

Changes in Protein Content in Micropropagated and Conventional Soybean Plants (*Glycine max* (L.) Merr.)

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Abstract: The experiment was to study the protein content in various parts such as roots, stems, leaves, fruits wall and seeds of micropropagated and conventional soybean plants. The seeds were cultured on thidiazuron (TDZ) supplemented B5 medium under the *in vitro* condition. The cotyledonary nodal explants were taken from the *in vitro* seedlings and cultured in the same medium. Fully developed plantlets were acclimatized into the field. At the same time, the surface sterilized seeds were propagated into the field. The amount of protein content in micropropagated plants roots and leaves was more than conventional plants, but the protein extracted from the micropropagated plants stems and fruits wall was lower than the conventional plants. Moreover, the seed protein content of conventional plants was slightly increased when compared to the parent and micropropagated plants. Eventhough some differences were observed in the protein contents of vegetative parts of micropropagated plant samples, the seed protein content was not found the differences in major level. These results indicate that the tissue culture process is not detrimental to plant performance.

Key words: Micropropagated plants and conventional plants % Protein content % Soybean

INTRODUCTION

Soybean is an economically important plant. It grows in varied agro-climatic conditions. Soybean has great potential as an exceptionally nutritive and very rich protein food. It can supply the much needed protein to human diets, because it contains above 40% protein of superior quality and all the essential amino acids particularly glycine, tryptophan and lysine, similar to cow's milk and animal proteins. Soybean also contains about 20 per cent oil with an important fatty acid, lecithin and Vitamin A and D. The 4% mineral salts of soybeans are fairly rich in phosphorous and calcium.

Its productivity also reduced by both biotic and/ or abiotic factors. The biotic and / or abiotic stress tolerant plants are needed to increase the productivity. The conventional breeding method has been unsuccessful in transferring the new traits to the target species. So we are alternatively using tissue culture and gene transfer method to produce the new traits containing species. Soybean plant regeneration from tissue culture has been difficult and recently it becomes routine. Plants regenerated from tissue culture have exhibited various

morphological and biochemical variation. A great potential of cell and tissue culture techniques in plant improvement provided plants can be readily regenerated in large numbers [1]. The vegetative propagation of plants using tissue culture may lead at certain level variation in phenotype compared to the original stock material i.e., somaclonal variation [2]. 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 [3-5].

Tissue culture technique could be successfully used in the improvement of rice for tolerance to salinity [6,7] or other abiotic stresses [8]. Somaclones have also been reported for various traits such as higher 1000 grain weight, protein concentration, sedimentation values and harder kernels [9]. 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, dwarf, sterility, maturity, height, leaf shape, variegation and isozymes

[10]. The adoption of new technologies such as plant tissue culture and recombinant DNA may help in achieving some of the goals to increase food production. The objective of this study was to test the presence of variation in protein content of micropropagation derived plants that were not detrimental to plant performance under the field conditions.

MATERIALS AND METHODS

Plant material and seed sterilization: The certified seeds of soybean (CO₃) were obtained from Tamilnadu Agricultural University, Coimbatore, India. The seeds were surface sterilized with 0.1% HgCl₂ solution for 5 min and 70% alcohol for 1 min and again washed thoroughly with distilled water for 5 times.

Production of micropropagated plants: The surface sterilized seeds were cultured in B5 [11] salts, 3% sucrose, B5 vitamins and 0.7% agar and B5 medium supplemented with 1.0 mg/L Thidiazuron (TDZ) in aseptic condition. The pH of the medium was adjusted into 5.8 by adding 0.1 N NaOH and / or 0.1 N HCl and then 16 h light and 8 h dark photoperiod was maintained by cool fluorescent lamp. 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). Once in two week interval shoot buds were sub-cultured continuously in the same medium and the percentage of regeneration and number of shoots per explants was recorded. After three weeks, the regenerated plants (micropropagated plants) were hardened in the mud cub and maintained in the growth chamber. The hardened plants were transferred into the mud pots contain soil

Production of conventional plants: The plants were propagated in 25 cm clay pots containing 6 kg of air dried red soil under 12 h photoperiod in natural conditions and the plants were watered regularly. The healthy and surface sterilized seeds were propagated in ten mud pots containing soil. Both micropropagated and conventional plants were maintained under the same environment. The experiment was repeated at three times.

Estimation of protein content: The protein content was recorded after seed maturation of both the micropropagated and conventional plants. The soybean roots, stems, leaves, fruits wall and seeds were homogenized using 70% (v/v) ethyl alcohol and were

utilized for the estimation of soluble proteins. For this the homogenate were precipitated by adding 20% (w/v) trichloroacetic acid. The precipitate was then dissolved in 1% (w/v) sodium hydroxide solution. Quantitative estimation of protein was done employing the method of Lowry *et al.* [12].

Statistical analysis: Data from the experiment, the protein content of roots, stems, leaves, fruits wall and seeds collected from micropropagated and conventional plants were analyzed by SPSS software, in which statistical significance was determined at the 0.05 probability level.

RESULTS AND DISCUSSION

The micropropagated and conventional plants protein content (roots, stems, leaves, fruits wall and seeds) were studied. The significant increase of the protein content 7.17 and 31.27% was observed in root and leaf samples of the micropropagated plants than that of conventional plants (Table 1). Todorovska *et al.* [13] investigated to find all tissue culture derived plants showing polymorphism in a protein level and an additional band (2.8 kb) was detected in the profile of barley cv. Jubiley. The protein content of micropropagated plant stems, fruits wall and seeds samples were decreased into 24.13, 27.84 and 3.64%, respectively than compared with conventional plants (Table 1). The micropropagated plant stem height, leaf area and number of immatured fruits were varied from conventional derived plants (data not shown). In oil palm a woody tropical crop species, the field evaluation of tissue culture derived plants revealed the occurrence of variant palms which show an abnormal flower development preventing fruit set [14].

Compared with parent seeds, the protein content was more in both of the seeds of conventional and micropropagated plant (Fig. 1). In micropropagated plant, only 3.64% of seed protein alone decreased from the conventional plants. Nguyen *et al.* [15] also observed the somaclonal variation for protein and yield character was decreased in soybean. The exogenous application of plant growth regulator (TDZ), medium and culture conditions might be the reason for decreased the protein contents. The somaclonal variations might be caused in soybean due to the hormonal concentrations in the tissue culture medium [16]. The highest variability was observed in micropropagated rhubarb PC49 might be triggered by the cytokinin during micropropagation [17]. The frequency of somaclonal variation would depend on

Table 1: Protein content in roots, stems, leaves and fruits wall of micropropagated and conventional plants

Plant samples	Protein content (mg g ⁻¹ dw)	
	in conventional plant	in micropropagated plant
Root	21.2±0.06	22.7±0.57
Stem	42.7±0.23	32.4±0.87
Leaf	30.7±0.64	40.3±0.18
Fruits wall	46.8±0.35	33.7±0.72

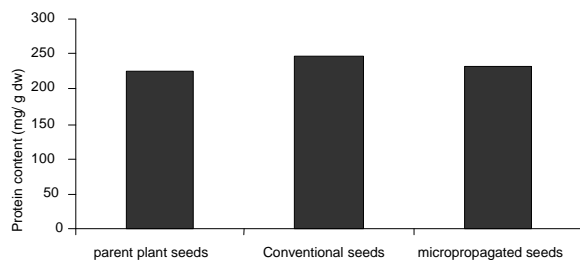


Fig. 1: Seed protein content in parent, micropropagated and conventional plants

the culture protocol applied during the *in vitro* process, particularly on the hormone composition of the medium and the number of subcultures [18]. If chromosome aberrations were present or if genes responsible for qualitative traits had been altered, we would expect to see abnormal plants and greater variation [16].

In conclusion, our study showed that the micropropagated plants were slightly changed the amount of protein content in roots, stems, leaves, fruits wall and seeds. However, the tissue culture derived plants did not cause any detrimental effects.

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