ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 10 (2): 203-208, 2011
ISSN 1682-296X / DOI: 10.3923/biotech.2011.203.208

Different Inducer Molecules and Strains of *Agrobacterium rhizogenes* on Enhancing Transformation Frequency in Host Plants

[1]R. Pratap Chandran and [2]V.P. Potty
[1]Department of Biotechnology and Research, K.V.M. College of Engineering and Information Technology, Kokkothamangalam P.O. Cherthala-688583, Kerala State, India
[2]Cashew Export Promotion Council, Lab and Technology Division
Mundakkal P.O. Kollam-691001, Kerala State, India

Abstract: The aim of the present investigation was to find the best *Agrobacterium rhizogenes* strain, which can induce hairy root initiation faster and the best inducer molecule which can potentiate hairy root induction in host plants. Four different wild strains of *A. rhizogenes,* namely *A. rhizogenes* 15834, A4, WS and WR were used for hairy root infection in four host plants (*Ipomoea batatas*, *Solenostemon rotundifolius*, *Vigna vexillata* and *Canavalia* sp). Agrobacterium rhizogenes strains initiated hairy roots from the host plants without inducer molecules (AS and sugars) to a lesser level. Phenolic compound acetosyringone (AS) and sugars (D-glucose, mannose and galactose) were used to activate the virulence genes of the root inducing (Ri) plasmid which initiates transcription of virulence genes for hairy root induction. AS and sugars activated the virulence genes of the root inducing (Ri) plasmid of *A. rhizogenes* strains, which further initiated the transfer of T-DNA region to the host plants and enhanced hairy root induction frequency. As enhanced hairy root induction percentage in the entire host plants ranges from 11 to 61.5% and sugars from 16.5 to 39.5%. *A. rhizogenes* 15834 was found to initiate hairy roots early when compared to other strains of *A. rhizogenes*.

Key words: Acetosyringone, *Agrobacterium rhizogenes*, infection, *Ipomoea batatas*, *Vigna vexillata,* virulence

INTRODUCTION

The genus, *Agrobacterium* can transfer DNA to a remarkably broad group of organisms including numerous dicot and monocot angiosperm species (Wordragen and Dons, 1992) and gymnosperms (Levee *et al*., 1999). In addition, *Agrobacterium* can transform fungi, including yeasts, ascomycetes and basidiomycetes (Piers *et al*., 1996).

Hairy roots are initiated by infecting the plant material with various strains of *Agrobacterium rhizogenes* (Shanks and Morgan, 1999). Hairy roots induced by Ri plasmid usually exhibited a vigorous growth and extensive lateral branching while growing in a media devoid of phytohormones. Hairy roots have several properties that which promoted their use for various plant biotechnological applications. Their fast growth and biosynthetic stability, low doubling time, ease of maintenance and their ability to synthesize a range of chemical compounds makes them a suitable system for *in vitro* production of secondary metabolites (Giri *et al*., 2001).

A. rhizogenes containing Ri (root inducing) plasmid respond more strongly to wound phenolic compounds, such as acetosyringone, which acts as a chemotactic agent at very low concentrations and it activates the *vir* (virulence) gene on the Ri plasmid, which initiate the infection process for the transfer of T-DNA (Rahimi *et al*., 2008) etosyringone (AS) acts as signal molecule for the *vir* gene induction and it is widely used in experiments aimed at increasing Ri transformation frequencies (Huang *et al*., 2001).

The virulence genes *vir* A/*vir* G two-component regulatory systems on the tumor-inducing plasmid of *Agrobacterium tumefaciens* enables this soil bacterium to cause tumors in dicotyledonous plants. When wounded, plant cells release specific phenolic signal molecules such as acetosyringone, which induce the *virA/virG* system and monosaccharides and acid pH in the surrounding environment further potentiate expressions by *vir* A and *vir* G (Gao and Lynn, 2005). The induction of *vir* A and *vir* G leads to transcription of *vir* genes and the resulting products generate and transfer a defined segment of DNA from the tumor-inducing plasmid to the plant nuclear

Corresponding Author: R. Pratap Chandran, Department of Biotechnology and Research, K.V.M. College of Engineering and Information Technology, Kokkothamangalam P. O. Cherthala, Kerala State, India

genome, transforming the plant cell and leading to tumorous growth (Winans, 1992). Acetosyringone or related compounds (eg., α-hydroxy acetosyringone) have been reported to increase *Agrobacterium tumifaciens* mediated transformation frequency in a number of plant species- *Salvia miltiorrhiza*, *Allium cepa*, *Glycine max, Nicotiana tabacum* etc. Acetosyringone acts as a chemotactic agent in very low concentrations and it activates the *vir* gene on the root inducing (Ri) plasmid, which initiates the infection process for the transfer of T-DNA. Various sugars were also known to induce high levels of *vir* gene expression in *Agrobacterium tumifaciens* and are also used for enhancing transformation frequencies (Cangelosi *et al*., 1990). In the case of *A. rhizogenes,* integration and expression of T-DNA genes in host cell lead to the development of hairy roots, which can be excised and grown *in vitro* as hairy root cultures.

In the present investigation hairy roots were induced from four host plants, Ipomoea batatas, *Solenostemon rotundifolius*, *Vigna vexillata* and *Canavalia* sp. through the mediation of *A. rhizogenes* ATCC 15834 for co-cultivating vesicular arbuscular mycorrhizal fungi in these roots (Chandran and Potty, 2008). VAM fungi are symbiotic biotrophs and it requires living roots for their growth and multiplication. The present study reports the efficiency of different strains of *A. rhizogenes* in eliciting hairy root induction and the role of AS and sugars in enhancing the hairy root induction frequency in host plants. The use of AS and sugars as inducer molecules for *A. rhizogenes* 15834 in these host plants is the first report.

MATERIALS AND METHODS

Host plants: Host plants, *Ipomoea batatas* (sweet potato), *Solenostemon rotundifolius* (chinese potato), *Vigna vexillata* and *Canavalia* sp. (sword bean) were used for hairy root induction. Cotyledons (cut into 0.5 cm^2 blocks) and hypocotyls (cut into 1.0 cm long) were used as explants in all the host plants except *S. rotundifolius*. Stem cuttings and *in vitro* plants were used in *S. rotundifolius* for *A. rhizogenes* infection. Good quality seeds of all the above-mentioned plants were collected from Central Tuber Crops Research Institute (CTCRI), Sreekariyam, Thiruvananthapuram, Kerala State, India These experiments were done during January 2007.

Explant sterilization: The seeds of *I. batatas, V. vexillata* and *Canavalia* sp. were surface sterilized by treating the seeds with 0.1% $HgCl_2$ for 10 min and washed well with sterile distilled water and poured 1% $NaOCl_3$ and kept for 10 min and washed well with distilled water. The surface sterilized seeds were placed in 1% agar and kept for incubation at 27°C in an incubator with continuous illumination under fluorescent lamps for 3 weeks.

Table 1: YEB Media composition

Media composition	Values
Beef extract	5 g
Yeast extract	1 g
Peptone	5 g
Sucrose	5 g
$MgSO_4$ $7H_2O$	0.49 g
Bacto Agar	12 g
Distilled water	1000 mL
pH	7.2

Bacterial strains and growth media: Different wild strains of *Agrobacterium rhizogenes* used for the hairy root induction are *A. rhizogenes* ATCC 15834, *A. rhizogenes* A4, *A. rhizogenes* WR and WC (obtained from National Chemical Laboratory, Pune, India). Bacterial culture was maintained in Yeast Extract Broth (YEB) medium (Table 1).

Activation of bacterial culture: AS and sugars were incorporated with YEB culture medium in varying concentrations of 50, 100, 150, 200 and 250 μm L^{-1} were poured separately into sterilized Petri plates and allowed to solidify. Fresh bacterial colonies were streaked on the medium containing AS and sugars and incubated at 24°C for 24 to 48 h in the dark. The 24 to 48 h old activated cultures were used for infecting explants.

Agrobacterium transformation: Surface sterilized explants were wounded with a sterile scalpel, which was smeared with activated culture of *A. rhizogenes*. The infected explants were transferred to petri plates containing sterilized modified Murashige and Skoog medium (½ strength MS salts with 15 g L^{-1} sucrose, full strength B5 vitamins (myoinositol-100, nicotinic acid - 1.0, pyridoxine HCl-1.0, thiamine HCl- 10 mg L^{-1}), 0.5 g L^{-1} cysteine HCl as antioxidant and pH 5.7). The plates were incubated at 24°C for two days in dark. After two days of incubation, the infected explants were transferred to fresh modified MS medium, containing 250 mg L^{-1} cefotaxime to eliminate bacterial growth (Danesh *et al*., 2006) for two days. Then the explants were transferred to fresh modified MS medium without antibiotics and kept for incubation at 24°C with 16 h photoperiod for thirty days for hairy root emergence. The hairy root initiation from the wounded explants was checked on a daily basis and the percentage of hairy root was also monitored in 100 explants each infected with AS and sugars activated *A. rhizogenes*. The percentage values were statistically analyzed.

Statistical analysis: One hundred explants were used in each case and the experiment was done in triplicates and

all the data were processed using t-test as per (Hsu and Lee, 2010).

RESULTS AND DISCUSSION

After host plant infection, *A. rhizogenes* 15834 was found to be the most virulent strain because it initiated hairy root induction in all the four host plants early even in the presence and absence of inducer molecules. The early emergence of hairy roots indicated the enhanced virulence of *A. rhizogenes*. Similarly, David and Tempe (1988) reported that agropine type strains (A4 and 15834) were more virulent than manopine type strains of *A. rhizogenes*. *I. batatas* hairy roots were brittle, light yellow, branched with lot of root hairs and were negatively geotropic. *V. vexillata hairy* roots were thin, soft and were highly branched with lateral branching. The newly formed roots were pure white and become dark brown subsequently. *Canavalia* sp. roots were thick, highly branched and brittle. The growing root tips were white and the basal portions were brown. Rapid proliferation and tumor formation was also observed. *S. rotundifolius* hairy roots were white and very slender. Differences in pathogenecity among the four wild strains of *A. rhizogenes* were observed (Fig. 1) and similar observations were also made by Vanhala *et al.* (1995). The difference in virulence and morphology of hairy root could be explained by the plasmids harboured by bacterial strains (Nguyen *et al.*, 1992). The genetic transformation mediated by *Agrobacterium* was affected by explant genotype (Fig. 1) and structure, chemical and physical factors, bacterial strains and signal molecules (Tao and Li, 2006). This could be due to variations in the concentration of phenolic compounds present in the respective host plants.

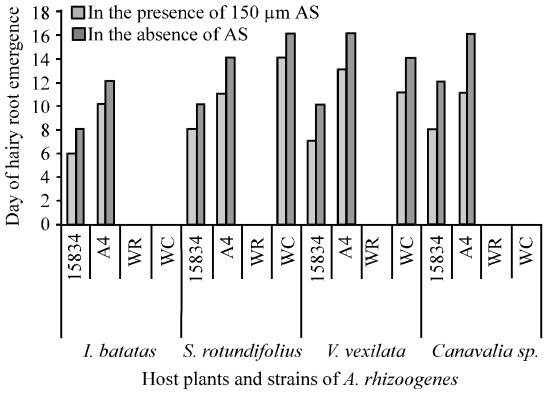

Fig 1: Hairy root induction by different strains of *A. rhizogenes* in the presence (150 µM) and absence of acetosyringone

A. rhizogenes 15834 was further used in this study because of its high susceptibility to induce hairy roots faster in all the host plants. In the presence of 150 µm acetosyringone, the hairy root induction time was reduced by 2 days in *I. batatas* and *S. rotundifolius* and by 3 days in *V. vexillata* and by 4 days in *Canavalia* sp. Giri *et al.* (2001) observed that AS induced *A. rhizogenes* strains reduced the time of hairy root induction by one week in *Artemisia annua* when compared to non AS induced *A. rhizogenes*. Hairy root emerged from the host plants within 15 days of incubation and found that 24°C was optimum for *vir* gene expression. The present results also correlate with the observations made by Jin *et al.* (1993), that *vir* gene induction was maximal at approximately 25 to 27°C and indicated a temperature effect on transformation, because the pilus of *Agrobacterium* strains is most stable at 18 to 20°C and pilus may function as a hook to seize the recipient cells and bring the bacteria and plant into close proximity to effect molecular transfer (Gelvin, 2003).

A. rhizogenes infected explants incubated at 24°C showed hairy root emergence from the 8th day of infection and the percentage of hairy root induction enhanced considerably in the presence of inducer molecules. Table 2 shows that 150 µm of AS enhanced maximum hairy root induction frequency on the cotyledon explants of *I. batatas* (92.5%), followed by *V. vexillata* (82.5%). In *Canavalia* sp., 150, 200 and 250 µM AS provided a root initiation percentage of 71.5. The response of *S. rotundifolius* was low to AS and 150 µm provided a maximum of 32.5% hairy root induction in *in vitro* plants. Ming *et al.* (2007) observed the frequency of rose hybrid nodal segment showed GUS expression was higher at 50 µM acetosyringone and found that the inclusion of acetosyringone in co-cultivation medium increased the transformation frequency. Rahimi *et al.* (2008) observed that the transformation frequency of explants of *V. sisymbriifolium* by *A. rhizogenes* (15834) doubled when 100 µM acetosyringone AS incorporated into the bacterial culture medium.

Hairy root induction frequency of different explants in modified MS medium with and with out acetosyringone is given in Table 2 and the cotyledons were found to be the preferred explants in modified MS medium without acetosyringone because of its highest percentage of hairy root induction. On modified MS medium sharp variations were observed in the preferences of explants to *Agrobacterium* infection. In the case of *I. batatas,* cotyledon was the maximal explants but hypocotyls were the preferred explants in *V. vexillata* and *Canavalia* sp. From these results it was confirmed that the types of explants also influenced root induction efficiency.

V. vexillata responded very well to 150 μm acetosyringone and showed a hairy root initiation frequency of 82.5% in cotyledon explants. The corresponding values of other host plants are also given in Table 3. Kumar *et al.* (2006) also reported 86% transformation frequency in *Nicotiana tabacum* by sonication of *A. rhizogenes* with 100 μM/L AS. Giri *et al.* (2001) reported that the incorporation of 50 μm acetosyringone in bacterial culture medium and the same concentration in the co-cultivation medium resulted in the hairy root induction frequency ranging from 75 to 100% in *Artemisia annua* by different strains of *A. rhizogenes*. In the present investigation AS was incorporated with the bacterial culture medium and a similar method was also adopted by Wang *et al.* (2001). They observed a higher transformation frequency of 59.2 to 84.7% in the presence of AS induced *A. rhizogenes* 15834 and a low of 10.6 to 22.4% in cotyledons and hypocotyls of *Alhagi pseudoalhagi* in the absence of AS (Table 2). In the present study AS activated *A. rhizogenes* 15834 showed hairy root induction frequency from 31-92.5% in *I. batatas*, 28-82.5 % in *V. vexillata*, *Canavalia* sp. 27-71.5%, *S. rotuntifolius* 20-32.5%. High concentration of acetosyringone, (200 μM) in the *A. rhizogenes* culture medium was used for transformation in three days old cotyledonary nodes of *Vigna unguiculata* and found that acetosyringone was indispensable for successful transformation (Raveendar and Ignacimuthu, 2010). The interaction among the host plants analyzed revealed that *Canavalia* sp. with *S. rotundifolius* is highly significant and the least significance was found with *V. vexillata* with *Canavalia* sp. The interaction of all other host plants was found to be statistically significant at 0.5% level (Table 2).

The explants of *Canavalia* sp. showed the higher hairy root induction frequency of 71.5% at 150 μM of AS and this concentration showed the highest hairy root initiation frequency in all the other host plants (Table 2).

Table 2: The effect of AS concentrations on hairy root induction frequency using *A. rhizogenes* 15834

		Percentage of hairy root induction frequency					
			Concentration of AS in MS medium				
Host plants	Explants used	MS medium without AS	50	100	150	200	250
I. batatas	Cotyledon(C)	31	64.500	70.500	92.500	76.500	77.500
V. vexillata	C	28	42.500	46.500	82.500	49.500	50.500
Canavalia sp.	C	27	58.500	62.500	71.500	71.500	71.500
S. rotundifolius	IVP (*in vitro* plants)	20	17.500	18.500	32.500	18.500	17.500
Mean			45.750	54.250	54.000	69.750	49.500

Interaction	F-value
I. batatas×*V. vexillata*	6.37*
I. batatas×*Canavalia* sp.	4.80*
I. batatas×*S. rotundifolius*	9.10*
V. vexillata×*Canavalia* sp.	4.80*
V. vexillata×*S. rotundifolius*	7.93*
Canavalia sp.×*S. rotundifolius*	9.20*

*Significatant at p = 0.05

Table 3: Effect of sugars on hairy root induction frequency using *A. rhizogenes* 15834

		Percentage of hairy root induction efficiency						
			Concentration of sugar on MS medium (μM)					
Host plants	Sugars	Absence of sugar on MS medium	50	100	150	200	250	Mean	% increase due to sugar
I. batatas (Cotyledons)	D-glucose	31	62.500	64.500	68.500	68.500	68.500	66.500	37.5
	Mannose	31	62.500	62.500	64.500	66.500	64.500	64.000	33.5
	Galactose	31	62.500	60.500	64.500	64.500	60.500	62.500	33.5
V. vexillata (Cotyledons)	D-glucose	28	40.500	40.500	44.500	46.500	50.500	44.500	16.5
	Mannose	28	41.000	44.500	48.500	54.500	54.500	48.500	20.5
	Galactose	28	40.500	44.500	48.500	48.500	50.500	46.500	20.5
Canavalia sp. (Cotyledons)	D-glucose	27	56.500	57.500	60.500	62.500	62.500	59.900	33.5
	Mannose	27	56.000	60.500	66.500	62.500	64.500	62.000	39.5
	Galactose	27	56.500	62.500	64.500	68.500	70.500	64.500	37.5

	F value		
Interaction	D-glucose	Mannose	Galactose
I. batatas×*V. vexillata*	9.10*	10.39*	10.9*
I. batatas×*Canavalia* sp.	7.00*	4.19*	3.26*
Canavalia sp.×*V. vexillata*	8.76*	9.73*	10.53*

*Significatant at p = 0.05

Further increase in AS concentration did not increase hairy root induction frequency and this may be due to the inhibitory effect of AS on transformation and it can radically affect the relative virulence of different strains and these effects are not consistent across species (Vanhala *et al*., 1995). Bond and Roose (1998) found that 250 μM of AS was the adequate concentration supplemented to the co-culture medium to induce hairy roots in citrus Washington navel orange.

Glucose, mannose and galactose also induced *vir* genes expression strongly, but to a lesser level than that of AS at concentrations ranging from 150 to 250 μm. Among the promising host plants, *I. batatas* was observed to be the most susceptible plant, both to acetosyringone and glucose with a 37.5% increase in hairy root induction frequency (Table 3). According to (Cangelosi *et al*., 1990) certain sugars induce *vir* genes synergistically with acetosyringone, even in low concentration of the latter they strongly induce *vir* gene expression in wild type cells of *A. tumifaciens*. The increase in hairy root initiation frequency attained due to the sugars activation of *A. rhizogenes* is in full agreement with the observation made by Shimoda *et al*. (1990). Besides increasing hairy root induction, sugars also promote rapid growth of hairy roots.

Glucose activated *A. rhizogenes* 15834 strain showed a high hairy root initiation frequency of 68.5% at concentration ranging from 150 to 250 μM in *I. batatas* and in *V. vexillata* explants, a maximum hairy root induction frequency of 50.5% in 250 μM of glucose. *Canavalia* sp. 200 to 250 μM glucose showed a similar hairy root initiation frequency of 62.5% and further increase in concentration of glucose did not elevate hairy root initiation frequency (Table 3).

In most of the cases 200 μM of mannose showed a maximum hairy root initiation frequency in *I. batatas* (66.5%) and *V. vexillata* (54%) explants and in *Canavalia* sp. explants a maximum of 66.5% was observed in 150 μM of mannose and further increase in concentration showed a negative trend in hairy root initiation frequency (Table 3).

In explants of *I. batatas,* galactose activated *A. rhizogenes* 15834 showed a higher hairy root induction frequency of 64.5% in both 150 μM and 200 μM galoctose. In *V. vexillata* and *Canavalia* sp. 250 μM galoctose showed a higher hairy root initiation frequency of 50.5 and 70.5% in the respective explants. The interaction of various sugars with host plants is given in Table 3 and all the sugars showed good increase in hairy root initiation and are statistically significant at 0.5% level.

From these results it was very clear that D-glucose, galactose and mannose activated the virulence genes of *A. rhizogenes* strain 15834 at different concentration and showed that the sugars can also contribute substantially to hairy root induction frequency. Citovsky *et al*. (1992) also reported that when AS concentration is low or not detectable, *vir* gene expression was significantly increased by monosaccharides (glucose or galactose) and various sugars can also act synergistically with AS to induce high levels of *vir* gene expression (Delmotte *et al*., 1991). Acetosyringone and sugar treatment of *A. rhizogenes* might be useful for eliciting hairy roots and also to enhance transformation frequencies of other host plants and this has immense potential in biotechnology. The inducer molecules were used to initiate hairy root from recalcitrant plant species, which are very difficult to propagate by known methods (Hansen *et al*., 1989) and after hairy root initiation they were planted as new plants. The inducer molecules are also used for genetic engineering experiments, to reduce the time of hairy root initiation and this has commercial significance also, hairy roots from medicinally important plants are used for the production of secondary metabolites (Shanks and Morgan, 1999). Hairy roots can also produce recombinant proteins from transgenic roots and they hold immense potential for the pharmaceutical industry (Guillon *et al*., 2006).

CONCLUSION

Because of the tremendous commercial potential of hairy roots in research and industry, the role of inducer molecule in enhancing hairy root initiation in host plants is vital in achieving success in transformation. The results obtained in this study highlighted the role played by inducer molecule AS and sugars in Agrobacterium mediated transformation.

REFERENCES

Bond, J.E. and M.L. Roose, 1998. Agrobacterium-mediated transformation of the commercially important citrus cultivar Washington navel orange. Plant Cell. Rep., 19: 229-234.

Cangelosi, G.A., R.G. Ankenbauer and E.W. Nester, 1990. Sugars induce the *Agrobacterium virulence* genes through a periplasmic binding protein and a transmembrane signal protein. Proc. Natl. Acad. Sci. USA., 87: 6708-6712.

Chandran, R.P. and V.P. Potty, 2008. Induction of hairy roots through the mediation of four strains of *Agrobacterium rhizogenes* on five host plants. Indian J Biotechnol., 7: 122-128.

Citovsky, V., J. Zupan, D. Warnick and P. Zambryski, 1992. Nuclear localization of *Agrobacterium vir* E2 protein in plant cells. Science, 256: 1802-1805.

Danesh, Y.R., E.M. Goltapeh, A. Alizadeh and M.M. Sanavy, 2006. Optimizing carrot hairy root production for monoxenic culture of arbuscular mycorrhizal fungi in Iran. J. Biol. Sci., 6: 87-91.

David, C. and J. Tempe, 1988. Genetic transformation of cauliflower (*Brassica oleracea* L. var. cotrytis) by *Agrobacterium rhizogenes*. Plant Cell Rep., 7: 88-91.

Delmotte, F.M., D. Delay, J. Cizeau, B. Guerin and J.C. Leple, 1991. *Agrobacterium* vir-inducing activities of glycosylated acetosyringone, acetovanillone, syringaldehyde and syringic acid derivatives. Phytochemistry, 30: 3549-3552.

Gao, R. and D.G. Lynn, 2005. Environmental pH sensing: Resolving the VirA/VirG two-component system inputs for *Agrobacterium* pathogenesis. J. Bacteriol., 187: 2182-2189.

Gelvin, S.B., 2003. *Agrobacterium*-mediated plant transformation: The biology behind the Gene-Jockeying tool. Microbiol. Mol. Biol. Rev., 67: 16-37.

Giri, A., S.T. Ravindra, V. Dhingra and M.L. Narasu, 2001. Influence of different stains of *Agrobacterium rhizogenes* in induction of hairy roots and artemisinin production in *Artemisia annua*. Curr. Sci., 81: 378-382.

Guillon, S., J. Tremouillaux-Guiller, P.K. Pati, M. Ridean and P. Gantet, 2006. Hairy root research: Recent scenario and exciting prospects. Curr. Opin. Plant Biol., 93: 341-346.

Hansen, J., J.E. Jorgensen, J. Stougaard and K.A. Marcker, 1989. Hairy roots-A short cut to transgenic root nodules. Plant Cell. Rep., 8: 12-15.

Hsu, C.L. and W.C. Lee, 2010. Detecting differentially expressed genes in heterogeneous diseases using half Student's t-test. Int. J. Epidemiol., 39: 1597-1604.

Huang, J.Q., Z.M. Wei, H.L. An and Y.X. Zhu, 2001. *Agrobacterium tumefacien* mediated transformation of rice with the spider insecticidal gene conferring resistance to leaf folder and striped stem borer. Cell Res., 11: 149-155.

Jin, S., Y.N. Song, W.Y. Deng, M.P. Gordon and E.W. Nester, 1993. The regulatory VirA protein of *Agrobacterium tumefacien* does not function at elevated temperatures. J. Bacteriol., 175: 6830-6835.

Kumar, V., A. Sharma, B.C.N. Prasad, H.B. Gururaj and G.A. Ravishankar, 2006. *Agrobacterium rhizogenes* mediated genetic transformation resulting in hairy root formation is enhanced by ultrasonication and acetosyringone treatment. Electron. J. Biotechnol., 9: 349-357.

Levee, V., E. Garin, K. Klimaszewska and A. Seguin, 1999. Stable genetic transformation of white pine (*Pinus strobes* L.) after co-cultivation of embryogenic tissues with *Agrobacterium tumefaciens*. Mol. Breed., 5: 429-440.

Ming, O.C., A.S. Wen, U. Sinniah, R. Xavier and S. Subramaniam, 2007. Cysteine and acetosyringone are the two important parameters in agrobacterium-mediated transformation of rose hybrid (*Rosa hybrida* L.) cv. nikita. J. Plant Sci., 2: 387-397.

Nguyen, C., F. Bourgaud, P. Forlot and A. Guckert, 1992. Establishment of hairy root cultures of *Psoralea* species. Plant Cell. Rep., 11: 442-447.

Piers, K.L., J.D. Heath, X. Liang, K.M. Stephens and E.W. Nester, 1996. *Agrobacterium tumefacien* mediated transformation of yeast. Proc. Natl. Acad. Sci. USA., 93: 1613-1618.

Rahimi, K., K. Haghbeen, J. Marefatjo, F.R. Jazii and R. Sheikhani, 2008. Successful production of hairy root of *Valeriana sisymbriifolium* by agrobacterium rhizogenes. Biotechnology, 7: 200-204.

Raveendar, S. and S. Ignacimuthu, 2010. Improved *Agrobacterium* mediated transformation in cowpea *Vigna unguiculata* L. walp. Asian J. Plant Sci., 91: 256-263.

Shanks, J.V. and J. Morgan, 1999. Plant hairy root culture. Curr. Opin. Biotechnol., 10: 151-155.

Shimoda, N., A. Toyoda-Yamamoto, J. Nagamine, S. Usami, M. Katayama, Y. Sakagami and Y. Machida, 1990. Control of expression of *Agrobacterium tumefacien* genes by synergistic actions of phenolic signal molecules and monosaccharide. Proc. Natl. Acad. Sci. U.S.A., 87: 6684-6688.

Tao, J. and L. Li, 2006. Genetic transformation of *Torenia fournieri* L. mediated by *Agrobacterium rhizogenes*. S. Afr. J. Bot., 72: 211-216.

Vanhala, L., R. Hiltunen and K.M. Oksman-Caldentey, 1995. Virulence of different *Agrobacterium* strains on hairy root formation of *Hyoscyamus muticus*. Plant Cell Rep., 14: 236-240.

Wang, Y.M., J.B. Wang, D. Luo and J.F. Jia, 2001. Regeneration of plants from callus cultures of roots induced by *Agrobacterium rhizogenes* on *Alhagi pseudoalhagi*. Cell. Res., 11: 279-284.

Winans, S.C., 1992. Two-way chemical signaling in *Agrobacterium*-plant interactions. Microbiol. Rev., 56: 12-31.

Wordragen, V.M.F. and H.J.M. Dons, 1992. *Agrobacterium tumefacien* mediated transformation of recalcitrant crops. Plant Mol. Biol. Rep., 10: 12-36.