JOURNAL OF PURE & APPLIED MICROBIOLOGY, Nov. 2014. Vol. **8**(Spl. Edn. 2), p. 461-466

Genetic Diversity of 41 Countries *Phaseolus vulgaris* L. Accessions, by ISSR Technique

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(Received: 06 August 2014; accepted: 31 October 2014)

The genetic diversity of 60 *Phaseolus* genotypes was investigated, which represented 41 countries around the world. *Phaseolus vulgaris* L. is the world's second most important bean after soybeans; used for human food and animal feed. ISSR effectiveness in achievement of the genetic diversity among 60 common bean accessions. Into 2 major groups the studied *Phaseolus vulgaris* accessions were divided. Accessions of Asia and Europe seem to be more genetically similar (99%) to each other as they clustered in the same sub-group. The American and African varieties showed similarities as well and clustered in the same sub-tree group. In contrast, Asian and American accessions No. 22 and 23 showed high level of genetic similarities, although these were isolated from different regions. The phylogenetic tree showed that all the Asian accessions (along with Australian No. 59 and 60) were similar except Indian and Yemen accessions No. 9 and 20. Only Netherlands accession No. 3 was different from the rest of European accessions. Morocco accession No. 52 was genetically different from the rest of the African accessions. Canadian accession No. 44 seems to be different from the other North American accessions including Guatemala, Mexico and USA.

Key words: Genetic Diversity, *Phaseolus vulgaris* L., Accessions, ISSR Technique.

Common bean (*Phaseolus vulgaris* L.); the world's second most important bean after soybeans; has generally been originated in Latin America then quickly distributed worldwide (Luthria and Pastor, 2006). Its dry seeds are broadly consumed as rich and inexpensive source of proteins, carbohydrates, fibers and vitamins in developed and developing countries. Today dry beans are receiving increasing attention as a functional food; its consumption has been linked to reduced risk of cardiovascular disease, diabetes mellitus, obesity, cancer and diseases of digestive tract (Cardador *et al*., 2002). In addition to human food and animal feed; these plants are capable of fixing soil nitrogen by the symbiotic bacteria present in their root nodules (Berber and Yasar 2011).

Advances in molecular biology have allowed the development of rapid, sensitive and specific screening methods for identification and assessment of genetic diversity between crop cultivars including common bean (Marotti *et al*., 2007; Sadeghi and Cheghamirza 2012). The use of molecular methods in assessing polymorphism and relationships between *Phaseolus vulgaris* L. cultivars have frequently been reported (Métais *et al*., 2000; Svetleva *et al*., 2006). Of those, Inter Simple Sequence Repeat Markers (ISSR) has widely been used for assessment of genetic diversity between common bean cultivars (Galvan *et al*. 2001; Bornet *et al*. 2002). ISSR was provided a reliable

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and efficient tool for assessment of genetic diversity both within and among sympatric populations of *Phaseolus vulgaris L.* (Galván *et al*. (2003; De la Cruz *et al.*, 2005; Gonzalez *et al*., 2005). Furthermore, it was successfully used to target diverse regions of the genome in different bean varieties (Nowosielski *et al*., 2002; Sicard *et al.* (2005).

This study aimed to investigate the effectiveness of ISSR in achievement of the genetic diversity among 60 common bean accessions; represent various germplasms around the world.

MATERIALS AND METHODS

Biological material

Phaseolus vulgaris L. tested seeds were obtained from the Agricultural Research in the Dry Areas (ICARDA) Aleppo, Syria and germplasm collection of the USDA, ARS, WRPIS Washington State University, Regional Plant Introduction Station, 59 Johnson Hall, P.O. 646402 Pullman, Washington, United States, 99164-6402. Sixty bean accessions represent various germaplasms around the world were used.

DNA extraction

Tested seeds were aseptically grown up to be a complete plant morphology seedlings, then harvested to keep in plastic bags at -20 °C until DNA extraction. DNA was isolated from bean seedlings using a modified CTAB method (Khan *et al*., 2007). The young seedlings were ground by liquid nitrogen into extraction buffer (100 mM tris buffer pH 8, 25 mM EDTA, 2 M NaCl, 3% CTAB, 3% PVP). The suspension was gently mixed and incubated at 65°C for 20 min with occasional mixing. The suspension was then cooled to room temperature and an equal volume of chloroform: isoamyl alcohol (24:1) was added. The mixture was centrifuged at 12,000 rpm for 5 min. The clear upper aqueous phase was then transferred to a new tube and _ volume of ice-cooled isopropanol was added and incubated at -20°C for 30 min. The nucleic acid was collected by centrifugation at 10,000 rpm for 10 min. The resulting pellet was washed twice with 70% ethanol. The pellet was air-dried under a sterile laminar hood and the nucleic acid was dissolved in TE (10 mM Tris buffer pH 8, 1 mM EDTA) at room temperature and stored at 4°C until used. The RNA from crude DNA was eliminated by treating the sample with RNase A (10 mg/ml) for 30 min at 37°C. DNA concentration and purity were determined by measuring the absorbance of diluted DNA solution at 260 and 280 nm. The quality of the DNA was determined using agarose gel electrophoresis stained with ethidium bromide and the template DNA concentration was adjusted to 50 ng/¼l of samples.

ISSR assay:

Ten of 45 ISSR primers synthesized from Metabion international AG, Master mixture purchased from Amerson Company (UK) were used (Table 1). PCR amplification was done with ISSR primers according to the protocol developed by Zeitkiewicz *et al*. (1994). The PCR reaction was carried out in 20 µL (consists of 4 µL master mixture (Amerson Company, UK), 0.5 µL of primer, 1 µL template DNA, enough quantity of deionized distilled water were added to give the total volume of 20 µL) in each tube, and briefly vortexed. The amplification was done on 48 well plates on a Primus PCR machine as the following programs: First denaturation at 94°C for 3 min, segment denaturation at 94°C for 1 min and then 30 cycles, annealing for 30 s at the tabled temperatures, extension at 72°C for 1 min and final extension at 72°C for 3 min was performed for amplification. PCR products were electrophoresed in 1.6 % Agarose gels and visualized by ethidium bromide. Gels were photographed using Universal Hood ii, Bio-RAD system. The bands were recorded as present (1) or absent (0) and assembled in a data matrix table (Table 2). Only clear and major bands were considered for this purpose. Pair wise comparisons based on the proportion of shared bands and bootstrap values (250 replications) were calculated using the program Free-Tree (Pavlícek *et al*., 1999; Zhao *et al.*, 2007). Nei-Li distances and neighbor-joining tree construction method were used for the analyses.

RESULTS AND DISCUSSION

Phylogenetic dendrogram inferred by neighbor-joining method is shown in Figure 1.In general, the 60 *Phaseolus vulgaris* geographic accessions were divided into 2 major groups. Most of the accessions (56 accessions out of 60; 91.7%) were clustered in one group except the accessions 25, 26, 28 and 29. All of the accessions in the

second major group were isolated from the regions of South-America. These accessions may have different genetic feature that is distinct from the rest of the accessions clustered in the major group.

Twenty accessions (1-20) of Asia and Europe seem to be more genetically similar as they clustered in one sub-tree group and supported by very high bootstrap value (99%). The American

NO.	Primers	Sequence	T
1	(AG)8T	5'-AGA GAG AGA GAG AGA GT-3'	50
4	(GA)8A	5'-GAG AGA GAG AGA GAG AA-3'	50
27	(GTG)4RC	5'-GTG GTG GTG GTG RC-3'	48
29	(CAC)4RC	5'-CAC CAC CAC CAC RC-3'	49
31	(CA)7YC	5'-CAC ACA CAC ACA CA YC -3'	49
33	(GT)8CA	5'-GTG TGT GTG TGT GTG TCA-3'	50
35	(CA)8A	5'-CAC ACA CAC ACA CAC AA -3'	50
36	(GA)8T	5'-GAG AGA GAG AGA GAG AT-3'	50
38	(GAG)4RC	5'-GAG GAG GAG GAG RC-3'	48
43	(AC)8AG	5'-ACA CAC ACA CAC ACA CAG-3'	54

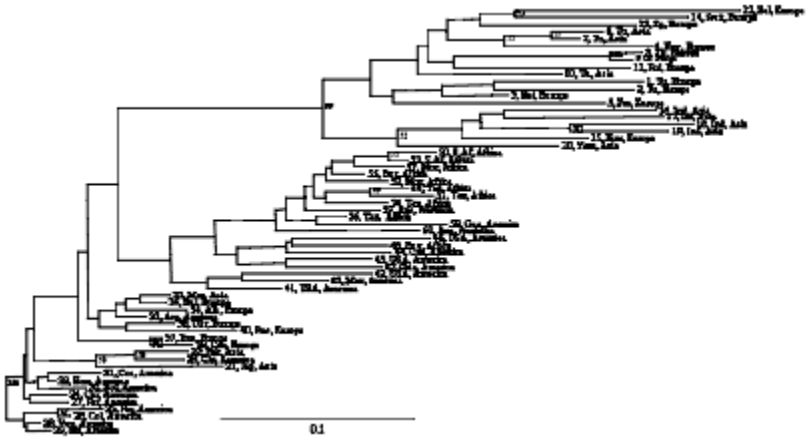

Fig. 1. Neighbor-joining phylogenetic tree of *P. vulgaris* geographic accessions (60 accessions). Bootstrap values (>50%; 250 replicates) are shown next to the branches

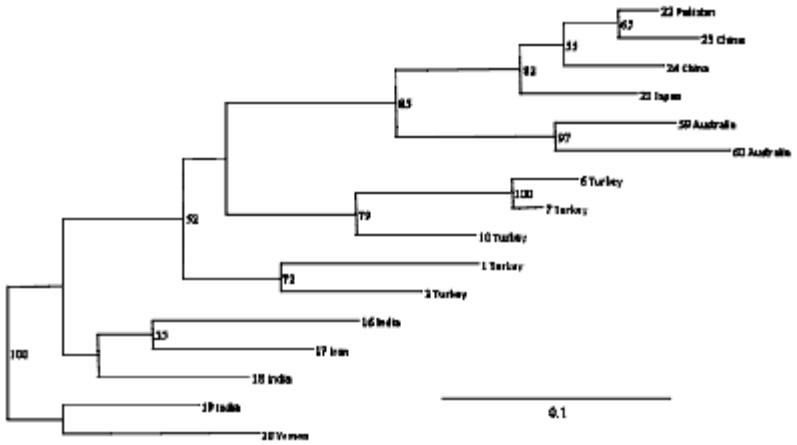

Fig. 2. Neighbor-joining phylogenetic tree of *P. vulgaris* showing the relationship among the Asian accessions. Bootstrap values (>50%; 250 replicates) are shown next to the branches

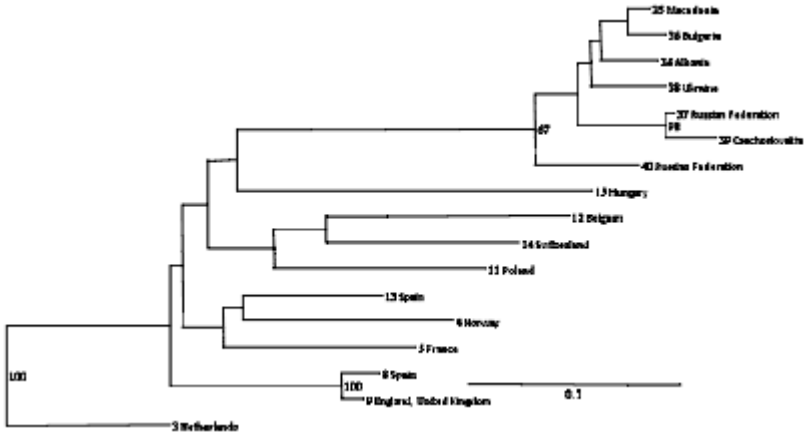

Fig. 3. Neighbor-joining phylogenetic tree of *P. vulgaris* showing the relationship among the European accessions. Bootstrap values (>50%; 250 replicates) are shown next to the branches

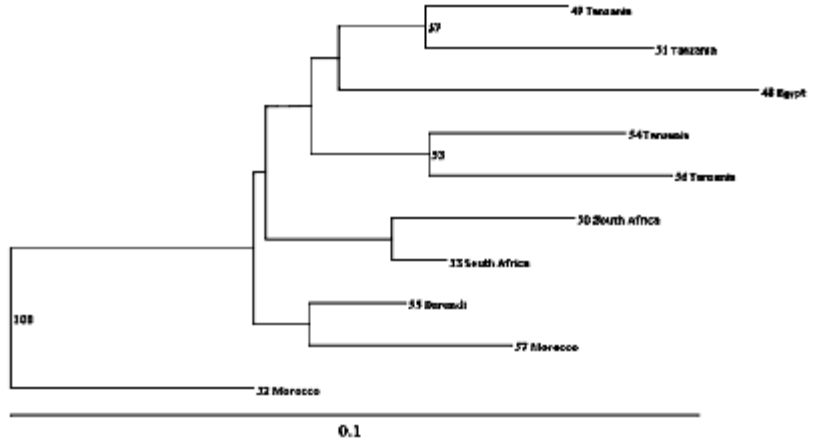

Fig. 4. Neighbor-joining phylogenetic tree of *P. vulgaris* showing the relationship among the African accessions. Bootstrap values (>50%; 250 replicates) are shown next to the branches

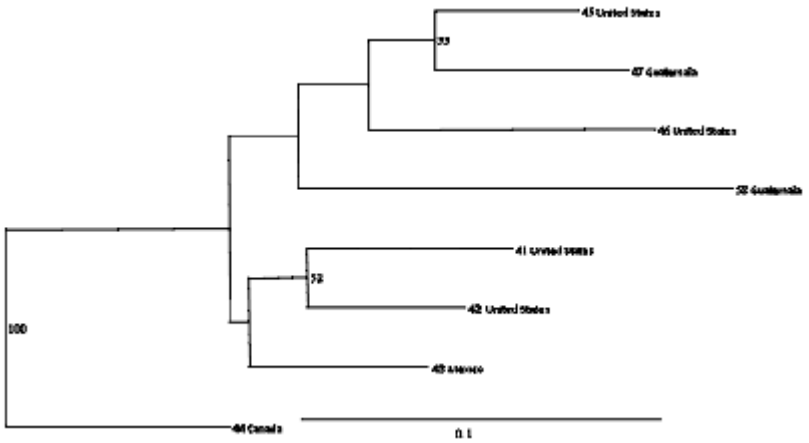

Fig. 5. Neighbor-joining phylogenetic tree of *P. vulgaris* showing the relationship among the North American accessions. Bootstrap values (>50%; 250 replicates) are shown next to the branches

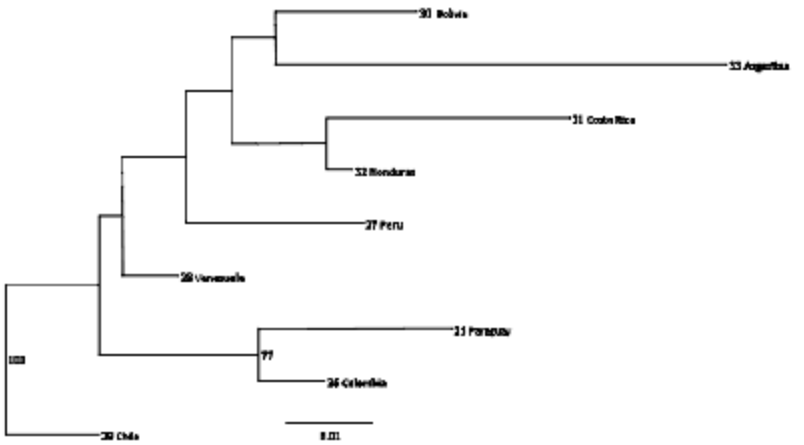

Fig. 6. Neighbor-joining phylogenetic tree of *P. vulgaris* showing the relationship among the South American accessions. Bootstrap values (>50%; 250 replicates) are shown next to the branches

and African varieties showed similarities as well and clustered in one sub-tree group (Accessions 41-60). Although this group is not supported by reliable bootstrap value.

Further, accessions 34, 35, 36 (Europeans) were more similar compared with the American isolate, 33. Distinct genetic relationship also observed between European accessions 38 and 40; 37 and 39. In contrast, accessions 22 and 23 showed high level of genetic similarities, although these were isolated from different regions (Asia and America).

We further analyzed the relationship among the 5 regional accessions (Asia, Europe, Africa, North America and South America). The phylogenetic tree showed that all the accessions of Asian region including Australia (59 and 60) are similar except 2 accessions from India and Yemen (9, 20) (Figure 2). Among the European accessions, only one accession from Netherlands (3) showed different from the rest of the European accessions (Figure 3). One accession from Morocco (52) showed very different genetically from the rest of the accessions of the African region (Figure 4). Canadian accession (44) seems to be different from the other North American accessions including Guatemala, Mexico and USA (Figure 5). One accession (29) from Chile was different from the other accessions of South America (Argentina, Bolivia, Colombia, Costa Rica, Honduras, Paraguay, Peru and Venezuela) (Figure 6). Further, accessions of Paraguay and Colombia showed very close relationship and this relationship was supported by high bootstrap value (77%).

ACKNOWLEDGEMENTS

This project was supported by the King Saud University, Deanship of Scientific Research, College of Science Research Center.

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