Sex Linked Polypeptides in Dioecious *Momordica dioica*

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Summary Soluble proteins from the specialised reproductive structure, the tuberous roots of *Momordica dioica* Roxb. through which it propagates, were analysed by SDS-PAGE to compare the protein profiles of the sex forms. Twenty eight bands with molecular masses ranging from ~15 kD protein to more than 94 kD proteins were found to be common in both sexes. The difference in their protein profiles was marked by the presence of a 22 kD polypeptide (p-22) in the female sex which was not detected in its male counterpart. Further studies by immunoblot assay demonstrated that antibody raised against p-22 not only cross-reacted at 22 kD antigen of the female sex but also with 29 kD and 32 kD polypeptides of the male and female sex forms. It indicates that these 3 polypeptides are electrophoretically distinct but antigenically similar. 22 kD protein found in female sex is, therefore, not sex specific rather sex linked. Moreover, variation in the intensity of 29 kD and 32 kD polypeptides of male and female sex forms suggests that the interplay of these 2 sex linked polypeptides may be a contributing factor in controlling sex mechanism of dioecious *Momordica dioica*.

Most of the flowering plants are bisexual having flowers with both male and female reproductive organs and only less than 4% of plant species are dioecious in nature. While many of these species have distinct X and Y sex chromosomes, in some species no chromosomal distinction has been recorded in relation to sex (Roy *et al.* 1966, Singh 1988). The chromosomal sex determination systems in flowering plants indicates that plant sex chromosomes have evolved recently through replicated, independent events (Mayer and Charlesworth 1992, Westergaard 1958) contrasting with ancient origins of mammalian and insect sex chromosomes (Guttman and Charlesworth 1998).

Momordica dioica, a dioecious perennial cultivar of Cucurbitaceae propagates vegetatively by means of specialised reproductive organs, the tuberous roots. It is obvious that the inherent genetic make up responsible for the expression of sex forms of *M. dioica* perpetuates, through the specialised reproductive structure from which sprouting occurs every year without failing, to reproduce their own kind. In the light of this knowledge, electrophoretic study of soluble protein patterns from the vegetative reproductive organ of dioecious *M. dioica*, was carried out to compare the protein profiles of the male and female plants. The present communication also reports a study on the antigenic response of a 22 kD polypeptide found in female sex to determine the sex specificity of the resolved polypeptide.

Materials and methods

Acrylamide, N,N'-methylenebisacrylamide, sodium dodecyl sulphate, ammonium persulphate and Bovine Serum Albumin (BSA) were purchased from SRL (India). All other chemicals used were of analytical reagent grade. Tuberous roots of dioecious *M. dioica* collected from a cultivated field of west Tripura were grown in the experimental garden, Department of Life Sciences, Tripura University for the present study.

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Extraction of soluble proteins

2 g fresh tuberous tissue were homogenised in 5 ml extraction buffer (EB) containing 0.25 M sucrose, 1 mM EDTA in 0.1 M Tris-HCl buffer (pH-6.8). The homogenates were then centrifuged at 18000 g for 45 min at cold. The supernatants were collected and immediately used for electrophoresis.

Protein estimation

Protein concentration was determined by the method of Lowry *et al.* (1951) using BSA as a standard.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis

The soluble protein obtained in EB was boiled with equal amount of 1 X strength electrophoresis sample buffer (12.5% glycerol, 1.25% SDS, 178 mM 2-mercaptoethanol, 0.005% bromophenol blue, 62.5 mM Tris-HCl, pH-6.8) for 5 min and was allowed to cool at room temperature before proceeding. Electrophoresis in 11% polyacrylamide slab gel, containing 0.1% SDS was carried out by the method of Laemmli (1970) and approximately 50 mg protein was loaded onto each lane. The protein patterns were visualised by staining the gel for overnight with 0.2% Coomassie brilliant blue R-250 in methanol:acetic acid:water (9:2:9) mixture and destained with several changes of mixture of isopropanol:acetic acid:water (2:1:7). The positions of the bands were expressed as relative mobilities (R_m) by measuring the distance migrated by the particular bands to that of the indicator bromophenol blue. The different R_m values of the protein bands were numbered serially. Using two cycle semilog graph paper, their molecular weights were determined from the standard curve.

Raising of antibody against p-22

From the stained gel strips of 22 kD polypeptide of the protein profile of female plant were taken out and homogenised in EB. The suspension containing 80–100 μ g proteins were injected into rabbits subcutaneously at 2–3 places on the thigh and back on day 0. The step was repeated on 8th, 15th and 22nd day from day 0. After a rest of 10 days blood was collected and allowed to stand at room temp for 2 h. It was then centrifuged at 1000 rpm for 10 min. The serum was collected, de-complemented at 56°C for 30 min, mixed with 0.3% Na-azide solution, aliquoted and stored in deep freeze until use.

Immunoblot experiment

After electrophoresis in 12.5% polyacrylamide slab gel, the resolved polypeptides were transferred electrophoretically onto nitrocellulose paper with 0.1 Amp. current for 90 min at 4°C (Towbin *et al.* 1979). The nitrocellulose sheet was then blocked with 0.5% Tween 20 in TBS buffer (20 mM Tris+50 mM NaCl, pH-7.5) for 2 h. at 37°C. After blocking the sheet was transferred to a fresh solution of TTBS (20 mM Tris+50 mM NaCl+0.05% Tween 20) containing anti p-22 at 1:3000 dilution and again incubated at room temperature for 90 min with shaking. Then the sheet was washed with TTBS for a period of 2 h, then incubated with 1:4000 dilution of anti-IgG alkaline phosphatase conjugate (Cat-A 8025, Sigma) in TBS for 1 h with gentle agitation. The paper was then washed with TTBS containing Tween 20 (0.05%) for 3 times each with a duration of 30 min. The bands were developed with 5 bromo-4-chloroindonyl phosphate and nitroblue tetrazolium (Blake *et al.* 1984).

Results and discussion

When the SDS-PAGE protein profiles of the tuberous roots of male and female plant of *M.*

dioica were compared on a single gel, the 2 sex forms could be readily distinguished by visual observation of their banding patterns (Fig. 1). A total of 29 bands with molecular masses ranging from ~15 kD to more than 94 kD were observed on the gel. Twenty eight bands were found to be common in male and female plants and only a single band difference was observed in relation to sex. However, a variation in the intensity of the band patterns between the sexes was also noted although equal amount of protein was loaded onto each lane. The difference between the sexes was distinctly marked by the presence of a 22 kD protein in female plants but not detected in its male counterpart. The constant appearance of this specific protein band at 22 kD region found in all the protein profiles of the female plant studied from the dormant and sprouting specialised reproductive structure, reveals its relation to a particular sex.

The genetic variability thus obtained when compared with chromosomal studies, it has been recorded that the somatic chromosome complement ($2n=56$) were more or less homogenous and no heteromorphism was recorded in relation to sex (Roy *et al.* 1966, Sinha *et al.* 1997). The data obliterate the possibility of XY mechanism and suggests the genic control of sex expression.

The question that might arise (i) whether 22 kD polypeptide found in female sex is antigenically unique without any common epitope(s) or (ii) 22 kD polypeptides is electrophoretically distinct but antigenically similar with a common epitope(s) to any of the protein profiles of the male and female sex forms. To test this, antibody was raised against p-22 and immunoblot experiment was performed to determine the cross-reactivity against anti p-22. It was found that in the female plant the antisera cross-reacted not only at 22 kD region (Fig. 2) but also with 29 kD and 32 kD regions. In male plant we have found that anti p-22 cross reacted at 29 kD and 32 kD regions only. Further no cross reaction was observed when the protein profiles of both the sex forms were treated with normal rabbit serum. It suggests that 22 kD antigen is present only in female plants and two more electrophoretically distinct but immunologically cross reacting antigens with anti p-22 are also present in both male and female sex forms of *M. dioica*. Evidently such cross reactivity of anti-p-22 with 29 kD and 32 kD polypeptides of male and female plants suggests that 22 kD polypeptide is not sex specific rather sex linked. Recently, Guttman and Charlesworth (1998) have shown that in the dioecious plant *Silene latifolia*, a gene encoding a male specific protein is linked to the X-chromosome and it has a degenerate homologue in the non-pairing region of the Y-chromosome. On the basis of common homology between the active X linked locus and the degenerate Y linked locus they have suggested a common ancestry for these two loci.

The intensity of 29 kD polypeptide in female sex form of *M. dioica* are more than that of the male plant which may be due to lower amount of anti p-22 cross reacting antigen in the male sex form. However, a reverse situation was observed in male plant where the amount of 32 kD antigen was more than that present in the female plant. The difference of antigenic cross reactivity observed

Figs. 1-2. 1) Electrophoregram of protein profile from tuberos roots of *M. dioica* in 11% polyacrylamide gel respectively (lanes 1, 3=male; 2, 4=female). Molecular weights standards (lane 5) are phosphorylase b (94 kD), albumin (67 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), trypsin inhibitor (20.1 kD), lactalbumin (14.4 kD). 2) Immunoblot showing antigen recognition by anti p-22. lane 1. *M. dioica* (male), lane 2. *M. dioica* (female).

between male and female sex forms suggests that an interplay of 29 kD and 32 kD polypeptide may play an important role in controlling the sex mechanism of *M. dioica*. The homology of the sex linked 22 kD polypeptide with 29 kD and 32 kD polypeptide of both male and female sex form indicates a common ancestry probably from which they have evolved through independent events.

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