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Article *in* Genetic Resources and Crop Evolution · May 2008

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DOI 10.1007/s10722-007-9252-3

RESEARCH ARTICLE

Genetic variations and interrelationships in *Vanilla planifolia* and few related species as expressed by RAPD polymorphism

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Received: 7 March 2006 / Accepted: 22 June 2006 / Published online: 14 July 2007

Abstract Vanilla is naturally distributed in Mexico and parts of Central America and the history of origin of cultivated vanilla suggests that the entire stock outside Mexico may be from a single genetic source. In the present study, RAPD polymorphism was used to estimate the level of genetic diversity and interrelationships among different collections of *Vanilla planifolia* Andr., and few related species, including both leafy and leafless types such as *V. tahitensis* J.W.Moore, *V. andamanica*, Rolfe, *V. pilifera* Holtt., and *V. aphylla* Blume. Studies revealed that there are very limited variation within collections of *V. planifolia*, indicative of its narrow genetic base, and of the related species we tested, *V. tahitensis* is nearest to *V. planifolia.* The species studied are diverse and have a similarity ranging from 1.2 to 57.3 %. Of the sampled taxa, *V. andamanica* is the most divergent and there is also reasonable variability within its collections, indicating the possibility of natural seed set. A total of 82 polymorphic bands expressed in the RAPD profiles were used to generate a genetic distance matrix, which was then used in cluster analysis. Specific groupings were revealed by the cluster analysis whereby the leafless forms (*V. aphylla, V. pilifera* and the new species) and *V. andamanica* formed separate clusters. This is the first report of species interrelationship studies, including both cultivated and wild vanilla species.

Keywords RAPD · Species relationships · *Vanilla andamanica* · *V. aphylla* · *V. pilifera* · *V. planifolia* · *V. tahitensis*

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Introduction

The genus *Vanilla* is widely distributed throughout tropical and subtropical regions around the world (Indonesia, South and Central America, Mexico and Africa), and this distribution supports the theory that it is very old genus. These and other *Vanilla* species have been treated in various monographic works (Correll 1953; Hooker 1973) including the life history of *V. planifolia* (Swamy 1947). This belief is also reinforced by the fact that these orchids share many similar characteristic features making researchers (Dressler 1981) conclude that the genus was differentiated when the primitive continent of Gondwanaland divided 120 million years ago, based on the plate tectonics theory.

Vanilla was introduced to Europe from Mexico, in about 1,500 and its reputation of being an aphrodisiac followed it to countries where it was introduced. The

importance of vanilla since early times in Mexico, is evident by the mention of offering vanilla as a medicinal beverage as part of a tribute during reign of Itzcóatl (Aztec Emperor) in 1427, and citing vanilla as a remedy for fatigue in Badianus manuscript in 1552 (Lubinsky 2004).

The genus *Vanilla* includes about 110 species. Cultivated vanilla, *V. planifolia* Andr. (syn. *V. fragrans* (Salisb.) Ames), is a tropical climbing orchid known for yielding the delicate popular flavouring material (Purseglove et al. 1981) and is the second most expensive spice traded in the world market (Spices Board 2000). The major vanilla producing countries are Madagascar, Comoro, Indonesia, Mexico and the Reunion. Of these, Madagascar holds the prominent position.

The history of origin of cultivated vanilla suggests that the entire stock outside Mexico may be from a single genetic stock and for the last 400 years, humans have been playing important role in the dispersal and spread of vanilla in the New World. The British introduced *V. planifolia* into India about 200 years ago where five other species are native viz, *V. pilifera* Holtt., *V. andamanica* Rolfe, *V. aphylla* Blume, *V. walkeriae* Wight and *V. wightiana* Lindl. *V. pilifera* originally described from Malaya, recorded in Thailand is found in the Mikir hills (Borthakur and Hajra 1976) of North East India. *V. andamanica* is endemic to Andaman group of Islands and is believed to be same as *V. albida* (Seidenfaden 1978). *V. aphylla*, an endangered species, previously known from Thailand, Vietnam is found in South India. *V. tahitensis* J.W. Moore, which is also commercially exploited (commonly called Tahiti vanilla) throughout the tropics, is indigenous to the Tahiti Islands. The presence and absence of leaves, and floral characters (flower colour, colour of lip and hairs on lip, colour of ovary-pedicel etc.,), morphologically distinguish these species (Fig. 1).

The higher-level relationships among orchids, as among most plant groups, have been a matter of speculation. The present study was conducted with a long term goal of elucidating a probable phylogenetic pattern for vanilla on which further evolutionary studies can be based. The study assessed the genetic diversity, using RAPD polymorphism, of the vanilla germplasm established at the Indian Institute of Spices Research, India, needed for establishing a core collection of genotypes that would capture the variability at the same time contain genotypes with useful traits like disease resistance, desirable floral and bean characters and understanding its genetic structure. Thus the aim was twofold; a) to investigate morphological and genetic diversity within cultivated types and between species, and b) to test the usefulness of RAPDs in discriminating different species that are usually discriminated by presence/absence of leaves and flower colour.

Materials and methods

Vanilla accessions established and maintained as in vitro cultures and in the field repository of Indian Institute of Spices Research, Calicut, India, were used in the present study. These specimens are deposited in the germplasm conservatory of Indian Institute of Spices Research, India, who hold the national mandate for collection, conservation of spices genetic resources. Six species of *Vanilla*, including wild and cultivated with distinct leaf characters (leafy and leafless species), were studied. The leafy species included were *V. planifolia*, *V. andamanica* and *V. tahitensis*, and the leafless species were *V. aphylla*, *V. pilifera* and an unidentified leafless 'New' species resembling *V. aphylla*.

Collections of *V. planifolia*, made from plantations in vanilla growing regions in India, consisting of two indigenous and two exotic collections (one each from Madagascar and Mauritius) and a variegated mutant, *V. planifolia* 'Marginata', were studied. Eight collections of *V. andamanica*, collected from Andaman Islands were also included.

Four interspecific hybrids, produced in the authors' laboratory, using *V. planifolia* as female parent and *V. aphylla* as male parent (VH1, VH4, VH5 and VH6), one colchicine-treated seedling progeny of *V. planifolia* (V161.c) and one callus-regenerated seedling progeny of *V. planifolia* (V161.1) (Nirmal Babu et al. 1998; Minoo 2002) were tested as well.

Isolation of DNA

The CTAB method (Ausubel et al. 1995) was used for the isolation of genomic DNA from vanilla leaf tissue. The stock solutions and buffers were

Fig. 1 Morphological variations in *Vanilla* species; (**a**) *V. aphylla*; (**b**) *V. pilifera*; (**c**) *V. planifolia*; (**d**) *V. andamanica*, (**e**) *V. planifolia* 'Marginata' (**f**) *V. tahitensis*, a variant among the *V. planifolia*, collections from Mauritius in (**g**) plant growth, (**h**) large pod size, (**i**) larger flowers

prepared for DNA isolation following Sambrook et al. (1989).

RAPD profiling

RAPD profiles were developed as per the method suggested by Williams et al. (1990) with minor modifications. The dNTPs, Taq polymerases and other chemicals were procured from Amersham Pharmacia Biotech, Sweden. Sixteen arbitrary primers from Operon Technologies Inc. Alameda, California were used for PCR reaction. Each primer contained at least 60–70% GC content and no self-complementary ends. The primers used and their base sequences are given in Table 2.

PCR amplification reaction volumes were 25 µl PCR each containing the reagents added sequentially to 25 µl of assay buffer—1 ×, 150 µM of dNTPs, 2 mM $MgCl_2$, 1 unit of Taq DNA polymerase (Amersham Pharmacia Biotech, Sweden), 40 ng of genomic DNA and 10 picomoles of primer (Operon Technologies Inc. Alameda, California). Amplification employed a programmable thermal cycler (Genecycler TM, BIORAD, USA) with cycling regimes of an initial denaturation temperature of 94°C for 5 min, followed by a 33 repeats of a PCR core cycle of 94°C for 1 min, 40°C for 1 min and 72°C for 1 min, followed by a final extension cycle of 72°C for 15 min.

The amplicons were resolved electrophoretically alongside a 1 kb ladder, on a 2% agarose gel stained with 0.5 µg ml^{-1} of ethidium bromide in a 1 × TAE buffer. Equal volumes of the completed reaction mix were loaded and run at 90 V for 3 h in an OWL separating system (OWL, USA). The completed gel was documented with the help of GelDoc 1000

documentation system from Biorad, USA. Twenty arbitrary 10-mer primers were tested and nine which gave polymorphic and scorable amplified products, were used for RAPD profiling, following the protocol of Williams et al (1990). Duplicate amplification was performed to confirm reliability of the bands. Amplified products were listed as discrete character states in a present (1) or absent (0) matrix. Relationships among genotypes was evaluated with a phenetic cluster analysis using the Unweighted Pair Grouping Mathematical Average (UPGMA) analysis and plotted in a phenogram using NTSYS pc version 2.0j (Rohlf 1997). The polymorphic loci exhibited by the different genotypes were consolidated in a chart (Microsoft Excel) to study the expressed loci of each genotype. Bootstrap analysis was performed using WinBoot (Yap and Nelson 1996) with 1,000 repetitive sampling using Dice's coefficient, of the data to compute bootstrap *P* values.

The similarity/differences between the genotypes using RAPD polymorphism was estimated by Paired Affinity Index (PAI). The PAI expressed as percentage indicated the similarity (%) between any two genotypes and was calculated by the formula;

$$\text{PAI} = \frac{\text{No. of similar bands}}{\text{Total no. of bands}}$$

Results and discussion

Species of vanilla

A preliminary analysis of the various characters of *Vanilla* species (Table 1) including the above species, showed presence and absence of leaves formed an important part in the classification of the genus which in general had the basic chromosome number; $x = 16$. Differences in floral characters existed in flower colour and lip characters. Most of the Indian species were leafless and the chromosome number in *V. aphylla* is $2n = 64$, whereas the cultivated vanilla and *V. tahitensis* had a somatic chromosome number of $2n = 32$ (Franklin 1963). In *V. pilifera* vines, leaves were not observed in the initial stages of growth but developed as the vine grew, flowers were narrower (2.8×0.8 cm) with distinct pure white ovary-pedicel, pale green tepals, purplish violet and longer (6 mm approx.) hairs on white lip. *V. aphylla* is leafless (with scales-1.8 cm) and yellowish-cream flowers (petal size 3×1.2 cm approx.) having tuft of hairs that are cream near tip, deep reddish inside (2–3 mm) and light green ovary-pedicel. *V. andamanica*, is endemic to Andaman islands and reported as the only orchid with glossy green leaves, having white fragrant flowers with pink lip with white patches (Dagar 1989) and most frequently observed chromosome number of $2n = 40$ (IISR 2005). *V. tahitensis* is characterized by narrow and long leaves (12–14 × 2.5–3 cm, approx.) and whitish flowers with narrow perianth.

Among the species studied, *V. tahitensis* (Fig. 1f) is most closely related to a *V. planifolia* collection from Mauritius with an average similarity index of 85.3%, while *V. andamanica* is the most distant of the wild species we studied. In spite of superficial morphological similarity, *V. andamanica* (Fig. 1d) is not closely related to *V. planifolia* or *V. tahitensis*, and its collections are the most divergent from all other species studied forming a separate and unique cluster. The leafless forms of vanilla, *V. aphylla, V. pilifera* and the new species resembling *V. aphylla* (Fig. 1a), formed a separate sub-cluster. All the other leafy vanilla types formed a separate sub-cluster. *V. pilifera* (Fig. 1b), which showed an intermediate leaf character, showed only 50–56.1% similarity to *V. planifolia* but closely resembled *V. aphylla* (76.8%).

The primers produced 82 fragments with a mean of 9.22 fragments/primer, ranging from 6 fragments (OPB 14) to 14 fragments (primer OPA 10) (Table 2). Eight fragments (9.6%) were found in all the genotypes. A few fragments were found to be exclusive to certain species, viz., OPA 10 fragment at 0.5 kb to *V. pilifera* and *V. aphylla* and OPB 20 fragment at 1 kb to *V. pilifera* and the new species. One fragment of primer OPA 10 at 1.4 kb was specific to *V. tahitensis*. A few species-specific bands/loci that could be identified in the present profiling are listed in Table 3.

Intraspecific variation in *V. planifolia*

The present study indicated that various collections of *V. planifolia* (Fig. 1c) showed only a few differences in RAPD profiles (Fig. 2). This reflects the narrow genetic base in cultivated vanilla, which

Table 1 Characteristics of few *Vanilla* species

No.	Botanical name	Origin	Purpose of collection	Type of material	Characters	Chromosome numbers	References
1	*V. planifolia* Andr.	South Eastern Mexico, Guatemala	Cultivated types	Cultivated	Fleshy, sub sessile (8–25 × 2–8 cm) leaves, Pale yellowish green flowers with green ovary-pedicel	$2n = 32$	Correll 1953
2	*V. tahitensis* Moore	Tahiti, Hawaii	Commercially exploited species	Cultivated	Narrow and long (12–14 × 2.5–3 cm) leaves, Whitish, flowers with narrow perianth	$2n = 32$	Straver 1989
3	*V. andamanica* Rolfe	Middle, South and Little Andamans	Endemic and rare species	Wild/ cultivated	Glossy green leaves, White flowers, fragrant, pink lip, petal with white patches	$2n = 40$	Dagar 1989; Seidenfaden 1978
4	*V. pilifera* Holtt.	Malaya, North Eastern India	Endemic, endangered and rare species	Wild	Leafless near base; leaves only near top, flowers, fragrant, pale green tepals, narrower with white ovary-pedicel, purplish violet and longer (6 mm approx.) hairs on white lip	x = 16	Borthakur and Hajra, 1976
5	*V. aphylla* Blume	Thailand, North Eastern India	Endangered species	Wild	Leafless, scales-1.8 cm, Yellowish cream flowers with tuft of hairs that are cream near tip, deep reddish inside (2–3 mm), light green ovary-pedicel	$2n = 64$	Satish and Manilal, 1993

Table 2 Operon primers and the polymorphism expressed in RAPD profiles

Sl.No	Primer code	Base sequence	% of GC	No. of scored fragments	No. of polymorphic markers	Polymorphism (%)
1	OPA 10	5′ GTGATCGCAG 3′	60	14	13	92.8
2	OPA 20	5′ GTTGCGATCC 3′	60	10	9	90.0
3	OPB 14	5′ TCCGCTCTGG 3′	70	6	6	100.0
4	OPB 20	5′ GGACCCTTAC 3′	60	8	8	100.0
5	OPD 03	5′ GTCGCCGTCA 3′	70	9	7	77.7
6	OPD 19	5′ CTGGGGACTT 3’	60	8	8	100.0
7	OPE 05	5′ TCAGGGAGGT 3′	60	10	8	80.0
8	OPE 14	5′ TGCGGCTGAG 3′	70	10	8	80.0
9	OPF 12	5′ ACGGTACCAG 3′	60	7	6	85.7
Total				82	73	806.2
Mean				9.11	8.1	89.5

may be due to its clonal origin from few genotypes, and propagated vegetatively.

The collection from Mauritius is most divergent from the rest of the group (PAI = 89–91.5%), whereas the other collections are more than 96.3% similar to each other. This was reflected in the bootstrap analysis too with high *P value* of 84%. This exotic collection differed in external morphology too, in that it had thicker stem, larger leaves, flowers and pods (Fig. 1g, h, i). When screened against disease causing organisms, *V. planifolia* collections were generally susceptible, however the Mauritius collection did not take up infection to both these pathogens. The *V. planifolia* collection (*V. planifolia* ‘Marginata’), which can be clearly distinguished morphologically from the others by its variegated leaves (Fig. 1e), could not be demarcated from other collections by RAPD markers.

Intra-specific variation in *Vanilla andamanica*

V. andamanica formed a separate cluster in the studies (Figs. 2, 3, 4, 5), and among the different collections similarities ranged from 47.8 to 100%, with highest resemblance between *V. andamanica*-4 and *V. andamanica*-7 and the most divergent being *V. andamanica*-3. This reflects considerable variability among different collections of *V. andamanica*, supporting the probability that this species did originate in the Andaman Islands where sexual reproduction is likely. Collection 3, which is most divergent from collections 1, 2 and 5 (91.4%) and 4, 7 and 8 (92.7%) and bears

Table 3 Species-specific bands expressed in RAPD profiles of *Vanilla*

No.	Vanilla species	No. of species-specific bands	Primer	Fragment size (kb)
1	*V. planifolia*	2	OPA 20	0.4
			OPD19	2.0
2	*V. tahitensis*	1	OPE 14	0.9
3	*V. andamanica*	9	OPE 14 (2)	1.45. 1.0
			OPE 5 (3)	0.95, 0.9, 0.4
			OPA 20	0.5
			OPA 12	0.4
			OPF 20	1.5
4	*V. pilifera*			
5	*V. aphylla*	1	OPA 10	0.5
6	New species	1	OPA 10	0.6

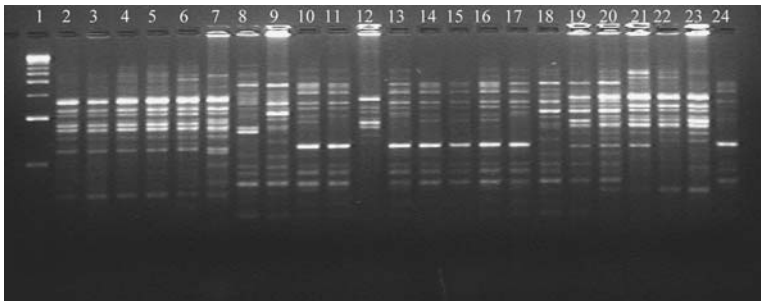

Fig. 2 RAPD polymorphism observed in vanilla using OPERON primer OPB14; 1 kb Ladder, 2: *Vanilla planifolia* 'Marginata' 3: *V. planifolia* IISR, 4: *V. planifolia* Madagascar, 5: *V. planifolia* IAHS, Coimbatore 6: *V. planifolia* Mauritius colln., 7: *V. tahitensis* 8*: V. pilifera* 9*: V. aphylla* 10*: V. andamanica* 1, 11: *V. andamanica* 2, 12: *V. andamanica* 3, 13*: V. andamanica* 4, 14*: V. andamanica* 5, 15: *V. andamanica* 6, 16*: V. andamanica* 17: *V. andamanica* 8, 18*: Vanilla* spp. (new), 19: Interspecific hybrid (VH1), 20: Interspecific hybrid (VH4), 21: Interspecific hybrid (VH5), 22: Interspecific hybrid (VH6), 23: V161c (*V. planifolia* colchicine treated), 24: V161.1 (*V. planifolia* callus regenerated)

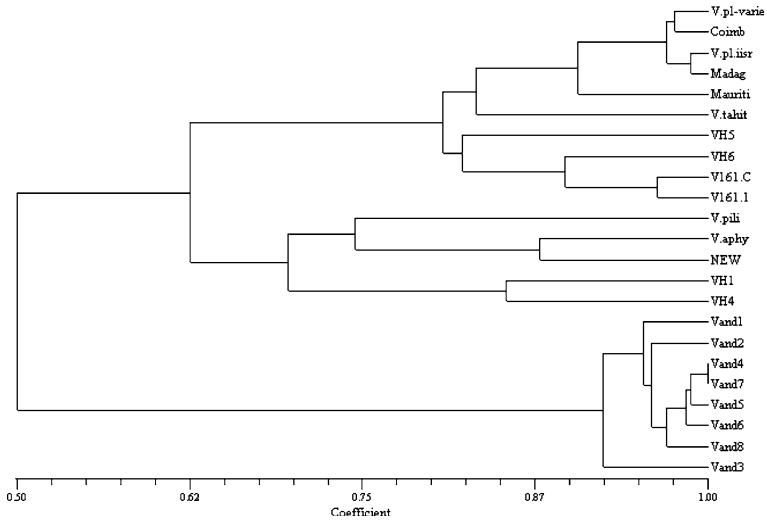

Fig. 3 Dendrograms constructed for the taxa (species, collections, interspecific hybrids and somaclones of *Vanilla*) obtained from genetic differences based on different similarity coefficients for the RAPD profiles

highest resemblance to collection 6 (94%), formed a separate group in the cluster of *V. andamanica* collections whereas all the others were together in the same group. Nine fragments (10.8%) were found exclusively in *V. andamanica* (Table 3). *V. andamanica* 3 was further characterized by the absence of two fragments (produced by OPB 14 at 1.5 and 0.6 kb and present in all other collections) and presence of a fragment at 0.9 kb produced by primer OPB 14, which was absent in all others.

Interspecific hybrids of *V. planifolia* (♀) × *V. aphylla* (♂)

The interspecific hybrids, VH1, VH4, VH5 and VH6, were approximately equidistant from their parents. Of the four, VH1 and VH4 clustered more closely with *V. aphylla* while VH5 and VH6 clustered along with *V. planifolia.* Morphologically, VH-1 and VH-6 are leafed '*V. planifolia'* types, and VH-4 and VH5 are leafless types. Taken together, the RAPD profiles and an analysis of morphological characters help confirm the true hybrid nature of these four genotypes. This is promising for possibilities of transfer of useful traits from related wild species to *V. planifolia*. Ravindran (1979) has studied the nuclear behavior of sterile pollen and discussed the possibility of the origin of *V. planifolia* as a hybrid. Ovule culture of *V. planifolia* has been utilized to exploit heterozygosity and develop a population of segregating progenies, which revealed differences in isozyme banding patterns (Minoo et al. 1997).

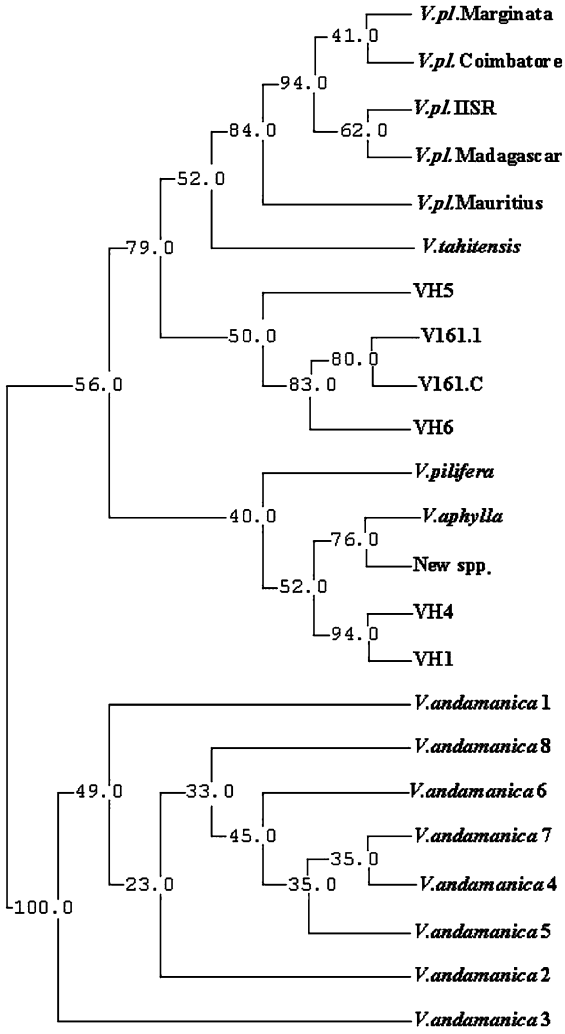

Fig. 4 Phenogram constructed for the taxa (species, collections, interspecific hybrids and somaclones of *Vanilla*) with bootstrap values based on similarity matrix developed from RAPD profiles

Colchicine-treated and callus-derived progenies of *V. planifolia*

The RAPD profiles of callus-regenerated and colchicines-treated progenies of *V. planifolia* seedling (V161) were 96.3% similar. Callus-regenerated (V161.1) and colchicines-treated (V161c) samples, though very similar to typical *V. planifolia*, two of the interspecific hybrids clustered together reflecting the induction of a certain amount of variability through these processes.

Paired affinity indices indicating the percentage of similarity between the genotypes studied were calculated (Table 4). The dendrogram (Fig. 3) showed the relationships among the genotypes. Only polymorphic bands were used for the construction of the binary value matrix, representing the absence and presence of bands by 0 and 1, respectively. Each band was considered a locus as has been done in *Zea mays* (Meyer et al. 2004). The polymorphic loci exhibited by the different genotypes were consolidated to depict and study the pooled data of each genotype and certain species-specific loci were identified (Fig. 5). Probability of mutations and recombination was found in this data. Commonality of many loci among the different species collected from distant geographical regions indicates their common origin and lineage. Loci 43 was absent in most of the leafy species viz., *V. planifolia*, *V. tahitensis* and *V. andamanica*, however, it was present in all the interspecific hybrids probably introduced from its male parent, *V. aphylla* and in the colchicine treated progeny of *V. planifolia* suggestive of a mutable form of the particular locus. The largest number of loci was in the new (leafless) species of *Vanilla* followed by *V. aphylla*. The interspecific hybrids also had the largest number of loci suggestive of inclusion of recombination products not present in the female parent. Locus 47 which was absent in *V. planifolia* and *V. aphylla* but was characteristically present in all *V. andamanica* collections and *V. pilifera* was found in two interspecific hybrids. However, correlation of these to evolution in the genus has to be studied further.

Bootstrap analysis divided the genotypes into two major clusters with significant *P* values (Fig. 4). The first cluster ($P = 56\%$) contained all genotypes except *V. andamanica* and showed two distinct subgroups. The first subgroup ($P = 79\%$) revealed clustering of all leafy genotypes including *V. planifolia* collections and species. Among the *V. planifolia* collections, exotic collection from Mauritius which had thicker stems and larger leaves, flowers and pods, formed an out-group ($P = 84\%$). The existing characterization of *Vanilla* species and collections are based on morphological characters only. Dressler (1981) suggested occurrence of two kinds of Vanilla plants based on plant morphology, that the plants with thick stems and succulent leaves are considered good producers of vanilla and those, with thinner stems, wider leaves and whose pods are not succulent are not good producers. *V. tahitensis*, which has fruits, that do not

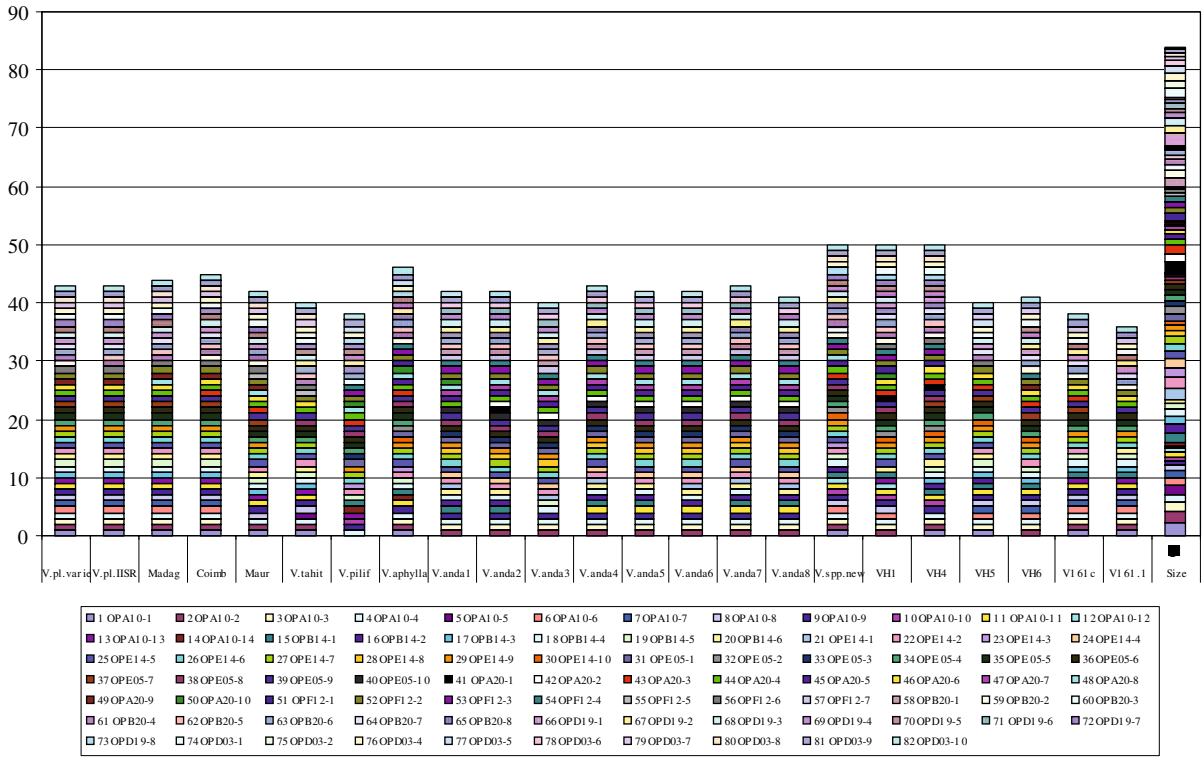

Fig. 5 Pooled data analysis of polymorphic loci expressed by the different species and collection of *Vanilla*

split open, with lower vanillin content (Straver 1989) and slender stem with long narrower leaves than *V. planifolia*, formed an outgroup in the leafy genotypes in the present study. Thus the molecular data was in agreement with differences in morphological characters. Earlier nuclear ribosomal ITS 1, 5.8 SrDNA and ITS 2 sequences have been studied and the phylogenetic analysis identified 7 major core orchidiod groups revealing morphological characters fully convergent with the well supported groups (Douzery et al. 1999). Neilsen and Siegismund (1999) have suggested, based on electrophoretic patterns of seven polymorphic enzymes of three native vanilla species of Puerto Rico, that genetic differences between species was mostly differences in allele frequency and the hierarchical differentiation shows that differentiation between species is only a little higher than differentiation between population and species, partially due to the nature of pollen mass and absence of wind dispersal affecting gene flow.

In the present study, few variations that could be utilized for genomic regions as molecular markers for the recognition of the different cultivars and species was expressed. Moreso, since the primary gene pool is evidently threatened (Lubinsky 2004), the secondary gene pool, that is, the close relatives of *V. planifolia*, become more important as a source of desirable traits to be incorporated in the crop. Desirable traits, such as tolerance to diseases caused by *Fusarium oxysporum* (*V. aphylla* and few collections of *V. andamanica*) and *Phytophthora meadii* has been observed (Minoo 2002). The species were screened against the infection of the causal agents of foot rot and wilt diseases in vanilla. The disease development was manifested as browning and water soaked patches at the axil, spreading out onto the either sides of the internode and at certain times into the leaf finally leading to death of the portion of stem above the inoculation point. Most of the collections of *V. andamanica*, gave resistant reaction to both the pathogens, except *V. andamanica* 8 (Table 5), which was susceptible to *Phytophthora* and *V. andamanica* 5, which was partially susceptible to *Fusarium*. *V. planifolia* collections were generally susceptible but the exotic collection from Mauritius did not take up infection to both these pathogens. *V. aphylla*

Table 4 Paired affinity indices based on RAPD profiles exhibited by species, collections, selfed progenies and interspecific hybrids of vanilla

	V.pl.varieg	V.pl.IISR	Madagas.col	Coimb.coll	Mauritius	*V.tahitensis*	*V.pilifera*	*V.aphylla*	*V.andaman1*	*V.andaman2*	*V.andaman3*	*V.andaman4*	*V.andaman5*	*V.andaman6*	*V.andaman7*	*V.andaman8*	New spec.	VH1	VH4	VH5	VH6	V161.C	V161.1
V.pl.varieg	x																						
V.pl.IISR	97.5	x																					
Madag	96.3	98.8	x																				
Coimb	97.6	97.6	96.3	x																			
Maurit	91.5	89.0	90.2	91.5	x																		
V.tahit	**81.7**	84.1	82.9	81.7	85.3	x																	
V.pili	50.0	50.0	51.2	50	56.1	56.1	x																
V.aphylla	53.6	53.6	54.9	56.1	57.3	57.3	76.8	x															
V.and1	42.7	45.1	46.3	42.7	43.9	48.8	61.0	64.6	x														
V.and2	42.7	45.1	46.3	42.7	43.9	48.8	58.5	62.2	95.1	x													
V.and3	43.9	46.3	47.6	43.9	45.1	50.0	62.1	61.0	91.4	91.4	x												
V.and4	43.9	46.3	47.6	43.9	45.1	50.0	57.3	63.4	96.3	96.3	92.7	x											
V.and5	45.1	47.6	48.8	45.1	46.3	51.2	58.5	64.6	95.1	97.6	91.4	98.8	x										
V.and6	45.1	47.6	48.8	45.1	46.3	51.2	56.1	62.2	95.1	95.1	94.0	98.8	97.6	x									
V.and7	43.9	46.3	47.6	43.9	45.1	50.0	57.3	63.4	96.3	96.3	92.7	100	98.8	98.8	x								
V.and8	43.9	46.3	47.6	43.9	45.1	50.0	59.7	63.4	93.9	93.9	92.7	97.6	96.3	96.3	97.6	x							
New spec.	61.0	61.0	62.2	63.4	62.2	62.2	71.9	**87.8**	59.7	57.3	56.1	58.5	59.8	57.3	58.5	58.5	x						
VH1	69.5	69.5	68.3	71.9	65.1	63.4	61.0	69.5	48.7	48.7	45.1	47.6	48.8	46.3	47.6	50.0	76.8	x					
VH4	71.7	72.0	70.7	74.4	70.7	65.8	58.5	76.8	48.7	51.2	45.1	50.0	51.2	48.7	50.0	50.0	74.4	85.4	x				
VH5	84.1	84.1	82.9	84.1	78.0	70.7	51.2	59.7	46.3	46.3	42.7	47.6	48.8	46.3	47.6	47.6	59.8	63.4	70.7	x			
VH6	82.9	80.5	79.3	82.9	76.8	67.1	59.8	63.4	50.0	50.0	51.2	51.2	52.4	50.0	51.2	51.2	65.9	67.1	69.5	81.7	x		
V161.C	86.6	84.1	82.9	86.6	80.5	73.1	60.9	62.2	46.3	46.3	47.6	47.6	48.8	46.3	47.6	47.6	62.2	63.4	68.3	82.9	91.5	x	
V161.1	85.4	83.0	81.7	85.4	79.3	74.4	59.7	61.0	47.6	47.6	46.3	48.8	50.0	47.6	48.8	48.8	61.0	67.1	69.5	81.7	87.8	96.3	x

Vp-1–Vp-5 : Collections of *Vanilla planifolia*, Vt : *V. tahitensis;* Vpi : *V. pilifera;* Vap : *V. aphylla;* Van-1–Van-8 : Collections of *V. adama*V? : Doubtful species of *Vanilla;*VH-1, VH-4, VH-5 : Inter-specific hybrids of *V. planifolia* (♀) × *V. aphylla* (♂) ;VH-6 V161–Col.: Colchicine treated seedling progeny of *Vanilla planifolia* (No. V161); V161.1–Ca: Callus regenerated; seedling progeny of *Vanilla planifolia* (No. V161)

Table 5 Reaction of a few *Vanilla* species and collections against infection to *Phytophthora* and *Fusarium*

Sl. No	Plant no.	Disease incidence (%)[a]	
		Phytophthora meadii Disease index	*Fusarium oxysporum* Disease index
1	*V. planifolia*	++++	+
2	*V. aphylla*	++++	0
3	*V. andamanica 1*	0	0
4	*V. andamanica 2*	0	0
5	*V. andamanica* 3	0	0
6	*V. andamanica* 4	0	0
7	*V. andamanica* 5	0	+
8	*V. andamanica* 6	0	0
9	*V. andamanica* 7	0	0
10	*V. andamanica* 8	++++	0
11	*V. tahitensis*	++++	+++
12	Madagascar collection	0	++++
13	Mauritius collection	0	0
14	Coimbatore collection	+++	++++
15	Calicut Univ. coll.	++	0

[a] Scored as 0: 0% infection; +: 0–25% infection; ++: 25–50% infection; +++: 50–75% infection; ++++: 75–100% infection

showed tolerance to *Fusarium*. In the absence of any resistant line to *Phytophthora* and *Fusarium* in the existing vanilla germplasm, the disease escapes identified from this study could be considered as potential candidates to be exploited for identification of disease resistant lines.

Occurrence of natural seed set in *V. wightiana*, the leafless wild species from coastal Andhra Pradesh in South India (Sudhakar and Rao 1982; Rao et al. 2000) have been reported. These studies are thus indicative of the presence/association of desirable characters in the wild species.

Only nine primers were needed to generate a similarity matrix, of which a phenogram was derived that separated *V. andamanica* from all other species. The significant variation noted among collections of *V. andamanica*, suggests possibility of natural seed set and seedling origin of the collections. Further collection three of the species formed an outgroup with *P value* = 100%, suggesting that it may be a hybrid, with *V. andamanica* as one of its parent or a closely related species. The studies also revealed that to enhance chances of capturing allelic diversity in a species or genus, especially for desirable traits, collection procedures would need to capture alleles at lower frequency. Hybridization between species with different chromosome numbers usually produce nonviable embryos, but the success of hybridization between cultivated *V. planifolia* ($2n = 32$) and wild *V. aphylla* ($2n = 64$), indicates that no incompatibility factors seem to exist in between these two geographically distant species (Minoo 2002) and that they have similar genomes, revealed by the significant similarity between the different species. The use of RAPDs earlier to assess genetic diversity among cultivated *Vanilla* species has been found to separate the three species (Besse et al. 2004). Data obtained in the present study was found to be consistent thus allowing it to be used as a reliable tool augmenting conventional taxonomic principles.

Acknowledgements We are thankful to Dr Johnson K George, Sr. Scientist, Indian Institute of Spices Research and Dr KJ Madhusoodanan, Sr. Scientist, Indian Cardamom Research Institute, Spices Board, for sharing their collections of vanilla. We are also grateful to Dr PN Ravindran former Project Coordinator (Spices) for his help and guidance.

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