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Short communication

DNA fingerprinting of *aonla* varieties using RAPD markers

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ABSTRACT

***Aonla* (*Emblica officinalis* L.) is known for its medicinal and therapeutic properties from the ancient time in India and considered as wonder fruit for health conscious population. DNA fingerprinting for cultivar or varietal identification has become important tool for genetic identification in plant breeding and germplasm management. With aim to understand relationship among different popular *aonla* cultivars developed by N.D.U.A.&T., Faizabad and popular ones existing in the region, fingerprinting using RAPD markers was undertaken. DNA was isolated from young leaves of ten *aonla* cultivars. Six RAPD markers (OPA-7, OPA-8, OPZ -4, OPZ-14, OPY-1and OPY-2) were used for the analysis for genetic variation. Out of six primers, OPZ-14 gave most distinct banding pattern for cv. Banarasi. Dendrogram represented four major groups, which was further divided into sub-groups IA and IB. Sub-group IA had NA-04 and NA-10, while IB had cv. Francis. Cluster IIA represented NA-5, NA-6 and NA-9, while IIB represents NA-7 and Chakaiya. Banarasi and Anand represented by group III and IV respectively.**

Key words: *Aonla*, DNA fingerprinting, diversity analysis, RAPD.

Aonla (*Emblica officinalis* L.) is a medium-sized deciduous tree belonging to the family Euphorbiaceae. It is commonly known as Indian Gooseberry and grows in the plains and sub-mountain tracts (Pathak, 3). *Aonla* is well known for its neutraceutical and pharmacological properties. Not only it has good antioxidants, but it has also proven anti-fungal, anti-bacterial, anti-viral (Balasubramaniam *et al.*, 1), anti-mutagenic, anti-hepatoxic, anti-inflammatory, immuno-modulator and hypotensive relieving properties. Varietal/genotype identification of *aonla* is therefore important in order to selectively process the variety as demanded by the customer. The conventional method used to identify varieties involves studies on leaf/fruit morphology, fruit colour etc. (Pathak, 3). However, these are based on morphological traits that undergo variations due to environmental and edaphic conditions. In order to develop an efficient identification method, molecular techniques have been used since these are reliable, unaffected by environmental conditions and can aid varietal identification. Genetic polymorphisms naturally prevailing in plants has been studied widely and is used to differentiate varieties that differ even marginally from each other.

A large number of reports have appeared in the literature using RAPD patterns for differentiating varieties, species, etc. of crop plants. These include studies on pomegranate (Sarkosh *et al.*, 5), pea (Koveza and Gostimskii, 2), Tunisian fig (Salhi-Hannachi *et al.*, 4) etc., wherein subtle differences in the banding patterns have been used as an index to differentiate varieties and assess genetic variability. Research on similar lines has been initiated to use molecular marker specifically RAPD to study genetic variability among *aonla* cultivars.

The experiment was carried out in Department of Plant Molecular Biology and Genetic Engineering, College of Agriculture, N.D.U.A.&T., Faizabad. Fifteen-day-old leaves were collected from tree and stored at -70°C till further use. Leaf samples were collected randomly from tree, so as to avoid location effect (soil texture, soil structure, soil fertility etc.). Ten *aonla* cultivars namely NA-04, NA-05, NA-06, NA-07, NA-09, NA-10, Chakaiya, Francis, Banarasi and Anand were used. Total genomic DNA from leaves was extracted using protocol suggested by Warude *et al*. (7) with some modifications to get the high yield of genomic DNA.

In most cases, by above methods, we could not get any DNA in the leaf samples, though often pellet was seen after final step. As we know that leaves are rich in tannins (Pathak, 3), the leaves have low pH value. Thus, it is essential to maintain the pH up to normal, *i.e.* pH. 7.0. For this, we added some amount of alkali. Therefore, addition of 2 ml NaOH in 4 g of crushed tissue was done as an additional step in this method. Modified DNA isolation yield good quality high molecular weight DNA, *i.e.* free of entrapments and coloured pigment and was suitable for PCR amplification especially for plants like *aonla*, *Terminalia belerica* and *T. chebula* containing low pH

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and high amount of secondary metabolites (Warude *et al*., 6). The RAPD primers used are listed in Table 1.

Table 1. Primers used for polymerase chain reaction.

Primer No.	Sequence
OPA-7	5`-GAAACGGGTG-3`
OPA-8	5`GTGACGTAGG-3`
OPY-4	5`-GGCTGCAATG-3`
OPY-5	5`-GGCTGCGACA-3`
OPZ-4	5`-AGGCTGTGCT-3`
OPZ-14	5`-TCGGAGGTTC-3`

Most of the bands generated by these primers were monomorphic, though some were unique to specific cultivar. The result clearly established the utility of RAPD markers in detecting polymorphism. Amplification by primer OPZ-14 gave total 20 bands, which ranged from 564 to 1372 bp in size. A common band 1016 bp size was present in all the cultivars except Banarasi. Banarasi had a distinct profile with this primer. It gave unique bands of 564 and 831 bp in size, which was absent in all the cultivars. NA-05, NA-06, NA-07 gave a band of 1372 bp, which was not present in other cultivars with primer OPZ-14. The amplification product by primer OPZ-4 gave total 40 bands, which range from 430 to 572 bp. Most of the bands obtained were common to all cultivars whereas a band of 564 bp was also common to all except NA-06. The maximum number of bands was amplified by primer OPA-7, which ranges from 400 to 1904 bp. Most of the bands were monomorphic, while some cultivars showed absence of some bands, like NA-09 did not gave the band of 831 bp similarly, bands of 1375 and 1900 bp were absent in NA-10. The primer OPA-8 gave a total 35 bands, which range from 392 to 1293 bp. Maximum numbers of bands were present in cv. NA-10, while Anand and Banarsi gave minimum bands. A band of 392 bp was common to all cultivars. Bands of 1169 and 129 bp were present in NA-06, NA-07, NA-09, NA-10, Chakaiya and Francis and were absent in NA-05, Banarsi and Anand. The primer OPY-5 gave total 45 bands, which ranged from below 250 to above 947 bp. A band of below 250 and 707 bp was common to all varieties. Whereas, a band of 698 bp was present only in Francis and Banarsi. Cultivars NA-04, NA-10, Francis and Anand had an additional band of 725 bp, which was absent in rest of the cultivars. The amplification product by primer OPY-4 showed total 56 bands, which were mostly monomorphic in nature. These bands ranged from 256 to 586 bp were monomorphic, while other bands from 759 to 1253 bp showed some variation among the cultivars. NA-04 had maximum number of bands. However, Banarsi and Chakaiya gave more intense bands than any other cultivars.

The PCR amplified product with different primers showed that different primers had different specificity towards the cultivar. Most informative result was obtained by primer OPZ-14, which showed a distinct polymorphism and unique band in Banarasi. Other primers gave mostly monomorphic bands but some unique bands were also present, which indicated about the diversity present among *aonla* cultivars.

Dendrogram generated showed the ten cultivars to group into four major groups. Cluster I represented NA-04, NA-10 and Francis, whereas Cluster II had NA-05, NA-06, NA-09, NA-07 and Chakaiya. Group III and IV were represented by Banarasi and Anand. Group I and II had further two sub-groups. Sub-group IA had NA-04 and NA-10, while, sub-groups IB contained Francis. In sub-group IIA contained NA-05, NA-06 and NA-09, whereas, IIB represented NA-07 and Chakaiya. The group III and IV contained no subgroups.The bands clearly represented that there was highest similarity amongst NA-05 and NA-06, while, Banarasi and Anand had maximum diversity. The similarity between NA-05 and NA-06 was also verified by the phenological/ morphological characters of these cultivars. Both cultivars were isolated as chance seedlings of Banarasi and collected

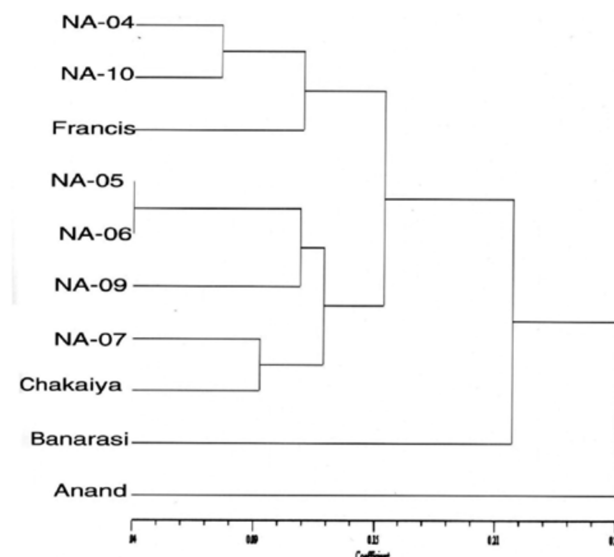

Fig. 1. UPGMA dendrogram of 10 *aonla* cultivars based on six random RAPD primers.

from Pratapgarh during 1982-83. Banarasi variety represented a distinct group. It originates from seedling selection which may be one of the possible reasons for its diverse nature. Like Banarasi, Anand did not match with any other cultivar. Thus, molecular findings support the morphological data that the two distinct cultivars, *i.e.* Banarasi and Anand may be used for further breeding programme and transfer of traits from one variety to other.

Earlier, Warude *et al.* (7) have reported on the development and application of RAPD-SCAR marker for the identification of *aonla* (at the species level), however studies on the genetic variability of *aonla* cultivars employing molecular markers have been rare. The RAPD patterns obtained from our study can serve as a vital input to the conventional method of varietal identification that relies solely on morphological characters. The powerful capability of molecular technique to distinguish closely related cultivars based on their RAPD patterns has been brought out by this study.

REFERENCES

1. Balasubramaniam, G., Sarathi, M., Rajesh K., Sahul, S. and Hameed, A.S. 2007. Screening the antiviral activity of Indian medicinal plants against white spot syndrome virus in shrimp. *Aquaculture*, **263**: 15-19.

2. Koveza, O.V. and Gostimskii, S.A. 2005. Development and study of SCAR markers in pea (*Pisum sativum* L.). *Genetica*, **41**: 1522-30.

3. Pathak, R.K. 2003. *Status report on Genetic Resources of Indian Gooseberry – Aonla* (*Emblica officinalis* L.) *in South and South East Asia*. IBPGRI, 26.

4. Salhi-Hannachi, A., Chatti, K., Saddoud, O., Mars, M., Rhouma, A., Marrakchi, M. and Trifi, M. 2006. Genetic diversity of different Tunisian fig (*Ficus carica* L.) collections revealed by RAPD fingerprints. *Hereditas*, **143**: 15-22.

5. Sarkhosh, A., Zamani, Z., Fatahi R. and Ebadi, A. 2006. RAPD markers reveal polymorphism among some Iranian pomegranate (*Punica granatum* L.). *Scientia Hort.* **111**: 24-29.

6. Warude, D., Chavan P., Joshi, K. and Patwardhan, B. 2003. DNA isolation from fresh and dry plant samples with highly acidic tissue extracts. *Pl. Mol. Biol. Rep.* **21**: 467a-467f.

7. Warude, D., Chavan, P., Joshi, K. and Patwardhan, B. 2006. Development and Application of RAPD-SCAR marker for identification of *Phyllanthus emblica* L. *Biol. Pharm. Bull.* **29**: 2313-16.

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