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Species-specific AFLP markers for identification of *Zingiber officinale*, *Z. montanum* and *Z. zerumbet* (Zingiberaceae)

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ABSTRACT. The *Zingiber* genus, which includes the herbs known as gingers, commonly used in cooking, is well known for its medicinal properties, as described in the Indian pharmacopoeia. Different members of this genus, although somewhat similar in morphology, differ widely in their pharmacological and therapeutic properties. The most important species of this genus, with maximal therapeutic properties, is *Zingiber officinale* (garden ginger), which is often adulterated with other less-potent *Zingiber* sp. There is an existing demand in the herbal drug industry for an authentication system for the *Zingiber* sp in order to facilitate their commercial use as genuine phytoceuticals. To this end, we used amplified fragment length polymorphism (AFLP) to produce DNA fingerprints for three *Zingiber* species. Sixteen collections (six of *Z. officinale*, five of *Z. montanum*, and five of *Z. zerumbet*) were used in the study. Seven selective primer pairs were found to be useful for all the accessions. A total of 837 fragments were produced by these primer pairs. Species-specific markers were identified for all three *Zingiber* species (91 for *Z. officinale*, 82 for *Z. montanum*, and 55 for *Z. zerumbet*). The dendogram analysis generated from AFLP patterns showed that *Z. montanum* and *Z. zerumbet* are phylogenetically closer to each other than to *Z. officinale*. The AFLP fingerprints of the *Zingiber* species could be

used to authenticate *Zingiber* sp-derived drugs and to resolve adulteration-related problems faced by the commercial users of these herbs.

Key words: DNA fingerprinting; AFLP; *Zingiber* spp; Adulteration; Molecular marker

INTRODUCTION

Since ancient times, the Zingiberous plants, curcuma and ginger, have been recognized as medicinally valuable. References of these plants are found in all systems of Indian traditional and folk medicine. Classic/taxonomic grouping of these plants has often caused confusion. About 50 genera and 1300 species of ginger species/varieties are known to exist worldwide. The plants occur mainly in Australia, Bangladesh, Haiti, Jamaica, Japan, Nigeria, Srilanka, and other south east Asian countries. In India, the indigenous species grow wild in the Western Ghat region particularly in the Malabar coasts of Kerala. Selected species within this group of plants are cultivated on a large scale in warm and moist regions, mainly around Chennai, Cochin, Himachal Pradesh, Meghalaya, Sikkim, Assam, Arunachal Pradesh, and to a lesser extent in Orissa, Uttar Pradesh (Nainital). In West Bengal these plants are cultivated in the plains as well as in the Darjeeling Hills.

Being vegetatively propagated by rhizomes, which constitute the part of medicinal importance, such plants run the risk of overexploitation in the wild. Notwithstanding cultivated varieties that stand protected from such loss, it is important to explore document and conserve the wild genotypes/land races that may possess valuable medicinal potential and/or stress tolerance genes.

Zingiber officinale Roscoe is a perennial herb belonging to the Zingiberaceae family. It is the most common ginger species in India and it is grown all over the country. The rhizome is horizontal, branched, fleshy, aromatic, white or yellowish to brown, which are generally effective in stomach disorders such as colic, spasms, vomiting, dyspepsia, flatulence, and other painful disorders. It accumulates high levels of important pharmacologically active metabolites, viz., [6]-gingerols, which are some of the products of the phenylpropanoid pathway. The other constituents in *Z. officinale* are the pungent vanilloids, and [6]-paradol. [6]-Gingerol is a biologically active component that may make a significant contribution towards medicinal applications of ginger and some products derived from ginger. The antioxidant, antitumor, and anti-inflammatory pharmacologic effects of ginger are mainly due to its pungent constituents (e.g., [6]-gingerol). Chewing a fresh ginger piece helps reduce these ailments. The rhizomes are also an effective remedy for cold and cough.

Zingiber montanum (J. Koeing) Link ex A. Dietr is a native of tropical Asia. In India it is found in Arunachal Pradesh, Assam, Sikkim, and Meghalaya. The plant has been proven to be extremely useful for human health and thus developed into creams and massage oils for relieving muscle pain. Furthermore, it is well known that the essential oils from *Z. montanum* have also been shown to cure acne, bruises, skin burns, skin inflammation, muscle pain, insect bite, and asthmatic symptoms. They are even proven to help cope with cough and respiratory symptoms as well. Rhizome extracts exhibit anti-inflammatory and anti-bacterial activities. A number of pure compounds isolated from the plants have been shown to possess anti-microbial, anti-inflammatory, analgesic, anti-tyrosinase, and anti-oxidative activities. It is also considered to have properties such as analgesic, anti-neuralgic, anti-inflammatory, antiseptic, antispasmodic, antitoxic, anti-viral, carminative, digestive, diuretic, febrifugal, laxative, rubefacient, stimulant, tonic, and vermifugal, and it has been used for aches and pains, asthma, chronic colds, colic, constipation, diarrhea, fevers, flatulence, heartburn, immune problems, inflammation, influenza, joint problems,

muscle spasms, nausea, respiratory problems, sprains and strains, and torn muscles and ligaments.

Zingiber zerumbet (L.) Roscoe ex Sm, also known as the "shampoo-ginger", is a vigorous ginger with leafy stems growing to about 1.2 m tall. This plant, originating in India, was distributed east-ward through Polynesia and introduced to the Hawaiian Islands in the canoes of early Polynesian settlers. For a toothache or a cavity, the cooked and softened Awapuhi rhizome is pressed into the hollow and left for as long as needed. To ease a stomach ache, the ground and strained rhizome material is mixed with water and drunk. Similarly, Awapuhi Pake is widely cultivated and eaten, or made into a tea for indigestion as well as increased circulation of the blood and to increase sense of well-being. An extract from *Z. zerumbet*, "Zerumbone", has been found to induce apoptosis, or programmed cell death, in human liver cancer cells.

Of the plants described above, *Z. montanum* and *Z. zerumbet* are morphologically almost identical. It is even almost impossible for classical taxonomists to differentiate between these two species in the non-flowering stage. Such life cycle/season-based difficulty in plant identification, through conventional morphological parameters, calls for more precise parameters of plant identification, viz., DNA fingerprinting patterns that provide the ultimate in individualization due to the stability of DNA in any plant part and also through variation in environment and also variation in phase of life cycle. DNA fingerprinting patterns thus provide a yardstick for precise identification of plants and for delimiting possible mixing of similar looking/named plants wittingly or unwittingly by plant collectors/vendors. Production of medicines from precisely selected prescribed plants through the use of DNA characterization protocols would ensure uniform efficacy of medicines produced in different batches.

Other important uses of DNA fingerprinting methods for the development of species-specific markers include the establishment of sovereignty rights of plant genetic resources, which is essential under the benefit-sharing regime of the Convention of Biological Diversity (CBD). Under the WTO regime, it is becoming increasingly important to develop passport data of plants to be exported, a need of universal concern particularly considering that the global herbal drug market is expected to grow to US$5 trillion by the year 2050 (Joshi et al., 2004).

Amplified fragment length polymorphism (AFLP) is a novel DNA fingerprinting technique that allows DNA characterization under stringent experimental conditions, thus allowing precision. This method also allows the examination of large segments of the DNA in each experiment thus helping to explore the entire genome within a short span of time. Thus, AFLP is a useful method for precise identification of genotype within a short period of time. This method also proves to be very important in plant taxonomy for species-specific identification (Lin et al., 1996, soybean; Waugh et al., 1997, barley; Degani et al., 2001, strawberry; Saunders et al., 1999, 2001, marijuana and opium, respectively; Percifield et al., 2007, *Hypericum perforatum* L.; Misra et al., 2010, *Swertia* sp).

The present study was undertaken to generate AFLP-based DNA markers for three species of the *Zingiber* genus (*Z. officinale* Roscoe, *Z. montanum* (J. Koeing) Link ex A. Dietr and *Z. zerumbet* (L.) Roscoe ex Sm). These plants are most commonly used in the herbal trade.

MATERIAL AND METHODS

Plant materials

The plant materials used in this study were collected from different parts of eastern and northeastern India, viz., West Bengal, Sikkim, Assam, and Meghalaya (Table 1). The samples consisted of 6 collections of *Z. officinale*, 5 collections of *Z. montanum* and 5 collec-

tions of *Z. zerumbet*. Voucher specimens of all samples are preserved at the Central National Herbarium (CAL), Botanical Survey of India, Howrah, for future reference.

Table 1. Name (scientific and common), family, GPS data, place, and date of collection of the experimental *Zingiber* germplasm.

Sl No.	Name of the plant	Common name	Family	Latitude, longitude and elevation	Place of collection	Date of collection
1	*Zingiber officinale* Roscoe	Garden ginger	Zingiberaceae	26° 22' N 89° 29' E 45 m	Kamat Abutara, Coochbehar, West Bengal	20.03.2010
2	*Zingiber officinale* Roscoe	Garden ginger	Zingiberaceae	26° 32' N 88° 46' E 75 m	Uttar Balaguri, Dist-Jalpaiguri, West Bengal	20.03.2010
3	*Zingiber officinale* Roscoe	Garden ginger	Zingiberaceae	26° 22' N 89° 29' E 45 m	Tufanganj Bazar, Coochbehr, West Bengal Source-Bhutan	20.03.2010
4	*Zingiber officinale* Roscoe	Garden ginger	Zingiberaceae	26° 32' N 88° 46' E 75 m	Birpara market, Dist-Jalpaiguri, West Bengal	20.03.2010
5	*Zingiber officinale* Roscoe	Garden ginger	Zingiberaceae	26° 49' N 87° 49' E 53 m	North Dinajpur Dist, Old Alluvial zone, West Bengal	20.03.2010
6	*Zingiber officinale* Roscoe	Garden ginger	Zingiberaceae	23° 80' N 93° 30' E 790 m	Chajing, 10 km from Imphal, Manipur	19.04.2010
7	*Zingiber montanum* (J. Koeing) Link ex A. Dietr	Cassumunar ginger	Zingiberaceae	27° 15' N 88° 35' E 922 m	East District, Aau, Sikkim	01.09.2010
8	*Zingiber montanum* (J. Koeing) Link ex A. Dietr	Cassumunar ginger	Zingiberaceae	27° 15' N 88° 35' E 922 m	East District, Aau, Sikkim	01.09.2010
9	*Zingiber montanum* (J. Koeing) Link ex A. Dietr	Cassumunar ginger	Zingiberaceae	27° 15' N 88° 35' E 922 m	East District, Aau, Sikkim	01.09.2010
10	*Zingiber montanum* (J. Koeing) Link ex A. Dietr	Cassumunar ginger	Zingiberaceae	27° 15' N 88° 35' E 922 m	East District, near Aau, Sikkim	11.05.2009
11	*Zingiber montanum* (J. Koeing) Link ex A. Dietr	Cassumunar ginger	Zingiberaceae	27° 15' N 88° 35' E 922 m	East District, near Aau, Sikkim	11.05.2009
12	*Zingiber zerumbet* (L.) Roscoe ex Sm	Shampoo ginger	Zingiberaceae	27° 11' N 88° 36' E 512 m	East District, near Rongli, Sikkim	05.09.2010
13	*Zingiber zerumbet* (L.) Roscoe ex Sm	Shampoo ginger	Zingiberaceae	25° 40' N 91° 54' E 973 m	East Khasi Hills, Barapani, Meghalaya	14.07.2010
14	*Zingiber zerumbet* (L.) Roscoe ex Sm	Shampoo ginger	Zingiberaceae	25° 40' N 91° 54' E 969 m	East Khasi Hills, Barapani, Meghalaya	12.07.2010
15	*Zingiber zerumbet* (L.) Roscoe ex Sm	Shampoo ginger	Zingiberaceae	24° 50' N 93° 20' E 356 m	Lakhipur, 45 km from Silchar, Assam	26.04.2010
16	*Zingiber zerumbet* (L.) Roscoe ex Sm	Shampoo ginger	Zingiberaceae	23° 43' N 92° 23' E 300 m	Karimganj, 54 km from Silchar, Assam	05.05.2010

DNA extraction

Genomic DNA from all plant samples was isolated from young leaves from each genotype. Total genomic DNA was extracted using the Qiagen total plant DNA extraction kit. The concentration of DNA in the samples was determined by the 260/280 O.D. value. The DNA samples were subjected to 0.8% agarose gel electrophoresis; genomic λ DNA (25 ng/μL) was used

as standard. All DNA samples taken for AFLP study showed a 260/280 O.D. value of 1.75-2.00.

AFLP fingerprinting

AFLP electropherograms were produced for each variety using the ABI prism fluorescent dye labeling and detection technology (Perkin-Elmer). AFLP analysis was performed using the kit supplied by Applied Biosystems (USA) and was used according to manufacturer instructions.

High-quality genomic DNA (500 ng) was digested with 1 U *Mse*I and 5 U *Eco*RI restriction endonucleases. *Eco*RI and *Mse*I adaptors were ligated with 1 U T4 DNA ligase (all enzymes were from New England Biolabs, Beverly, MA, USA). Restriction and ligation were done simultaneously (Vos et al., 1995) in a single step by incubating at 37°C for 2 h in a thermocycler (Applied Biosystems).

Polymerase chain reaction (PCR) amplification and selective amplification were carried out according to instructions provided in the kit. Pre-amplifications were evaluated running pre-amplified samples on a 1.5% agarose gel. A smear of product from 100-1500 bp was clearly visible.

Selective amplification was carried out using seven primer pairs for three species of the *Zingiber* genus, viz., *Z. officinale* Roscoe, *Z. montanum* (J. Koeing) Link ex A. Dietr and *Z. zerumbet* (L.) Roscoe ex Sm. The amplified products were mixed with Size Standard Gene Scan 500 ROX, and the samples were then analyzed on an automated DNA sequencer (ABI Model 3130 XL genetic analyzer, Applied Biosystems).

Scoring and data analysis

Fragment analysis was carried out for bands in the range of 35-500 bp. For diversity analysis, bands were scored as presence (1) or absence (0) to form a raw data matrix. A square symmetric matrix of similarity was then obtained with the Jaccard's similarity coefficient (Jaccard, 1908). The average similarity matrix was used to generate a tree for cluster analyses by UPGMA (unweighted pair group method with arithmetic mean) using the NTSys v 2.1 software.

RESULTS AND DISCUSSION

In the present study, seven pairs of primer pairs were used in AFLP fingerprinting for all landraces (of three species of the *Zingiber* genus, viz., *Z. officinale*, *Z. montanum* and *Z. zerumbet*). Of a total of 837 peaks, only 2 peaks were monomorphic and 835 were polymorphic. A polymorphism of 99.7% was detected among the species.

The study could identify species-specific AFLP markers for the three *Zingiber* species, where all landraces within a single species showed similarity in pattern. The AFLP alleles common to all the landraces of *Z. officinale*, using seven primer pairs, and their respective size of alleles in bp are shown in Table 2. Similarly, alleles, specific for *Z. montanum* and *Z. zerumbet* with their respective size (in bp) are shown in Table 3 and Table 4, respectively. Such data on DNA characterization reveal variation among the three species, landraces of each species being similar regardless of their place of collection. This suggests that DNA fingerprinting (by AFLP) may be used as a dependable identifying parameter for species identification even if collected from different locations, at least within the range of distance of this collection. The fact that this study relates to the study of leaves also establishes that in DNA fingerprinting methods flowers are not essential for plant identification as is needed for classical taxonomic studies. Some representative AFLP patterns of the three species are given in Figures 1A-C and 2A-C.

Table 2. Unique/common alleles, number and size (in bp) of *Zingiber officinale*.

Primer combination M-CAG/E-ACG		Primer combination M-CTT/E-AAG		Primer combination M-CTG/E-ACA		Primer combination M-CTC/E-AAG		Primer combination M-CTG/E-ACT		Primer combination M-CAG/E-ACA		Primer combination M-CTC/E-AAC	
Allele No.	Allele size (bp)	Allele No.	Allele size (bp)	Allele No.	Allele size (bp)	Allele No.	Allele size (bp)	Allele No.	Allele size (bp)	Allele No.	Allele size (bp)	Allele No.	Allele size (bp)
16	54	2	38	4	45	10	55	5	46	10	45	5	43
35	91	42	106	23	72	22	68	19	76	11	46	27	77
40	100	72	186	31	86	23	70	24	84	37	77	28	79
52	130	73	187	37	95	32	94	36	124	38	78	57	159
56	132	95	264	62	161	37	118	37	126	127	292	91	279
57	134	86	235	96	247	41	126	40	128	136	328	92	280
58	144	56	132	61	159	45	139	46	141	142	344	102	327
61	157	58	135	29	82	50	159	49	145	143	346	110	345
62	158	84	227	91	237	51	165	54	169	150	381	111	346
63	159	92	253	102	293	56	180	59	180			117	392
64	160			105	304	73	295	61	181			118	393
65	170			106	312	74	303	64	188				
66	171			107	314	76	315	71	203				
92	432			110	350	85	391	76	228				
				117	437	91	450	87	256				
								102	312				
								108	374				

Table 3. Unique/common alleles, number and size (in bp) of *Zingiber montanum*.

Primer combination M-CAG/E-ACG		Primer combination M-CTT/E-AAG		Primer combination M-CTG/E-ACA		Primer combination M-CTC/E-AAG		Primer combination M-CTG/E-ACT		Primer combination M-CAG/E-ACA		Primer combination M-CTC/E-AAC	
Allele No.	Allele size (bp)	Allele No.	Allele size (bp)	Allele No.	Allele size (bp)	Allele No.	Allele size (bp)	Allele No.	Allele size (bp)	Allele No.	Allele size (bp)	Allele No.	Allele size (bp)
27	71	34	93	9	51	5	45	8	51	31	70	8	46
28	72	44	108	11	53	18	64	9	52	40	81	11	51
32	81	88	243	26	74	26	74	23	84	47	95	25	75
46	115	83	225	30	84	42	130	28	97	53	102	30	83
48	122	101	303	45	110	44	131	29	98	89	171	42	116
55	132			55	131	62	197	38	126	91	173	53	144
88	335			56	132	63	204	58	180	116	255	54	145
				67	178	64	222	63	186	118	262	58	160
				79	212	72	285	65	191	119	279	62	170
				81	216	81	370	66	193	129	299	63	171
				85	222	82	371	68	196	133	312	85	245
				118	442	95	482	77	229			88	253
								82	243			89	255
								85	250			94	285
								104	324			100	300
								105	326			104	331
								116	500			116	379
								86	251				

Table 4. Unique/common alleles, number and size (in bp) of *Zingiber zerumbet*.

Primer combination M-CAG/E-ACG		Primer combination M-CTT/E-AAG		Primer combination M-CTG/E-ACA		Primer combination M-CTC/E-AAG		Primer combination M-CTG/E-ACT		Primer combination M-CAG/E-ACA		Primer combination M-CTC/E-AAC	
Allele No.	Allele size (bp)	Allele No.	Allele size (bp)	Allele No.	Allele size (bp)	Allele No.	Allele size (bp)	Allele No.	Allele size (bp)	Allele No.	Allele size (bp)	Allele No.	Allele size (bp)
80	284	No specific allele is present for this primer pair		8	49	8	49	20	77	49	95	17	64
68	180			10	52	52	166	25	90	60	111	18	66
				28	79	75	311	39	128	64	122	19	67
				39	99	77	321	56	176	69	132	22	71
				86	223	89	445	99	297	71	139	23	72
				112	353			113	435	74	144	37	105
				114	374					80	158	45	121
				121	466					52	216	46	123
										105	343	50	138
										141	360	51	139
										144	364	55	157
										145	365	60	167
										146	448	65	176
										160	101	66	177
												80	230
												87	253
												108	343
												114	360
												120	448

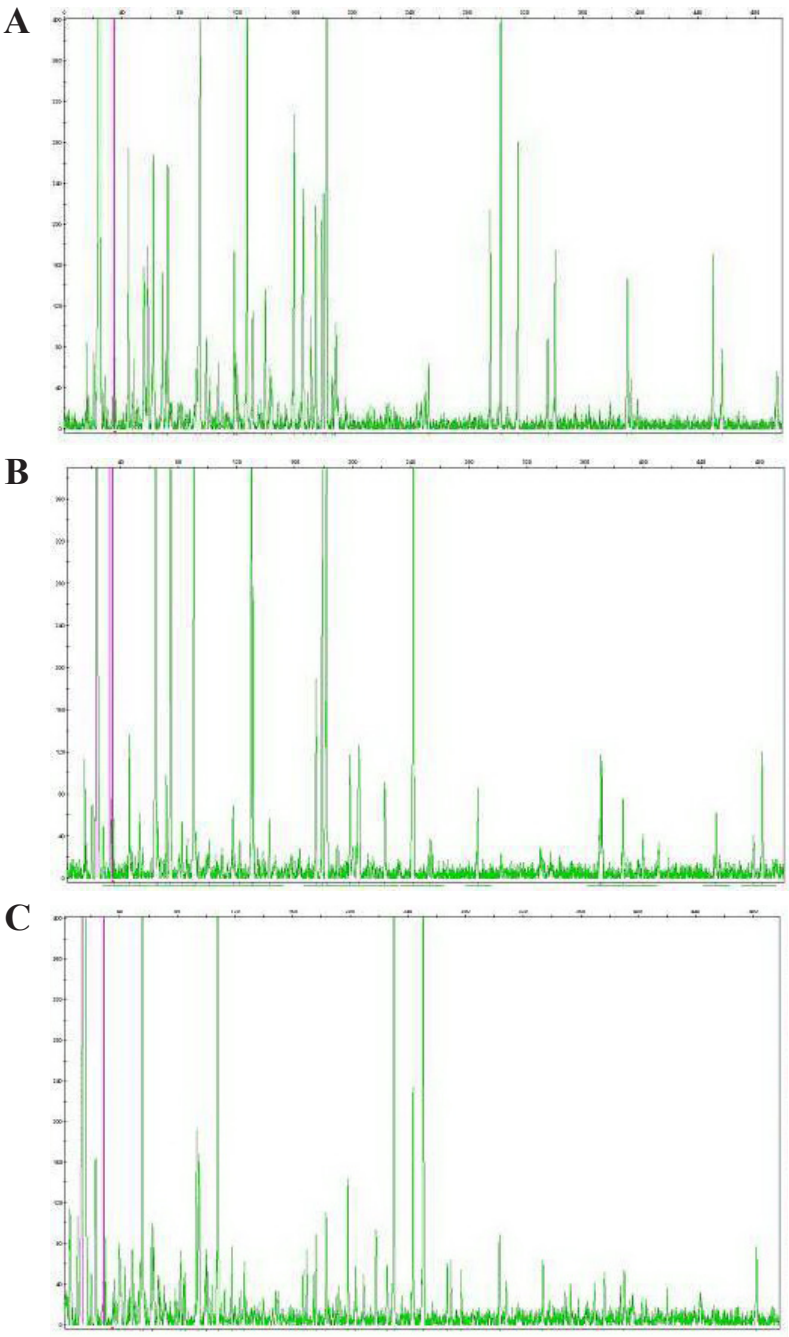

Figure 1. A. AFLP patterns of *Zingiber officinale* using primer pairs *Eco*RI-AAG and *Mse*I-CTC. **B.** AFLP patterns of *Z. montanum* using primer pairs *Eco*RI-AAG and *Mse*I-CTC. **C.** AFLP patterns of *Z. zerumbet* using primer pairs *Eco*RI-AAG and *Mse*I-CTC. X-axis = base pair of alleles; Y-axis = intensity of alleles.

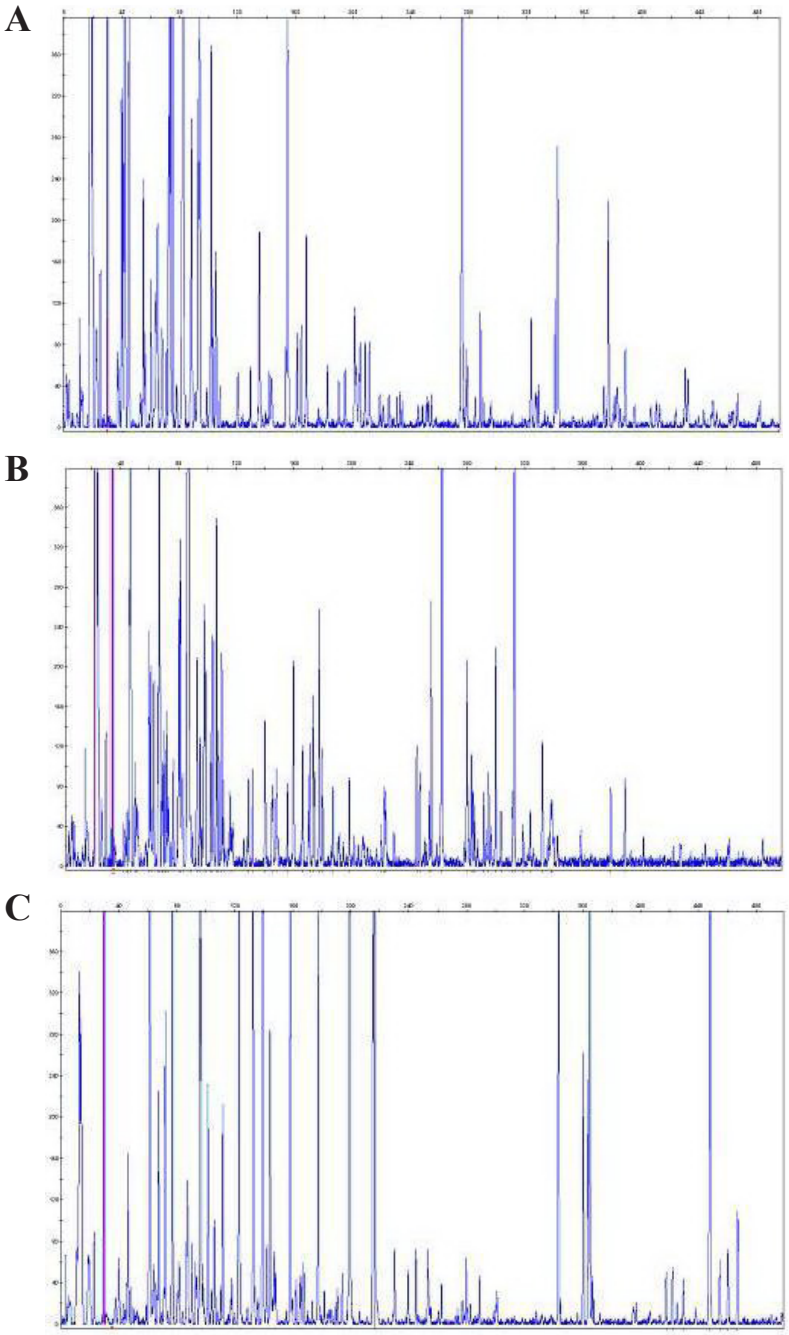

Figure 2. A. AFLP patterns of *Zingiber officinale* using primer pairs *Eco*RI-ACA and *Mse*I-CAG. **B.** AFLP patterns of *Z. montanum* using primer pairs *Eco*RI-ACA and *Mse*I-CAG. **C.** AFLP patterns of *Z. zerumbet* using primer pairs *Eco*RI-ACA and *Mse*I-CAG. X-axis = base pair of alleles; Y-axis = intensity of alleles.

The dendogram obtained after analysis using the NTSys v 2.1 software indicated 3 major clusters, each representing one of the 3 different species of the *Zingiber* genus used in the study (Figure 3). In the first cluster, six landraces of *Z. officinale* clustered together, showing 92.5% similarity among them. The second group consisted of five landraces of the *Z. montanum* grouping together with 95% similarity within the cluster. Similarly, in the third group, five landraces of *Z. zerumbet* clustered together with 95.5% similarity within them. From this dendogram, it appears that *Z. montanum* and *Z. zerumbet* are phylogenetically more closely linked to each other than to *Z. officinale*.

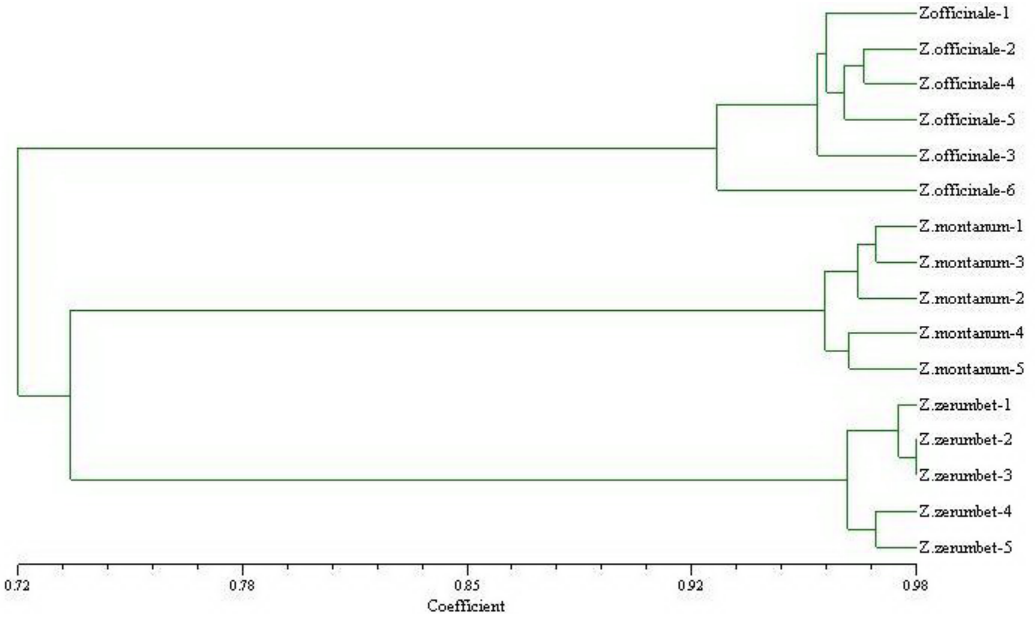

Figure 3. Cluster diagram showing relationship among three *Zingiber* species.

The DNA molecular markers represented by unique peaks for each of the three species of the *Zingiber* genus generated in the present study suggest these as a useful reference tool for species identification that circumvents problems associated with morphological or biochemical markers. The frequency of occurrence of unique peaks in AFLP analysis of DNA isolated from crude drug (plant) preparation (Misra et al., 2007) could be used to assay for the presence of a specific species population. AFLP, in particular, has been the method of choice for discriminating between closely related species and authentication of herbs, as demonstrated for the *Plectranthus* genus in an earlier study (Passinho-Soares et al., 2006). In the present study, too, a well-defined grouping pattern was obtained for the three *Zingiber* species analyzed. The significance of this study stems from the fact that it provides an authentication tool to detect adulterants in the crude drug preparations and to maintain the quality standards in the herbal drug industry.

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REFERENCES

Degani C, Rowland LJ, Saunders JA, Hokanson SC, et al. (2001). A comparison of genetic relationship measures in strawberry (*Fragaria ananassa* Duch.) based on AFLP's, RAPD's, and pedigree data. *Euphytica* 117: 1-12.

Jaccard P (1908). Nouvelles recherches sur la distribution florale. *Bull. Soc. Vaudoise Sci. Nat.* 44: 223-270.

Joshi K, Chavan P, Warude D and Patwardhan B (2004). Molecular marker in herbal drug technology. *Curr. Sci.* 87: 159-165.

Lin JJ, Kuo J, Ma J, Saunders JA, et al. (1996). Identification of molecular markers in soybean comparing RFLP, RAPD and AFLP DNA mapping techniques. *Plant Mol. Biol. Rep.* 14: 156-169.

Misra A, Shasany AK, Shukla AK, Sundaresan V, et al. (2007). AFLP-based detection of adulterants in crude drug preparations of the 'Safed Musli' complex. *Nat. Prod. Comm.* 2: 93-97.

Misra A, Shasany AK, Shukla AK, Darokar MP, et al. (2010). AFLP markers for identification of *Swertia* species (Gentianaceae). *Genet. Mol. Res.* 9: 1535-1544.

Passinho-Soares H, Felix D, Kaplan MA, Margis-Pinheiro M, et al. (2006). Authentication of medicinal plant botanical identity by amplified fragmented length polymorphism dominant DNA marker: inferences from the *Plectranthus* genus. *Planta Med.* 72: 929-931.

Percifield RJ, Hawkins JS, McCoy JA, Widrlechner MP, et al. (2007). Genetic diversity in *Hypericum* and AFLP markers for species-specific identification of *H. perforatum* L. *Planta Med.* 73: 1614-1621.

Saunders JA, Pedroni MJ and Daughtry CS (1999). DNA fingerprinting of marijuana by the AFLP technique. *Focus* 20: 10-11.

Saunders JA, Pedroni MJ, Penrose L and Fist AJ (2001). AFLP DNA analysis of opium Poppy. *Crop Sci.* 41: 1596-1601.

Vos P, Hogers R, Bleeker M, Reijans M, et al. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.* 23: 4407-4414.

Waugh R, Bonar N, Baird E, Thomas B, et al. (1997). Homology of AFLP products in three mapping populations of barley. *Mol. Gen. Genet.* 255: 311-321.