cient C sequestration in some forests, observed recently[6]. Increased C sequestration due to N fertilization and soil faunal activities may control further C sequestration as a feedback, by means of increased root exudation[7] and consequent incorporation of available N into the soil. Such control mechanisms could act as signalling systems to induce litterfall, etc. for improved efficiency of the ecosystems' functioning.

It is warned that the large-scale use of nitrogen-based fertilizers to boost the capacity of C sinks could actually increase climate change by releasing other greenhouse gases, particularly N_2O (ref. 1). This may be overcome by proper timing of the fertilizer application with the litterfall and turnover patterns, because soil-surface litter cuts down soil N_2O emission considerably[8]. In addition, environment-friendly fertilizer formulations could also contribute to this[8]. In conclusion, litter turnover and root exudation extensively modify soil N availability to plants and microbes in terrestrial ecosystems. Therefore, those factors determine C sequestration and open a room to increase the C sink strength of the biosphere. It is recommended that these basic parameters should be incorporated into computer simulations for reducing uncertainties of their predictions.

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Received 24 September 2001; revised accepted 14 November 2001

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Female sex-associated RAPD marker in pointed gourd (*Trichosanthes dioica* Roxb.)

Pointed gourd (*Trichosanthes dioica* Roxb.), a perennial vegetable crop is cultivated widely in West Bengal, Bihar and Eastern Uttar Pradesh. It is one of the most nutritive cucurbit vegetables highly used in India due to its availability for about eight months in a year. Pointed gourd is morphologically distinct from the other cucurbitaceous species due to its well-established dioecism (Figure 1) and vegetative means of propagation.

In the plant kingdom dioecy is found only approximately in 4% of the angiosperm. Dioecism has arisen independently in different families and genera, and several distinct genetic mechanisms regulating dioecy have been found in different plant species[1,2]. In contrast to the situation found in most animals, where highly differentiated sex chromosomes typically control dioecy, chromosomal heteromorphism is the exception, rather than rule in dioecious plant species. The presence of sex chromosome has been claimed for some dioecious angiosperms, but only in few cases have these claims been documented[3–5]. More often the sex ratio in dioeceous plant species is controlled by the expression of allele at one to several loci[1]. In *T. dioica*, the male and female plants strictly maintain their respective sexual phenotypes. This indicates clear genetic basis of difference between male and female individuals of this species.

Pointed-gourd breeding programme has recently been initiated to develop new cultivars. Dioecy represents an inconvenience in pointed-gourd breeding. Currently there is no method for distinguishing between male and female plantlets prior to flowering in *T. dioica*. A method to determine the gender of plants before flowering would facilitate breeding and selection, by enabling screening for gender at an early stage, thereby simplifying the breeding of male and female plants for different objectives, and saving time and economic resources. Hence the present investigation was carried out to understand the molecular basis of differentiation between male and female genotypes based on RAPD markers.

The plant material for this study comprised 16 female and 4 male accessions of pointed gourd. The materials were collected from isolated parts of Bihar and West Bengal and grown at the Research Farm in our institute. Young leaves collected from a random sample of ten field-grown plants from each accession were used for DNA extraction. Leaves were harvested and immediately stored at $-80°C$ until total genomic DNA was extracted, using the protocol of DNeasy plant mini-kit (QIAGEN). The DNA concentration was estimated with UV/VIS spectrophotometer (Lambda Bio20, Perkin Elmer, USA). The DNA concentrations were rechecked by visual assessment of band intensity in comparison with Lambda DNA of known concentration using 0.8% agarose gel. Bulk samples were prepared by pooling equal amount of genomic DNA purified from 10 individuals of each accession and aliquot from these combined samples was used for PCR reaction

DNA amplification was performed using a thermal cycler (Perkin Elmer, Cetus) programmed to 1 cycle of 4 min at 94°C (initial strand separation) followed by 40 cycles of 1 min at 94°C (denatura-

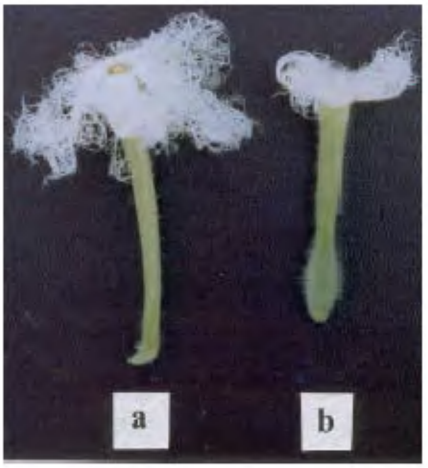

Figure 1. Photograph of male and female flowers of pointed gourd. ***a***, Male flower; and ***b***, Female flower.

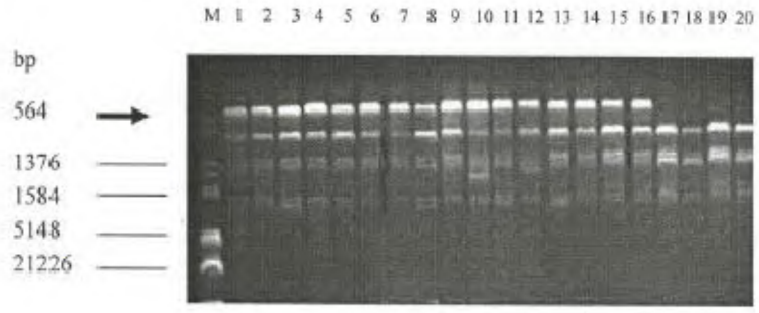

Figure 2. RAPD pattern of male and female lines of pointed gourd generated by primer OPC-7. Lane M, Molecular weight marker; lanes 1–16, RAPD products from DNA of female plants; lanes 17–20, RAPD products from DNA of male plants. Arrowhead indicates female-related marker.

tion), 1 min at 36°C (annealing) and 2 min at 72°C (primer extension) ending with 1 cycle of 72°C (final extension). Total reaction volume for DNA amplification was 25 µl. Reaction mixture contained 1× PCR buffer (10 mM Tris HCl pH 8.8, 50 mM KCl and 15 mM $MgCl_2$), 3 mM $MgCl_2$, 300 µM each dNTPs, 0.5 µM primer, 1 unit of Taq DNA polymerase and approximately 60 ng of genomic DNA. The reaction mixtures were overlaid with one drop of mineral oil to prevent evaporation. After amplification PCR product was stored at 4°C till electrophoresis. Reaction products were mixed with 2.5 ml of 10× loading dye (0.25% bromophenol blue, 0.25% xylene cyanol and 40% sucrose; w/v) and spun briefly in a micro centrifuge before loading[6]. PCR products were resolved by electrophoresis in 1.4% agarose gel at 60 volts followed by staining with ethidium bromide and photographed in Gel documentation system (UVP, GDS-8000).

Sex-linked genetic marker may not only be useful in breeding programmes, but would also allow the understanding of the genetic and molecular basis of dioecism in *T. dioica*. In the present study such differences were examined using RAPD technique. Though fertile male and female plants are produced, progeny of male and female plants from a cross was not available. Hence to study genetic differences, a germplasm collection consisting of 16 female and 4 male lines/entries collected from different geographical areas was used.

The experiment was carried out in two stages. In the first stage the DNA was pooled from all the male and female cultivars, separately and screening of primer was done on the pooled DNAs. Hundred decamer primers were screened for differences in male and female cultivars. In this way 5 primers were identified which produced probable female-related bands. In the next stage, the 5 selected primers were used to confirm the presence and absence of bands in all the male and female entries, individually. In this stage 567 bp band amplified by the OPC07 primer from the genomic DNA of all the female entries was absent in the PCR products of the DNAs from all the male entries (Figure 2). Thus within the limits of male and female genotypes available in this study, the RAPD band $OPC07_{567}$ appeared to be the female sex-related DNA marker in *T. dioica*. So far sex-linked RAPD markers have been reported from three other plant species as well. A RAPD band $OPA\ 08_{945}$ was shown to be female-specific in *Pistachio vera*[7]. Alstrom Ranagport *et al.*[8] reported that RAPD band $UBC\ 354_{560}$ was linked to a sex-determination locus in *Salix viminalis*. For male sex-associated genetic factors, Y-chromosome-specific restriction fragment has been reported from white campione[9] and two RAPD bands, $OPA\ 10_{908}$ and $OPC\ 12_{757}$ have been found to be male-specific in *Piper longum*[10].

Several attempts to find molecular differences between male and female plants such as immuno chemistry, isozyme pattern, RNA typing and RNA hybridization[2] have been undertaken. Those studies were related to gene expression and could show a differential expression of shaped genes between males and females. A much better approach is to study such differences at the DNA level. The availability of markers linked to sex-determining genes would allow to clone the gene(s) involved in this process. Promising results have already been obtained in *Asparagus* using RFLPs[11]. In this paper we report the finding of a RAPD marker associated with females, that is not present in males of *T. dioica*. The importance of this finding is the early assessment of gender in *T. dioica*, as well as the possible implication in understanding the molecular basis of sex determination in this dioecious species.

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Received 11 June 2001; revised accepted 22 November 2001

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