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Short Communication

Genetic diversity among omani basil (ocimum basilicum l.) landraces using RAPD markers

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Basil (Ocimum L.), a medicinal and aromatic plant genus, is widely used in food and pharmaceutical industry for ages. Our previous studies reported high potential of essential oils in the unexplored and un-exploited landraces of Omani basil. In the present study, nine Omani landraces of common basil (Ocimum basilicum L.) were analyzed for the assessment of genetic diversity with RAPD markers. Out of fifteen tested seven primers generated a total of 25 alleles with an average of 3.57 per locus. Maximum six alleles were amplified with the primer OPK16 while primers OPK1 and OPK10 amplified only two alleles. Mean polymorphic information content (PIC) was 0.5. Dendrogram based on similarity matrix grouped landraces 1 and 3; 4and W together showing similarity coefficient of 1.

Keywords: Landraces, Basil, Genetic Diversity

INTRODUCTION

Importance of basil, also known as Rehan, cannot be over-emphasized because of its wide use in food and pharmaceutical industry. It is still used in gastronomy and as traditional medicine by the poor people of rural areas in Pakistan and India. Its importance is due to presence of essential oils which result a unique flavor and medicinal properties (Al-Maskari et al. 2011). The aromatic leaves of basil are used fresh and dried as spices or flavoring in a wide variety of foods. Volatile oils of basil are used in dental and oral products and in fragrances. Basil extracts are also used as insecticide, nematocide, fungicide and antimicrobials (Hanif et al. 2011).Taxonomically, basil belongs to the genus Ocimum, originated in Africa and Asia, consisting of 30 species distributed in tropical and subtropical areas of new and old world. The main center of diversity of Ocimum spp. is in Africa and secondary center is in Asia (Vieira and Simon, 2000). Among 30 species of the genus Ocimum, common basil Ocimum basilicum L. is the most exploited species because of its economic importance.

The assessment of genetic diversity in germplasm is often exploited to develop improvedcrop varieties for changing needs and environments. The estimation of genetic diversity can be used onpedigree records, morphological traits and/or molecular markers (Sajjadet al., 2011). Molecular markers are more reliable as compared to pedigree and morphological data. Among several other molecular markers, Polymerase Chain Reaction (PCR) based Randomly Amplified Polymorphic DNA (RAPD) has been successfully used to assess genetic diversity in many crop plants. A major advantage of RAPD markers over many other DNA-based markers is that they require no prior sequence information and no prior knowledge about any particular gene in the target taxon. Therefore, RAPD markers are the best option to assay landraces, wild plants or new plant species for their characterization and assessment of genetic diversity. Furthermore, PCR method uses lower amount of genomic DNA, are non-radioactive, relatively low costly and can be developed rapidly. Because of these characteristics several studies have highlighted the

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Figure 1: Phenotypic variation among basil landraces collected from Oman (SQU-1, SQU-2, SQU-3, SQU-4, SQU-G, SQU-K, SQU-U, SQU-W and SQU-X). Lanes 1, 2 and 3 show variations in branching, inflorescence, and leaves respectively.

usefulness of RAPD markers to assess genetic diversity in almost all crops under cultivation. RAPDs have also been successfully used in basil for genetic diversity assessment among cultivars and species. The present study was designed to characterize and assess genetic diversity among the landraces collected from different regions of Oman. Seeds of characterized landraces are currently maintained in the depository of Sultan Qabos University(SQU), Oman.

MATERIALS AND METHODS

Plant material:Seeds of nine landraces(SQU-1, SQU-2, SQU-3, SQU-4, SQU-G, SQU-K, SQU-U, SQU-W and SQU-X)collected from different regions of Oman showing variation in branches, inflorescence and leave types (as shown in figure 1)were collected and grown at the AES farms of SQU following RCBD design. Standard cultural practices were followed throughout the growth of plants. For DNA extraction, 1 g tissue samples of fresh young leaves of each landrace were collected in Eppendorf tubes dipped in liquid nitrogen and maintained at -80 oC until used.

DNA extraction:DNA was extracted according to the method developed by Doyle and Doyle (1987). Tissue were ground in the frozen eppendorf tubes and 700 ul cetyltrimethyl ammonium bromide (CTAB) was added and mixture was incubated for 30min at 65 oĊ.An equal volume of chlorofom: isoamyl alcohol (24:1 v/v) was added to each tube and mixed. Samples were centrifuged for 10 min at 13000rpm. Supernatant was taken carefully to new eppendorf tubes. Ice cold isopropanol (500µl) was added and centrifuged at 1300rpm for 10min. The supernatant was discarded and pellets were washed with 70% ethanol. Tubes were inverted for 30min and then placed in hood until pellets were dried. After drying pellets, 150µl of TE buffer was added to dissolve the pellets by keeping overnight at 4 oĊ.Dissolved DNA pellets were checked by running 2µl of each sample on 1%agarose and then quantified using spectrophotometer at 260nm. For PCR reaction DNA was diluted to 25ng/µl in TE buffer.

PCR amplification: Fifteenarbitrary decameroligonucleotide primers were tested and seven polymorphic were used in analysis. Amplifications were performed in 25ul volume containing 50 to 200ng DNA, PCR buffer (50mM KCl, 10mM Tris-HCl, pH 8.8, 1.5mM MgCl2,and 1% Triton X-100), 0.1mM of each dNTP, 0.2mM primer and 1 unit Taq DNA polymerase. Amplification were performed inthermocycler programmed 40 cycles of denaturing for 20s at 94℃ annealing for 40s at 37℃ and extension for 1 min at 72℃. PCR products were electrophoresed on 1.5% agarose gel at 100V for 2h, stained with ethidium bromide, and visualized under ultraviolet light. A negative control lacking template DNA was also used in each set.

DATA analysis: The presence of each amplified band of DNA was scored as 1 and absence as 0. The data was analyzed using power marker v3.2. Numerical taxonomic system NTSYS was used to perform the cluster analysis using similarity matrix.

Nine Omani landraces of Ocimum basilicum L. were assayed with RAPD markers for genetic diversity. Out of fifteen tested seven primers generated a total of 25 alleles polymorphic with an average of 3.57 per locus Figure. 2), while, Vieira et al. (2003) found 98 alleles with mean of 8.9 alleles per locus in 37 accessions using 11 RAPD markers. The size ranged from 450 to 1200bp showing narrow size range compared to the findings

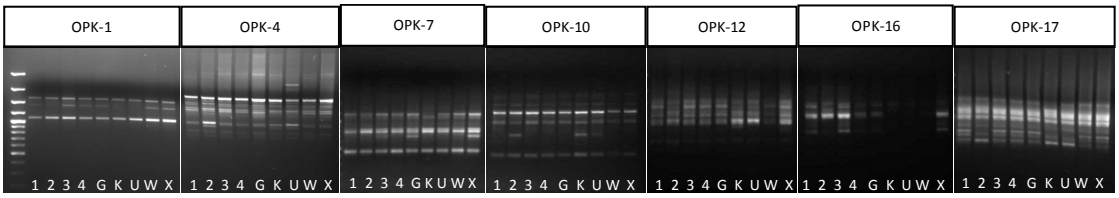

Figure 2: RAPD PCR of Omani landraces using OPK primers 1, 4, 7, 10, 12, 16 and 17.
Reults and Discussion

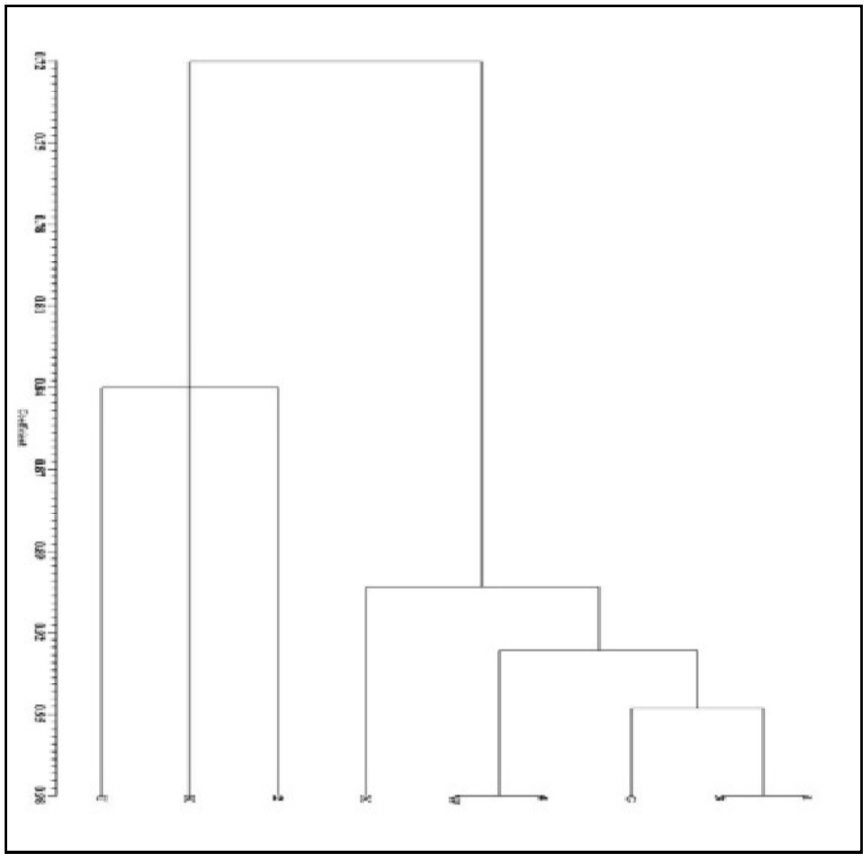

Figure 3: Dendrogram derived from 9 Omani landraces of Osmumbasilicum L. using 25 RAPD alleles. Similarity matrix was used to develop the dendrogram in NTSYS Program.

(300-2000bp) of Vieira et al. (2003). Maximum six alleles were amplified with the primer OPK16 while primers OPK1 and OPK10 amplified only two alleles. Mean polymorphism information content (PIC) was 0.5.Primer OPK16 showed maximum PIC value of 0.76 followed by OPK4 having PIC value of 0.69.These two primers OPK16 and OPK4 were also major contributor to total genetic diversity.Primers OPK1 and OPK10 showed minimum PIC value of 0.34 and contributed minimum to the total genetic diversity. Dendrogram based on similarity matrix grouped landraces in two groups. Landraces 1 and 3; 4and G, W and X were grouped together. In this group the minimum similarity was 91% between landrace X and subgroup of 1, 3, 4, G and W. Furthermore, high similarity coefficient (72%) between two groups indicates the narrow genetic diversity in local landraces under study. The leaves, inflorescence and branching patterns of all members of this group are similar except landrace 1. The inflorescence and branching pattern of landrace 1 is similar to the landraces 2 and U falling in other group (Figure.1 and 3).The landraces 2, K and U formed the second cluster with 84% similarity among themselves (Figure 3). The inflorescence, leaves and branching pattern of landraces

2 and U are similar, while, inflorescence of landrace K differs with those of 2 and U (Figure. 1). The results have demonstrated the utility of RAPD markers for analyzing the genetic divergence useful for subsequent exploitation of landraces in industrial applications. The genetic variation obtained by RAPD analysis partially confirms the phenotypic variation.

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