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# Characterisation of Chloroplast Protein in *Camellia* spp.

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## ABSTRACT

Recent molecular biological technique like SDS-PAGE of chloroplast protein can provide useful information on genetic make-up of crop. The chloroplast was isolated from actively growing young tea leaves using discontinuous sucrose density gradient (20%-45%-60%). The isolated chloroplast was lysed with 2% SDS solution. SDS being an anionic detergent breaks the disulphide linkages of the peripheral protein and thus causes lysis. SDS-PAGE of chloroplast protein isolated from different clones of *Camellia assamica* showed identical characteristic banding pattern. In total 11 bands could be detected in the gel. The molecular weight of which ranged from 13 Kd to 42.5 Kd.

Tea is one of the world's most popular hot beverage. The Indo-Burma region including the North-East part of India is considered as the primary centre of origin of the crop. Very little information regarding genetics and cytogenetics of tea are available. Taxonomic classification of tea is primarily based on morphological, anatomical and biochemical parameters. Due to perennial nature and inbreeding depression, the conventional methodology cannot be applied to know the genetic constitution of the crop. Characterisation of chloroplast protein can provide useful information regarding genetic make up of the crop. Markwell et al. (1979) worked on higher plant chloroplast and proved that chlorophyll exists at chloroplast protein complex. The fractionation of the phytochemical systems of photosynthesis was carried out by Anderson and Boardman (1966). Markwell and Thornber (1982) worked on the treatment of the thylakoid membrance with supernatants. They reported that the different membrances have different lipid and protein composition, so supernatants may vary in its efficacy between different membrane systems. This paper reports the chloroplast protein characterisation in different clones of *Camellia.*

## MATERIALS AND METHODS

The chloroplast protein was isolated from 10 TV clones of *Camellia* (TV1, TV2, TV3, TV4, TV5, TV6, TV7, TV8, TV9 and TV10). Fifty gram of actively growing young and fully expanded tea leaves were rinsed with tap water and surface sterilised with 0.1% HgCl2 solutions and was finally washed with several flush of distilled water (MilliQ, Millipore, USA). Mid-ribs were removed and the leaves were cut into small pieces. Leaves were then crushed with liquid nitrogen in a mortar and homogenized with 250 ml of buffer A (50 mM Tris, 0.35 M sucrose, 7 mM EDTA, 0.1% PVP, 0.1% BSA, 5 mM morcaptoothanol, pH 8.0) was added. The extract was further homogenized in a polytron homogeniser (Ploytron PT 3000, USA). The homogenate

was filtered through 8 layers of muslin cloth. The filtrate was centrifuged at 5000 rpm (Remi C-24, Bombay) for 10 min. The pellet was then suspended in minimal volume of buffer B (50 mM Tris, 0.35 M Sorbitol, 7 mM EDTA, pH 8.0). The sample was then loaded on a discontinuous sucrose gradient 20%-45%-60% and centrifuged for 1 hr at 17000 rpm (Rotor RP-80-AT-207, Hitachi CS-120, Japan). The green colour chloroplast fraction was collected from 20-45% interphase and further diluted with 3 volumes of buffer B. The extract was then centrifuged at 5000 rpm for 10 min. and the peller was collected. The chloroplast pellet thus obtained was lysed and solubilised following the method of Remy and Bretterille (1987) for solubilisation of organelle polypeptides. The chloroplast protein sample was electrophoresed on a 10% polyacrylamide gel using 0.025 M Tris-glycine buffer, pH 8.3 containing 0.1% SDS (Laemmli, 1970). A mixture of 6 standard protein of known molecular weight (Albumin egg, 45 kd; Glyceraldehyde 3-phosphate dehydrogenase, 36 Kd, Carbonic anhydrase, 29 Kd; Trypsinogen PMSF treated, 24 Kd; Trypsin inhibitor soybean, 20.1 Kd and a-lactalbumin, 14.2 Kd) was also run along with the sample to find out the molecular weight of the organelle polypeptides.

## RESULTS AND DISCUSSION

The SDS-PAGE analysis of chloroplast protein isolated from 10 TV clones of C. *assamica* showed identical banding pattern (Fig.1). In all, 11 bands could be detected in the gel. They were labeled as I (slowest moving) to 11 (farthest moving). The molecular weight ranged from 13 Kd to 42.5 Kd in all the clones (Table 1). Among all the bands, two bands stained more intensely. These two bands might be the major polypeptide in all the clones of molecular weight 40.5 Kd (Subunit 2) and 23.5 Kd (subunit 6) (Table 1). Based on the Rf values the polypeptides can be

**Table 1. SDS-PAGE characteristics and molecular weights of subunit fractions of chloroplast proteins from 10 TV clones of *Camellia assamica.***

| Subunit | Molecular weight (Kd) | SDS-PAGE band characteristics |
|---|---|---|
| 1 | 42.5 | + |
| 2 | 40.5 | ++++ |
| 3 | 38.0 | + |
| 4 | 28.5 | + |
| 5 | 27.0 | + |
| 6 | 23.5 | ++++ |
| 7 | 21.5 | ++ |
| 8 | 19.5 | ++ |
| 9 | 17.5 | ++ |
| 10 | 14.5 | ++ |
| 11 | 13.0 | ++ |

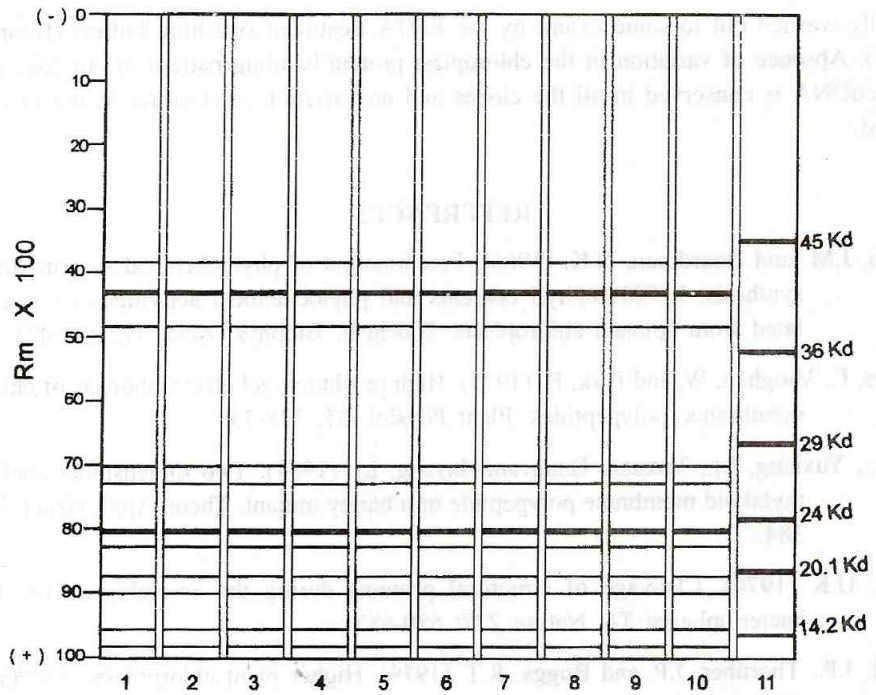


**Fig. 1. SDS-PAGE profile of chloroplast proteins in different clones of *Camellia assamica***

**Lane: 1. TV1 2. TV2 3. TV3 4. TV4 5. TV5 6. TV6**
**7. TV7 8. TV8 9. TV9 10. TV10 11. Marker**

grouped into three zones (Fig. 1). Zone I represents slow migrating band with Rf value between 0.2 to 05, zone II represents band with Rf value 0.6 to 0.8 and the third zone (Zone III) represents polypeptide with Rf value 0.8 to 1.00. Jigeng et al. (1984) found about 20 bands of thylakoid membrance polypeptide in barley and also reported that some of the bands are not stable and may change under the conditions of the separating procedures. Henriques et al. (1975) could detect 50 clearly district bands in spinach chloroplast membrance polypeptide. According to Henriques et al. (1975) solubilisation conditions produce only depolymerisaion of oligomeric proteins with the consequence that several polypeptide species may be present at the same band and few bands are seen. In our case, a long tract of bands could be seen in the gel. Henriques *et al.* (1975) also found a 23 Kd major band in spinach chloroplast which was similar to our results. Thornber and Highkin (1974) found that 23 Kd polypeptide is associated with photosystem II (PS II) chloroplast protein complex. Thornber and Highkin (1974) also reported that this polypeptide is the major location for chlorophyll b in higher plants and functions as light harvesting centre for PS II. Three other major polypeptides with molecular weight 60 Kd, 56 Kd and 54 Kd region was found to be associated with the proteins attached to the outer surface of chloroplast membrances. These were found to be absent in our investigation. These compounds

are usually washed out to some extent by the EDTA treatment (washing buffer) (Henriques et al., 1975). Absence of variation in the chloroplast protein banding pattern in our case suggests that the coDNA is conserved in all the clones and no variation exist as far as the organelle is concerned.

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