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Identification of Plant Food Raw Material by RAPD-PCR: Legumes

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Random amplified polymorphic DNA (RAPD) profiles were used to differentiate between several food and feed legume species. Template DNA was extracted from 63 seed meal samples representing 27 legume species (common, scarlet runner, lima, moth, and adzuki beans; broadbeans; soybeans; swordbeans; jackbeans; Florida velvetbeans; common, pigeon, and broad-leaved peas; chickpeas; grasspeas; green, black, and Bengal grams; horsegram; lentils; alfalfa; lupins; common sainfoin; and birdsfoot and sweet trefoil) with sodium dodecyl sulfate-containing buffer and purified by chloroform/isoamyl alcohol treatment and precipitation with 2-propanol. Amplification was performed with commercial RAPD-PCR beads and six 10-mer primers. Three primers (1, 5, and 6) generated RAPD profiles with all 63 templates. Two of these primers produced identical profiles only for two species of the same genus. Identification of all 27 species in homogeneous food or feed samples such as meals was demonstrated by applying either primer 5 or primer 6 and primer 3 to differentiate between swordbeans and jackbeans.

KEYWORDS: Species identification; RAPD-PCR; food legumes; feed legumes; Leguminosae; beans; soybeans; peas; lentils

INTRODUCTION

Seed meals and protein or carbohydrate fractions of many legume species are used in or discussed for the production of more or less complex foods because of their nutritional and/or functional properties. Identification of the raw material is necessary to scrutinize the authenticity and the correct labeling of the food, which should inform the consumer of the identity and quality of the product and the presence or absence of unwanted components, for example, lectins or allergens. In cases in which morphological characters are lacking, such as in seed meals or fractions thereof, characteristic components of the raw material are used for their identification.

In contrast to various components recommended for that purpose such as proteins, enzymes, carbohydrates, lipids, polyphenols, and pigments (*1*, *2*), DNA offers the advantage of being present in amounts sufficient for amplification even in purified fractions. For example, template DNA has been amplified by PCR of extracts from soy protein isolate, soy lecithin (*3*), and cornstarch (*4*). Amplicons suitable for species identification are produced by applying specific, universal, or arbitrary primers. For food legumes, specific primers have been used to detect soy protein concentrates in processed meat products (*5*) and genetically modified soybeans in food (*6*). Amplicons obtained with universal primers need further characterization such as single-strand conformation polymorphism (SSCP) or restriction fragment length polymorphism (RFLP) analysis. The latter technique has been used to detect guar gum in locust bean gum and the two thickening agents in dairy products, ice cream, finished roasting sauce, and fruit jelly, but not in tomato ketchup with highly degraded DNA (*7*). Arbitrary primers have the advantage that no information on the gene fragments to be amplified is needed to generate random amplified polymorphic DNA (RAPD) patterns. These PCR techniques have been used successfully in botany to detect phylogenetic relationships and genetic diversity (*8*).

Because PCR is well-introduced in food analyses for warm-blooded animal and fish species identification (*9*) and reports on their application to plant food analysis, in particular to legume species identification, are limited to specific problems, this study was started to investigate the potential of PCR to identify legume species in food raw material, complex foods, and processed foods. First, the identification of legume species was studied with the example of seed meal samples, and RAPD-PCR was chosen for the study. Preliminary experiments have shown that not all protocols recommended in the literature for plant DNA isolation, generally from leaf material, are equally well suitable for seed meals (*10*). The extraction of defatted seed meal with sodium dodecyl sulfate (SDS)-containing buffer followed by purification with chloroform/isoamyl alcohol treatment as well as the isolation from seed meal using a commercial membrane column kit produced the best results with RAPD-PCR. Small differences have been observed in the suitability of six 10-mer nucleotide primers tested, and both Taq DNA polymerase and

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Table 1. Sample Designation and Origin

sample		origin	year[a]
common bean (*Phaseolus vulgaris* L.)			
1	white bean cv. Beryl	Puerto Rico[b]	1994
2	Brazilian pink bean cv. Carioca	Brazil[c]	1995
3	Brazilian pink bean cv. Rosinha G2	Brazil[c]	1983
4	pinto bean	Puerto Rico[b]	1991
5	pinto bean	Mexico[d]	1983
6	Anasazi bean	Nebraska[e]	1990
7	Anasazi bean	Colorado[e]	1990
8	black turtle soup bean cv. Midnight	North Dakota[f]	1994
9	black turtle soup bean cv. La Vega	North Dakota[f]	1992
10	dark red kidney bean cv. Montcalm	North Dakota[f]	1994
11	red kidney bean	England[d]	1983
common pea (*Pisum sativum* L.)			
1	green pea	Germany[g]	1985
2	yellow pea	Germany[g]	1985
3	green pea cv. Solara	Germany[h]	1994
4	yellow pea cv. Erbi	Germany[h]	1994
5	brown pea cv. Golf	Germany[h]	1994
lentil (*Lens culinaris* Medik.)			
1	lentil	Germany[g]	1987
2	lentil	Germany[g]	1987
3	Syrian local small lentil ILL 4401	Syria[i]	1981
4	Syrian local large lentil ILL 4400	Syria[i]	1983
broadbean (*Vicia faba* L.)			
1	broadbean	Austria[g]	1995
2	broadbean cv. Condor	Germany[h]	1994
3	broadbean cv. Con Amore	Germany[j]	1985
soybean [*Glycine max* (L.) Merr.]			
1	soybean	Germany[g]	1987
2	soybean	Germany[g]	1987
3	soybean cv. Labrador	Germany[h]	1987
4	soybean cv. Maple Arrow	Germany[h]	1987
chickpea (*Cicer arietinum* L.)			
1	chickpea, Kabuli type cv. UC5	Australia[k]	1990
2	chickpea, Kabuli type cv. SP1-563	Australia[k]	1990
3	chickpea, Desi type cv. Dooen	Australia[k]	1990
4	chickpea, Desi type cv. Tyson	Australia[k]	1990
green gram [*Vigna radiata* (L.) Wilczek]			
1	green gram	Germany[g]	1987
2	green gram	Germany[g]	1987
alfalfa (*Medicago sativa* L.)			
1	alfalfa	Germany[g]	1987
2	alfalfa	Germany[g]	1987
3	alfalfa cv. Kara	Germany[h]	1987
4	alfalfa cv. Warotte	Germany[h]	1987
European yellow lupin (*Lupinus luteus* L.)			
1	European yellow lupin cv. Borsaja	Germany[h]	1994
2	European yellow lupin cv. Jantar	Germany[j]	1975
blue lupin (*Lupinus angustifolius* L.)			
1	blue lupin cv. Kubesa	Germany[j]	1975
2	blue lupin cv. Maresa	Germany[j]	1976
white lupin (*Lupinus albus* L.) cv. Neuland		Germany[j]	1976
common sainfoin (*Onobrychis viciifolia* Scop.)			
1	common sainfoin	Germany[h]	1994
2	common sainfoin	Germany[h]	1975
birdsfoot trefoil (*Lotus corniculatus* L.) cv. Oberhaunstädter		Germany[h]	1994
scarlet runner bean (*Phaseolus coccineus* L.)		Germany[g]	1975
lima bean (*Phaseolus lunatus* L.)		Ecuador[d]	1975
moth bean [*Vigna aconitifolia* (Jacq.) Marechal]		India[l]	1976
black gram [*Vigna mungo* (L.) Hepper]			
1	black gram	India[l]	1975
2	black gram	India[l]	1975
adzuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi]			
1	adzuki bean	China[l]	1975
2	adzuki bean	Brazil[l]	1975
horsegram [*Macrotyloma uniflorum* (Lam.) Verdc.]		Afghanistan[l]	1962
swordbean [*Canavalia gladiata* (Jacq.) DC.]		Philippines[l]	1964
jackbean [*Canavalia ensiformis* (L.) DC.]			
1	jackbean	Germany[m]	1975
2	jackbean	Germany[m]	1974
Bengal gram [*Mucuna aterrima* (Piper et Tracy) Holland]		Mexico[n]	1975
Florida velvetbean (*Mucuna deeringiana* Merr.)		Mozambique[n]	1975
pigeon pea [*Cajanus cajan* (L.) Millsp.]			
1	red gram	India[n]	1975
2	red gram	India[n]	1975
grasspea (*Lathyrus sativus* L.)		Germany[o]	1974
broad-leaved pea (*Lathyrus latifolius* L.)		Germany[p]	1974
sweet trefoil [*Trigonella coerulea* (L.) Ser.]		Germany[m]	1974

[a] Year of harvest or acquisition. [b] USDA Mayagüez, Puerto Rico. [c] Universidade de São Paulo, Brazil. [d] Donation from private persons. [e] Adobe Milling Co., Dove Creek, CO. [f] NDSU, Fargo, ND. [g] Local market. [h] Bayerische Futtersaatbau, Ismaning. [i] ICARDA, Aleppo. [j] Bayerische Landesanstalt für Bodenkultur und Pflanzenbau, München, Germany. [k] National Chickpea Breeding Program, Tamworth, NSW, Australia. [l] USDA, Pullman, WA. [m] Botanical Gardens, München, Germany. [n] USDA Experiment. [o] Institut für Pflanzenbau und Pflanzenzüchtung, TUM, Weihenstephan, Germany. [p] Benary, Hannoversch-Münden, Germany.

RAPD-PCR beads yield distinct and reproducible amplicon patterns. These experiments have been performed with two seed meal samples from two legume species each, soybeans and lentils, and some problems have been observed with the latter species. Thus, the study was extended to a larger number of legume species to prove the general applicability of RAPD-PCR for legume species identification. Some feed legumes were included in the study because protein and carbohydrate fractions from those legumes are discussed as ingredients for foods and because protein concentrates such as Lopino from sweet European yellow lupins (*Lupinus luteus* L.) are already on the market. The present paper deals with the identification of 27 legume species represented by 63 seed meal samples using the SDS buffer extraction−chloroform/isoamyl alcohol purification protocol for isolation of template DNA and RAPD-PCR analysis beads for amplification.

MATERIALS AND METHODS

Origin of Samples. Seed samples were selected from the author's collection to cover a broad range of legume species, geographical origin, and age. The seeds had been stored in closed jars at ambient temperature until analyzed. Details of the 63 seed samples representing 27 legume species are given in **Table 1**.

Extraction of DNA. Seeds were ground, and 150 mg of seed meal was defatted twice with 1.5 mL of cold acetone each and then dried over paraffin flakes. The further procedure was a modification of the method published for DNA extraction from fish samples (*11*). Samples were incubated overnight at 50 °C in 1.0 mL of SDS buffer (10 mM Tris-HCl buffer, pH 8.0; 1% w/v SDS; 2 mM EDTA; 10 mM NaCl) containing 200 μg of proteinase K, heated for 10 min at 70 °C, and centrifuged for 5 min at 3000*g* after cooling. The supernatant (800 μL) was mixed first with 200 μL of 5 M NaCl solution and then with 1000 μL of Chisom (chloroform/isoamyl alcohol 24:1, v/v) (*12*) by vortexing. After horizontal shaking at ambient temperature for 20 min, samples were centrifuged for 20 min at 8000*g*. The supernatant was transferred into a new reaction tube and the Chisom treatment repeated until no interphase was visible. Then the DNA was precipitated by the addition of 10 parts of 3 M sodium acetate/10 mM EDTA solution (pH 7.0) and 54 parts of 2-propanol to 90 parts of the supernatant. After 20 min at ambient temperature, the samples were cooled in an ice bath and centifuged for 30 min at 8000*g*. The resulting pellet was washed with iced ethanol/water (7:3, v/v) and centrifuged for 10 min at 8000*g*. The residue was dried in vacuo and dissolved in 50 μL of 10 mM Tris-HCl buffer, pH 8.0, 1 mM in EDTA. All reactions were performed in 2.0 mL Dolphin microcentrifuge tubes.

RAPD-PCR. Amplification was performed with Ready-To-Go RAPD Analysis Beads (Amersham Pharmacia Biotech, Freiburg,

Table 2. Effectiveness of RAPD-PCR with Different Primers

	primer	RAPD profiles (no. of samples) distinct	poor	none	identical profiles (no. of species)
1	5′-d[GGTGCGGGAA]-3′	59	4	0	2 × 2
2	5′-d[GTTTCGCTCC]-3′	60	2	1	2 × 2
3	5′-d[GTAGACCCGT]-3′	58[a]	4	1	2
4	5′-d[AAGAGCCCGT]-3′	57	5	1	2 × 2
5	5′-d[AACGCGCAAC]-3′	61	2	0	2
6	5′-d[CCCGTCAGCA]-3′	58	5	0	2

[a] RAPD profiles of four samples identical with that of blank and profiles of some other samples very similar to blank.

Germany) in a UNO-Thermoblock 40 with a heated lid (Biometra, Göttingen, Germany) as recommended by the producers. The primers used were the 10-mer RAPD Analysis primers 1−6 (Amersham Pharmacia Biotech) listed in **Table 2** (*13*). Template DNA concentration was determined from absorbance at 260 nm, and the samples were diluted to 10 μg of DNA/mL. Into each tube in which the beads were delivered, 19 μL of double-distilled water, 5 μL of primer solution containing 25 pmol of primer, and 1 μL of template DNA were pipetted with thorough mixing after each addition. After 4 min of preheating at 95 °C, 45 cycles were run with 1 min at 95 °C, 1 min at 36 °C, and 2 min at 72 °C. The CleanGel DNA Analysis Kit and the PlusOne DNA Silver Staining Kit from Amersham Pharmacia Biotech were used for PAGE and staining of the amplicons, respectively, following the producer's recommendations.

RESULTS AND DISCUSSION

Sixty-three legume seed samples representing 27 species were selected for this study (**Table 1**). The samples are arranged in **Table 1** in the same order as analyzed by PCR and submitted to PAGE (**Figures 1−6**). Template DNA was isolated from defatted seed meal by extraction with SDS-containing buffer, followed by purification with chloroform/isoamyl alcohol treatment and precipitation with 2-propanol. Amplification was performed with commercial RAPD-PCR beads using commercially available 10-mer primers. All six primers used generated RAPD profiles (**Table 2**). Best results were obtained with primers 1, 5, and 6, which yielded RAPD profiles with all 63 templates tested. A closer view of the profiles showed that primer 1 produced identical profiles for two species twice, whereas primers 5 and 6 did so only once (see below). As expected, the age of the samples did not influence the results; the two European yellow lupin samples differing by ~20 years showed nearly identical RAPD profiles (**Figures 2** and **5**, lanes 19 and 20), and two samples stored for >30 years (horsegram and swordbean) yielded clear profiles (clear profiles are defined as RAPD profiles with mainly prominent bands, as in **Figures 3** and **6**, lanes 13 and 14).

Blanks run through the whole procedure including extraction and purification without sample and diluted 1:100 prior to amplification yielded patterns of bands (**Figures 1** and **4**, lane 13; **Figures 2**, **3**, **5**, and **6**, lane 12), which, however, were different from those obtained with the seed samples (diluted between 1:100 and 1:600 prior to amplification). The problem that "no template" controls usually give some bands and/or smears in RAPD reactions is already mentioned by the producer of the RAPD beads and primers (*13*) and discussed in the literature (*14*). These bands probably arise from small amounts of DNA contamination in the polymerases or the blank extracts. They are not observed when 10 ng of template DNA is present in the reaction, which, at this level, will out-compete any contaminant DNA that may be present. In the present study, difficulties arose only in one set of experiments in which identical or similar profiles were obtained from several samples

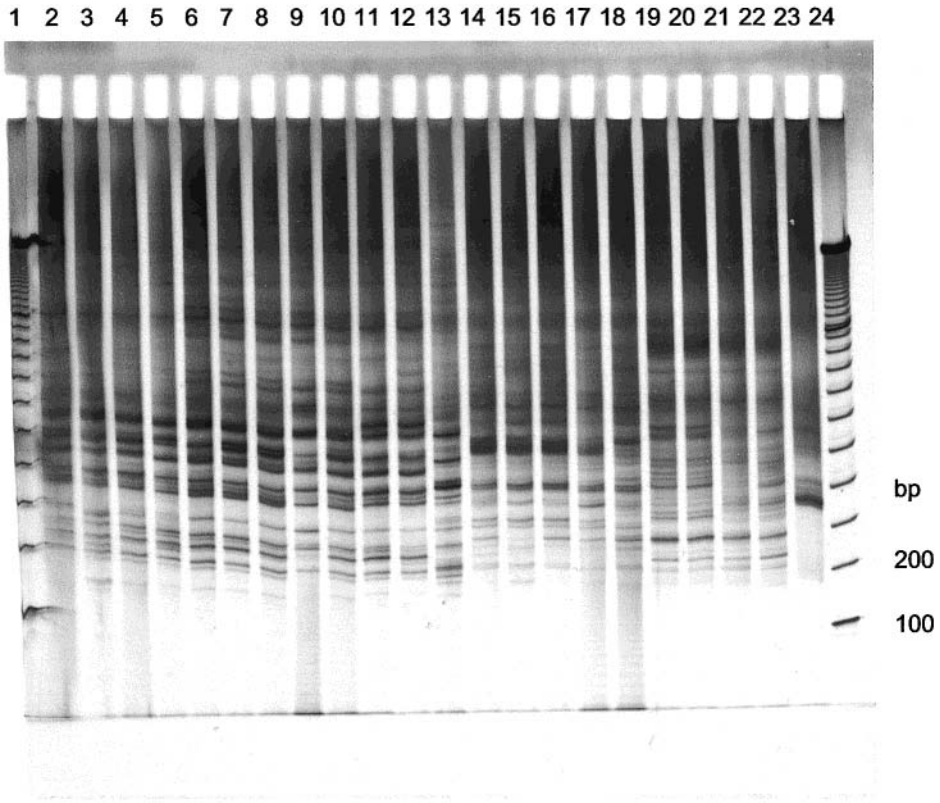

Figure 1. RAPD profiles of common beans, common peas, and lentils obtained with primer 5: (lanes 1 and 24) 100 bp ladder; (lanes 2–12) common beans 1–11; (lane 13) blank run through the whole procedure; (lanes 14–18) common peas 1–5; (lanes 19–22) lentils 1–4; (lane 23) broadbean 1 (sample details in **Table 1**).

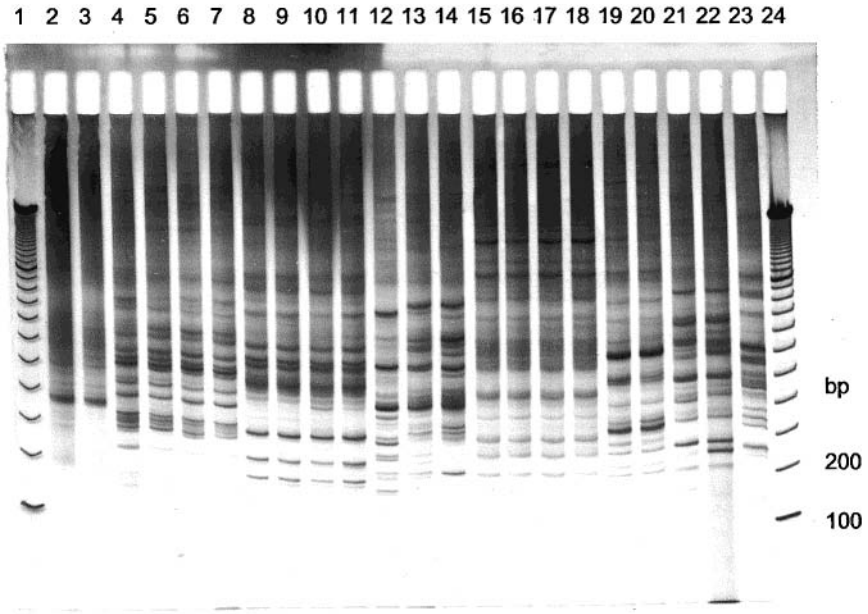

Figure 2. RAPD profiles of broadbeans, soybeans, chickpeas, green gram, alfalfa, and lupins obtained with primer 5: (lanes 1 and 24) 100 bp ladder; (lanes 2 and 3) broadbeans 2 and 3; (lanes 4–7) soybeans 1–4; (lanes 8–11) chickpeas 1–4; (lane 12) blank; (lanes 13 and 14) green grams 1 and 2; (lanes 15–18) alfalfas 1–4; (lanes 19–23) European yellow lupins 1 and 2, blue lupins 1 and 2, and white lupin, respectively (sample details in **Table 1**).

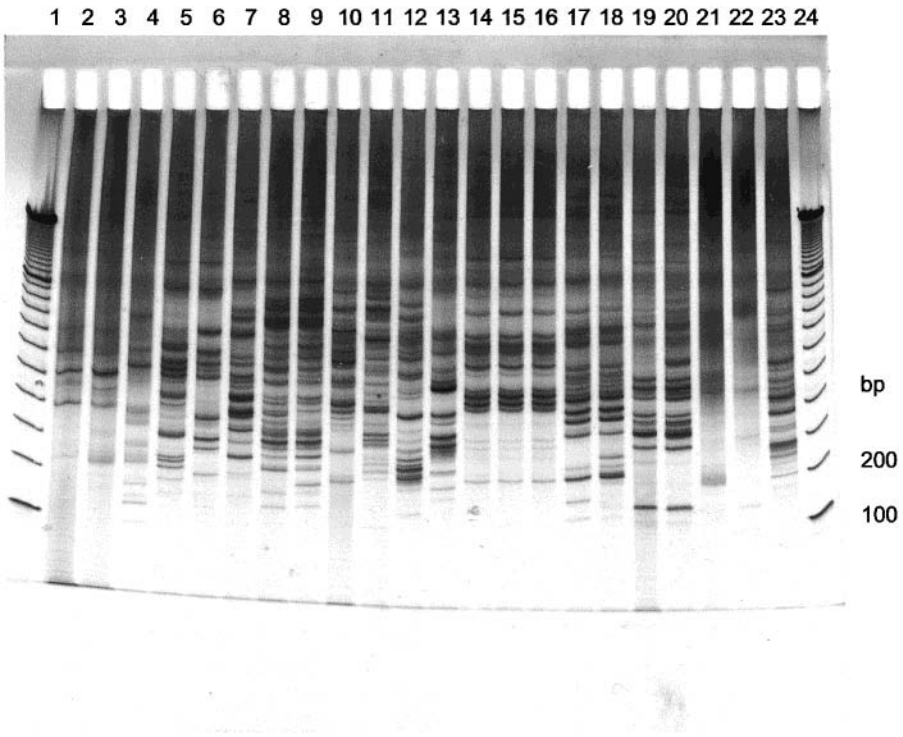

Figure 3. RAPD profiles of less common beans, peas, and some feed legumes obtained with primer 5: (lanes 1 and 24) 100 bp ladder; (lanes 2 and 3) common sainfoins 1 and 2; (lane 4) birdsfoot trefoil; (lane 5) scarlet runner bean; (lane 6) lima bean; (lane 7) moth bean; (lanes 8 and 9) black grams 1 and 2; (lanes 10 and 11) adzuki beans 1 and 2; (lane 12) blank; (lane 13) horsegram; (lane 14) swordbean; (lanes 15 and 16) jackbeans 1 and 2; (lane 17) Bengal gram; (lane 18) Florida velvetbean; (lanes 19 and 20) red grams 1 and 2; (lane 21) grasspea; (lane 22) broad-leaved pea; (lane 23) sweet trefoil (sample details in **Table 1**).

and the blank. Such experiments have, of course, to be repeated from the beginnning using freshly prepared reagents.

Reproducibility of the RAPD profiles obtained from the same sample in different experiments has been investigated in a previous study (*10*) with the example of soybeans and lentils. Identical RAPD profiles were generated in duplicate from two soybean samples with the three primers tested. Lentils yielded identical profiles in duplicate with two samples and one primer, identical profiles with duplicates of one sample and similar profiles with duplicates of the other sample and the second primer, and similar profiles in duplicate with two samples and the third primer tested. In the present study, identical RAPD profiles obtained from four samples of the same species are even better proof for reproducibility, as shown for soybeans

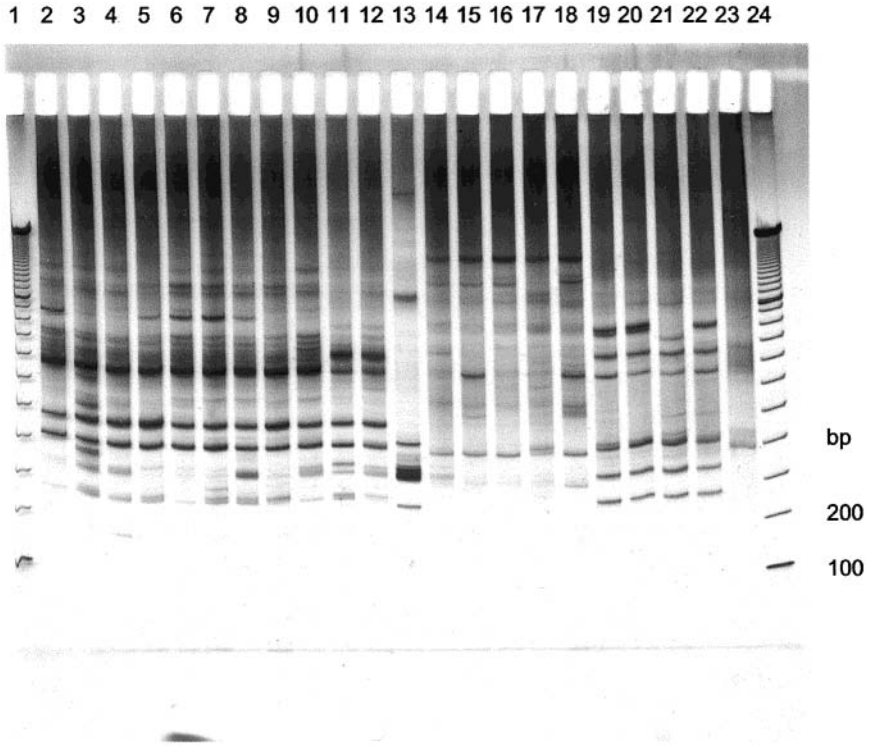

Figure 4. RAPD profiles of common beans, common peas, and lentils obtained with primer 6: (lanes 1–24) as in **Figure 1**.

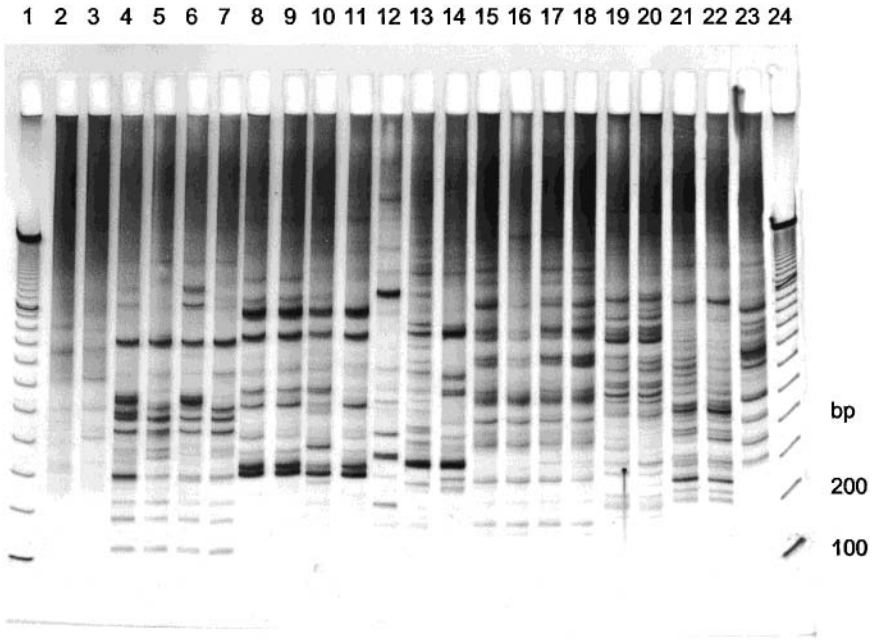

Figure 5. RAPD profiles of broadbeans, soybeans, chickpeas, green gram, alfalfa, and lupins obtained with primer 6: (lanes 1–24) as in **Figure 2**.

(**Figure 2**, lanes 4−7), chickpeas (**Figure 2**, lanes 8−11), and alfalfa (**Figure 2**, lanes 15−18), by applying primer 5 (identical profiles defined as RAPD profiles with all prominent bands identical). In addition, the RAPD profiles generated from the 11 common bean samples with primer 5 in a completely independent experiment are shown in **Figure 7**, lanes 2−12. Comparison of these profiles, which were obtained 1 year earlier, with those of **Figure 1**, lanes 2−12, demonstrates that almost identical profiles were produced for each of the samples (group of bands between 350 and 550 bp; additional band at ∼580 bp for common beans 10 and 11, lanes 11 and 12). The reproducibility of the majority of bands in RAPD experiments has also been shown by others (*14*, and references therein).

All common bean samples showed a characteristic common basic RAPD pattern with both primers 5 and 6, which can be used for their identification (common basic pattern defined as RAPD profiles with many prominent bands identical, as in **Figures 1** and **4**, lanes 2−12). The differences between the individual profiles obtained with the same primer were caused by intraspecies polymorphism, which was less pronounced when primer 5 was used (**Figure 1**, lanes 2−12). RAPD profiles different from those of common beans were generated from the other *Phaseolus* species studied, lima beans and scarlet runner beans. This interspecies polymorphism was clearly exhibited with primers 5 and 6 (**Figures 3** and **6**, lanes 5 and 6). Intraspecies polymorphism of common bean

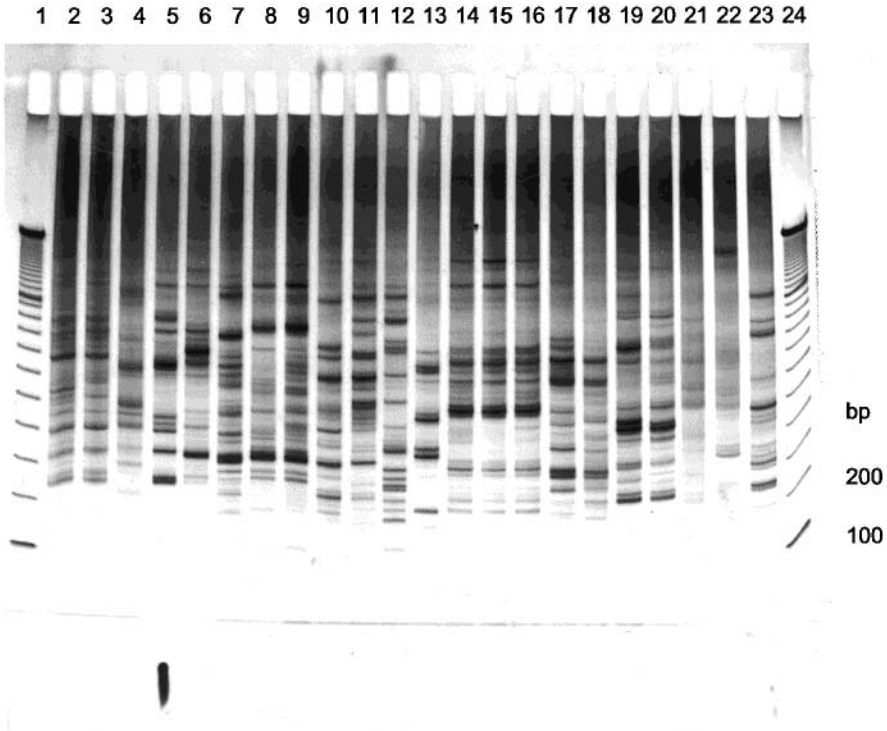

Figure 6. RAPD profiles of less common beans, peas, and some feed legumes obtained with primer 6: (lanes 1–24) as in **Figure 3**.

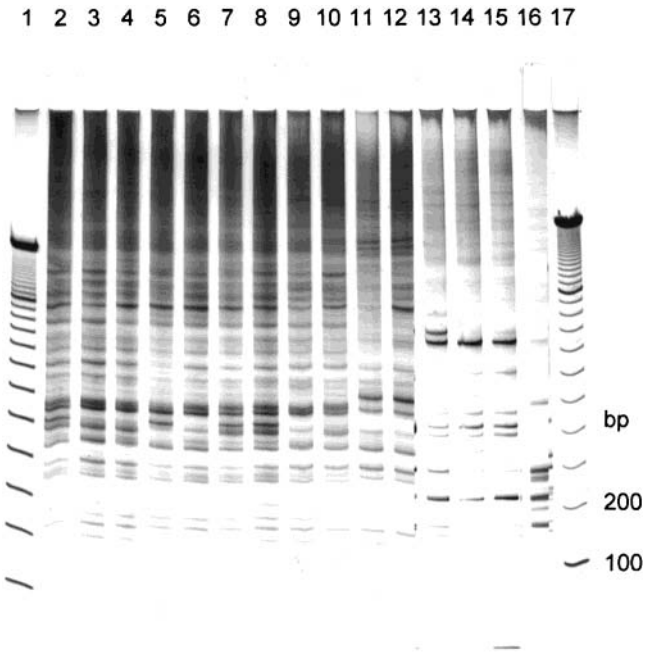

Figure 7. RAPD profiles of common beans with primer 5 and of swordbean and jackbeans with primer 3: (lanes 1 and 17) 100 bp ladder; (lanes 2–12) common beans 1–11; (lane 13) swordbean; (lanes 14 and 15) jackbeans 1 and 2; (lane 16) blank.

RAPD profiles has also been reported by others (*15*−*17*) who have used the technique to study the genetic diversity of the species.

Common pea samples displayed a common basic RAPD pattern with primer 5 as well as primer 6 (**Figures 1** and **4**, lanes 14−18). Similar genetic diversity trees obtained by amplified fragment length polymorphism (AFLP), microsatellite-AFLP, and RAPD-PCR have been reported in the literature (*18*). The same authors have differentiated *Pisum sativum* from *Pisum fulvum* by ALFP, which needs incubation with an endonuclease prior to amplification. Thus, RAPD-PCR is more convenient to identify common peas.

Similarly, lentils showed a common basic pattern with both primers 5 and 6 (**Figures 1** and **4**, lanes 19−22). The profiles of the two commercial samples from Germany and the two Syrian samples were somewhat different with both primers, indicating the possibility for geographic differentiation. To study inter- and intraspecific variations of the genus *Lens*, RAPD-PCR has already been used earlier (*19*, *20*), and the discrimination between the four *Lens* species can be derived from one of these studies. In a very recent study (*21*), a lentil sample from Ethiopia is clearly separated from the other samples by cluster analysis of RAPD and intersimple sequence repeat (ISSR) data. However, although the Italian accessions, the main topic of the study, show a trend to group together, they are not clearly separated from the other Mediterranean samples.

Whereas the species discussed so far exhibited distinct common basic RAPD patterns with the two primers, which can be used for their identification, the three broadbean samples showed common basic patterns, but with diffuse or weak bands, after amplification with primers 5 and 6 (**Figures 1** and **4**, lane 23; and **Figures 2** and **5**, lanes 2 and 3). These poor results are thought to be related to the primers used, because Potokina et al. (*22*) have used RAPD-PCR to clarify phylogenetic relationships among 31 *Vicia* species, whereas Shiran and Raina (*23*) have failed to do so among the 7 species of the *Vicia sativa* species complex that includes *V. faba*.

The four soybean samples resulted in identical RAPD profiles with primer 5 (**Figure 2**, lanes 4−7) and in a common basic pattern with primer 6 (**Figure 5**, lanes 4−7). Soybeans have also been identified by PCR using specific primers to amplify the soy lectin gene (*5*), and RAPD-PCR has been used to differentiate between two soybean species, *Glycine max* and *Glycine soja* (*24*), whereas both techniques have been used to study taxonomic relationships among wild soybean species (*25*).

Similarly, chickpea samples displayed identical RAPD profiles with primer 5 (**Figure 2**, lanes 8−11) and a common basic

pattern with primer 6 (**Figure 5**, lanes 8−11). The two chickpea types, the larger, more rounded, cream-colored Kabuli and the smaller, angular, colored Desi type, showed somewhat different patterns with primer 6, indicating the possibility for type differentiation. RAPD-PCR and microsatellite-primed PCR have been reported to be inadequate methods to detect intraspecific polymorphism (*26*), which is in agreement with the present study, the differences related to the two types excepted.

Alfalfa seed samples showed identical RAPD profiles with primer 5 (**Figure 2**, lanes 15−18) and almost identical profiles with primer 6 (**Figure 5**, lanes 15−18). The contrasting results of Mengoni et al. (*27*), who have found a high degree of intraspecific polymorphism using RAPD-PCR in their studies on genetic relationships, may be explained by the use of different primers.

From the seeds of the three lupin species, European yellow, blue, and white lupin, RAPD profiles were generated with both primers 5 and 6 that allowed differentiation between the lupin species as well as from other legume species (**Figures 2** and **5**, lanes 19−23). Some intraspecies polymorphism was observed with primer 6 (**Figure 5**, lanes 21 and 22). Intraspecies polymorphism in blue lupins has also been detected by a combination of AFLP and the microsatellite-anchor primer technique (*28*).

The two seed samples of common sainfoin showed almost identical profiles with primer 6 (**Figure 6**, lanes 2 and 3) and a common basic pattern with primer 5 (**Figure 3**, lanes 2 and 3).

The seeds of birdsfoot trefoil (one sample only) generated distinct RAPD profiles with primers 5 and 6 (distinct profiles defined as RAPD profiles with mainly clear but less prominent bands, as in **Figures 3** and **6**, lane 4) that differed from those obtained from the other species. Phylogenetic relationships among five *Lotus* species, birdsfoot trefoil and four possible diploid ancestors, have been studied using also RAPD-PCR and 20 10-mer primers in order to clarify the origin of the tetraploid *L. corniculatus* (*29*).

The four *Vigna* species, green gram, moth bean, black gram, and adzuki bean, showed RAPD profiles with the two primers that allowed differentiation between the species as well as from the other legume species (**Figures 2** and **5**, lanes 13 and 14; and **Figures 3** and **6**, lanes 7−11). Some intraspecies polymorphism was observed for adzuki beans with both primers 5 and 6 (**Figures 3** and **6**, lanes 10 and 11) and for green gram with primer 6 (**Figure 5**, lanes 13 and 14). Differentiation among *Vigna* species has also been studied by Yu et al. (*30*), who have found 12 microsatellites distributed in three *Vigna* species (*V. radiata*, *V. aconitifolia*, and *V. unguiculata*) in 120 *Vigna* DNA sequences from databases, which may be useful for amplification to differentiate the species.

Horsegram (one sample only) displayed a clear RAPD profile with primer 5 (**Figure 3**, lane 13) and a distinct profile with primer 6 (**Figure 6**, lane 13), both differing from those of the other legume species.

Swordbeans and jackbeans, both *Canavalia* species, showed identical RAPD profiles with primer 5 as well as primer 6 (**Figures 3** and **6**, lanes 14−16), but both primers can be used to differentiate the *Canavalia* species from the other legume species. Only primer 3 rendered possible the differentiation between swordbeans and jackbeans (**Figure 7**, lanes 13−15). Although many identical bands in the profiles demonstrated the close phylogenetic relationship of the two species also with primer 3, one clear band at ~800 bp was present only in the swordbean (lane 13).

The two *Mucuna* species, Bengal gram and Florida velvetbean, showed different RAPD profiles with primers 5 and 6 (**Figures 3** and **6**, lanes 17 and 18), which also differed from those of the other legume species.

The two pigeon pea samples displayed almost identical RAPD profiles with primer 6 (**Figure 6**, lanes 19 and 20). On the contrary, primer 5 produced different RAPD profiles with red grams 1 and 2 (**Figure 3**, lanes 19 and 20). However, all profiles differed from those obtained from the other legume species. The application of RAPD-PCR to identify pigeon peas and 13 of their related wild species (8 *Cajanus* species and 5 species from three other genera) has already been reported (*31*). On the contrary, application of PCR-RFLP has failed to differentiate the samples even at genus level in a study on 15 *Cajanus*, 10 *Rhynochosia*, and 3 other related species, despite the analysis of four gene regions using 15 restriction enzymes (*32*).

The two *Lathyrus* species, grasspea and broad-leaved pea, produced weak RAPD profiles with both primers 5 and 6 (weak profiles defined as RAPD profiles with mainly diffuse and/or weak bands, as in **Figures 3** and **6**, lanes 21 and 22). However, the profiles of grasspea and broad-leaved pea obtained with the same primer differed from each other and from the profiles of the other legume species.

Sweet trefoil (one sample only) showed a distinct RAPD profile with primer 5 (**Figure 3**, lane 23) and a clear profile with primer 6 (**Figure 6**, lane 23), which differed from those obtained from the other legume species.

In conclusion, identification of 25 food and feed legume species can be performed by RAPD-PCR using commercial RAPD analysis beads after the isolation of DNA templates by an extraction and precipitation technique and only one primer, either primer 5 or 6. Although these primers differ also between the two remaining species studied, swordbean and jackbean, and the 25 other legume species, differentiation between these two species is possible only with primer 3. To unambiguously identify the species, RAPD profiles of samples should be compared to those of authentic seed samples with analyses and references run in parallel to avoid the influence of isolation and amplification conditions. The application of commercial RAPD analysis beads offers a convenient procedure with a minimum of pipetting for amplification. Although the results of the present study clearly demonstrate that the identification of all 27 legume species investigated is possible in homogeneous food or feed samples such as seed meals, their identification in mixtures, more complex or processed foods, and feeds will be more difficult due to the multibanded nature of the RAPD profiles and demands further studies.

ABBREVIATIONS USED

AFLP, amplified fragment length polymorphism; DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetraacetate; ISSR, intersimple sequence repeat; PAGE, polyacrylamide gel electrophoresis; PCR, Polymerase Chain Reaction; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; SDS, sodium dodecyl sulfate; SSCP, single-strand conformation polymorphism; Tris, tris(hydroxymethyl)aminomethane.

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LITERATURE CITED

(1) Ashurst, P. R., Dennis, M. J., Eds. *Food Authentication*; Blackie: London, U.K., 1996; 399 pp.

(2) Ashurst, P. R., Dennis, M. J., Eds. *Analytical Methods for Food Authentication*; Blackie: London, U.K., 1998; 368 pp.
(3) Hurst, C. D.; Knight, A.; Bruce, I. J. PCR detection of genetically modified soya and maize in foodstuffs. *Mol. Breed.* **1999**, *5*, 579−586.
(4) Hupfer, C.; Hotzel, H.; Sachse, K.; Engel, K.-H. Nachweis gentechnischer Veränderungen in Lebensmitteln aus transgenem Bt-Mais. *Lebensmittelchemie* **1998**, *52*, 103−104.
(5) Meyer, R.; Chardonnens, F.; Hübner, P.; Lüthy, J. Polymerase Chain Reaction (PCR) in the quality and safety assurance of food: detection of soya in processed meat products. *Z. Lebensm. Unters. Forsch.* **1996**, *203*, 339−344.
(6) Meyer, R. Development and application of DNA analytical methods for the detection of GMOs in food. *Food Control* **1999**, *10*, 391−399.
(7) Meyer, K.; Rosa, C.; Hischenhuber, C.; Meyer, R. Determination of locust bean gum and guar gum by Polymerase Chain Reaction and restriction fragment length polymorphism analysis. *J. AOAC Int.* **2001**, *84*, 89−99.
(8) Wolfe, A. D.; Liston, A. Contributions of PCR-based methods to plant systematics and evolutionary biology. In *Molecular Systematics of Plants II. DNA Sequencing*; Soltis, D. E., Soltis, P. S., Doyle, J. J., Eds.; Kluwer: Boston, MA, 1998; pp 43−86.
(9) Lockley, A. K.; Bardsley, R. G. DNA-based methods for food authentication. *Trends Food Sci. Technol.* **2000**, *11*, 67−77.
(10) Weder, J. K. P. Influence of experimental conditions on the reproducibility of RAPD-PCR identification of legumes and cereals. *Food Sci. Technol. (Lebensm.-Wiss. Technol.)* **2002**, in press.
(11) Scheider, M.; Mandorf, T.; Rubach, K. Tierartbestimmung von Fisch durch DNA-Analyse mittels RAPD-Technik. *Dtsch. Lebensm. Rundsch.* **1997**, *93*, 137−140.
(12) Graf, E. Untersuchung molkereispezifischer Mikroorganismen mit molekularbiologischen Methoden. Dissertation, Technische Universität München, Germany, 1998.
(13) Pharmacia Biotech. *Ready-To-Go RAPD Analysis Beads−Instructions*; XY-065-00-08, 1997.
(14) Hadrys, H.; Balick, M.; Schierwater, B. Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Mol. Ecol.* **1992**, *1*, 55−63.
(15) Skroch, P. W.; Nienhuis, J.; Beebe, S.; Tohme, J.; Pedraza, F. Comparison of Mexican common bean (*Phaseolus vulgaris* L.) core and reserve germplasm collections. *Crop Sci.* **1998**, *38*, 488−496.
(16) Métais, I.; Aubry, C.; Hamon, B.; Jalouzot, R.; Peltier, D. Description and analysis of genetic diversity between commercial bean lines (*Phaseolus vulgaris* L.). *Theor. Appl. Genet.* **2000**, *101*, 1207−1214.
(17) Galván, M. Z.; Aulicino, M. B.; García Medina, S.; Balatti, P. A. Genetic diversity among northwestern Argentinian cultivars of common bean (*Phaseolus vulgaris* L.) as revealed by RAPD markers. *Genet. Resour. Crop Evol.* **2001**, *48*, 251−260.
(18) Lu, J.; Knox, M. R.; Ambrose, M. J.; Brown, J. K. M.; Ellis, T. H. N. Comparative analysis of genetic diversity in pea assessed by RFLP- and PCR-based methods. *Theor. Appl. Genet.* **1996**, *93*, 1103−1111.
(19) Abo-elwafa, A.; Murai, K.; Shimada, T. Intra- and inter-specific variations in *Lens* revealed by RAPD markers. *Theor. Appl. Genet*. **1995**, *90*, 335−340.
(20) Sharma, S. K.; Dawson, I. K.; Waugh, R. Relationships among cultivated and wild lentils revealed by RAPD analysis. *Theor. Appl. Genet.* **1995**, *91*, 647−654.
(21) Sonnante, G.; Pignone, D. Assessment of genetic variation in a collection of lentil using molecular tools. *Euphytica* **2001**, *120*, 301−307.
(22) Potokina, E.; Tomooka, N.; Vaughan, D. A.; Alexandrowa, T.; Xu, R.-Q. Phylogeny of *Vicia* subgenus *Vicia* (Fabaceae) based on analysis of RAPDs and RFLP of PCR-amplified chloroplast genes. *Genet. Resour. Crop Evol.* **1999**, *46*, 149−161.
(23) Shiran, B.; Raina, S. N. Evidence of rapid evolution and incipient speciation in *Vicia sativa* species complex based on nuclear and organellar RFLPs and PCR analysis. *Genet. Resour. Crop Evol.* **2001**, *48*, 519−532.
(24) Williams, J. G. K.; Kubelik, A. R.; Livak, K. J.; Rafalski, J. A.; Tingey, S. V. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* **1990**, *18*, 6531−6535.
(25) Taylor-Grant, N.; Soliman, K. M. Detection of polymorphic DNA and taxonomic relationships among 10 wild perennial soybean species using specific and arbitrary primers. *Biol. Plant.* **1999**, *42*, 25−37.
(26) Sharma, P. C.; Hüttel, B.; Winter, P.; Kahl, G.; Gardner, R. C.; Weising, K. The potential of microsatellites for hybridization- and Polymerase Chain Reaction-based DNA fingerprinting of chickpea (*Cicer arietinum* L.) and related species. *Electrophoresis* **1995**, *16*, 1755−1761.
(27) Mengoni, A.; Gori, A.; Bazzicalupo, M. Use of RAPD and microsatellite (SSR) variation to assess genetic relationships among populations of tetraploid alfalfa, *Medicago sativa*. *Plant Breed.* **2000**, *119*, 311−317.
(28) Yang, H.; Sweetingham, M. W.; Cowling, W. A.; Smith, P. M. C. DNA fingerprinting based on microsatellite-anchored fragment length polymorphisms, and isolation of sequence-specific PCR markers in lupin (*Lupinus angustifolius* L.). *Mol. Breed.* **2001**, *7*, 203−209.
(29) Campos, L. P.; Raelson, J. V.; Grant, W. F. Genome relationships among *Lotus* species based on random amplified polymorphic DNA (RAPD). *Theor. Appl. Genet.* **1994**, *88*, 417−422.
(30) Yu, K.; Park, S. J.; Poysa, V. Abundance and variation of microsatellite DNA sequences in beans (*Phaseolus* and *Vigna*). *Genome* **1999**, *42*, 27−34.
(31) Ratnaparkhe, M. B.; Gupta, V. S.; Ven Murthy, M. R.; Ranjekar, P. K. Genetic fingerprinting of pigeonpea [*Cajanus cajan* (L.) Millsp.] and its wild relatives using RAPD markers. *Theor. Appl. Genet.* **1995**, *91*, 893−898.
(32) Lakshmi, M.; Senthilkumar, P.; Parani, M.; Jithesh, M. N.; Parida, A. K. PCR-RFLP analysis of chloroplast gene regions in *Cajanus* (Leguminosae) and allied genera. *Euphytica* **2000**, *116*, 243−250.

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