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Electrophoretic Distinction Between Sexes of *Hodgsonia macrocarpa*

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Abstract: Soluble protein profiles, fractionated by reducing and non-reducing SDS-PAGE, were carried out in dioecious *Hodgsonia macrocarpa* (Bl.) Cogn. Though the reducing SDS-PAGE protein profiles from the tuberous roots of the male and female plants did not show any qualitative distinction, the protein profile in non-reducing SDS-PAGE reveals a clear distinction when compared on a single gel. The difference is marked by the presence of two disulphide linked tertiary or folded protein at 17 KD and 35 KD regions detected in male sex. However, at the level of primary structure the qualitative expression is similar indicating a common ancestry.

Key words: Dioecious · Reducing and Non-Reducing SDS · PAGE

INTRODUCTION

Most of the flowering plants are bisexual having flowers with both male and female reproductive organs and only less then 4% plant species are dioecious in nature [1]. Chromosomal sex determination system in flowering plants also indicates that the plant sex chromosomes have evolved recently through replicated independent events [2]. *Hodgsonia macrocarpa* is a dioecious plant species belonging to Cucurbitaceae and is distributed in Eastern Himalayas, Sikkim, Assam, Bangladesh, Nepal, China, Burma and Malaya [3, 4]. In Tripura, it is mainly restricted to hilly region of West, Dhalai and North district. Seeds of *H. macrocarpa* are used as raw vegetables by the local tribal people. This plant is also medicinally very important, viz., seeds are used in antifertility and armament sterility, fruit juice used in skin disease, leaves are used for treating bleeding nose and to reduce body heat [5-7]. Each sex form of *H. macrocarpa* strictly maintains their sexual phenotypes and every year sprouting occurs from their respective vegetative reproductive structure, i.e. the tuberous root, without failing to reproduce their own kind. Plantlets germinated from seeds usually generates flower after 5 years which is a barrier in sex identification for cultivators. Till date there is a single report by Xiluan and Wengin [8] on the somatic chromosome number of *H. macrocarpa* and according to them the chromosome count was 2n = 18. In the absence of detailed chromosomal information, electrophoretic study of soluble protein profile of the vegetative reproductive structure could be useful in understanding the genic expression of the sexual phenotypes. The present investigation has, therefore, been aimed at SDS-PAGE analysis from the tuberous root with or without 2-mercaptoethanol to resolve the differences, if any, between the sexes of *H. macrocarpa*.

MATERIALS AND METHODS

Tuberous roots of the sex forms of *H. macrocarpa* growing in wild condition were collected from hilly region of North District of Tripura, for the experimental purposes. Two grams of fresh tuberous tissue for each sample were homogenized in 4ml of extraction buffer containing 0.25M sucrose and 1mM EDTA in 0.1M Tris - HCl buffer (pH 6.8). The homogenates were then centrifuged at 12000 rpm for 45 minutes at cold. The supernatants were collected and immediately used for electrophoresis. The protein concentration was estimated by the method of Lowry [9] using BSA as a standard. The soluble protein obtained in extraction buffer was boiled with equal amount of 1 X strength electrophoresis sample buffer (12.5% glycerol, 1.25% SDS, 0.005% bromophenol blue, 62.5%Tris-HCl, pH- 6.8) in presence and absence of 178 mM 2-mercaptoethanol for 5 minutes & allowed to cool at room temperature before proceeding to the next step.

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All the reagents used were of electrophoresis grade (SRL & MERCK). Electrophoresis in 12% polyacrylamide slab gel containing 0.1% SDS [10] using a discontinuous system [11] was carried out and approximately 10 µg, 15 µg and 30 µg soluble proteins of each sex form was loaded onto each lane in different experimental sets. Protein patterns were visualized by staining the gel for overnight with 0.2% Coomassie Brilliant Blue R-250 in methanol: glacial acetic acid: DDH_2O (9:2:9) mixture followed by destaining repeatedly with mixture of iso-propanol: acetic acid: DDH_2O (2:1:7). The position of the bands was expressed as relative mobility (Rm) and was determined by measuring the ratio of the distances traveled by a particular band and the indicator Bromophenol Blue. The different Rm values of the protein bands were numbered serially. Using five cycle semi log graph paper their molecular weights were determined from the standard curve.

RESULTS AND DISCUSSION

The sexual phenotypes of *H. macrocarpa* differ and it has been observed that the male flowers of *H. macrocarpa* are in racemes whereas female flowers are solitary axillary (Fig. 1 & Fig. 2). The soluble protein profiles of the sex forms of *H. macrocarpa*, fractionated by reducing SDS – PAGE (in presence of mercaptoethanol), did not show any marked distinction but only a variation in the intensity of staining pattern was observed (Fig. 3). The non- reducing (without mercaptoethanol) SDS – PAGE protein profile obtained showed a total of 23 bands which were common in both the sex forms. However, the non-reducing protein profile

Fig. 1: Female flower of *H.macrocarpa*.

Fig. 2: Male flower of *H.macrocarpa*.

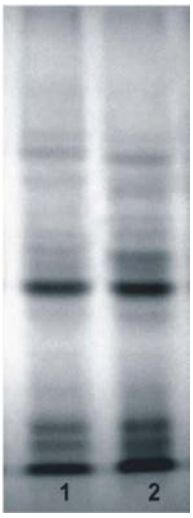

Fig. 3: Electrophoregram of reducing SDS – PAGE protein profile from tuberous roots of *H. macrocarpa* (lane 1- female, lane 2 – male) {Marker lane is in middle}.

of male plant also contained two bands at 17 KD and 35 KD regions which were not detected in female sex (Fig. 2 and Fig. 5). Moreover, variation in the intensity of band patterns was also observed although equal amount of protein was loaded onto each lane. The electrophoretic distinction between two sexes is, therefore, marked by the presence of two disulphide linked tertiary or folded protein at the 35 K D and 17 KD regions in male sex. The variability thus obtained suggests that such tertiary or folded proteins are formed in the vegetative reproductive structure of male sex which is not detected in female plant. Evidently at the level of primary structure, the qualitative expression is similar in both sexes indicating a common ancestry.

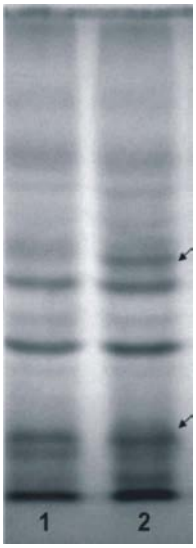

Fig. 4: Electrophoregram of non-reducing SDS – PAGE protein profile from tuberous roots of *H. macrocarpa* (lane 1- female, lane 2 – male) {Arrow indicates 35 &17 KD protein respectively}.

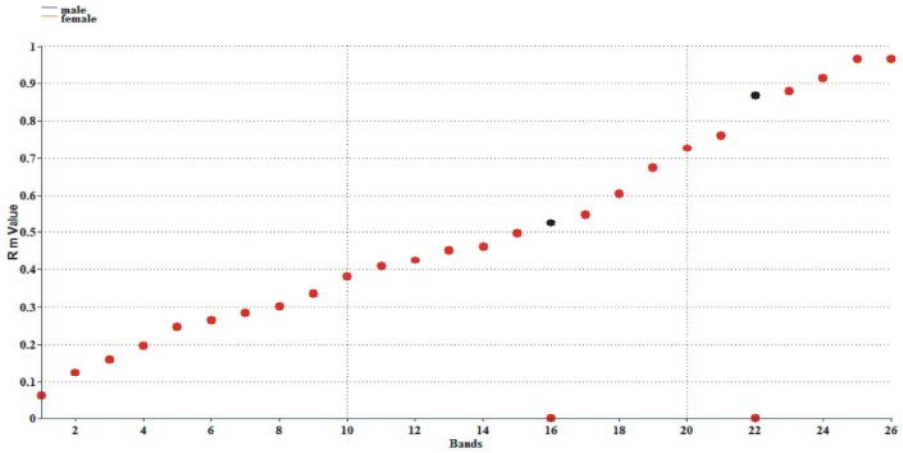

Fig. 5: Graph showing non-reducing SDS-PAGE Profile which indicates presence of two extra bands only in male *H. macrocarpa*. – Female - Male

CONCLUSION

SDS-PAGE seed protein profile is usually used to resolve the taxonomic and evolutionary implications of several crop plants. The present finding suggests that the non-reducing SDS-PAGE profile, of vegetative reproductive structure i.e. the tuberous roots, could be used to resolve the distinction between the sexes of dioecious *Hodgsonia macrocarpa*.

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