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Detection of Genetic Diversity in *Jasminum* species through RAPD Techniques

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Abstract

Randomly Amplified Polymorphic DNA (RAPD) technology was used to work out the genetic relationship among the twenty one accessions of *Jasminum* spp. Forty decamer primers from OP-S and OP-V were used for this purpose out of which 14 primers, ten from OP-V and four from OP-S series produced 1173 reproducible, bright and clear bands which ranged from 250 bp to 10,000 bp. Overall 195 alleles were detected. The PIC value calculated for these primers ranged from 0.438 to 0.698 which indicates a good level of genetic diversity among the accessions as also revealed by generated similarity matrix. The similarity values among the genotypes ranged from 0.263 to 0.776. The resulting dendrogram divides the accessions into two distinct main clusters (A and B) at 0.40 similarity value. Cluster A has the most diverse jasmine accessions, while cluster B further divided the accessions into smaller groups. The results clearly indicated that RAPD analysis provide a good tool to detect and classify the genetic diversity of *Jasminum* spp. It is believed that these findings are helpful in further exploration, classification and improvement of *Jasminum* spp.

Keywords: *Jasminum* spp.; Genetic diversity; RAPD

Introduction

Jasminum one of the largest genus of family *Oleaceae* comprised of more than 200 species is well known due to its medicinal importance. It is mainly cultivated in sub continental region to obtain fragrant flowers and essential oil extracts (Gallo *et al*., 2006). Flowers of species like *Jasminum sambac, J. officinale, J. grandiflorum* and many others are used in the manufacturing of perfumes and aromatizing (Abdoul-Latif *et al*., 2010). It is also used in the form of liana to decorate the hair and the neck in the form of collar (Isa, 2005).

The pre-requisites for the improvement of any plant species are germplasm collection and its characterization to find out the diversity among the accessions. Characterization can be done by several ways which include morphological, enzymatic and genetical methods. The best way to assess the diversity is based on DNA markers which allow differentiation among genotypes without any environmental influences (Newbury and Ford-Lloyd, 1993). These markers have a great potential to identify the genetic diversity within and among the accessions, which can help the breeders to optimize the collections, the planning of seed regeneration and the successful implementation of pre-breeding approaches (Borner *et al*., 2000). Among different techniques available to unreveal the genetic diversity, RAPD is the simplest one (Semagn *et al*., 2006).

RAPD technique has been widely used to uncover the mysteries of different aspects in floricultural and horticultural crops which include estimation of genetic diversity (Nowbuth *et al*., 2005; Khan *et al*., 2005; Mohapatra and Rout, 2006; Hadian *et al*., 2008) DNA fingerprinting, mapping of genes controlling important horticultural traits (Matsumoto and Fukui, 1996; Lee *et al*., 2005), making of genetic and linkage maps (Zhang *et al*., 2010), molecular assisted selection, studying of the complex inheritance mechanism of interspeicific hybrids (Ye *et al*., 2008), identification of mutants (Teng *et al*., 2008) and characterization of germplasm (Kiani *et al*., 2008).

In *Jasminum* species a very little work has been reported in literature regarding its genetic aspect. Joy and Raja (2010) reported the use of RAPD technique to detect the somaclonal variation in *J. auriculatum*. Since no molecular information regarding the genetic diversity of *Jasminum* species available, this study was aimed at determining the relationship among the available accessions/species of *Jasminum* in Pakistan using RAPD technique. The information generated from this work will be useful for the better identification of accessions and in future breeding program.

Materials and Methods

Plant Material

The experiment was conducted in the Tissue Culture

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Laboratory of Horticulture Department, Pir Meher Ali Shah Arid Agriculture University Rawalpindi during the year 2011-2012. Twenty one jasmine accessions were used for this work, which were collected from the *Jasminum* Germplasm Unit of Horticultural Research Institute for Floriculture and Land Scaping, Rawalpindi.

Total Genomic DNA Extraction

Total genomic DNA of twenty one jasmine genotypes were isolated by Cetyltrimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle, 1990). For this purpose tender leaf from field grown plants were sequentially washed under running water and Tween-20 for 10 min each. Approximately 1.5 g of sterilized leaves were taken for further extraction. After drying with blotting paper the leaves were crushed to a fine ground powder in liquid nitrogen and extracted with CTAB, hot extraction buffer [100 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 1% w/v PVP (polyvinyl pyrrolidone) and 2% (v/v) ß-mercaptoethanol]. The mixture was then incubated at 60°C for 1 hour, followed by two extractions with chloroform/isoamyl alcohol (24:1). Isopropanol was used to precipitate nucleic acids and the pellet obtained was dissolved in Tris-EDTA (TE) buffer (10 mM Tris-HCl, pH 8.0 and 1 mM EDTA, pH = 8.0). Co-precipitated RNA was removed by digestion with RNAase. Remaining impurities were extracted with processed phenol and chloroform. Total DNA was precipitated using sodium acetate and cold ethanol. The precipitates were washed twice with 10 mM Ammonium Acetate in 76% ethanol and the pellet was dissolved in TE buffer. The purified total DNA was quantified by gel electrophoresis, and its quality verified by spectrophotometry. DNA sample was stored at 4°C.

PCR Amplification and Product Electrophoresis

Two sets of decamer random primers (OPS and OPV) from Operon Technologies Inc., Alameda, California, USA were used to determine genetic variation. Amplification reactions were performed in 25 µL volumes containing 2 µL primers, 3 µl genomic template DNA of jasmine, 1 unit of Taq DNA polymerase, 5 µL Magnesium Chloride, 4 µL Deoxyribonucleotide Triphosphate (DNTPs), 2.5 µL 10x Taq buffer and 8 µL distilled water. DNA amplification reaction was carried out with a thermocycler (Creacon) programmed for initial pre-denaturation step at 94°C for 5 min followed by 40 cycles of 1 min. at 94°C, 1 min at 38°C and 2 min. at 72°C lastly 5 min incubation period at 72°C.

The amplified fragments were separated on 1% agarose gel in 0.5 x Tris-borate-EDTA (TBE) buffer stained by Ethidium Bromide (Sambrook *et al*., 1989) visualized under Ultraviolet light (UV) and photographed by using the gel documentation system. All PCR reactions were repeated at least twice.

Data Interpretation

The photographs of gels were used to score data for RAPD markers. RAPD behave as dominant markers, thus they tend to be bistate (Present-absent) type of scoring. The molecular size of the amplification products were measured by comparing with 1 kb DNA ladder. Each DNA fragment amplified by a given primer was considered as a unit character and the RAPD fragments were scored as a binary variable (1) for presence and (0) for absence of each of the primer accession combinations. Only bright bands were scored and faint bands were not considered for further analysis. Allelic polymorphism information content (PIC) was calculated according to following formula as described by Botstein *et al*. (1980):

$$\text{Polymorphism Information Content PIC} = 1 - \Sigma (P_{ij})^2$$

Where P_{ij} is the frequency of the i^{th} pattern revealed by the j^{th} primer summed across all patterns revealed by the primers. Genetic similarity between the genotypes was estimated using similar matching coefficient.

Results and Discussion

A total of forty decamer RAPD primers from S and V series (Operon Technologies Inc. Almeda CA, USA) were screened to get amplification of *Jasminum* genotypes. Out of these forty primers only fourteen primers, ten from OP-V and four from OP-S series amplified reproducible, bright and clear bands. OPS series primers are OPS-5, OPS-7, OPS-8 and OPS-19, while the name of OPV series primers were OPV-01, OPV-03, OPV-06, OPV-07, OPV-08, OPV-10, OPV-15, OPV-17, OPV-18 and OPV-20. Thus these primers were used to analyze the whole set of *Jasminum* accessions available in Pakistan. Population genetic analysis showed that these primers detected 195 alleles in the 21 accessions of *Jasminum* spp. The number of alleles per primer ranged from seven to twenty. Maximum numbers of alleles (20) were amplified by OPS-19, while OPV-03 amplified only 07 alleles, which were recorded to be the minimum. On an average 13.9 alleles per primer were detected. These fourteen primers yielded about 1173 bright, countable and reproducible bands, which ranged from 250 bp to 10,000 bp. Highest numbers of bands (192) were produced by OPS-19, which ranged from 250 bp to 4000 bp. Whereas, OPV-03 could produce only 08 countable bands which were recorded to be the minimum while other primers OPS-7, OPV-15 and OPS-5 produced 167, 145 and 132 countable and reproducible bands respectively.

The allele size amplified by these primers ranged from 250 bp to 10,000 bp however, most of the alleles had the size ranged from 500 bp to 4000 bp. Smallest alleles of size 250 bp were amplified by primers OPV-08 in the genotype V18, OPS-08 in the genotype V19 and OPS-19 in the genotypes V6, V11, V12, V13 and V14. Largest alleles were amplified by only OPV-18 in the genotypes V8 and

V15. In this study different primers amplified different number of genotypes. The number of genotypes or accessions amplified by these RAPD primers varied according to the primer used, which mainly depends upon the presence of amplification site. Primer OPV-7, OPV-15, OPS-7 and OPS-19 amplified all the 21 accessions of jasmine however OPV-3 could amplify only 2 genotypes while OPV-01 amplified 14 accessions, OPV-6 amplified 12 accessions, OPV-08 and OPV-20 amplified 18 genotypes, OPV-10 amplified 17 accessions, OPV-17 amplified 7 accessions, OPV-18 amplified 6 accessions, OPS-5 amplified 19 accessions and OPS-8 amplified 9 accessions.

Like our results recent study on *J. auriculatum* revealed the use of RAPD to detect the genetic variation among the somaclonal variants of this species (Joy and Raja, 2010). They used 10 RAPD primers to screen the somaclonal variants and reported the formation of total 60 bands by 5 primers, which exhibit 1.2% polymorphism among the bands. Ye *et al*. (2008) used RAPD along with ISSR to determine the genetic diversity between the inbred lines of Zinnia (*Zinnia elegans*). In this study out of 79 RAPD primers 12 primers produced 147 DNA fragments and exhibited 68.03% polymorphism loci. Similarly 08 RAPD primers were used to detect the genetic diversity in 24 cultivars of *Anthurium* (Nowbuth *et al*., 2005). The band size ranged from 300 bp to 2600 bp, while most of the bands were concentrated between 500 bp to 1500 bp. They concluded that the RAPD provides a quick mean to assess the genetic variation as compared to morphological markers. Same results were also reported for *Anthurium* by Ranamukhaarachchi *et al*. (2001). Lee *et al*. (1996) classified the *Lilium* species on the basis of RAPD primers and reported that these primers could be used as an important tool for the classification of *Lilium*. They observed 51 distinct bands amplified by three primers, which vary in size from 300 bp to 1.7 kbp. Similarly 15 cultivars of chrysanthemum were successfully identified on the basis of RAPD analysis by Martin *et al*. (2002). Teng *et al*. (2008) and Lema-Ruminska *et al*. (2004) reported the usefulness of RAPD to detect mutation and genetic variation in Chrysanthemum. Teng *et al*. (2008) used 18 primers which produced 167 reproducible bands, which ranged from 0.3 to 0.2 kb. These results are in comparison with the present study. Mohapatra and Rout (2006) evaluated the genetic relationship among 34 rose cultivars by the help of 4 RAPD series (A, B, D and N), selected primers produced 162 bands which ranged from 100 bp to 3400 bp in size. Trindade *et al.* (2009) used 17 RAPD primers along with ISSR to characterize the aromatic plant species of *Thymus caesptitus* and reported 127 polymorphic bands in its 31 accessions with wide range of genetic diversity.

Polymorphism information content (PIC) value indicates the level of polymorphism assessed by the primers and hence is a good indicator to select the polymorphic primer. Results (Table 1) indicated that the value of PIC ranged from 0.438 to 0.698. The average value of PIC was computed to be 0.637 for each primer. Maximum PIC (0.698) value was recorded for the primer OPS-19 followed by OPS-07 which has the PIC value of 0.693 (Table 1). These primers were able to exhibit the maximum level of polymorphism among the jasmine genotypes. On other hand, Primer OPV-03 was able to detect the minimum level of polymorphism as the PIC value for this primer was computed to be the minimum i.e., 0.438 (Table 1). The results show that a wide range of diversity is detected by the RAPD primers and the accessions evaluated in this experiment have wide genetic base with great diversity of genes among the jasmine accessions. The results reported here are in accordance with the results reported in other Horticultural crops especially with reference to aromatic floral crops. Kiani *et al.* (2008) evaluated the *Rosa damascena* from Iran and Bulgharia for genetic diversity by the help of 31 RAPD primers. They reported wider genetic diversity among the accessions to be used in breeding program. Adiguzel *et al.* (2006) examined phenotypic and genotypic relation among eight species of larges genus of flowering plants (*Astragalus*) with the help of fatty acid and RAPD profile and concluded that both these profiles could be used to differentiate the species of *Astragalus*. Persson *et al*. (1998) analyzed the genetic diversity among different populations of Turk's Cap Lily (*Lilium martagon*) by using RAPD primers. Debener *et al.* (1996) studied the genetic variability of 10 cultivated and 9 wild species of rose by RAPD analysis. They concluded that these molecular markers can be used to know the relationship among rose species. Kumar *et al*. (2006) reported that RAPD markers could reveal polymorphism in radio-mutants of Chrysanthemum and a useful tool assess the distinctness and stability analysis in breeding work. Li *et al*. (2006) reported the low level of genetic diversity of *Eichchornia crassipis* (an ornamental garden plant) in China by analyzing its accessions with RAPD profile. Lee *et al.* (2005) studied the segregation pattern of morphological traits in an interspecific cross of Carnation, while Zhang *et al*. (2010) used RAPD along with AFLP and ISSR to construct the

Table 1: Values of Polymorphism Information Content (PIC) of 14 primers

Primer Name	Sequence (5' to 3')	PIC Value
OPS-05	TTTGGGGCCT	0.6817
OPS-07	TCCGATGCTG	0.6937
OPS-08	TTCAGGGTGG	0.6058
OPS-19	GAGTCAGCAG	0.6989
OPV-01	TGACGCATGG	0.6781
OPV-03	CTCCCTGCAA	0.4387
OPV-06	ACGCCCAGGT	0.6575
OPV-07	GAAGCCAGCC	0.6356
OPV-08	GGACGGCGTT	0.6796
OPV-10	GGACCTGCTG	0.6866
OPV-15	CAGTGCCGGT	0.6797
OPV-17	ACCGGCTTGT	0.5706
OPV-18	TGGTGGCGTT	0.5487
OPV-20	CAGCATGGTC	0.6691

Table 2: Similarity matrix for Nei and Li's coefficient of 21 accessions of jasmine obtained from RAPD analysis

Acces.	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20
V2	0.462																			
V3	0.558	0.581																		
V4	0.557	0.536	0.557																	
V5	0.320	0.610	0.488	0.473																
V6	0.364	0.330	0.427	0.491	0.364															
V7	0.464	0.467	0.505	0.542	0.447	0.550														
V8	0.449	0.467	0.517	0.550	0.448	0.556	0.582													
V9	0.438	0.644	0.575	0.449	0.506	0.520	0.444	0.496												
V10	0.396	0.366	0.516	0.481	0.292	0.547	0.526	0.520	0.383											
V11	0.345	0.413	0.413	0.505	0.386	0.495	0.442	0.476	0.430	0.424										
V12	0.470	0.404	0.404	0.480	0.309	0.551	0.415	0.527	0.417	0.463	0.633									
V13	0.411	0.325	0.341	0.448	0.303	0.485	0.389	0.408	0.306	0.385	0.481	0.649								
V14	0.436	0.383	0.417	0.540	0.378	0.563	0.456	0.456	0.345	0.426	0.529	0.699	0.776							
V15	0.547	0.465	0.566	0.691	0.379	0.517	0.550	0.556	0.520	0.547	0.495	0.551	0.485	0.563						
V16	0.414	0.327	0.551	0.514	0.362	0.541	0.521	0.500	0.404	0.610	0.404	0.349	0.430	0.504	0.504					
V17	0.397	0.469	0.592	0.606	0.511	0.559	0.504	0.591	0.424	0.514	0.481	0.413	0.415	0.504	0.522	0.618				
V18	0.440	0.341	0.488	0.409	0.359	0.379	0.330	0.362	0.337	0.472	0.364	0.345	0.336	0.396	0.364	0.468	0.553			
V19	0.291	0.263	0.303	0.382	0.358	0.375	0.383	0.406	0.320	0.415	0.324	0.315	0.338	0.359	0.414	0.378	0.432	0.400		
V20	0.333	0.396	0.458	0.449	0.283	0.459	0.342	0.462	0.371	0.447	0.333	0.387	0.361	0.416	0.478	0.481	0.500	0.391	0.440	
V21	0.602	0.400	0.484	0.585	0.352	0.574	0.483	0.512	0.458	0.451	0.416	0.407	0.394	0.419	0.375	0.467	0.486	0.330	0.426	0.400

genetic linkage map of Chrysanthemum. Sunil *et al*. (2011) assessed the genetic diversity in 34 *Jatropha* species by means of RAPD, ISSR and phenotypic traits.

The values for these similarities are shown in Table 2. The values of similarity coefficient ranged from 26.30 percent to 77.60%. Minimum similarity was recorded between V-02 and V-19, which showed that these are genetically most diverse genotypes of the studied jasmine germplasm. These two genotypes have only 26% genetic similarity. On other hand genotypes V-13 and V-14 showed the maximum similarity between them. These two genotypes are 77.60% genetically at par to one another. The genotypes V-12 and V-14 also showed much similarity between them (Table 2). The values computed for similarity between these two genotypes are 0.699, which means that these are 69.90% genetically similar to one another. In fact these accessions (V-12, V-13 and V-14) belong to the same species of *J. sambac*. The minimum difference among these genotypes might be due to the varietal difference. Overall the values of similarity matrix (Table 2) show that good amount of genetic diversity is present among the accessions/genotypes of the germplasm.

Cluster analysis resulted grouping of 21 genotypes of jasmine into two main clusters A and B at the similarity level of 40 percent (Fig. 2). Clusters are the small groups which combine the genetically similar genotypes and make the interpretation of dendrogram easy.

Cluster A

This cluster consists of three accessions V18, V19 and V20 which are not only quite diverse from rest of the accessions of jasmine but the similarities among these accessions are also very less. Accession V-18 is 40% and 39% related (similar) to V-19 and V-20, respectively which shows the presence of good amount of genetic diversity. Similarly V-19 and V-20 which are the closest genotypes in this cluster has the similarity index value of 44%. This cluster represents the most diverse accessions of jasmine available in the present germplasm.

Cluster B

This cluster can be further divided into two smaller sub-clusters named as B1 and B2 at a similarity level of about 42%.

Sub-cluster B1

It has five accessions, which are V-6, V-11, V-12, V-13, and V-14. All these five accessions belong to *J. sambac* that's why cluster analysis combine these accessions under a common cluster. Among these accession V-13 and V-14 are less genetically diverse as the similarity between these two accessions is computed to be 77.6%. The figure shows that the accession V-6 is most diverse among these five accessions as it has the highest genetic distance as compared to rest of the accessions. The results of RAPD are in accordance to the phenological study as the accession has many phenological features, which are different from the accessions of *J. sambac*.

Sub-cluster B2

This biggest cluster of dendrogram is further subdivided into three groups named as I, II and III (Fig. 2). Four accessions are present in Group I of sub-cluster B2. These are V-2, V-3, V-5 and V-9. Among these three genotypes viz V-2, V3 and V-9 are wild accessions of *J. grandiflorum* collected from the Rawalpindi and Murree region of Pakistan. Moreover these accessions also share the large number of morphological traits such as plant habitat, leaf pattern, flowering structure etc. (data not shown). RAPD analysis revealed that these are much interlinked with one another. Among these accessions V-2 and V-9 are

genetically very close to one another as the similarity index value between them is 64.4% similar. Five accessions viz. V-7, V-8, V-10, V-16 and V-17 are present in group II of sub-cluster B2. Among these, V-10 is the most diverse of this group and more related to V-16 as compared to rest of the accessions. Accessions V-16 and V-17 are 61 percent similar to one another, while the value of similarity index is 0.582 (58.2% similar) between V-7 and V-8. In group III of sub-cluster B2 four accessions were present viz. V-1, V-4, V-15 and V-21. Data from molecular amplicons of primers revealed that among these accessions V-4 and V-15 were genetically very close to one another while accession V-1 is related to V-21 to an extent of 60.2 percent. Though these accessions are morphologically not so similar but closeness detected by primers might be due to the fact that these accessions occupy the same geographic region.

The importance of classification of diversity cannot be denied both for plant breeding and germplasm collection however the method of detection of this genetic diversity depend upon the tool available to researchers (Hadian *et al*., 2008). RAPD is universally known for its detection of genetic diversity and classification of germplasm of any organism (Williams *et al*., 1990). The results of this study also proved this fact and in accordance to other studies. Joy and Raja (2010) concluded that the RAPD approach is convenient, fast and reproducible to detect somaclonal variations in *J. auriculatum*. Person *et al*., 1998 classified the Turk's Cap Lily on the basis of RAPD analysis. Adiguzel (2006) classified the eight species of *Astragalus*, while Kiani *et al.* (2008) and Hadian *et al.* (2008) classified the different species of rose using the same molecular technique.

The results of present study showed that RAPD analysis provides a good tool to detect and classify the genetic diversity of *Jasminum* spp. collected from the different areas of Pakistan. Cluster analysis based on RAPD primers clearly classified the different species of jasmine into different clusters or groups, while low level of similarity value index among accessions authenticated the relatedness among the species. Wide genetic diversity as detected in this study has provided a solid plat form for further improvement of jasmine flower as aromatic floricultural crop. Based on this conclusion further exploration of genetic resources of jasmine, its taxonomic classification and improvement in Pakistan is needed.

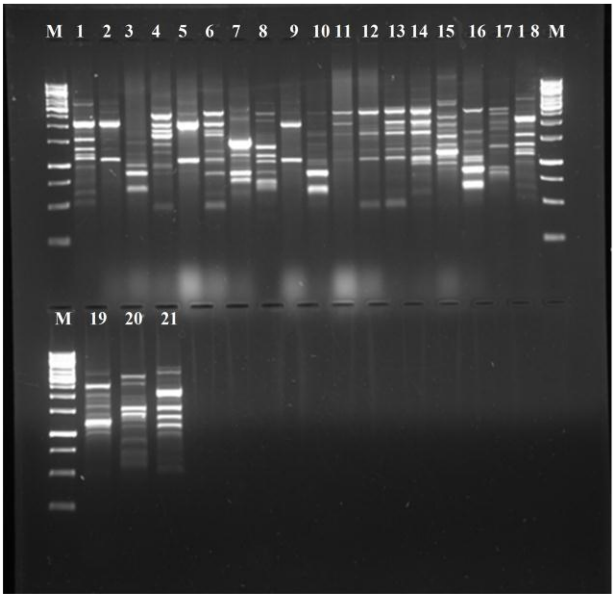

Fig. 1: Agrose gel showing RAPD banding pattern produced with OPV-15 in 21 accessions of jasmine from Lane 1-12. Lane M is 1kb Ladder Molecular Weight Standard

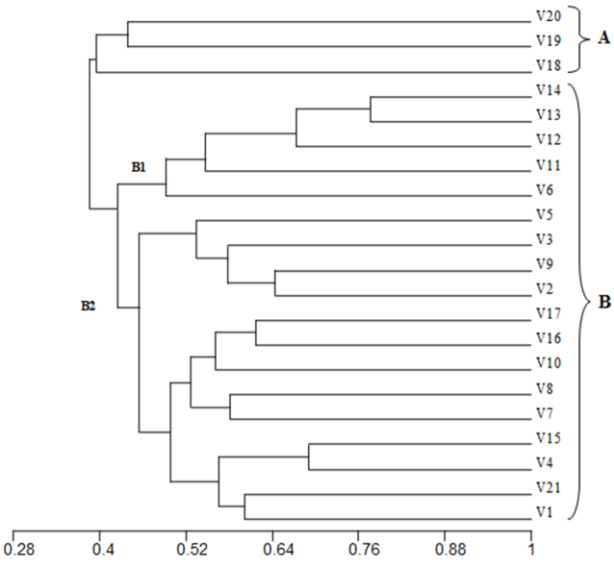

Fig. 2: RAPD Based Cluster Formation of 21 jasmine accessions

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