TRANSIENT GUS EXPRESSION STUDIES IN ONION (*ALLIUM CEPA* L.) AND GARLIC (*ALLIUM SATIVUM* L.)

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Abstract

Three genotypes of onion B-780, N-2-4-1, Hisar 2 and garlic HG-1, G-41 and G-1 were used to study the transient gus expression. Also different explants in onion, callus, shoot tip and roots and in garlic, roots, basal portion and young etiolated leaves along with two plasmids were used to study the effect of different plasmids (pTOK 233 and pCAMBIA 1301) and suitability of explants towards transformation. Results revealed that in onion callus obtained from different explant sources performed significantly superior than shoot tip whereas in root tip no *gus* expression was observed. In garlic, only basal portion showed transient expression whereas leaves and roots did not show any response. There were no significant differences among genotypes but plasmids showed significant differences. Genotypes differences were not significant; however, the two plasmids showed variables response transient *gus* assays. Plasmid pCAMBIA 1301 show significant superiority over pTOK 233 in these experiments.

Keywords: Transformation, *gus* expression, *Allium cepa*, *Allium sativum*

Soğan (*Allium cepa* L.) ve Sarmısakda (*Allium sativum* L.) Transient Gus Expression Çalışmaları

Özet

Bu çalışmada, transient gus ekpressiyonu amacıyla, 3 soğan (B-780, N-2-4-1 ve Hisar-2) ve 2 sarmısak (HG-1, G-41 and G-1) genotipi kullanılmıştır. Bu amaçla, soğanda kallus, sürgün ucu ve kök; sarmısakta ise kök, basal kısım ve genç etiyolleşmiş yapraklar kullanılarak 2 farklı plazmidin (pTOK 233 ve pCAMBIA 1301) transformasyon için uygunluğu araştırılmıştır. Soğanda eksplant kaynağı olarak kallusun kullanımı sürgün ucuna göre daha başarılı bulunmuş ve kök uçlarında gus epressionu gözlenmemiştir. Sarmısakta, sadece basal kısımda transient ekpression belirlenmiş, yaprak ve kökler her hangi bir yanıt göstermemişlerdir. Genotipler arasında gus transient ekspression bakımından bir farklılık belirlenmemiş, fakat plasmidler arasında farklılık kaydedilmiştir. Plasmid pCAMBIA 1301, pTOK 233'e göre önemli bir üstünlük göstermiştir.

Anahtar Kelimeler: Transformasyon, *gus* ekspression, *Allium cepa*, *Allium sativum*

1. Introduction

Alliums, comprising of onion, leek, garlic, shallot etc, is a diverse taxon encompassing nearly 500 species and belongs to family *Alliaceae*. Among them, onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) enjoy a place of privilege because of their antiquity and importance towards culinary purposes and medicinal uses. Both onion and garlic, are monocots with the haploid number of chromosomes n=8 and the diploid number 2n=16. The main method of propagation is seed in onion whereas garlic, being sexually sterile is propagated vegetatively.

India ranks second by area next to China for both of the crops (4.08 and 1.03 lakh ha, respectively). However, by production it ranks second for onion (57.32 lakh tons) and third for garlic (4.73 lakh tons) in the world (Anonymous, 2001). As monocotyledons, the *Allium* species were thought to be recalcitrant to transformation. They have, therefore, been relatively understudied with respect to the application of biotechnology (Eady, 1995). There are only a few reports of DNA delivery to *Allium*s (Klein *et al*., 1987; Eady *et al*., 1996; Barandiaran *et al*., 1998). Recently some reports of transformation have appeared in onion (Eady *et al*., 1998, Zheng *et al*., 2001) and garlic (Barandiaran *et al*., 1998; Kondo *et al*., 2000).

In India, no transformation work in onion and garlic except a few reports in

garlic (Bhojwani, 1980; Koul *et al*., 1994) towards protocol standardization on Indian varieties has been done. Keeping in view all the above facts, the present investigation was undertaken in three cultivars of onion (B-780, N-2-4-1, Hisar-2) and garlic (G-41, G-1 and HG-1) for preliminary transformation studies.

2. Materials and Methods

In vitro grown onion and garlic seedlings were used as the explant source for all the three genotypes of onion and garlic. The *Agrobacterium* strain containing plasmid pTOK 233 and pCAMBIA 1301 were streaked on LB medium having Kanamycin at 50 mg/ml for 3 days to multiply the bacterium. Afterwards, the three day old culture was inoculated on solidified AB medium supplemented with Kanamycin at 50 mg/ml and hygromycin at 50mg/ml concentration and cultured for three days. The bacterial growth so obtained was inoculated in liquid co cultivation medium 1 and kept for 2 hours on continuous agitation in incubator shaker to increase the bacterial population. Optical density of the bacterial culture of the bacterial suspension was measured at 600 nm. Shoot tip, roots and basal or portion of both onion and garlic was immersed in bacterial suspension for one hour. Agro-infected calli and other tissues were blotted dry on sterile Whatman number 1 filter paper and incubated in dark for three days at 28 ± 2^0C. Histochemical *gus* assay (Jefferson *et al*., 1987) was carried out using *Gus* assay solution consisting of sodium phosphate buffer and X-gluc (5-bromo, 4 Chloro-3-indolyl glucuronic acid) as the substrate. Callus and other explants were dipped in *Gus* assay solution in multiwell plates and incubated at 37^0C for 24 hours. Observations were recorded after 3 days.

3. Results and Discussion

Based on the results, it was observed that no significant differences existed between the genotypes, but the plasmids used exhibited significant differences in exhibiting transformation efficiency. Plasmid pCAMBIA 1301 exhibited significant transformation frequency (21.8%) over plasmid pTOK233 (13.2%). In terms of explants used, callus seemed to be better target for transformation and the *gus* expression ranged from 40% (Hisar-2 & N-2-4-1) to 43.33% (B-780) (Table 1). This was followed by shoot tip where the percentage explant showing *gus* expression was highest in B-780 (15.8%) (Table 1) followed by N-2-4-1 (10.0%) and Hisar-2 (10.0%). Root explant was not amenable to transformation and did not show any *gus* expression (Table 1).

In garlic, three genotypes i.e., HG-1, G-41 and G-1 having explants basal portion, leaves and roots were used for transformation studies. It was observed that the basal portion was more suitable for transformation with G-41 recording 18.3% transformation efficiency followed by G-1 (18.3%) and HG-1 (15.0%) showing *gus* expression (Table 2). Root and leaf explant did not show any *gus* expression in all the three genotypes studied. Based on statistical analysis it was observed that no significant differences existed among the genotypes for *gus* expression. But the *Agrobacterium* strains exhibited significant differences in their ability to transform the explants. *Agrobacterium* strain carrying plasmid pCAMBIA 1301 was significantly superior with a mean transformation frequency of 7.4% over the strain pTOK 233 with a mean transformation frequency of 4.1%.

The present study involves the use of two plasmids pTOK 233 and pCAMBIA 1301 for attempting genetic transformation in onion and garlic. In onion, callus obtained from different explant sources performed significantly superior than shoot tip. In root tip, no *gus* expression was observed. In garlic, only basal portion showed transient expression whereas leaves and roots did not show any response. There were no significant differences among genotypes but plasmids showed significant differences with pCAMBIA 1301 exhibiting significant superiority over pTOK 233. Genotypes differences were not significant, however, the two plasmids showed variables

Table 1: Percent transient *gus* expression in *Agrobacterium* mediated transformation studies in onion.

Genotypes	Explant	Percent explants showing *gus* expression P1	P2	Mean
B-780				
	Callus	30.00±5.77	56.66±3.33	43.33±6.66
	Shoot tips	15.00±2.88	16.66±3.33	15.83±2.01
	Roots	0.00±0.00	0.00±0.00	0.00±0.00
N-2-4-1				
	Callus	23.33±3.33	56.66±3.33	40.00±6.83
	Shoot tips	6.66±3.33	13.33±3.33	10.00±2.58
	Roots	0.00±0.00	0.00±0.00	0.00±0.00
Hisar-2				
	Callus	26.66±6.66	53.33±3.33	40.00±6.83
	Shoot tips	6.66±3.33	13.33±3.33	10.00±2.58
	Roots	0.00±0.00	0.00±0.00	0.00±0.00
Mean		13.52±2.38 b	21.85±6.41 a	
CD(0.05): Genotype x Plasmid= 5.23, Genotype x Explant= 6.41				

P1: Plasmid pTOK233, P2 : Plasmid pCAMBIA 1301

Table 2: Percent transient *gus* expression *Agrobacterium* mediated transformation studies in garlic.

Genotypes	Explant	Percent explants showing *gus* expression P1	P2	Mean
HG-1				
	Basal portion	10.00±0.24	20.00±0.42	15.00±2.23
	Leaves	0.00±0.00	0.00±0.00	0.00±0.00
	Roots	0.00±0.00	0.00±0.00	0.00±0.00
G-41				
	Basal portion	13.33±3.33	23.33±3.33	18.33±3.07
	Leaves	0.00±0.00	0.00±0.00	0.00±0.00
	Roots	0.00±0.00	0.00±0.00	0.00±0.00
G-1				
	Basal portion	13.33±3.33	23.33±3.33	18.33±3.07
	Leaves	0.00±0.00	0.00±0.00	0.00±0.00
	Roots	0.00±0.00	0.00±0.00	0.00±0.00
Mean		4.07±1.22 b	7.41±2.10 a	
CD(0.05): Genotpe x Medium= 2.45, Genotype x Explant= 3.01				

P1 : Plasmid pTOK233, P2 : Plasmid pCAMBIA 1301

response transient *gus* assays. Plasmid pCAMBIA 1301 show significant superiority over pTOK 233 in these experiments.

The results are in confirmation with findings of Barandiaran *et al.,*(1998) who reported less DNA expression in garlic because of nuclease activity preventing exogenous DNA expression. Moreover, co-cultivation period, use of lower temperature (19-20ºC) is important for garlic transformation (Kondo *et al.,* 2000). Regarding superiority of plasmid, Barandarian *et al.,* (1998) also reported the relative superiority of plasmids in genetic transformation experiments. Superiority of callus for transformation in onion (Zheng *et al.,* 2001) and in cereals, from immature embryos and calli induced from scutella (Hiei *et al.,* 1994; Aldemita and Hodges, 1996, Eady *et al.* 2000) due to their excellent morphogenetic competence was observed.

In the present investigation, certain explants did not show any *gus* expression and it seems that further optimization of transformation procedure is needed in these explants such as addition of acetosyringone in medium and/or bacterial culture or other pre-treatments. Further optimization in root

explants will be of high value since these show good regeneration potential too.

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