International Journal of Engineering, Science and Mathematics
Vol. 7 Issue 2, February 2018,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: **http://www.ijesm.co.in, Email: ijesmj@gmail.com**
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

AFLP analysis reveals exceptionally narrow genetic background in ginger (*Zingiber officinale*Rosc.)

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Abstract

Ginger (*Zingiber officinale*Roscoe), whose rhizome is globally valued as both a medicine and as a spice, is an important cash crop in tropical and sub-tropical countries. India is a leading producer and exporter of ginger in the world. As many as 50 commercial cultivars of ginger are known in India. The gene pool of this clonally propagated crop species has not been well characterized. We analysed the genetic variability among a collection of 17 ginger clones in India, consisting of released varieties and prominent traditional cultivars, using amplified fragment length polymorphism (AFLP) markers. The 10 AFLP primer pairs tested failed to detect any polymorphism at the 485 loci they screened among the clones examined. The results indicate an extremely narrow genetic base of ginger germplasm in India and suggest that the clones examined are the descendants of a single genotype with wide ecological tolerance. The results are discussed in the context of exclusive asexual multiplication in ginger and its long history of cultivation and trade in India.

Keywords:

AFLP;
Clonal multiplication;
Genetic diversity;
Ginger;
Isoclonal.

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1Introduction

Ginger (*Zingiber officinale*Roscoe, Zingiberaceae), a rhizomatous herbaceous perennial, is an important cash crop of tropical and subtropical countries. The rhizomes of ginger plants are used world over in medicine, culinary preparations, as spice and a home remedy [1],[2], [3]. India is one of the largest producers of ginger, contributing to about one-fifth of the world's production [4]. Ginger flowers regularly, but never sets seed [5], [6], [7]. It is obligatory asexual, propagated exclusively using rhizomes. Although grown throughout India, Kerala state ranks first in terms of area and production, and the local product, traditionally known as Cochin ginger, is considered to be one of the best in the world [3]. As many as 50 different commercial ginger clones are known in India [1], [8], but these are poorly discriminated and for the most part, have been named after the localities or villages in which they were collected or cultivated.

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Morphological characteristics are not a reliable indicator of genetic diversity in ginger. AFLP analysis of Indonesian ginger clones showed that morphologically dissimilar small and big ginger were highly similar genetically, and several accessions of small ginger were genetically closer to red ginger [9]. Molecular markers are powerful tools for the accurate quantification of genetic diversity in a species and for cultivar/clone identification [10],[11], [12]. Comparatively little is known about the genetic diversity of ginger germplasm and is poorly characterized at the molecular level. Genetic diversity was found to be very low for ginger in earlier studies. Wahyuniet al.[9] found a very low genetic diversity among 22 Indonesian ginger accessions with AFLPs. The genetic base of ginger accessions in United States of America is yet narrow. Ten ginger accessions sampled from different regions in USA were indistinguishable based on the sequence variations at *trn*L-F and *rpsl*6 regions [12]. However, Leeet al.[13]reported a moderate level of microsatellite diversity in a set of eight ginger accessions sampled from Malaysia. In this study, we used AFLPs to evaluate the extent of genetic variability in a set of 17 Indian ginger clones, including prominent traditional cultivars and released varieties.

2Materials and methods

2.1 Plant materials

The plant materials used in this study are listed in Table 1 and their approximate geographical origins shown in Figure 1. Code numbers 1 - 15 in Table 1 are maintained at the Department of Plantation Crops, Kerala Agricultural University, Thiruvananthapuram and 16 and 17 at the Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram. Varada and Suprabha are clonal selections released by the Indian Institute of Spices Research (IISR), Kerala and the High Altitude Research Station (HARS), Orissa, respectively. The latter, released in 1988, was the first ginger variety released in India, and is recommended for cultivation in Orissa state, whereas the former is recommended for cultivation throughout India [14], [15]. Maran, Wayanad Kunnamangalam, Wayanad Mananthody, Kuruppampady and Himachal are among the more prominent traditional Indian cultivars [16], [17],[8], [3]. Clones 1 and 2 were collected from two distinct homestead gardens in Kottayam, Kerala. Only the state of origin, but not the precise location of collection of Maran, Himachal, PGS and SG series is known. PGS and SG were originally procured from, respectively, the High Altitude Research Station, Orissa and Y.S. Parmar University of Horticulture and Forestry, Himachal Pradesh.

Table 1Name and origin of the ginger clones used

Code	Clone name	Origin
1	Varada	Released variety, IISR*
2	Suprabha	Released variety, HARS**
3	Nedumangad	Nedumangad, Kerala
4	Maran	Assam
5	Kunnamangalam	Kunnamangalam, Wayanad, Kerala
6	Mananthody	Mananthody, Wayanad, Kerala
7	Kuruppamapady	Kuruppampady, Kerala
8	Himachal	Himachal Pradesh
9	SG-551	Andhra Pradesh
10	SG-547	Andhra Pradesh
11	PGS-667	Orissa
12	SG-639	Himachal Pradesh
13	SG-557	Andhra Pradesh
14	PGS-9	Orissa
15	SG-603	Himachal Pradesh
16	Clone 1	Kottayam, Kerala
17	Clone 2	Kottayam, Kerala

*Indian Institute of Spices Research, Calicut, Kerala, India
** High Altitude Research Station, Orissa, India

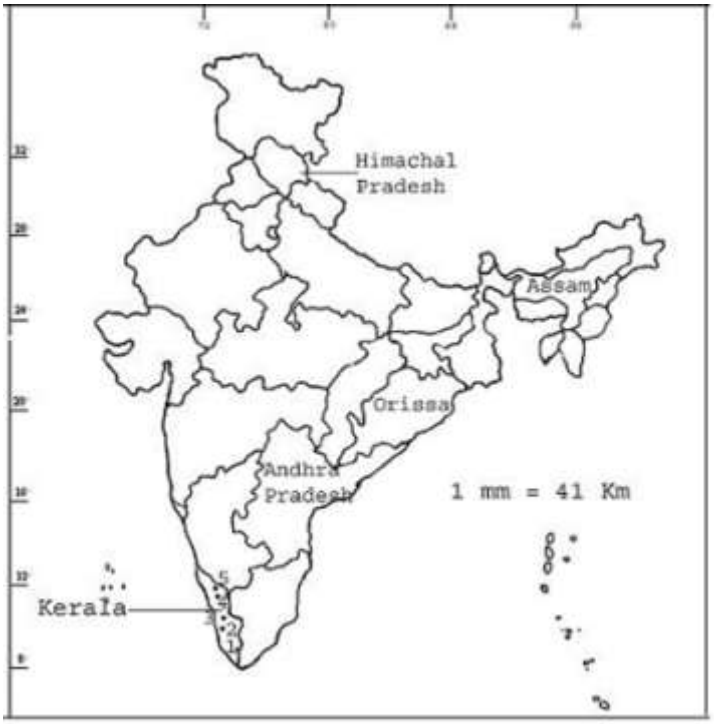

Fig. 1 Map of India showing the approximate geographical locations of the ginger genotypes used in the analysis. 1-Nedumangad; 2-Kottayam; 3-Kuruppampady; 4-Kunnamangalam; 5-Mananthody

2.2 DNA profiling

DNA was extracted from young leaves using the GenElute Plant DNA Kit following the manufacturer's instructions (Sigma, St. Louis, USA). AFLP analysis was performed using the AFLP Analysis System II Kit following the manufacturer's protocol (Invitrogen, Carlsbad, CA). The ten primer combinations used were E-AA x M-CTA, E-AC x M-CAA, E-AC x M-CTA, E-AT x M-CTC, E-AT x M-CTT, E-AT x M-CAA, E-TA x M-CTG, E-TT x M-CTA, E-TT x M-CAG and E-TT x M-CTT. The pre-amplification and selective amplification were carried out in a Mastercycler Gradient thermal cycler (Eppendorf). The PCR products were fractionated on a 6% denaturing polyacrylamide gel ('Hoefer SQ3 Sequencer', Amersham Pharmacia Biotech) at 45 W for 2.5 h. The gel was transfered onto Whatman 3MM filter paper and dried under vacuum in a gel dryer (BioRad). The dried gel was exposed to a Phosphor Imager screen (Kodak), the exposed screen was scanned using a Phosphor Imager (Personal FX, BioRad) and the images were subjected to two independent readings, and clear and unambiguous bands were scored.

3. Results and Discussion

Ten AFLP profiles yielded altogether 485 scorable bands, all of which were monomorphic. A representative AFLP profile is given in Fig. 2.

Despite surveying a large number of marker loci, no polymorphism was detected among the ginger clones sampled from different regions in India. Similar level of genetic narrowness has been earlier reported in American ginger clones [12]. Absence of segregation and recombination predicts reduced levels of genetic variation in an asexual species [18], [19]. An exceptionally narrow genetic diversity as observed in the present study has been previously reported in other asexual plant species also [20], [21][22], [23]. However,Wahyuniet al.[9]and Leeet al. [13] reported genetic variation in Indonesian and Malaysian ginger, respectively, although the level of variation was verylow. Together, these reports are suggestive of a very narrow genetic base of world ginger germplasm and that the level of diversity varies among different ginger gene pools. Somatic mutations, which are known to generate DNA polymorphisms in other asexual plants [24], [25], seem to have occurred at extremely low frequencies in the materials examined in the present study. Background selection, which drastically reduces variability at neutral loci in asexual plants [26] may counterbalance many of the mutational events in ginger.

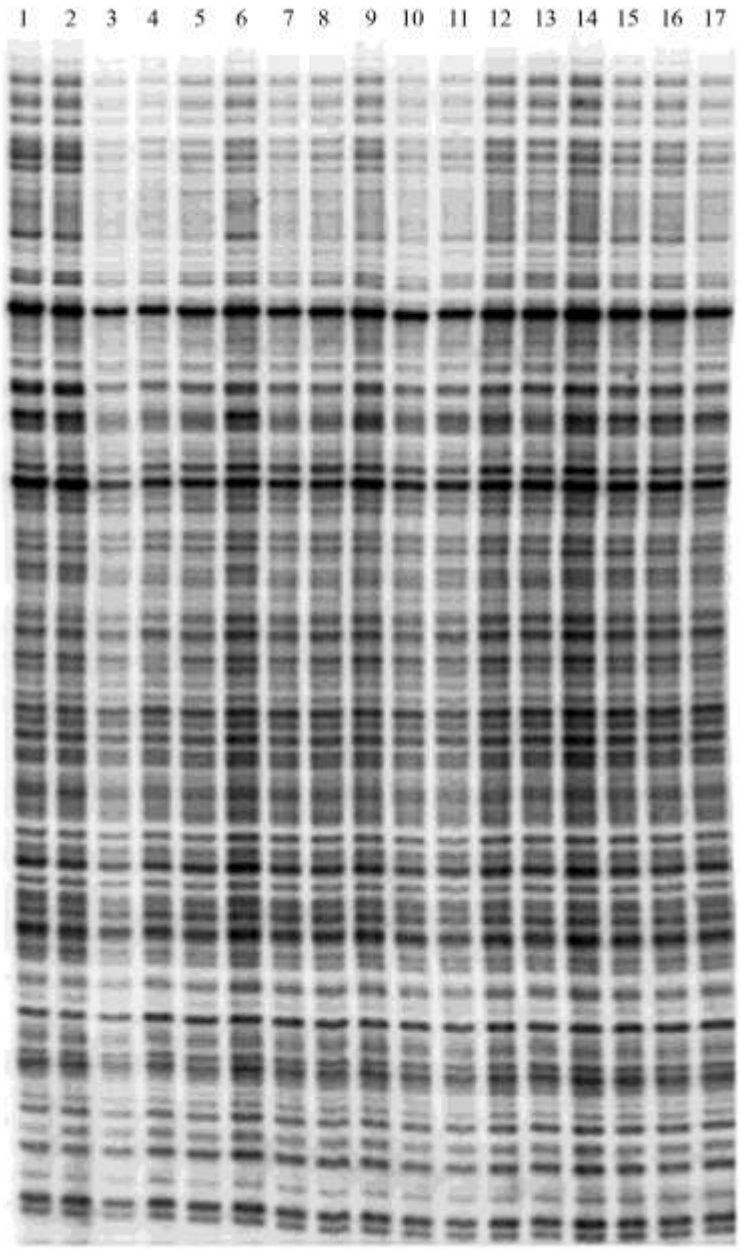

Fig. 2 A representative AFLP profile of 17 ginger clones using the primer combination E-AA x M-CTA. Lane numbers corresponds to clone code in Table 1

Our data indicate the possibility of an isoclonal lineage of the material examined, i. e. they represent descendants of a single genotype, got dispersed over a wider geographical area. Interestingly, all the Indian ginger clones are equally susceptible to major diseases, such as rhizome rot, bacterial wilt and leaf spot [27], [28], indicating a lack of intrinsic genetic variation.

Ginger has been extensively cultivated and traded since ancient times [1],[29]. The cultivation of a single preferred genotype is very common in commercial agriculture[20]. It is possible therefore that a single commercially important and widely adapted genotype was identified in the past, and that this genotype was then adopted for cultivation in different regions. A parallel example has arisen in *Morus laevigata* in India, where Chatterjeeet al.[30]have shown that geographically distant accessions were the descendants of a single gene pool dispersed in the past for sericultural purposes, and propagated primarily through stem cuttings. The exclusive reliance on vegetative reproduction can preserve a clonal genotype for a very long period, as has been shown in the case English elm that has been preserved over 2000 years [31].

Another plausible reason for the observed lack of genetic diversity in Indian ginger relates to the clonal ancestry. Hooker [5] described ginger as a species that never sets seeds and later reports support this view [6], [7], [8]. Thus the genetic diversity present in the early evolutionary history of ginger may have been completely eroded due to an exclusive reliance on asexual reproduction, leading to the fixation of a single genotype, as predicted for a strictly asexual population [32].

The understanding that the Indian ginger clones are so monomorphic is important for the future planning for germplasm characterization and conservation in ginger. The narrow genetic base and the paucity of discriminatory traits in ginger demand the conservation of as many accessions as possible. It has been suggested that phenotypic plasticity plays a role in ensuring the survival of clonal plants in heterogeneous environments [33], [34], [35]. The chemical composition of ginger genotypes is known to be dependent on the agroclimatic conditions where the crop is grown [36], [37]. This underlines the importance of defining the extent to which plasticity can contribute to quality variation of the ginger rhizome. Inadequate levels of nuclear DNA polymorphism requires surrogate measures of genetic variability in ginger for the characterization of its germplasm and varietal identification. Variation in the chemical profile of rhizome can be explored, since the rhizome is rich in various chemical compounds [1].

4. Conclusion

In the present study we analysedgenetic variability among a collection of 17 ginger clones in India, consisting of released varieties and prominent traditional cultivars, using amplified fragment length polymorphism (AFLP) markers.The results indicate an extremely narrow genetic backgroundof ginger germplasm in India and suggest that the clones examined are the descendants of a single genotype with wide ecological tolerance.

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