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Short Communication

Callus mediated multiple shoot organogenesis from stem explant of grasspea (*Lathyrus sativus* L.)

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Grasspea (*Lathyrus sativus* L) is a potential grain legume crop for adoption to dry land agriculture and saline soil. Seeds of grasspea are rich in protein (26-30%) as well as lysine. However, this crop remains underutilized due to the presence of a neurotoxin, β-N-oxalyl-L-α, β-diaminopropionic acid (BOAA) in seed storage protein, which causes neurolathyrism and osteolathyrism to human beings and farm animals [1]. Unsuccessful for development of varieties with low BOAA content through conventional breeding methods due to its narrow genetic base; advance biotechnology techniques such as somaclonal variation, mutagenized tissue culture, somatic hybridization and genetic engineering have been utilized as potential tools in addition to conventional plant breeding for genetic improvement of many crop plants. Present experiment was conducted for genetic improvement of grasspea with low BOAA content and for agronomically desirable types.

The seeds of two grasspea genotypes (Nayagarh local and 1C-120487 procured from Pulse Research Center, Nayagarh, Orissa and NBPGR, New Delhi, respectively) were treated with 0.1 % (w/v) aqueous solution of $HgCl_2$ (Hi-media, Mumbai) for 5 min and rinsed five times with sterilised double distilled water. The sterilised seeds were germinated aseptically on half-strength Murashige and Skoog's basal medium (1/2 MS) [2] supplemented with 30.0 g/l sucrose and gelled with 8.0 g/l agar powder with pH-5.8 at a temperature of 25 ± 1°C, l6hr photoperiod (18 μ mol m^{-2} s^{-1}) provided by white fluorescent tubes (Phillips, India). Different explants such as epicotyl (upto 1 cm below from the cotyledonary node), hypocotyl (small portion between cotyledonary node and first leaf), stem (portions above the first leaf) and leaves were excised from seven-day-old axenically grown seedlings and cultured on MS medium supplemented with different concentrations of α-naphthalene acetic acid (NAA) 1.0-5.0 mg/l and N^6-benzylaminopurine (BAP) 0.1-0.5 mg/l for callus induction and proliferation (25 combinations tested). The calli were sub-cultured at 15 days interval and 45 days old calli were used for morphogenesis. Green compact calli were cultured on MS with different concentrations of BAP 0.5-2.0 mg/l or kinetin (Kn) 1.0-6.0 mg/l for caulogenesis (15 combinations tested). For rhizogenesis, twenty days old *in vitro* shoots with 3-4 fully expanded leaves were excised and cultured on 1/2 MS medium supplemented with different concentration (0.25-2.0 mg/l, 8 combinations tested) of auxins i.e. indole-3-acetic acid (IAA) or Indole-3-butyric acid (IBA).

For callus induction, each treatment consisted of 20 explants with three replications and observations were recorded for callus induction frequency (%), fresh weight of calli after 15, 30 and 45 days of inoculation. The relative growth rate (RGR) of callus was estimated as per [3] as under;

$$\frac{\text{Change in weight}}{\text{Initial weight} \times \text{Time interval}}$$

For caulogenesis, 10 callus cultures per treatment were used with three replications and observations on caulogenesis frequency (%), number of shoots/callus and shoot length were recorded. For rhizogenesis, 10 *in vitro* derived shoots were used per treatment and observations on rhizogenesis frequency (%), number of roots/shoot and root length was recorded. The data were analyzed statistically by randomized block design to study the significance of various parameters.

Out of 25 combinations of growth regulators, MS + 3.0 mg/l NAA + 0.3 mg/l BAP found to be the best for callus experiment regardless to explants and genotypes Among the explants, stem explant was found most suitable for callus induction in genotype 1C-120487 (Fig. 1A) followed by leaf, hypocotyl and epicotyl explant (Table 1). Though the fresh weight of calli increased with days to inoculation, RGR values were maximum at 30 days. Statistical analysis showed significant differences between the genotypes, callusing media and explants. Compact green calli derived from stem explant were sub-cultured on the same medium at 15 days interval for shoot initiation. It was observed that MS + NAA + BAP/Kn did not show any sign of shoot induction in both the genotypes. Shoot bud initiation was achieved by the complete elimination of NAA and singular supplement of higher concentration of BAP or Kn. It revealed that auxin-cytokinin combinations did not induce shoot, while only cytokinins promoted shoot induction.

Table 1. Explant evaluation of grasspea genotypes with respect to different combinations of growth regulators (NAA & BAP) on the basis of callus induction frequency, callus fresh weight gain and relative growth rate (RGR) values.

Parameters	Genotype: IC-1 20487				Genotype : Nayagarh Local			
	Epicotyl	Hypocotyl	Leaf	Stem	Epicotyl	Hypocotyl	Leaf	Stem
Average callus induction frequency %	60.8	63.4	68.6	72.7	59.4	63.0	63.5	69.5
	(51.5)	(52.8)	(56.2)	(58.8)	(50.6)	(52.7)	(53.0)	(568)
Average fresh weight gain (gm) of callus:								
(a) at 1 5 days	0.008	0.014	0.016	0.025	0.006	0.011	0.018	0.019
(b) at 30 days	0.086	0.117	0.152	0.216	0.081	0.113	0.138	0.183
(c) at 45 days	0.090	0.123	0.156	0.219	0.087	0.120	0.150	0.187
Average RGR value of callus growth								
(a) at 1 5 days	0.06	0.11	0.15	0.29	0.04	0.09	0.18	0.19
(b) at 30 days	0.33	0.53	0.98	1.41	0.29	0.49	0.89	1.19
(c) at 45 days	0.29	0.42	0.67	0.95	0.24	0.38	0.65	0.81
Maximum callus induction frequency %	78.3	80.1	90.0	91.7	76.7	83.3	86.7	90.0
on MS + 3.0mg/l NAA + 03 mg/l BAP	(62.3)	(63.4)	(72.0)	(73.4)	(61.1)	(66.0)	(68.7)	(72.0)
Maximum RGR of calli/day on MS + 3 0 mg/l NAA + 0.3 mg/l BAP on 30 days	1.39	2.03	3.53	6.15	1.12	1.92	2.80	5.52

(Figures in parenthesis indicate angular values)

In contrary to these present findings, auxin-cytokinin combinations supplemented to the BM [4] and SS-B-8 [5] basal media were reported to be beneficial for shoot induction. On average, BAP showed a better response to caulogenesis as compared to Kn. The superior response of BAP over Kn for shoot induction due to endogenous production of zeatin [6]. MS + 1.0 mg/l BAP showed highest caullogenesis percentage 63.3 and 70.0, shoots/callus 4-6 and 6-8 and mean shoot length 3.1 cm and 4.3cm in Nayagarh local and 1C-120487, respectively (Fig. 1B).

No root was formed when 20 days old *in vitro* shoots of 3.0 4.0 cm length were inoculated to the basal media (full, half and quarter strength) lacking auxins where as, 1/2 MS + IAA/IBA showed root initiation. 1/2 MS + 0.5 mg/l IAA showed highest rhizogenesis frequency % (63.3), roots/shoot (3-4) and root length (4.5-4.9 cm) in the genotype 1C-120487 (Fig. 1C) Increase in the concentrations of auxins beyond 0.5 mg/1 for IAA and 1.0 mg/1 for IBA reduced the rooting response. It is because auxins may promote root initiation but they can inhibit subsequent root growth, therefore a transient exposure to hormone was more effective.

The rooted plants were maintained on the same medium for 10-15 days. Then the plantlets were transferred to pots containing vermi-compost moistened with water, covered with polyethylene bags with minute holes and the potted plants were hardened inside a plant growth chamber set at 25 ± 1°C temp, 85-90% relative humidity and l6hr photo-period 50 μ mol. M^{-2} s^{-1} provided by cool white fluorescent tubes for three weeks. The hardened plants were then transferred to large pots containing garden soil and kept under shade for another 2 weeks before their transfer to the garden with a survival rate of 82 % (Fig. 1D).

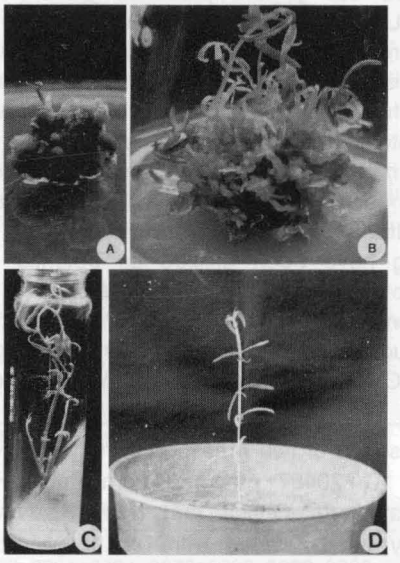

Fig. 1. (A) Callus initiation and proliferation on MS + 3.0 mg/l NAA + 0.3 mg/l BAP from stem explant of grasspea genotype IC-120487 (B) Shoot bud multiplication and elongation on MS + 1.0 mg/l BAP, (C) Rooted in vitro shoot on 1/2 MS + 0.4 mg/l IAA, (D) Acclimated plant in plastic pot

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