*Chemistry of Natural Compounds, Vol. 45, No. 6, 2009*

# FRACTIONAL COMPOSITION OF PROTEINS FROM SEEDS OF TRANSFORMED (Trn) *Gossypium hirsutum*

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UDC 581.3:633.511

Accelerated methods of selecting invariable forms that are based on biochemical markers cannot be developed without comparing proteins from various cotton varieties and species and studying the inheritance of individual protein components [1].

Wild cotton species exhibit many economically valuable properties. The transfer of these properties to cultivated plants would allow the production of new high yielding and wilt-stable cotton varieties [2].

We studied seeds of *Gossypium hirsutum* L. (Namangan-77 variety) and *G. arboreum* L. (D-1331 variety) and seeds of cotton transformed forms ($Trn_1$ and $Trn_2$) that were produced by micro-injection of sperm cells isolated from sprouted *G. arboreum* L. pollen tubes into ovaries of *G. hirsutumm* flowers. Pollen tubes were obtained by sprouting fresh pollen in liquid medium. Sprouted pollen tubes were disintegrated in buffer (pH 7.3) containing saccharose (20%), EDTA (5 mM), and Triton X-100 (0.05%). The whole mixture was filtered through a nylon filter and layered into Percoll [3] or saccharose [4–6] gradients. The layer in which sperm cells collected was determined using a microscope.

Changes were made to the literature method [3] for the samples that we used. Thus, nutrient medium was supplemented with Triton X-100 (0.05%), EDTA (5 mM, pH 5.3), and $CaCl_2$ (0.1 M). This mixture was mixed gently for 30 min. Then, the suspension was filtered through a 20–50 μm filter and rinsed with saccharose solution (20%). Each portion of filtrate (5 mL) was treated with Percoll (500 μL). The whole mixture was layered into a gradient of 15% and 40% Percoll in 20% saccharose. The gradient was centrifuged at 9,000 g and 4°C for 60 min. Starch grains, remnants of pollen grain coatings, cell organelles, and other contaminants were precipitated. Sperm cells that accumulated in the 15% Percoll layer were collected, washed, and concentrated by centrifugation at 3,000 g (4°C, 15 min).

The viability of the isolated sperm cells was determined. The resulting material was introduced by microinjection into the ovaries of preliminarily castrated flowers. Seeds from ripe pods were used for electrophoretic analysis of proteins.

Seeds were ground to a powder. The powder was defatted with acetone. Proteins were dissolved in PS-buffer and heated for 3 min on a boiling water bath. Bromphenol blue (0.01%) was added. Samples were placed on a gel in a minimum volume (30 μL). Electrophoresis of proteins was carried out at room temperature calculated at 5 mA per tube in a PAAG linear concentration gradient from 10 to 18% [7]. The gel was fixed after the electrophoresis was finished in coumassie P-250 solution (0.1%) in EtOH:AcOH:$H_2O$ (25:5:70). The excess of dye was removed with acetic acid (7%). Bovine albumin (67,000 Da), ovalbumin (45,000 Da), and cytochrome C (11,700 Da) were used as marker proteins. Molecular weights of subunits were calculated from the relative electrophoretic mobility of standard and studied proteins.

Our goal was to compare electrophoresis of total proteins from seeds of parent forms and first and second generation transformed plants ($Trn_1$ and $Trn_2$).

Fertile plants grown from seeds that ripened after microinjection of *G. arboretum* sperm cells into *G. hirsutum* flower ovaries had a monotypic morphology for the vegetative and generative organs that was intermediate between the initial parent forms [8].

Electrophoretic analysis showed significant differences among fractions of parent specimens (*G. hirsutum* and *G. arboreum*) with respect to the distribution of major fractions (Table 1).

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0009-3130/09/4506-0941  

TABLE 1. Electrophoretic Mobility ($R_f$) of Polypeptide Fractions of Seeds from Parent and Transformed (Trn) Forms

| *G. hirsutum* L. Namangan-77 (parent) | *G.arboreum* L. D-1331 (sperm-cell donor) | $Trn_1$ | $Trn_2$ No. 15 (intermediate form) | $Trn_2$ No. 20 (close to parent form) |
|---|---|---|---|---|
| 0.05 mj. | 0.05 mj. | 0.05 mj. | 0.05–**0.07*** mj. | 0.05–**0.07** mj. |
| | | **0.10** | **0.11** | **0.11** |
| 0.15 | 0.15 mj. | 0.15 | 0.15–**0.17** | 0.15–**0.17** |
| 0.23 | | – | 0.23 | 0.23–0.24 |
| | | **0.27** | – | – |
| 0.37 | 0.30–0.35 | 0.35–0.37 | 0.36–**0.38** mj. | 0.37–**0.39** mj. |
| | | | **0.40** | **0.42** |
| 0.45 | 0.45 mj. | 0.45 mj. | 0.44–0.46 mj. | 0.47 mj. |
| | | | **0.54** | **0.55** |
| | | | | **0.57** |
| 0.59 w | | 0.59–**0.60** mj. | 0.59–**0.60** mj. | 0.59–**0.61** mj. |
| 0.65 w | 0.64 | | **0.62** | |
| | 0.67 | 0.68 | 0.68 | – |
| | | | **0.71** | **0.69–0.71** |
| 0.76 w | | 0.75 | 0.76 | 0.76 mj. |
| | | | **0.78** | **0.79** |
| | 0.83 | 0.83 | 0.82 | – |
| | | **0.84** | **0.84** | **0.84** |
| | | | **0.86** | **0.86** |
| 0.90 w | 0.89 | 0.89 | – | – |
| 0.93 | | | **0.95** | **0.90** mj. |
| 0.98 w | 0.97 | 0.97 mj. | 0.97 mj. | – |
| | 0.10 | 0.10 | 0.10 | – |

mj., major proteins; **0.07***, proteins not expressed in parent specimens; (–), proteins not appearing in following generations; w, weakly appearing proteins.

Five major fractions with relative electrophoretic mobility (REM, $R_f$) 0.5, 0.10, 0.15, 0.30–0.35, and 0.45 were found in the spectrum of *G. arboreum* seed proteins.

For *G. hirsutum*, five major fractions with *Rf* 0.5, 0.15, 0.22, 0.35, and 0.45 and two minor ones with $R_f$ 0.59 and 0.92 were characteristic.

The spectrum of *G. arboreum* seed proteins had minor fractions with $R_f$ 0.20, 0.25, 0.47, 0.59, 0.67, 0.75, 0.88, and 0.97 and fractions with intermediate contents with $R_f$ 0.63 and 0.82 that were not found for *G. hirsutum*.

The electrophoregrams showed that *G. arboreum* typically had more low-molecular-weight proteins whereas *G. hirsutum* contained polypeptides with molecular weights from 12 to 27 kDa. Therefore, the parent specimens, *G. hirsutum* and *G. arboreum*, differed sharply from each other with respect to qualitative composition of low-molecular-weight proteins. The spectrum of total seed proteins of the first and second generations of transformed plants ($Trn_1$ and $Trn_2$) was different from that of the parent proteins (*G. hirsutum* and *G. arboreum*).

Electrophoregrams of total seed proteins of this generation contained fractions not found for the parent specimens. These were a major high-molecular-weight polypeptide with $R_f$ 0.10; fractions with $R_f$ 0.75, 0.90 and 0.97; and minor ones with $R_f$ 0.42, 0.53, and 0.70 that were missing for *G. hirsutum* and *G. arboreum* (Table 1).

The quantitative ratio of major proteins for the next generation did not typically repeat the distribution spectrum of the parent specimens (*G. hirsutum* and *G. arboreum*). They had several differences among low-molecular-weight protein fractions in the $R_f$ range from 0.50 to 0.100.

The distribution pattern of polypeptides extracted from $Trn_1$ seeds was analogous to those of seed proteins from $Trn_2$. However, there were some differences. For example, proteins with $R_f$ 0.54, 0.55,0.57, 0.62, 0.69–0.71, 0.78, 0.79, 0.86, 0.90, and 0.95 appeared. These had not been observed previously for any of the parents or $Trn_1$ (Table 1).

The results suggested that foreign genetic information of sperm cells that incorporates into a donor organism affect the biosynthesis and inheritance of total proteins in subsequent $Trn_2$ generations.

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