J. Sci. Trans. Environ. Technov. 2009, 3(2): 79-82

Variations in morphology, photosynthetic pigments and protein content of micropropagated soybean (*Glycine max* (L.) Merr) CO3, compared to conventional plants

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Abstract

Micropropagated plants of soybean (*Glycine max* (L.) Merr) CO3 had reduced plant height and leaf area than the conventional plants under the same growth conditions. However, the chlorophyll and protein content were found to be high in micropropagated plants when compared to conventional plants. The results indicated that the tissue culture process is not detrimental to plant performance and this technique could be useful in genetic transformation for the production of plant varieties containing new traits.

Keywords : chlorophylls, leaf area, micropropagation, plant height, protein, soybean

INTRODUCTION

Cell and tissue culture techniques have a great potential in plant improvement, as the plants can be readily regenerated in large numbers (Jain, 1998). For e.g. tissue culture technique could be successfully used in the improvement of rice for tolerance to salinity (Wintcov, 1996, lutts *et al*., 1999) or other abiotic stresses (Bertin *et al*., 1996). In some regenerated soybean progenies the chromosomes number was found to 80 (Ranch and Palmer, 1987). Barwale and Widholm (1989) reported mixaploidy with chromosome counts ranging from 10 to 60. Somaclonal variation can occur from pre-existing or from *in vitro* induced variability due to the unorganized callus proliferation. It can lead to chromosome alterations, gene amplifications, mitotic crossing over, point mutations or DNA hypomethylation (Kaepller *et al*., 2000; Jaligot *et al*., 2000; Jain, 2001).

The vegetative propagation of plants using tissue culture may lead, at certain level, to variations in phenotype when compared to the original stock material e.g. somaclonal variation (Larkin and Scowcroft, 1981). Freytag *et al.* (1989) described changes in leaf morphology and growth habit from indeterminate to determinate in organogenitically regenerated soybean plants. Variation in morphological characters among callus regenerated plants were observed in rice for grain size, tiller number, leaf number, maturity (Sun and Zheng, 1990), panicle number, seed weight and mature plant height (Lal and Lal, 1990). Variations in potato with regard to maturation time, shape, size, number and colour of tubers, leaf shape and size and yield were observed in micropropagated plants (Karp, 1990). Plants regenerated from the embryogenic calli of wheat have revealed variants for various agronomic and quality characters such as plant height, stem thickness, leaf size, spike shape pollen fertility, gliadin storage protein, presence or absence of awns, maturity, plant type, etc. (Ahloowalia, 1982; Maddock *et al*., 1985; Carver and Johnson, 1989; Cheng *et al*., 1992). Somaclones have also been reported for various traits such as higher grain weight, protein concentration, sedimentation values, harder kernels (Hanson *et al*., 1994). Somaclonal variants of wheat for yield traits and disease resistance suitable for heat stressed and zero-till conditions have been discussed by Arun *et al.* (2007). While the frequency of somaclonal variation is relatively low in soybean in comparison to some crop species, a number of reports have described a large number of variants, including maternally inherited wrinkled leaf, chlorophyll-deficiency, dwarfness, sterility, maturity, height, leaf shape, variegation and isozymes (Widholm, 1996). The objective of this study was to test the presence of variations in morphological traits, photosynthetic pigments and protein content of tissue culture derived soybean *Glycine max* under field conditions.

MATERIALS AND METHODS

Plant material

The certified seeds of soybean were obtained from Tamilnadu Agricultural University, Coimbatore, India and used as initial explants for plant source.

Seed sterilization and seed seedling growth

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The seeds were surface sterilized with 0.1% $HgCl_2$ solution for 5 min, then with 70% alcohol for 1 min and washed thoroughly five times with distilled water. The seeds were cultured in B5 medium (Gamborg *et al.* 1968) supplemented with 1.0 mg/L Thidiazuron (TDZ) in aseptic condition with the pH of the medium adjusted to 5.8 by adding 0.1 N NaOH or 0.1 N HCl and in 16 h light and 8 h dark photoperiod maintained by cool fluorescent lamp.

Production of micropropagated plants

The cotyledonary node with axillary buds were excised from seven days old seedlings and transferred into 1.0 mg/L TDZ treated B5 medium (B5 salts, 3% sucrose, B5 vitamins and 0.7% agar). The shoot buds were sub-cultured continuously at an interval of two weeks in the same medium and the percentage of regeneration and number of shoots per explants were recorded. After three weeks, the regenerated plants were hardened in mud cup and maintained in a growth chamber. The hardened plants were transferred to the field after one week. At the same time, the healthy seeds were propagated in ten mud pots. The experiment was repeated three times.

Analysis of variation between micropropagated plants compared with conventional plants

In both the micropropagated and conventionally reared plants, plant height, leaf area, chlorophyll content and protein contents were measured after the seed maturation in the pod. Photosynthetic pigments including the chlorophyll a, chlorophyll b, and total chlorophylls were extracted and then estimated adopting the procedure described by Arnon (1949). The quantitative estimation of soluble protein was done employing the method of Bradford (1976).

Statistical analysis

Data from the experiment, the height of the plants, leaf area, chlorophyll and protein content of both micropropagated and conventionally reared plants were analysed by SPSS software, in which statistical significance was determined at the 0.05 probability level.

RESULTS AND DISCUSSION

The plantlets that were regenerated from micropropagation had high variability in plant size, morphological and biochemical traits. Yipeng *et al.* (2005) while observing high variability in micropropagated rhubarb PC49 opined that it might be triggered by the cytokinin during micropropagation. The height of the micropropated plants was reduced to 57.8% of the conventional plants in the present study (Figure 1). Earlier number of other researchers have also reported a decrease in the size of micropropagated plants when compared to parent plants (Karp, 1990;

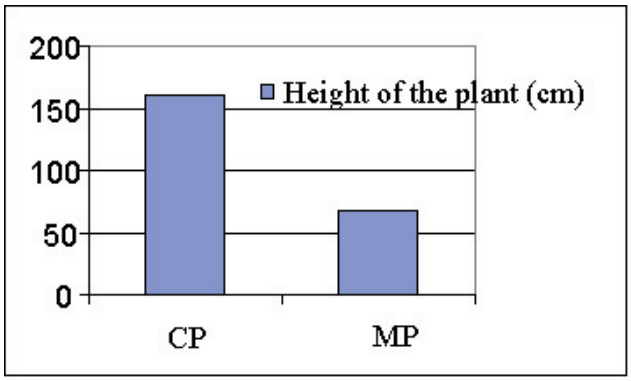

Figure 1. Mean Height (cm) of the micropropagated plants (MP) and conventionally reared soybean *Glycine max* (CP)

Jain, 2001). Furthermore, tissue culture derived plant leaves were darker green in color than conventional plants, and the leaf area was decreased to 24.8% when compared to normal plants in the present study (Figure 2). Ravindra *et al.* (2004) attributed such changes

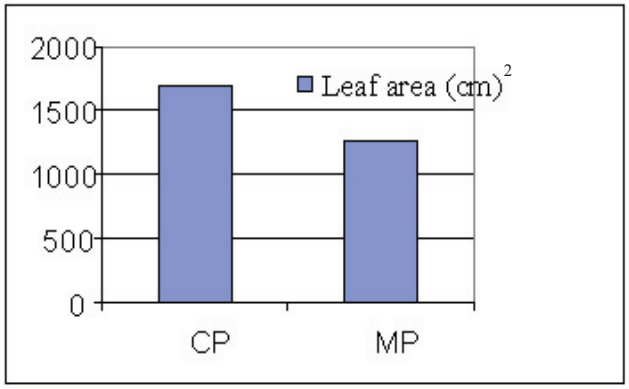

Figure 2. Mean Leaf area (cm^2) of the micropropagated (MP) and conventionally reared soybean *Glycine max* (CP)

in plant height and leaf size among micropropagated plants of the Indian cultivar of rose-scented geranium to be the effect of somaclonal variations.

Increased levels of photosynthetic pigments *viz.*, chlorophyll a (46%), chlorophyll b (27.6%) and total chlorophyll (42.1%) contents were observed in the micropropagated plants of the present study (Table 1). The chlorophyll content might probably be related to the activity of the enzyme chlorophyllase perhaps enhanced by the supplementation of thidiazuron to the tissue culture medium (Reddy and Vora, 1986). On the contrary Yipeng *et al.* (2005) found no difference in chloroplast number and chlorophyll content between micropropagated and conventional rhubarb plants.

Protein content was higher (23.8%) in somaclonal lines than the conventionally reared plants (Table 1). Todorovska *et al.* (1997) found the tissue culture derived plants of barley cv. 'Jubiley' to show polymorphism

Table 1. Photosynthetic pigments and protein contents in leaf samples of micropropagated and conventionally reared soybean *Glycine max*

Leaf samples	Photosynthetic pigments (mg g-1fw)			Protein content (mg g-1fw)
	Chlorophyll a	Chlorophyll b	Total chlorophyll	
Micropropagated plants	0.850±0.009	0.410±0.016	1.275±0.017	4.004±0.182
Conventional plants	0.459±0.014	0.297±0.012	0.756±0.008	3.072±0.143

in protein level, with an additional band (2.8 kb) in the protein profile. However, the report of Nguyen *et al*. (2001) was contrary to the present findings as they found the somaclonal variation for protein and yield to decrease in soybean. Stephens *et al*. (1991) evaluated 86 lines derived from soybean plants regenerated through organogenesis and demonstrated that all plants that regenerated from tissue cultures do not carry deleterious genetic changes.

ACKNOWLEDGEMENT

We gratefully acknowledge Bharathidasan University, Tiruchirappalli, India for providing us the financial support through the University research studentship.

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