Annals of Agrarian Science xxx (2017) 1–5

Contents lists available at ScienceDirect

Annals of Agrarian Science

journal homepage: http://www.journals.elsevier.com/annals-of-agrarian-science

Sequence-related amplified polymorphism (SRAP) markers based genetic diversity analysis of cumin genotypes

Jahnvi Bhatt [a], Sushil Kumar [a, *], Swati Patel [a], Ramesh Solanki [b]

[a] Department of Agricultural Biotechnology, Anand Agricultural University, Anand 388 110, India
[b] Division of Plant Improvement, Propagation and Pest Management, Central Arid Zone Research Institute (CAZRI) Jodhpur, 342 003, India

ARTICLE INFO

Article history:
Received 5 August 2017
Received in revised form
11 September 2017
Accepted 18 September 2017
Available online xxx

Keywords:
Cumin
DNA marker
Diversity
Spices
SRAP

ABSTRACT

Cumin (*Cuminum cyminum* L.) is an industrially important seed spices crop of family Apiaceae. Because of its diverse usage in food and pharma industries, it is essential to document and characterize the cumin genotypes to assess genetic diversity which is prerequisite for its genetic improvement. With this aim, a set of 10 sequence-related amplified polymorphism (SRAP) markers were deployed to examine the genetic diversity among 16 cumin genotypes including five released varieties. Out of 65 amplified bands, a total of 60 polymorphic bands were detected which demonstrated with polymorphism information content (PIC) value of 0.34 with a range from 0.14 to 0.51. The bands size ranged from 120 to 500 bp. The distinctive value of MI (marker index) for studied markers was 2.43 while average resolving power for all markers was 8.49. The UPGMA based grouping showed three distinct clusters at a cut-off value of 0.62. In the present study, the average Jaccard similarity coefficient was 0.59 which indicated a moderate level of genetic diversity in studied cumin genotypes. The result of current investigation suggested that there is need to increase the genetic base of cumin germplasm using different breeding approach *viz* mutagenesis, germplasm introduction, wide hybridization or somaclonal variation.

Introduction

Cumin (*Cuminum cyminum* L.) with 2n = 14 chromosomes is an important seed spices of subtribe Carinae of the tribe Ammineae of family Apaiaceae. This one of the old cultivated aromatic and herbaceous plant has a plenty of medicinal, pharmaceutical and nutraceutical properties. It has a wide usage in foods, beverages, liquors, medicines, toiletries and perfume industries. The centre of origin of cumin is not clear but it is native to northern Africa, and has spread via west Asia to Central Asia. It is extensively used in foods, beverages, liquors, medicines, toiletries and perfumery, and is grown in the mild climates of India, Syria, Pakistan, and Turkey [1].

Cumin is a culinary and flavouring agent due to strong and aromatic flavour. Cumin seeds contain up to 2.5–5% of a volatile oil composed primarily of aldehydes (up to 50-60%) and hydrocarbons (30–50%) [2]. Oleoresin from cumin seeds finds application in sauces, crackers, meat and sausages. Cumin seeds have diuretic, emmanogogic, carminative and antispasmodic properties consequently has role in traditional medicine to cure diseases like toothache, whooping cough, dyspepsia, diarrhea, epilepsy and jaundice.

This crop agonizes with inherent hitches of low germination and weak seedling establishment. Similarly, due to monotytpism genetic base in cumin for economic traits like yield, quality (volatile oil content) and biotic and abiotic stresses resistance is narrow. Further the lack of information on genetic diversity and intraspecific relatedness in cumin has offered limited scope for genetic improvement especially through hybridization [3]. Studies on genetic relationships among cumin ecotypes through morphological traits have been reported previously [4]. But inadequacy of polymorphic qualitative and quantitative traits has hampered the knowledge of diversity in real manner. Moreover, traditional phenotypic based variability assessment is time and energy consuming and costly, and exhibits genotype × environment interactions [5]. Therefore, during the past decades, classical methods of genetic variability evaluation have been complemented by DNA markers.

SSR is marker of choice for crop breeding as provides an extensive dispersion around the genome, and high polymorphism,

* Corresponding author.
E-mail address: sushil254386@yahoo.com (S. Kumar).
Peer review under responsibility of Journal Annals of Agrarian Science.

https://doi.org/10.1016/j.aasci.2017.09.001

but is costly to perform and unfortunately not available in cumin. Till date, no sequence based molecular markers like simple sequence repeat (SSR) and single nucleotide Polymorphic (SNP) has been developed in cumin [6]. Though, cross-amplification of SSR has been reported in cumin [1]. Among various markers, RAPD is the widely used for cumin diversity study [1]. However, poor consistency and low reproducibility limits the effectiveness of RAPD. In last decade, Sequence-related amplified polymorphism (SRAP) marker has emerged as a useful PCR based molecular marker system for diversity study and linkage mapping in various crops [7]. In cumin, no information is available on the deployment of SRAP marker-a moderately co-dominant marker for diversity study. Hence, first time a study has been conducted to assess the extent of genetic diversity and relationship among different genotypes of cumin using SRAP markers.

Materials and methods

Plant material and DNA extraction

A set of 16 genotypes including five released varieties were collected from National Research Centre on Seed Spices, Ajmer. Using bulk strategy, genomic DNA of 16 cumin accessions was isolated from fresh and healthy leaves 15 days old seedling using the protocol of Doyle and Doyle [8]. The extracted DNA was quantified using Nanodrop N.D.1000 (V.3.3.0, Thermo Scientific, USA). The quality DNA was determined on 0.8% agarose gel as per Rukhsar et al. [5]. DNA was diluted to 30 ng/μl and stored at 20 °C for SRAP marker amplification.

SRAP-PCR parameters and gel analysis

Primers reported by Ferriol et al. [9] were used for SRAP amplification in thermal cycler (Biometra). SRAP- PCR reactions were conducted in 15 μL reaction mixture containing 1.5 μL g DNA (45 ng), 7.5 Master Mix (2× Genei, Bangalore, India), and 1 μL of 10pMol primer and 5 μL nuclease free water. The polymerase chain reactions (PCR) was carried out with following thermal profile: an initial denaturalization at 94 °C for 5 min; followed by five cycles of 1 min at 94 °C (denaturation), 1 min at 35 °C (annealing), and 1 min at 72 °C (extension); followed by 35 cycles of 1 min at 94 °C (denaturation), 1 min at 50 °C (annealing), and 1 min at 72 °C (extension); followed by a final extension at 72 °C for 10 min. The amplified SRAP products were separated on non-denaturing polyacrylamide gels (6% PAGE) as per Rukhsar et al. [5]. The amplified bands were silver stained and PAGE gel were scanned and photographed by an automatically imaging system (Bio-6000, Microtek, Taiwan) at 600 dpi.

Data analysis

Due to multiple bands per primer (Fig. 1), the amplified products of SRAP markers were scored as 1/0, where 1 = presence of band and 0 = absence of band. From these 0/1 data, PIC value (Polymorphism information content) and primer index and resolving power (Rp) value were calculated as per Sharma et al. [10]. Multiplex ratio (MR = TB/TP where TB = total band; TP = total primers used), effective multiplex ratio (EMR = MR × FP where, MR = multiplex ratio and FP = polymorphism fraction) and marker index (MI = EMR × Mean PIC) were calculated as proposed by Powell et al. [11] and followed by Varshney et al. [12]. Genetic similarity between genotypes was calculated using on jaccard's similarity (J) coefficient by SIMQUAL program and unweighted pair group method average (UPGMA) dendrogram was constructed using SAHN clustering algorithum of NTSYSpc v. 2.20 [13].

Results and discussion

The extent and distribution of genetic variability in germplasm are keys of success of breeding programs [14]. Though, cumin is an important crop but very scarce efforts have been made to characterize it, which delays its improvement through breeding. The key objective of cumin characterization using phenotypic markers is to find high yielding genotype with high quality essential oil which can be further exploited for cumin improvement. Earlier, sufficient morphological variation in cumin has reported by different researchers [4,15–17]. However, expression of phenotypic traits varies with environment and edaphic conditions as well as with developmental stages. To cope up with such difficulties RAPD, ISSR and AFLP have been deployed to conceal genetic diversity of cumin [1]. However, RAPD is low reproducible while AFLP is time-demanding and cumbersome. Therefore, to describe the diversity

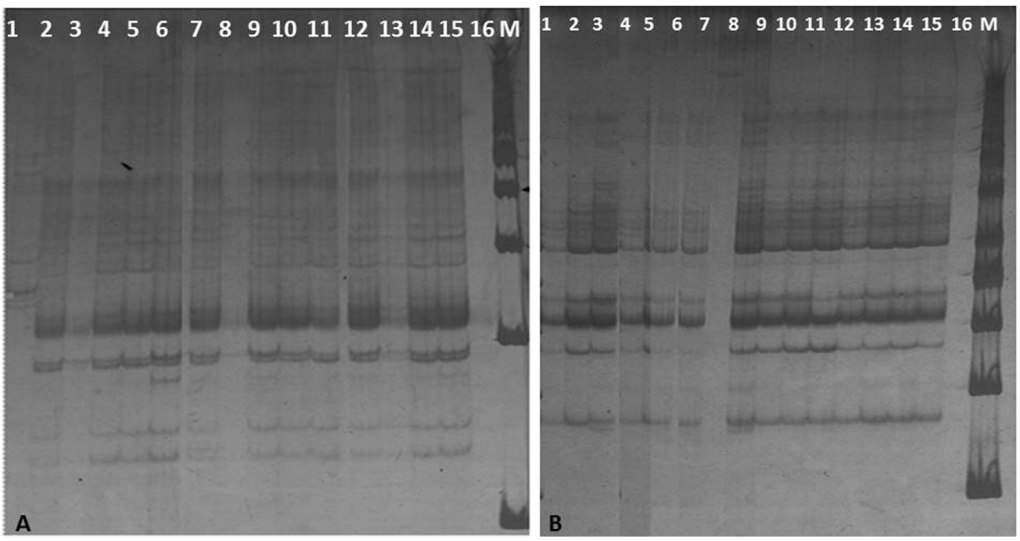

Fig. 1. PCR amplification products of 16 genotypes of 16 cumin with primer combination A) Me1+Em5 and B) Me1+Em4; M = 100bp ladder.

Please cite this article in press as: J. Bhatt, et al., Sequence-related amplified polymorphism (SRAP) markers based genetic diversity analysis of cumin genotypes, Annals of Agrarian Science (2017), https://doi.org/10.1016/j.aasci.2017.09.001