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Short Communication

Molecular characterisation of dioecious *Trichosanthes dioica roxb.* using RAPD markers

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Pointed guard (*Trichosanthes dioica* Roxb.) belonging to Cucurbitaceae is a perennial vegetable crop cultivated widely in different regions of Asia including India which is claimed to be the land of origin of this species. *T. dioica* is well represented in the eastern part of India and as many as 20-24 species of *Trichosanthes* have been recognized [1]. However, *T. dioica* remained one of the less explored crops of this region.

Morphological characters have been used extensively to study diversity of different forms in the past. In recent years, attempts to study biodiversity at molecular level have gained importance. Randomly Amplified Polymorphic DNA (RAPD) has been extensively used to study diversity in different crops [2]. Present study reports the extent of diversity between different accessions of *T. dioica*.

T. dioica accessions MI, MII (males) and NP260, NP307, NP604, NP602, NP701, RP (females) were collected from Narendra Dev Agriculture University Kumarganj, U.P. India and were grown as companion crop in betel vine plantations in botanical garden of National Botanical Research Institute Lucknow. Young leaves were harvested, washed, dried and their length and breadth were measured using 30 cm scale. Twenty leaves from each accession were punched to give 10 cm discs; the discs were dried in oven to constant weight and weighed individually to determine specific leaf area (SLA) (Table 1). Young leaf tissue was washed; adhering water was dried on paper towel and quickly powdered in liquid Nitrogen. The powder was either stored at –70°C or used for DNA isolation immediately.

Total genomic DNA was isolated from the powdered and frozen young leaf tissue of *T. dioica* cultivars using procedure of Dellaporta *et al.* [3] with some modifications. At least three independent DNA preparations were made for each cultivars of *T. dioica*. The quality of DNA was checked on 0.8% agarose gel and quantification is done by using Pharmacia Biotech DNA Quanta TM200 florimeter and diluted to 50ng/µl for polymerase chain reaction (PCR) amplification.

Twenty-four decamer primers of AB and AP Kit (Operon Technologies, Alameda, California, USA) were used as primers (Table 2). DNA was amplified by PCR amplification reaction as per the procedure [2] with minor modifications. The 20µl of reaction mixture contained 50ng genomic DNA and 100µM each of dATP, dGTP, dCTP and dTTP (Pharmacia Biotech) 1.5mM $MgCl_2$, 10 pmoles primer (Operon primers, Alamoda, California) and, 0.75units of Taq DNA polymerase (Bangalore Genei, Banglore India). The reaction mixture was overlaid by mineral oil prior to the amplification. The reaction consisted of 44 cycles each of 1 min at 98°C for denaturation, 1.5 min. at 36°C for primer annealing and 1.5 min at 72°C for extension, followed 1 cycle of 5 min at 72°C for final extension. Amplified product was checked on 1.2% Agarose gel incorporated with 0.01% ethidium bromide in 0.5 x TBE buffer [4], Gel was visualized and imaged using gel BIORAD Documentation system.

RAPD bands were scored as 1 (present) or 0 (absent) in a binary matrix for each primer. Fragments size of all the amplification products, estimated from

the gel by comparison with standard molecular mass marker, λDNA double digested with Hind III and EcoRI. Only reproducible and well-defined bands in each of the three replication were considered as potential polymorphic markers. From the band data monomorphic and polymorphic bands were identified for each type of landraces.

A pair wise matrix of distance between landraces was determined for the cumulative RAPD data. Using Jaccard formula in the program free Tree available from the URL:http://www.natur.cuni.cz/~Flegr/freetree.htm.cluster analysis from the pair group method of arithmetic means (UPGMA). *Trichosanthes dioica* has explicit dioecy and gender-based differences in leaf characteristics are well marked (Table 1). Female plants are more vigorous than male, which is evident by the bushy nature of female plants. The specific leaf area is nearly twice in male plants than female plants. Similar trends were also observed in leaf area per unit weight (SLA) where male plants required greater area than female plants. This is yet another evidence of vigor in female plants. Thus in terms of growth and so also in productivity female plants appear to be better equipped for greater light perception as well as utilization than male plants as reported earlier [6].

Studies have shown that in natural distribution the preponderance of the individuals of one sex is more in a given location. Earlier investigations have shown that there is indeed differences in the performance of individuals of one sex is better than the other or in competition one sex performs better under a given set of conditions [7]. Thus the sex specific spatial segregation (SSS) has been hypothesized to result from both proximate (ecological) and ultimate (evolved) causes. The differences in physiological features also give the species a greater flexibility and wider area under its occupation or habitation, which should not have been the case in its absence [7]. Thus based on the leaf characteristics it can be deduced that for a given location female plants will have dominant presence by occupying greater space. Since there is no data on their relative stress tolerance no inference can be drawn in their ability to stress tolerance and survival under experimental extremities.

Amplification of total genomic DNA from the accessions were carried out using forty Operon primers out of which only twenty four primers gave polymorphic bands. However, good amplification with the rest of sixteen Operon primers was not obtained. Highest number of polymorphic bands was obtained with OPAB-

Table 1. Gender based differences in leaf characteristics in *T. dioica* accessions. Each data point represents n = 20 ± SD

Accessions	Length cm	Width cm	Leaf area cm^2	Depth of notch	SLA mm^2mg^{-1}
NP-307	6.74 ±0.83	7.36 ±0.72	49.60 ±0.60	1.46 ±0.18	1.16 ±0.06
NP-602	7.96 ±0.74	7.60 ±0.92	60.50 ±0.68	1.40 ±0.18	1.54 ±0.38
NP-260	8.08 ±0.58	7.51 ±0.89	60.68 ±0.52	2.73 ±0.37	1.19 ±0.09
RP	7.65 ±0.56	8.74 ±1.68	66.86 ±0.94	2.71 ±0.38	1.30 ±0.19
NP-604	7.89 ±0.96	9.06 ±1.68	71.48 ±1.61	2.01 ±0.34	1.46 ±0.36
NP-701	7.56 ±0.98	8.64 ±0.71	65.31 ±0.70	1.74 ±0.89	1.75 ±0.48
Male I	5.59 ±0.67	6.32 ±0.39	35.32 ±0.26	1.53 ±0.21	2.20 ±0.29
Male II	6.07 ±0.65	6.15 ±0.65	37.33 ±0.42	1.54 ±0.21	2.21 ±0.24

Table 2. RAPD primers with total numbers of bands, polymorphic and monomorphic bands for each primer among eight landraces of *T. dioica*

S.No.	Random primer	Total amplified fragments	Poly-morphic fragments	Monomorphic fragments
1.	OPAB-01	6	2	4
2.	OPAB-02	9	6	3
3.	OPAB-03	11	4	7
4.	OPAB-04	7	7	0
5.	OPAB-06	8	6	2
6.	OPAB-07	8	7	1
7.	OPAB-08	4	4	0
8.	OPAB-09	12	12	0
9.	OPAB-10	8	8	0
10.	OPAB-11	11	7	4
11.	OPAB-12	11	11	0
12.	OPAB-13	9	8	1
13.	OPAB-14	11	7	4
14.	OPAB-15	12	12	0
15.	OPAB-16	11	11	0
16.	OPAB-17	10	8	2
17.	OPAB-18	14	13	1
18.	OPAB-20	9	9	0
19.	OPU-1	7	6	1
20.	OPAP-11	11	6	5
21.	OPAP-12	8	1	7
22.	OPAP-3	3	2	1

18 and the lowest was with OPAP-12. All eight landraces in this study revealed a unique profile with the primers and thus can be used for the DNA fingerprinting. Generally, the chromosomes have a large number of repetitive sequences which provide a greater chance for primer to find homology and give more than one and different size amplified fragments. Different primers produced different level of polymorphism among eight landraces (Fig. 1). Each primer of RAPD reaction was repeated twice to ensure reproducibility and reliability of RAPD markers. Total 200 bands were amplified by RAPD primers, out of which 157 bands were polymorphic and 43 bands were monomorphic.

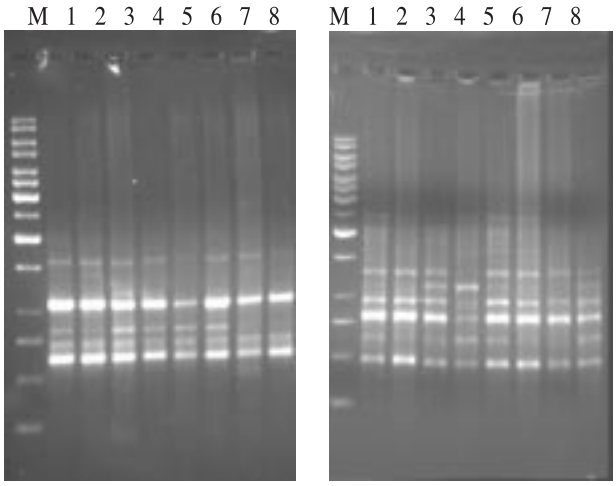

Fig. 1. RAPD agarose gel profile in *Trichosanthes dioica* using primers OPAB-01 and OPAB-03. Lanes M contained 1 Kb DNA ladder as the molecular weight marker; Lane 1 - Male I, lane 2 - Male II, lane 3 - NP260 lane 4 - NP307, lane 5 - NP604, lane 6 - NP701, lane 7 - Rajendra parval (RP), lane 8 - NP602

The pair wise similarity between the eight landraces of *T. dioica* was analyzed. The genetic distance amongst the accessions ranged from 0.38 to 0.59. Maximum similarity according to similarity index was between NP260 and NP604 (0.38) and the minimum similarity was between RP and NP604 (0.59) (Fig. 2).

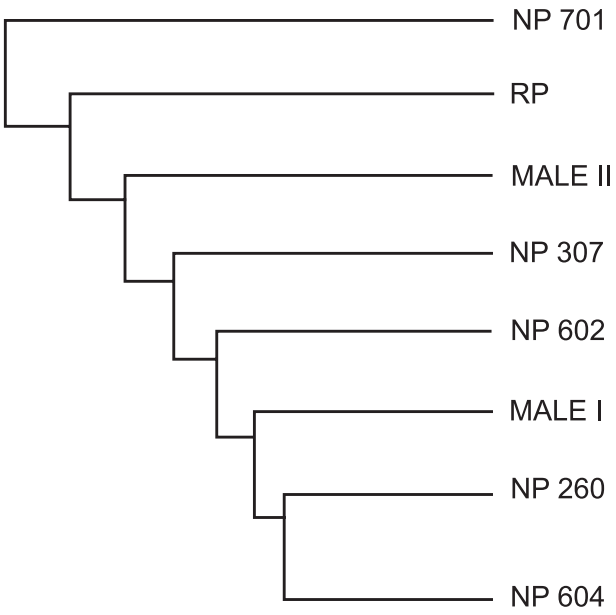

Fig. 2. Cluster analysis of cumulative RAPD for *Trichosanthes dioica*

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