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Genetic diversity of *Polygonum barbatum* in Yamuna water by using RAPD Molecular marker

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Abstract:

The genetic diversity of *Polygonum barbatum* was analyzed using a RAPD primer. Three plants and water samples were collected from three different sites of Uttar Pradesh, viz. Shergarh ghat, Auraiya, from the banks of Yamuna and Poiya Ghat, Agra, again on the banks of Yamuna. These two plants were studied for variations from the plant from the third site, on the banks of Keetham Lake, used as a control. There was a large variation seen in these samples.

Keywords: *Polygonum barbatum*, Genetic diversity, RAPD.

Introduction: As stated by Bashir Ahmad *et al.* (2013), the genus Polygonum (Polygonaceae) comprises of about 150 species. Out of which, *P. perfoliatum*[Lian 1983], showed the antihypertensive property, *P.multiforum* [Yim *et al.* 1998], showed the property of myocardial protective action, *P. punctum*[Kott *et al.*1999] showed the antiviral property, whereas, a commonly found species, *Polygonum barbatum* has not been reported in any biological activities. So, I look into the genetic variation of the plant species. This plant showed carminative, astringent and cooling effects in folk medicine.

Recently, randomly amplified polymorphic DNA (RAPD) analysis has become a general method for estimating genetic diversity and variation among plant and cultivars (Ha *et al.*, 2001; Oiki *et al*., 2001; Wang *et al*., 2001; Shaw, 1995) and also characterizing genetic polymorphisms since it does not require probe DNA and detailed information about the genomic and population polymorphism (Hartl, 1999). Furthermore, the RAPD technique has several advantages over

restriction fragment length polymorphism (RFLP) analysis, namely speed, low cost and the ability to analyze small amounts of samples (Um *et al*., 2001; Tochila-Komatsu *et al*., 2001).

RAPD analysis has been used to investigate the genetic diversity and phlogenetic relationships among the populations in many cultivated plant species (Hormaza *et al*., 1994; Arias and Rieseberg, 1995; Bonnim *et al*., 1996; Mimura *et al*., 2000). The number of studies using DNA analysis to clarify evolutionary relationships and classify species has proliferated over the past 25 years (Chris Brinegar, 2009). In the present study, RAPD molecular markers were used to investigate the genetic diversity of *Polygonum* L. 15 populations from different regions in Southern Western Ghats were sampled and analyzed.

The purpose of the present study was to characterize the natural variations of *Polygonum barbatum* at molecular levels using a RAPD marker.

Materials and Methods:

During the survey of sites, there were three locations selected for water samples during the pre-monsoons season. These three locations were:

1. Yamuna at Auraiya, near Etawah (coded as AU), the second site was;
2. Yamuna at Poiya Ghat at Dayalbagh, Agra (coded as PG), and the third location was;
3. Keetham Lake (coded as KL), situated at the outskirts of Agra district.

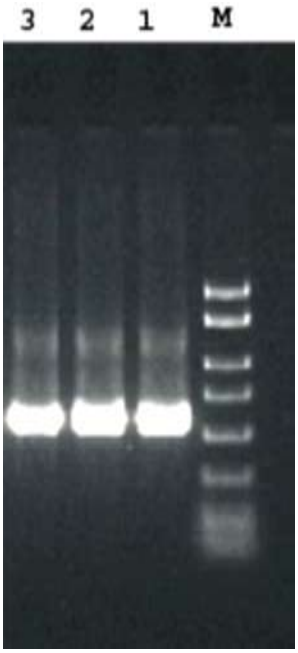
These three locations were chosen as based on the pollution load. Among all three sites chosen, Poiya Ghat at Dayalbagh, Agra, was the most polluted, as it is the direct cremation ground for the Hindus.

The next location was set on to the banks of Yamuna at Auraiya District. Auraiya is basically a rural place. The main use of water is in the irrigation. There is comparatively less pollution load in this area.

The third location is the conserved bird Sanctuary of Keetham Lake. This Lake is under the bird Sanctuary, so, it is under Govt. surveillance. That’s the reason the pant chosen as a control was the plant collected from this site.

DNA from all the collected samples was extracted separately and RAPD was performed using the primer OPP-11 5' AAC GCG TCG G-3'. Now the water samples collected from all the three samples were tested for a pH test using a pH testing machine and the results were re-analyzed using the traditional pH strips.

Observations and Results: There were two photographs taken: One after the isolation of genomic DNA and the other After the RAPD analysis. The Photographs are underneath followed by the results.



Lane M = Marker (50, 100, 300, 500, 750, 1000bp, 1.5kb and 2kb)
Lane 1 = Keetham (KL)
Lane 2 = Poia Ghat (PG)
Lane 3 = Auraiya (AU)

Figure 1: Genomic DNA isolation

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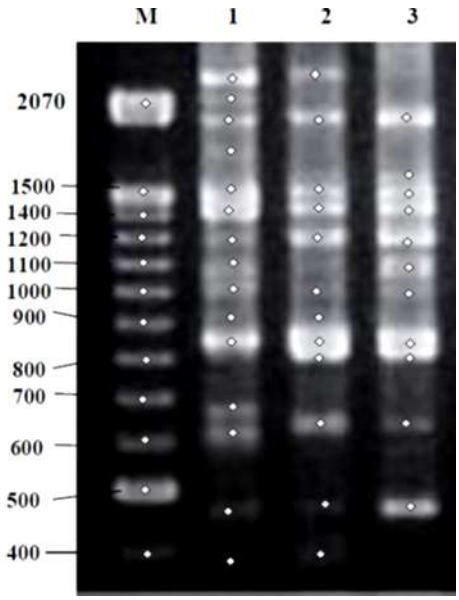

Lane M: Marker (600 -2070 bp)

Lane 1: Keetham (KL)

Lane 2: Poia Ghat (PG)

Lane 3: Auraiya (AU)

Figure2: RAPD profile of *P. barbatum*

S.N.	Molecular Weight	Marker	Lane 1 (KL)	Lane 2 (PG)	Lane 3 (AU)	Distance migrated By The Band D_b (in cms.)	Distance migrated By The Dye D_d(in cms.)	R_f Value (D_b/ D_d)
1	2300*	-	+	+	-	8.7	8.7	1
2	2070	+	+	-	-	8.3	8.7	0.9540
3	1960*	+	+	+	+	8.0	8.7	0.9195
4	1740*	-	+	-	-	7.5	8.7	0.8620
5	1620*	-	-	-	+	7.1	8.7	0.8161
6	1500	+	+	+	+	6.8	8.7	0.7816
7	1400	+	+	+	+	6.4	8.7	0.7356

8	1200	+	+	+	+	5.1	6.7	0.6926
9	1100	+	+	-	+	5.5	8.7	0.6322
10	1000	+	+	+	+	5.0	8.7	0.5747
11	900	+	+	+	-	4.5	8.7	0.5172
12	880*	-	+	+	+	4.4	8.7	0.5057
13	840*	-	+	+	-	4.2	8.7	0.4828
14	800*	+	-	+	+	3.9	8.7	0.4483
15	700	+	-	-	-	3.2	8.7	0.3678
16	640*	-	+	-	-	3.0	8.7	0.3448
17	620*	-	-	+	+	2.7	8.7	0.3103
18	610*	-	+	-	-	2.6	8.7	0.2989
19	600	+	-	-	-	2.4	8.7	0.2759
20	500	+	-	-	-	1.6	8.7	0.1839
21	480*	-	+	+	+	1.4	8.7	0.1609
22	400	+	+	+	-	0.5	8.7	0.0575

Table: 1 Table Showing Variations in the bands formed in the gel when run by the primer

*Values Obtained from Graph between molecular Weight and R_f Value after plotting

Conclusion: Thus the variation was seen in the 1^{st}, 2^{nd}, 4^{th}, 5^{th}, 9^{th}, 11^{th}, 13^{th}, 14^{th}, 16^{th}, 17^{th}, 18^{th}, and 22^{nd} band. Since all the three species are same, there should be no variation seen. But, these variations determine the natural inter-species variation.

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