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OPTIMIZATION OF A NON-TISSUE METHOD OF AGROBACTERIUM MEDIATED GENE TRANSFER IN COWPEA (*VIGNA UNGICULATA* L. WALP.)

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ABSTRACT

Agrobacterium infiltration is a common method used for effective gene transfer in many cultivated plants. In this study, the various concentrations (0.5, 1.0 and 1.5 × 10^9 cells ml^{-1}) of *A.tumefaciens* was injected into the young leaves and germinated seeds of Cowpea by syringe method and vacuum infiltration. Then the morphological and biochemical variations were observed. The results show that the vacuum infiltration method was more efficient then the syringe infiltration. Also the survival rate of transgenic plant of non-tissue culture method was increased then the tissue culture method.

INTRODUCTION

Vigna unguiculata (L.) Walp is commonly known as Cowpea, Southern pea and Black-eyed pea. It is originated from African Continent. It is a warm weather and drought resistant crop. It is well adapted to low rainfall, heat and wide range of soil conditions (Kochhar SL, 2009). Nowadays, many crop improvement programs are used to increase the disease resistance in cowpea. Genetic transformation is one of the advanced crop improvement method then the other traditional Mendelian methods (Chaudhary *et al*, 2007). Genetic transformation is divided into direct and indirect gene transfer methods. Direct gene transfer method includes particle bombardment, Polyethylene glycol – mediated transformation, Electroporation and Silicon carbide fibres. But this method gives very low (8%) transformation frequency in explants of cowpea (Ikea *et al*, 2003) and requires more financial support. In Indirect gene transfer method, many vectors used to transfer the desirable genes into plant genome. *Agrobacterium tumefaciens* is the soil – borne, Gram – negative bacteria and widely used as a vector for effective gene transformation. Agrobactrium mediated gene transfer or Agroinfiltration is effective and low cost gene transfer method (de la Riva GA *et al*, 1998).

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The simplicity of Agrobacterium gene transfer makes it is a 'poor man's vector. This method gives low incidence of transgene silencing (Pradhan, 2010). This method gives high (88.4%) transformation frequency in explants of cowpea (Bakshi *et al*, 2011). But the explants regeneration is only 44% in cowpea (Muthukumar *et al*, 1996).

MATERIALS AND METHODS

Cowpea seeds were collected from the sales centre of TNAU, Coimbatore, Tamilnadu, India. *A.tumefaciens* strain EHA105 (a non-virulent strain) containing CAMBIA2301 with a β-glucuronidase gene (Reporter gene) and a neomycin phosphotransferase gene (Marker gene). 2.5 g of LB medium was dissolved with 100 ml of distilled water and autoclaved at 120 ^oC for 15 minutes. Then few drops of *A.tumefaciens* strain EHA105 were added into LB medium. Then, medium was agitated at 120 rpm with the help of shaker for 2 days at room temperature. The bacterial culture was centrifuged at 5000 rpm for 5 minutes. The infiltration medium contains 10 mM MES buffer (pH – 5.7), 10 mM $MgCl_2$ and 100 µM acetosyringone were prepared. Then various proportions of bacterial culture were mixed with infiltration medium and various cell densities like as 0.5, 1.0, 1.5 × 10^9 cells ml^{-1} (OD_{600} = 0.5, 1.0, 1.5). All the above mentioned Agrobactrium cell densities were infiltrated in the germinated seeds and young leaves of cowpea by using vacuum pressure and syringe. Then, the plants were

Table 1. The results of morphological and biochemical analysis in Agrobacterium infiltrated plants

S.No.	Methods	Vacuum infiltrated plants				Syringe infiltrated plants			
		control	0.5 OD	1.0 OD	1.5 OD	control	0.5 OD	1.0 OD	1.5 OD
1	GUS positive plants (%)	0	30	74	94	0	18	39	67
2	Shoot length (cm/plant)	9.6	8.9	7.8	6.9	7.2	5.9	5.2	4.6
3	Dry weight (mg/g)	745	703	624	518	520	453	379	294
4	Total leaf area (cm^2)	10.62	8.46	6.83	5.43	8.58	7.24	5.58	3.78
5	Stress tolerance index	1.00	0.81	0.72	0.63	1.00	0.92	0.81	0.71
6	Chlorosis rate (%)	0	10	30	50	0	30	70	90
7	Death rate (%)	0	10	50	80	0	30	80	100
8	Total Carbohydrates (mg/100mg)	86	81	69	56	80	72	59	45
9	Buffer soluble poteins (mg/g)	3.32	2.67	2.13	1.79	2.80	2.01	1.72	1.35
10	Total free amino acids (mg/500mg)	8.23	09.76	13.20	15.30	07.00	10.40	15.00	18.80
11	Total phenols (mg/500mg)	47	74	95	126	40	65	83	115
12	Total chlorophyll content (mg/g)	0.88	1.05	1.23	1.38	1.18	1.31	1.50	1.67

allowed to grow in the normal environment. The rate of transformation efficiency was determined by GUS biochemical assay (Jefferson *et al*., 1987). The morphological and physiological parameters like as shoot length, dry weight, total leaf area (Yoshida *et al*., 1972), stress tolerance index (Turner and Marshal, 1972), chlorosis rate, death rate were calculated. The biochemical parameters like total carbohydrates, buffer soluble proteins, free amino acid, total phenols, total chlorophyll content (Sadasivam and Manickam, 1991) were estimated.

RESULTS

The Agrobacterium infiltrated plants were confirmed by GUS assay. The results of GUS biochemical assay was showed in Figure 1.

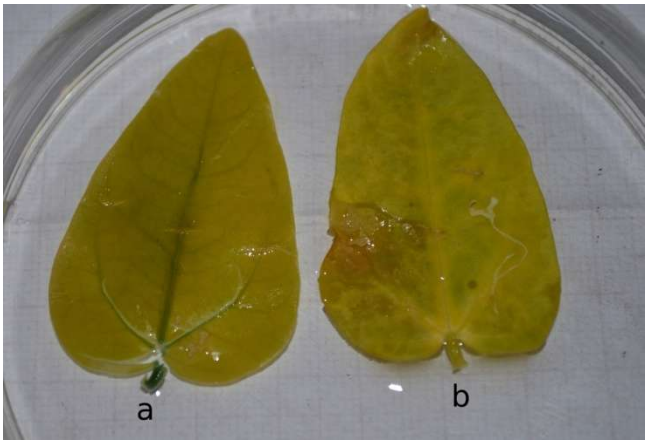

Fig.1. The GUS positive (a) and negative (b) plant leaves were showed. The leaf of GUS positive plant (a) indicates the dark blue color in veins. But GUS negative plant leaf (b) not show any blue color

The results of above mentioned morphological and biochemical methods were collected and tabulated (Table 1).

DISCUSSION

The present study has been carried out to find the morphological and biochemical changes due to the infiltration of Agrobacterium into cowpea. The different cell densities (OD_{600} = 0.5, 1.0, 1.5) were infiltrated in the seeds and young plants by vacuum and syringe.

The morphological characters like shoot length and total leaf area decreased when the Agrobacterium cell density was increased. However, there is no abnormal structure or somoclonal variations were noted. The carbohydrate and protein contents of the plant also decreased with the increased amount of cell density of Agrobacterium. The chlorosis rate, death rate, amino acid content and phenol content of the plant increased when the cell density was increased. The Agrobacterium cell density of 0.5×10^9 cells ml^{-1} (OD_{600} = 0.5) with vacuum infiltration method gives the most effective genetic transformation in cowpea. On the other hand, vacuum infiltration method increases the transformation efficiency and reduced the biotic stress in cowpea.

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