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Short communication

Callus induction and proliferation from *Rosa hybrida* leaf explants

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ABSTRACT

To establish a resourceful tissue culture system to induce anthocyanin pigments, research was conducted to obtain high quality callus from leaf explant of *Rosa hybrida* cv. Pusa Ajay. Of the different treatments employed for callus induction using Murashige and Skoog (MS) medium, a treatment combination of 19.60 µM indole-3-butyric acid (IBA) + 4.65 µM kinetin (Kin) + 108.6 mM adenine sulphate (AdS) gave the maximum induction coefficient (97.75 ± 1.11%) along with maximum gain in callus biomass accumulation. This treatment also resulted in the lowest fresh- and dry- cell weight ratio (26.52) suggesting actual gain in callus biomass accumulation. Correlation analysis indicated a significant positive correlation between callus induction coefficient and cell fresh- (r = 0.939) and dry- (r = 0.951) weight.

Key words: Callus, secondary metabolites, plant growth regulators, *Rosa hybrida*.

Plant tissue culture offers lot of opportunities in ornamental crops including roses. Tissue culture response in roses which include callus induction capacity is influenced by the genotype, explant source and physiological status of the donor plants, the culture medium and the interactions between them (Özgen *et al.*, 5). There are few reports on tissue culture of rose especially on the callus induction and its subsequent use for the secondary metabolite production. The present work was, therefore, undertaken in order to optimize the tissue culture medium for profuse callus induction from leaf explant of *Rosa hybrida* cv. Pusa Ajay. The comparative effects of PGRs on callus induction were assessed for the rapid callusing, callogenesis coefficient and callus growth.

The newly emerged shoots mother plants were collected from the Floriculture Research Farm, IARI, New Delhi. Freshly harvested young leaves were immersed in tap water containing 0.1% Tween20® and later on rinsed under running tap water for 10 min. These were then agitated in 0.1% carbendazim, 0.1% mancozeb-45 and 200 mg l^{-1} 8-HQC for 3 h on a horizontal shaker (100 rpm). Surface sterilization was carried out using 0.1% $HgCl_2$ for 4 min. The sterilized explants were excised into 5 × 5 mm in size and transferred aseptically onto the culture medium in the test tube. For callus induction, Murashige and Skoog (4) basal medium supplemented with 30 g l^{-1} sucrose was used. The potential of five different PGRs (Table 1), *viz.*, BAP, 2,4-D, kinetin, IBA and adenine sulphate were analyzed for callus induction. The media pH was adjusted to 5.8 ± 0.1 before solidifying with 5.5 g l^{-1} agar-agar and autoclaved. All the cultures were kept in controlled environment in a culture room at 24 ± 1°C under complete darkness.

Table 1. Concentrations and combinations of plant growth regulators used for callus induction.

2,4-D (µM)	IBA (µM)	kinetin (µM)	BAP (µM)	AdS (mM)
–	MS (control)			
–	19.60	4.65	–	–
–	19.60	4.65	–	108.60
18.08	–	–	4.44	–
18.08	–	–	4.44	108.60
18.08	–	–	8.88	–
18.08	–	–	8.88	108.60

The relative induction of callus was determined as: induction coefficient = (total No. of induced calli/ total No. of cultured explants) × 100. To assess the growth rate of callus, 42-day-old callus masses were harvested and fresh- and dry-cell weight (FCW, DCW) were taken. Dry cell weight was obtained after drying calluses in a hot air oven at 45°C for the first 24 h and 55°C for the next 24 h so as to achieve constant the weight. Callus growth status was classified as described by Matkowski (2). The data were subjected to analysis of variance using the SPSS 16.0 programme (SPSS Inc., Chicago, USA). Means were separated by Tukey's Honestly Significant Difference (HSD) test at $P \leq 0.05$. Correlation between days to callus initiation, induction coefficient percentage,

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fresh- and dry-cell weight and their ratio were also computed.

In this experiment, coefficient for callogenesis recorded approximately in the range of 0 to 98% of explants, depending on auxin type, concentration and their combination with cytokinins. Leaf segments cultured on MS medium containing IBA + Kin + AdS formed early and more callus than the other treatment combinations (Table 2). This particular treatment combination gave rise to luxuriantly growing callus with the highest induction coefficient (97.75 ± 1.11%) with minimum days (≈6) for callus induction.

It was noted that addition of adenine sulphate (AdS) showed a synergistic effect with other tested PGRs which increased the induction coefficient and callus, therefore, enhanced the process of callogenesis (Table 2, Fig. 1). It is suggested that adenine acts as a precursor for natural cytokinin synthesis or enhances natural cytokinin biosynthesis. Furthermore, it may also act as a synergist of cytokinins such as kinetin and zeatin. Auxin: cytokinin combination produces a superior, healthy and vigorous callus with high induction response (Table 2, Fig. 1). Such responses, in many species have been established previously (Shen *et al.*, 7). It is proposed that the callogenesis in *R. hybrida* cv. Pusa Ajay could be promoted by combining intermediate auxin and low cytokinin concentrations. The growth status of callus culture on MS medium supplemented with different PGR treatments, only IBA + Kin +AdS treatment combinations gave the Type-IV callus (Table 2). The fresh- and dry- cell weight were significantly affected by the various concentrations and combinations of tested PGRs in the culture medium. Fig. 1 shows that treatment combination of IBA + Kin + AdS resulted in a high cell dry biomass production (14.40 ± 0.05 mg).

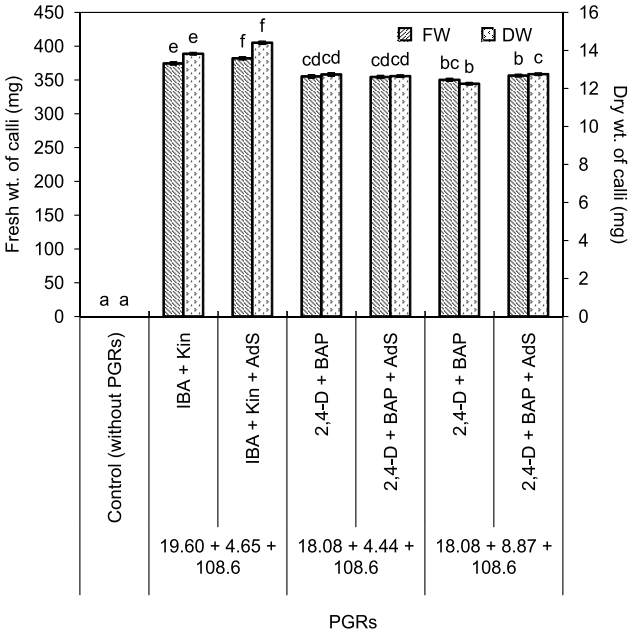

Fig. 1. Callus biomass accumulation from leaf explants, after 42-day of culture. Same letters on the bar figures did not differ significantly. The concentrations of 2,4-D, Kin, IBA and BAP are in µM and AdS is in mM.

Notwithstanding only with the fresh- and dry-cell weight, the ratio of fresh- to dry-cell weight (FCW: DCW), an index of cell water content, was also calculated to know the actual gain in callus biomass accumulation. Between the different concentrations and combination of PGRs tried, treatment combination of 19.60 µM IBA + 4.65 µM Kin + 108.60 mM AdS resulted in the lowest FCW: DCW ratio (26.52), which indicated that the callus produced was not watery and gained better biomass accumulation. The high correlation

Table 2. Effect of different PGRs on callus induction and multiplication. Means within a column that did not differ significantly are followed by the same superscript letters. The value in parenthesis indicates the concentrations of PGRs used in unless otherwise stated.

Treatment (µM)		Days to callus initiation	Induction coefficient (%)	Growth status
MS (without PGRs)		0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	–
IBA + Kin + AdS (mM)	(19.60) + (4.65)	7.50 ± 0.29^{c}	92.50 ± 1.19^{bc}	III
	(19.60) + (4.65) + (108.6)	5.75 ± 0.25^{b}	97.75 ± 1.11^{d}	IV
2,4-D + BAP + AdS (mM)	(18.08) + (4.44)	11.25 ± 0.48^{de}	88.25 ± 1.55^{b}	II
	(18.08) + (4.44) + (108.6)	11.00 ± 0.41^{de}	89.50 ± 1.50^{b}	III
	(18.08) + (8.87)	10.50 ± 0.29^{de}	88.75 ± 1.49^{b}	II
	(18.08) + (8.87) + (108.6)	10.25 ± 0.25^{de}	89.00 ± 1.35^{b}	III
HSD ($P \leq 0.05$)		1.04	4.69	

observed between the callus induction coefficient per cent, fresh- and dry-cell weight and weight ratio indicate that explants which provide good callus induction constitute a good index for callus biomass accumulation (Table 3). The direct correlations between different parameters such as explant type, callus induction, callus biomass accumulation and regeneration capacity were reported earlier in some plant species by researchers (Gandonou *et al.*, 1; Mohebodini *et al.*, 3).

Table 3. Pearson's correlation coefficient between different parameters recorded for leaf derived callus induction and multiplication of *R. hybrida* cv. Pusa Ajay.

Parameter	DCI	ICP	FCW	DCW	FCW: DCW R
DCI	1.000	0.496	0.711[b]	0.652[a]	0.828[b]
ICP		1.000	0.939[b]	0.951[b]	0.879[b]
FCW			1.000	0.996[b]	0.980[b]
DCW				1.000	0.958[b]
FCW:DCW R					1.000

DCI = Days to callus initiation, ICP = Induction coefficient per cent, FCW = Fresh cell weight, DCW = Dry cell weight, FCW: DCW R = Fresh cell weight: dry cell weight ratio.
[a,b] Correlation is significantly different at $P \leq 0.05$ and 0.01, respectively.

In conclusion, the present investigation reports an efficient, simple and easy-to-handle protocol for callus induction and multiplication from leaf explant. Given the present cognition developed through this experiment on the basis of results, it is amply evident that not all the PGRs and their combinations are as much efficient in callus induction and biomass accumulation as IBA + Kin + AdS. In future, this protocol could be very useful in widespread application of plant tissue culture technique for the secondary metabolites production.

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