, Bhubaneswar, India). Potted plantlets were covered with polyethylene bags to maintain high humidity and kept in the culture room at tmperature of 25 ± 1 °C and photon flux density of 50 μmol m^{-2} s^{-1}. After 15 d plantlets were transferred to larger earthenware pots (18 cm diameter) containing soil:compost (1:1) and kept under shade for another 10 d before transplanting in the field.

None of the five different explant types regardless of cultivar was able to regenerate shoots on MS basal medium free of growth regulators. A cytokinin supplement to MS was essential for inducing adventitious shoot proliferation. BA alone at the range of 4.44 - 22.19 μM was effective; 17.76 μM elicited the highest response. A combination of NAA with BA improved the percentage of shoot regeneration as well as the shoot number and shoot length. The highest response was obtained in MS fortified with 17. 76 μM BA + 10.74 μM NAA. Shoot development was enhanced with increments in the concentration of NAA and BA upto a certain threshold beyond which the frequency decreased as was observed in another grain legume *Vigna unguiculata* (Pellegrineschi 1997).

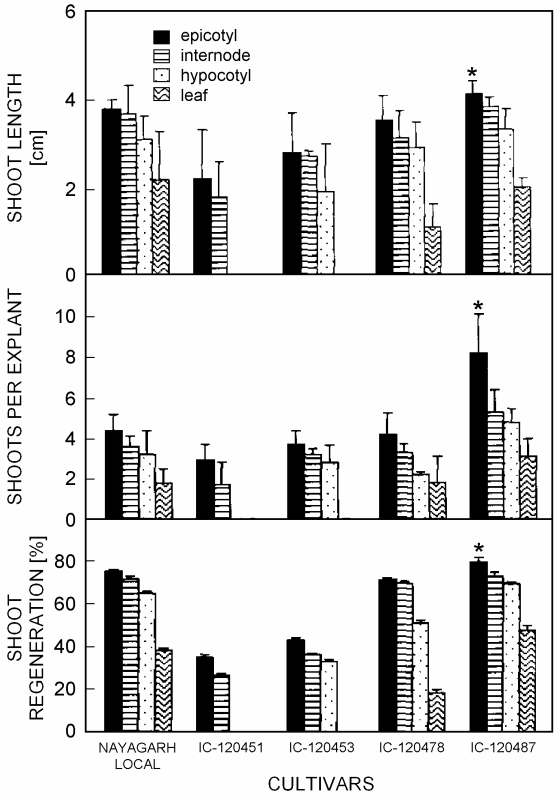

Fig. 1. Response evaluation of explants and cultivars of grasspea with respect to shoot regeneration on MS + 17.76 μM BA + 10.74 μM NAA. Means ± SE, * - values significantly different at $P \leq 0.05$ from corresponding ones.

Of the five different explant types evaluated for culture response on the shoot regeneration medium (MS + 17.76 μM BA + 10.74 μM NAA) epicotyl segments showed the highest shooting efficiency (80 %) with maximum shoot number (8 - 12 shoots per explant) and longest average shoot length (4.1 cm) (Fig. 1). Cotyledon segments failed to produce shoots. The five grasspea cultivars examined in the present study exhibited various response to the shoot regenerating culture medium (Fig. 1). The cultivar IC-120487 had the highest recorded performance with respect to shoot development frequency, shoot number and length. A relationship between plant regeneration capacity and cultivar was drawn in case of cowpea (*Vigna unguiculata*) in which it was related to the differential endogenous hormone contents (Pellegrineschi 1997).

Highest frequency of rhizogenesis (78.7 %), with maximum root number (3.6 roots per shoot) and longest average root length (3.9 cm) was obtained with IAA at 2.85 μM. Auxin concentrations exceeding 5.7 μM showed a reduction in rooting response. No root formation was observed when shoots were cultured in the

medium devoid of auxins. Nevertheless, roots were able to elongate only after the rooted shoots were transferred to half-strength MS lacking the auxin. In general, auxins are known to promote root induction, but they inhibit subsequent root growth if allowed to persist in the culture medium (Gulati and Jaiwal 1994, Polisetty *et al.* 1997).

The plantlets regenerated *in vitro* were successfully acclimatized in *Vermi-compost* and eventually in soil. Sixty three percent of plantlets survived following transfer to *Vermi-compost* and 75 % of these plants transferred to soil survived.

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