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Short Communication

An assessment on intraspecific transferability of SSR markers on fennel (*Foeniculum vulgare* Mill.) varieties

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Abstract

The amplification potential of 27 SSR primer pairs originally reported on exotic fennel (*Foeniculum vulgare* Mill.) was studied on Indian fennel. Only 13 primer pairs developed amplicons (only one amplicon per primer pair) of sizes ranging from 100 to 450 bp. To determine their polymorphic potential, a set of 20 diverse fennel germplasm lines was used. Four primers exhibiting polymorphism, did segregate the 20 diverse lines in 9 clusters. However, these primer pairs did not differentiate between 17 improved varieties of fennel.

Key words : Fennel germplasm, microsatellite markers, fennel varieties.

Fennel (*Foeniculum vulgare* Mill.), a member of Apeaceae family (chromosome number 2n=22) is one of the important seed spice crops of India. Although, every aerial part of plant is aromatic but its seeds are largely used in medicinal formulations, perfumery, beverages, pickle preparation, condiments to culinary and as mouth freshener. Globally, India ranks first in fennel production followed by Mexico and China (FAO 2016). With a large domestic consumption and bright export potential it was cultivated on an area of 65810 ha during 2017-18 (www.indianspices.com). In India, fennel is mainly grown in the states of Gujarat and Rajasthan. Gujarat contributes 57% of area and 76% of production in contrast to 37% of area and only 20% of production by Rajasthan. The higher productivity of fennel in Gujarat is largely due to its cultivation as a *kharif* crop with extended crop stand and favourable soil type, while in Rajasthan, it is grown as a *rabi* (winter) season crop. A number of high yielding fennel varieties have been developed (Singh et al. 2015) in India. But considering the harsh and fragile climatic conditions of Rajasthan, there is fairly a good scope and need for improvement of productivity of fennel varieties.

Although, a relatively small collection of germplasm lines (260 lines) is available for undertaking breeding research work, however, in the era of modern breeding approaches, it is important that data on molecular genotyping is also available for more precise use of the germplasm lines. In this context, availability of genomic sequences of food crops is becoming a necessity as this enables the researchers to identify microsatellite sequences for development of simple sequence repeats (SSR) markers which are inherently codominant. However, besides their use in gene tagging, gene mapping or pyramiding etc, such SSR markers are generally relatively more useful in plant breeding programmes if these are polymorphic too. In fennel, the first draft genomic sequences was published in the year 2018 by Palumbo et al. (2018). These authors have identified and reported 27 SSR primer pairs which may be used for multiplexing PCR for diversity studies on fennel genotypes of Italian origin. Aillo et al. (2020) has studied the interspecific transferability of wild carrot (*Dacus carrota*) SSR markers to fennel varieties and reported that only 23% markers were transferable. It is therefore, important to assess the amplification pattern of these SSR markers on fennel genotypes grown in India.

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Palumbo et al. (2018) had assessed amplification potential of 27 SSR primer pairs of exotic fennel genotypes of Indian origin and reported the robustness of amplification potential with little influenced by quality and quantity of genomic DNA used. A study was therefore, conducted to test these primer primers for their amplification potential of genomic DNA of 20 germplasm lines and 17 improved varieties comprising of 8 released and 9 pipeline varieties originated from India. These lines were considered as diverse on the basis of multiple traits like yield potential, plant height, days to flowering and seed storage protein profile (data not shown). The seeds of all fennel genotypes were obtained from ICAR-All India Coordinated Research Project on Spices, Department of Plant Breeding & Genetics, S.K.N. College of Agriculture, SKNAU, Jobner. The genomic DNA was isolated from young leaves of approximately one month old field grown fennel plants by CTAB method described by Saghai-Maroof et al. (1984) with some modifications (Saravanaperumal and Terza 2012; Krizman et al. 2006). The integrity of genomic DNA was electrophoretically tested on 1% agarose gel and stored at -20°C for further use. All 27 SSR primers were procured from Eurofins Genomic India Pvt. Ltd., Banglore and its 10 fold dilution yielding 10 pmoles/μl was directly used for PCR reaction mix. Genomic DNA (~50 ng/μl) was used to perform PCR with T*aq* Polymerase (Taq-MBT060A, HiMedia) using conditions of denaturation at 95°C for 3 min followed by 45 cycles consisting of 95°C (30s), 55°C (30s) and 74°C (45s) and final extension at 74°C for 10 min using a thermocycler of HiMedia Make (The Taq polymerase used in this investigation polymerizes optimally at 74°C). The PCR products were separated on high resolution agarose gel (3%) and the image taken on a gel documentation system of Biometra make.

Results revealed that out of the 27 SSR markers reported as polymorphic and suitable for diversity studies on exotic fennel (Italian) by Palumbo et al. (2018), only 13 showed amplification with the genomic DNA of one of the pipeline line varieties UF289 indicating that the SSR loci identified by these authors on their fennel genotypes have major differences with Indian fennel genotypes with respect to DNA base sequence arrangement of at least concerned loci. Out of the 11 possible linkage groups (LG) of *F. vulgare* Palumbo et al. (2018) could assign 9 linkage groups to these 27 SSR loci (some SSR loci are still not assigned to any linkage group and no loci assigned on LG 4 and 8. In the present study, amplicons (discrete or diffused) resulting from the 13 SSR loci (functional on our fennel genotypes) could be associated to all the linkage groups indicating coverage of most of the chromosomes. On the basis of amplicon size of these 13 SSRs reported by Palumbo et al. (2018) the amplification pattern on studied fennel genotypes seemed variable (Fig. 1). In the present study, the amplicons size of SSR primer 2, 3, 7 and 17 were similar to that reported by Palumbo et al. (2018) but those of SSR primer 8 to 13 and SSR 15 were larger. These results may enable to infer that the two types of fennel, i.e. Indian and Italian, share both identical and un-identical base sequences on the concerned loci. Out of these 13 SSR primers, five SSRs producing discrete amplicons were employed to study their polymorphic potential using the set of chosen diverse germplasm lines. The results revealed that SSR 2,4,8 and 9 showed visibly detectable differences in amplicons on some of the germplasm lines (Fig. 2). The SSR 3 seemed monomorphic. The binary data

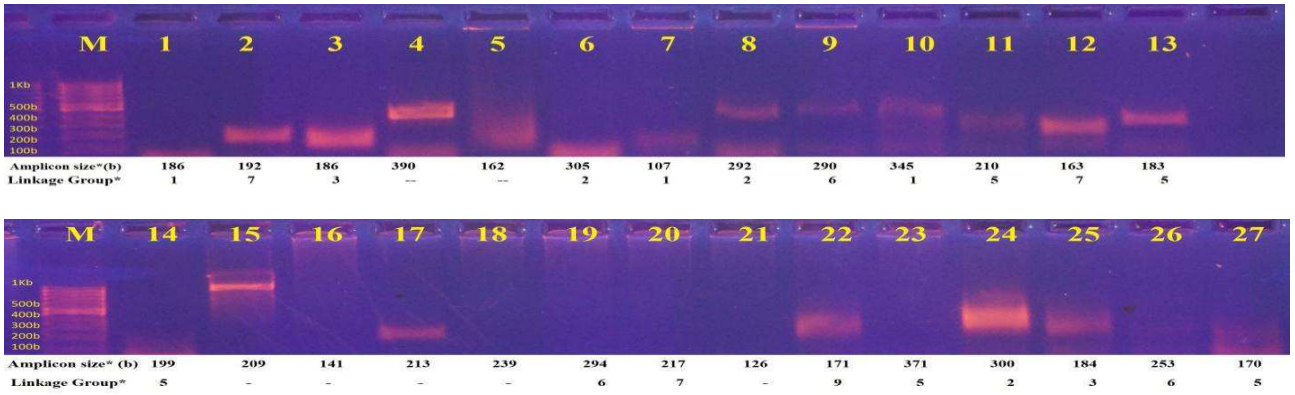

Fig. 1. PCR amplification pattern of fennel genomic DNA with fennel SSR primers (M=DNA Ladder) Palumbo et al. (2018)

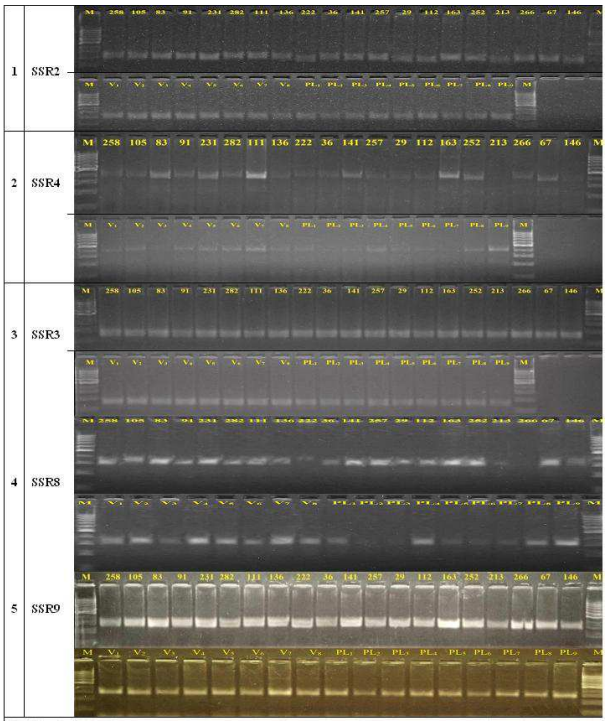

Fig. 2. Amplification pattern of 20 diverse fennel germplasm lines (Upper lane) and 17 developed varieties (lower lane) with five different SSR primers. V1=RF 101,V2=RF 125, V3=RF143, V4=RF 178, V5=RF 145, V6=RF 281, V7=RF 157, V8=RF 205; Pipeline varieties:PL1=UF 291, PL2=UF 290, PL3=UF 289, PL4=UF 288, PL5=UF 287, PL6=UF 286, PL7=UF 285, PL8=UF 284, PL9=UF 283

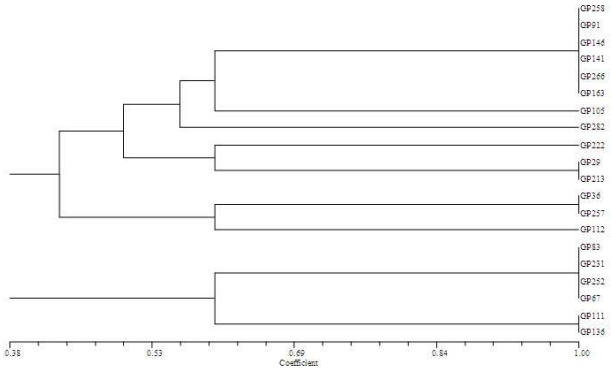

Fig. 3. Dendrogram resulting from an UPGMA cluster analysis of 20 diverse germplasm lines of fennel based on data of 4 SSR primer pairs

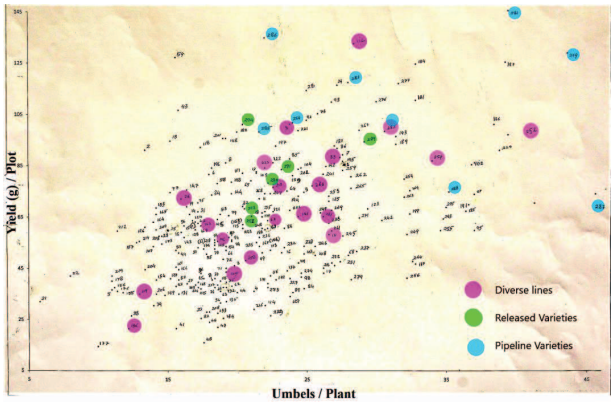

Fig. 4. Scattering of diverse germplasm lines, released and pipeline varieties of fennel

generated with alleles at the loci of four polymorphic SSR primer pairs was used to construct a dendrogram using UPGMA cluster analysis of these 20 diverse germplasm lines using NTSyspc software as described by Rohlf (2000). Thus, with these 4 polymorphic SSR markers, the 20 germplasm lines could be segregated into 9 groups (Fig. 3). Germplasm lines GP 258, GP 91, GP 146, GP 141, GP 266 and GP 163 were identified as identical. Similarly, germplasm lines GP

Table 1. SSR markers used for the determination of molecular variations in a set of 20 fennel germplasm lines using 5 primer pairs

SN	SSR	Primer	Sequence name	Linkage group	Amplicon size (bp)	Poly-morphism	No. of alleles	PIC value
1	2	**FV_253**	**Forward** :TTGTAGAGATACAGGGTCGAA **Reverse** : GAGGGGAGTCAGTTAAACAAC	7	192	Polymorphic	3	0.34
2	3	**FV_462**	**Forward** :ATGGCTGAGAATTAGGGTTAC **Reverse** : CGATCTACGCCTTAGAGGTAT	3	186	Monomorphic	1	Nil
3	4	**FV_18902**	**Forward** :GTTTGAACTCGAATGACCACC **Reverse** : GGGTCTATCATCACTCTCGC	-	390	Polymorphic	2	0.42
4	8	**FV_217218**	**Forward** :ACAAACGTACCTCTGTACGAA **Reverse** : TCAGAAGGTGAGTTATGTTGC	2	410	Polymorphic	2	0.18
5	9	**FV_290063**	**Forward** :TGATTTCTCAAAGGCATTCTA **Reverse** : TCTTCCTGTCATCTCAAGGTA	6	450	Polymorphic	2	0.26

83, GP 231, GP 252 and GP 67 were found identical. Germplasm lines GP 111 and GP 136 appeared as identical and most diverse from the rest of the lines although both GP 111 and GP 112 were of exotic origin (Fig. 3). We have attempted to compare SSR marker based similarity/diversity pattern with Meteroglyph Scatter diagram of germplasm lines plotted on the basis of seed yield and umbels per plant following Anderson (1957). Figure 4 depicts scattering of 260 germplasm lines of fennel from which 20 diverse lines were selected (data not shown). In the scatter diagram, the 20 diverse germplasm lines and developed varieties used in the present study have been shown in coloured glyphs. A perusal of the diagram reveals that the set of 20 germplasm lines are adequately diverse as per the requirement of the present study. However, in case of improved varieties one may observe that while both released and pipeline varieties have a distinguishable scattering pattern but such a distinction between the varieties is not apparent with SSR marker based approach. Earlier, with the help of seed storage protein profile (using SDS-PAGE) it was shown that it is possible to distinguish between the same set of varieties (Ram Krishna et al. 2019). These improved varieties have been developed through various breeding approaches involving selections made in exotic population and recurrent half sib selections made in diverse indigenous populations and F_2 recombinants of many selected crosses involving diverse parental populations (Singh et al. 2015). It is possible that these varieties represent more or less same combination of desirable alleles therefore escaped any distinction possible through studied SSR markers. In a recent study by Aillo et al. (2020) it was found that transferability of *Dacus carrota* SSR markers to fennel varieties was only 23% whereas in the present study it was close to 50% though it's a case of intraspecific transferability. Therefore, it becomes important that more number of SSR primers be examined to obtain polymorphic primers in fennel specifically to achieve fine probing between closely evolving fennel genotypes.

Authors' contribution

Conceptualization of research (KRK); Designing of the experiments (KRK); Contribution of experimental materials (KRK, NP, DS); Execution of field/lab experiments and data collection (KRK, NP, DS, GKM); Analysis of data and interpretation (KRK, DS, GKM); Preparation of manuscript (KRK, DS).

Declaration

The authors declare no conflict of interest.

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