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Research Article

Study Of Phytohormone Autonomy In *In Vitro* In *Petunia Hybrida*

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ABSTRACT

Petunia x *hybrida* is used as noble genetic material for somatic hybridization, molecular studies and hence is of concern in phylogenetic significance to serve as a useful models of physiology and host - parasite interaction. In the present study using two variants
of Petunia hybrida, the phytohormone autonomy on different formulation of MS medium was carried out. The type of response on hormone free MS medium was rhizogenesis in both the variants. When hormones IAA was substituted, the results of phytohormone independent growth in culture was a different in purple and white variants. Molecular level investigation for the role of *Agrobacterium rhizogenes rol* A,B,C genes in rhizogenesis and autonomous behavior is confirmed with specific amplification pattern. Using the combination of cyclin *GTcyc* gene isolated from tumorous hybrid GGLL (*N.glauca* x *N. langsdorfii*) and D3 cyclin primers, the cytogenetic behavior is known to be a cumulative effect of activation of these genes at molecular level.

Keywords- Agrobacterium rol genes, D3 cyclins, Phytohormone autonomy, Petunia hybrida.

Abbreviations

MS medium : Murashige and Skoog's medium.
IAA: Indole-3- acetic acid
2,4- D: 2,4- Dichlorophenoxyacetic acid
BAP : Benzylamino purine
MS_{1} ::(MS + IAA ,0.1 mg/L),
$MS_{2:}$ (MS +IAA, 0.4 mg/L),
$MS_{3:}$ (MS + 2,4-D 0.5 mg/L),
$MS_{4:}$ (MS + BAP,2 mg/L).

I..INTRODUCTION

AGROBACTERIUM TUMEFACIENS carry tumor (crown gall) Ti plasmid, while *Agrobacterium rhizogenes* carry Ri plasmid and cause hairy root disease in plants (Offringa et al., 1986). Infection of dicotyledonous plants by *Agrobacterium* induced neoplastic growth (*Agrobacterium tumefaciens* carry tumor (crown gall) Ti plasmid, while *Agrobacterium rhizogenes* carry Ri plasmid and cause hairy root disease in plants (Offringa et al., 1986). Watson et al., 1925), and autonomy for

phytohormones in plant cells was observed when Ti plasmid harbored by the bacteria was integrated into host plant hormones (Van larbeke et al., 1974, Schroder, 1985)

Single *rol* genes from the *Agrobacterium rhizogenes* TL-DNA alter some of the cellular responses to auxin in *Nicotiana tabacum*.(Schmulling, T.,et al.,1988).Phytohormone independent growth of tobacco interspecific hybrids is comparable to the indefinite maintenance of crown gall tissue without *Agrobacterium* on MS medium.

It was shown that Ri TL DNA introduced into plant genome can cause hairy root syndrome and autonomous productions of hairy roots without hormone requirement (Tepfer, 1984). The Ri plasmid of agropine strains of *Agrobacterium rhizogenes* contain, two distinct region which can be transferred TL and TR DNA (Frundt et al.,, 1998; Furner et al., 1986). There are 4 loci in the TL DNA: rol A, rol B, rolC and rol D (root loci) or orfs (open reading frames) 10,11,12,and 15 respectively (White et al., 1985; Slightom et al., 1986). The TR region consists of two genes which are homologous to aux-1 and aux-2 genes of PTi oncogenes, suggesting that TR DNA directs auxin synthesis and is involved in root induction (Cardarelli et al., 1987a; Spena et al., 1987). When rol A, B, C were introduced either individually or in combinations cause different phenotype effects.

The rol genes of *Agrobacterium* were shown to have synergistic action in growth, cell proliferation and root formation to plant hormones. Introduction of rol genes into plant caused increase in auxin sensitivity (Spena et al., 1987). Protoplasts of tobacco that contain rol genes showed autonomy for auxins and cytokinins (Walden et al ., 1993). This feature is not restricted to interspecific hybrids of tobacco. Nirmala kumari et al., 2004 reported phytohormone independent growth in seven species of tobacco which look normal and do not produce tumors in field.

The presence of different length of T-DNA in different species of *Nicotiana* and other genera like *Petunias* has been reported by southerns (Furner et al., 1986); wherein the presence of bacterial genes being incorporated into *Nicotiana* and other genera during the course of evolutions. In *N. tabacum* large region corresponding to *Ngrol* and rol C; *N glauca* homologue of TL DNA gave positive in southern blot with *Petunia* genomic DNA (Furner et al., 1996).

II. PROCEDURE FOR IN VITRO STUDY

A. *Sample used*

Leaves from 4-week old field grown plants of *Petunia hybrida* garden varieties purple variant and white variant which produces purple flowers and white flowers respectively, were used. These leaves were surface sterilized and leaf discs were placed on the predesigned MS medium and MS with different hormone combination to study the phytohormone independent growth and differential response under the influence of different hormone in vitro cultural conditions.

B. *Plant tissue culture method*

Different medium were formulated with phytohormone IAA, 2,4-D and BAP substituted in MS medium. There were designated as MS_1 (MS + IAA ,0.1 mg/L), MS_2 (MS +IAA, 0.4 mg/L), MS_3 (MS + 2,4-D), MS_4 (MS + BAP). Medium without any hormone combination is designated as MS_0 (MS basal medium).The cultures were maintained at $20^oC \pm 1^oC$ in dark conditions. Response was observed and recorded on regular basis. The experiment was carried out in 4 sets of 50 explant in each set. The explant which showed some morphogenesis were subcultured after 3 week time interval.

C. *Molecular method*

Genomic DNA was isolated from leaves and cultured samples of *Petunia hybrida* purple variant and white variant. Using CTAB method with required modifications, DNA was isolated and used for PCR. The concentration and purity of genomic DNA were measured by reading optical density at 260nm, 280nm and 320nm using UV light spectrophotometer (Systronics 117).

The plant DNA samples, PCR amplified DNA samples were separated and visualized as clear,

distinct band as and when compared with the standard marker in agarose gel electrophoresis. PCR amplification pattern of specific genes were obtained by using 5 set of primers viz., *rol A, rol B, rol C* and cyclin *D* (GT*cyc* cyclin), D3 cyclin. The genomic DNA of *Petunia hybrida* garden varieties purple and white respectively have been amplified with the respective primers, *[rol A, rol B rol C* and cyclin *D* (GT*cyc* cyclin), D3 cyclin].

III. RESULTS

The type of response on hormone free MS medium was rhizogenesis in both the variants. When hormone IAA was substituted it results in phytohormone independent growth in culture with a differential response of purple and white variants. This indicates the difference in the level of auxin in these varieties, results in varaiation in the type of rooting. Such a response is an indication of the differences in auxin translocation to different regions in tissues. Thus, it can be interpreted that when IAA, 2,4-D and BAP are substituted, there is difference in phenotype, although the overall response is favoring rooting in both the variants. This indicates the expression of auxin like genes of *Agrobacterium rhizogenes* in these varieties.

Table I: Morphogenic Response Of *Petunia Hybrida* Purple Variety (A) And *Petunia Hybrida* White Variety (B) On Ms Basal Media (Mso) And Ms With Different Hormone Combinations.

S.No.	Medium Used	% Response		Days Required For response		Type of Response	
		A	B	A	B	A	B
1.	MS basal medium (MSo)	90	85	7	10	Rhizogenesis from periphery	Rhizogenesis from periphery of leaf disc.
2.	MS+1AA$_1$ 0.1 mg/L(MS1)	83.3	53.3	15	18	No callus, tuft of roots from single point with root hairs	No callus. 2-3 individual roots without root hairs
3.	MS + 1AA$_2$ 0.4 mg/L (MS$_2$)	70	90	16	14	Very scanty callus with 3-5roots without root hairs	No callus, with roots with or without root hairs
4.	MS + 2,4-D 0.5 mg/L (MS$_3$)	67.5	81	14	16	Scanty callus, white in color	Profuse callus, short roots, callus white-green in color
5.	MS+BAP (MS$_4$) 2 mg/L	88.5	65	15	20	Scanty callus, tuft of roots from single point, with or without root hairs	Scanty callus, Individual roots without root hairs, tuft of roots with branching

Table 2: Amplification Pattern Of Rol Genes And Cyclin Genes In *Petunia Hybrida* Purple And White Varieties Field Grown And Cultured Samples

Primers	Petunia hybrida purple variety field grown	Petunia hybrida white variety field grown	Petunia hybrida purple variety cultured sample	Petunia hybrida white variety cultured sample
Rol A	284 bp	284 bp (faint)	284 bp	284 bp
Rol B	800 bp (faint)	800 bp	800 bp (Other Sizes) 891 bp 663 bp	800 bp 1000 bp 540 bp
Rol C	1235	1260	1320 bp 1235 bp 1125 bp	1375 bp 1260 bp 1180 bp
Cyclin D	Faint	Faint	540 bp	540 bp
Cyclin D$_3$	200 bp 780 bp 580 bp 540 bp 430 bp	200 bp 831 bp 780 bp 580 bp 540 bp	200 bp (Faint) 430 bp 400 bp	200 bp (Faint) 420 bp

		430 bp		

IV. CONCLUSION

Petunia Hybrida varieties used in the study have proved that the *Agrobacterium* genes present in hybrids are well expressed in cultural conditions and they are responsible for phytohormonal independent growth of tissues (autonomy) in cultural condition. The same is confirmed by specific amplification pattern of rol genes and cyclin genes. By this, it is concluded that there might be a possibility of *Agrobacterium* genes interaction with the plants earlier, during evolution.

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