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Varietal differences for storage seed protein profile in fennel (*Foeniculum vulgare* Mill.)

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Abstract

An investigation was carried out on 8 released and 9 pipeline varieties of fennel to determine their seed storage protein subunit profile variation through SDS-PAGE. Optimal buffer for extraction of proteins which revealed maximum protein subunit bands was worked out. The results of the SDS-PAGE revealed a total of 26 protein subunit bands in the range of 116 kDa to ~5 kDa of molecular weight (MW). These bands appeared to fall in three distinct regions. The bands of region B and C were largely monomorphic but that of region A (with bands between 45-116 kDa MW) were relatively polymorphic. The binary data generated from the polymorphic bands over the varieties were used to compute Jaccard's similarity coefficients. The similarity matrix thus prepared was used to construct a dendrogram by UPGMA. The dendrogram distributed the 8 varieties in two major clusters. Cluster II was represented by RF 125 (a selection from exotic fennel population). The cluster I exhibited two sub-clusters. With 9 pipeline varieties also the dendrogram revealed two clusters with 2 sub clusters in each. Results indicate that seed storage protein subunit profile variation may be utilized to differentiate between the collection of fennel varieties/genotypes as a consequence of selection.

Key words : Apeace, crop improvement, fennel, SDS-PAGE, storage proteins.

Introduction

Fennel (*Foeniculum vulgare* Mill.) which belongs to the family Umbelliferae (Apeaceae) is an annual herb and an important seed spice crop (chromosome no. 2n=22). Every part of the plant is aromatic and has multifarious uses (Badgujar *et al*., 2014). Its seeds are mainly used in medicinal formulations, perfumery, beverages, pickle preparation, condiments and culinary and as mouth fresher. India is the largest producer of fennel in the world after Mexico and China (FAO, 2016). Within India, it is grown on an area of 89540 ha and its cultivation is concentrated mainly in the states of Gujarat, Rajasthan and Uttar Pradesh. In Rajasthan, it is an important *Rabi* season crop occupying 45200 ha of area and 56240 tones of production (*www.indianspices.com/statistics/2016-17*).

Although most of its production is consumed in the country itself, but, during last 10 years efforts have been made to promote its export. With an increasing trend every year, during 2016-17, 30875.50 lakhs worth of fennel was exported.

Considering its multifarious uses, its domestic demand and bright export potential it is imperative to improve its productivity. Therefore, full characterization of available genetic resources is important as it enables one to choose diverse lines for making hybridization. For an effective breeding programme, genetic or a heritable variation is a prerequisite. There may be substantial variation in the germplasm of a crop with respect to different characters. However, it is being increasingly realized that selection of parents on the basis of molecular typing (besides morphogenetic data) which may include DNA or protein based data would be more useful in breeding programs for making choice of parents for hybridization. Keeping these points in view the present study aims at exploring the possibility of using seed storage protein profile detected by Sodium Dodecyl Sulphate- Poly Acrylamide Gel Electrophoresis (SDS-PAGE) on certain released and promising lines of fennel.

Materials and methods

The seeds of 8 released varieties and 9 pipeline varieties of fennel developed under the auspices of ICAR-All India Coordinated Research Project on Spices, Jobner Center were obtained from Department of Plant Breeding & Genetics, S.K.N. College of Agriculture, SKNAU, Jobner. The work on SDS-PAGE of fennel seed storage protein was initiated with the help of two released varieties of fennel crop, namely, RF 101 and RF 125. The seed were ground to get a fine powder and defatted with defatting solution containing Chloroform: Methenol:Acetone in the ratio of 2:1:1 as described by Singh *et al*. (2013). Different

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seed protein extraction procedures available in the literature were reviewed and the different combination of its component reagents were examined for obtaining maximum number of protein subunit bands. One of the combination of extraction buffer that contained 0.5M Tris, 5M Urea, 2.5% SDS and 5.0% Mercaptoethenol (added at the time of use), adjusted to pH 6.8, yielded maximum and clear subunit bands, and hence, was used for extraction of proteins from the defatted sample. For extraction of the proteins 50 mg of defatted sample was suspended in 1.0 ml of extraction buffer in a 2.0 ml microcentrifuge tube and incubated for 6 h with intermittent shaking/vortexing. After incubation the tubes were centrifuged at 10000 rpm for 15 min and 0.5 ml of supernatant was then transferred to a fresh centrifuge tube and kept in boiling water in a water bath for 10 min. The samples so prepared were directly used for electrophoresis or stored at -20°C for 2-3 days for repeated use. Bromo phenol blue (BPB) added to the extraction buffer served as tracking dye to monitor the movements of protein bands in the gel. The optimum sample volume for loading was also worked out as 20 µl per lane of the gel (data not shown). The polyacrylamide gel slab was prepared for electrophoresis as described by Laemmli (1970). The electrophoresis was carried out at 30 mA constant current in a BioRad, USA, electrophoresis equipment (Model Protean II Cell). After electrophoresis the gels were stained overnight. The staining solution contained 0.1% Coomassie Brilliant Blue stain -R250 (SRL,India) in 40% Methenol and 10% Glacial Acetic Acid. De-staining of the stained gel was done in 40% Methenol and 10% Glacial Acetic Acid solution to get clear background gels. The gels with stained protein bands were kept on an illuminated platform and the images captured using Biometra Gel Doc system, Gottingen, Germany for further studies. The molecular weights of the dissociated polypeptides were determined by using standard marker proteins obtained from Puregene, Genetix, Biotech Asia Pvt. Ltd., New Delhi (Range 14.4 to 116 kDa). The molecular weight of the unknown protein subunits was extrapolated from plotting its Rf value and that of marker protein bands by developing a standard curve on a semi-log graph. Gels were scored for the presence (1) and absence (0) of every protein subunit band. In some cases distinctly intense band was scored as 1 and as 0 if the same band was less intense in other genotype. The binary data so obtained were analyzed using NTSYS-pc [Numerical Taxonomy System, Version 2.1, Rohlf (2000)]. The SIMQUAL sub-program was used to calculate the Jaccard's coefficient using following formula (Jaccard 1908). Jaccard's coefficient= NAB/ (NAB+NA+NB). Similarity matrices as computed by the programme were used to construct the dendrogram to elucidate the diversity among the varieties studied using un- weighted pair group method with arithmetic average (UPGMA) as described by Sneath and Sokal (1973).

Results and discussion

Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis (SDS–PAGE) as originally described by Laemmli (1970) is a powerful and dependable technique for characterization of proteins (or its subunits). In principle, it involves extraction of proteins from any target material, e.g. whole seeds, cotyledons, leave etc, followed by its denaturation into polypeptide subunits. The resulting protein subunits are then separated through a porous gel (polyacrylamide) in an electric field according to their masses. Characterization of genotypes on the basis of seed storage proteins/subunits is well documented in different groups of crops such as, blackgram (Ghafoor and Ahmad, 2005; Ghafoor *et al*., 2002), *Capsicum annuum* L. (Anu and Peter, 2003), *Solanum* (Menella *et al.,* 1999), *Vigna* spp (Rao *et al.,* 1992; Sharma and Ram Krishna, 2017; Choudhary, 2018), wheat (Siddiqui and Naz, 2009) including fenugreek (Erum *et al.,* 2011; Hora *et al.,* 2013; Pareek *et al*., 2018),

The present investigation was focused on 8 varieties of fennel crop which have been released for cultivation since year 2002 along with 9 pipeline varieties developed recently at the ICAR-All India Coordinated Research Project on Spices at SKN College of Agriculture Jobner, Rajasthan. These varieties represented 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). The major long term objective was to explore the possibility of application of storage seed protein profile for fennel germplasm characterization.

The results of electrophoresis revealed that there were a maximum of 26 detectable bands and the variety RF 125 represented 25 of these (Fig.1). These subunit bands seemed to be spread over in three distinct regions, namely, A, B and C (Fig.1); the A region comprised the bands between 116.2 kDA to 45 kDa of molecular weight (MW), the B region contained the bands between 45-25 kDa and the C region had the bands with less than 25 kDa MW. A band corresponding to 116 kDa seemed to specific to the released variety RF 101 and RF 281 and the pipeline variety UF290 where it is only faintly seen (Fig. 2 and 3). The

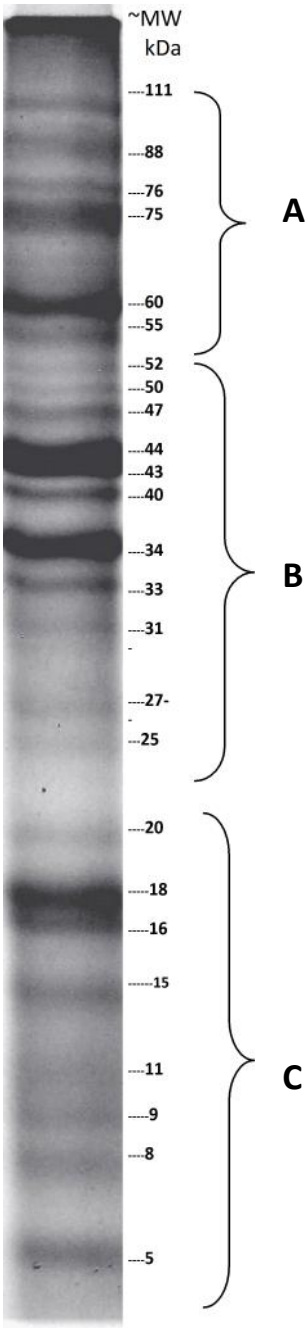

Fig. 1. MW of the protein-subunit bands.

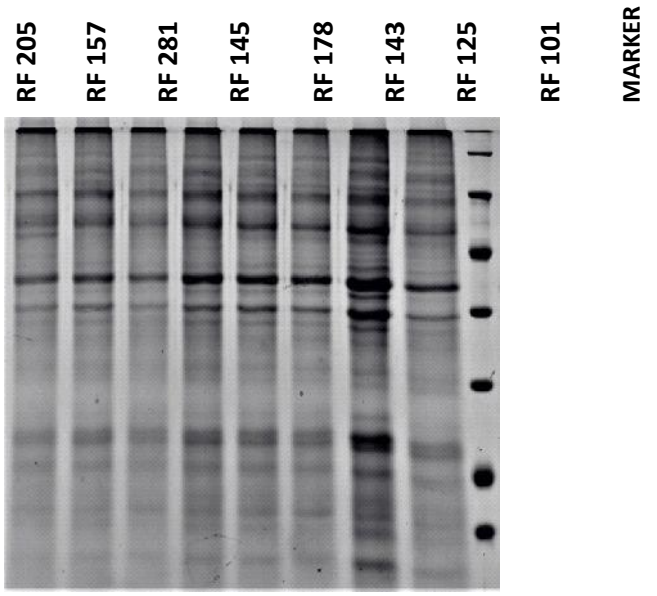

Fig. 2. SDS-PAGE Profiles of storage seed proteins of 8 different released varieties of Fennel (Variety designation is indicated on the top of each lane)

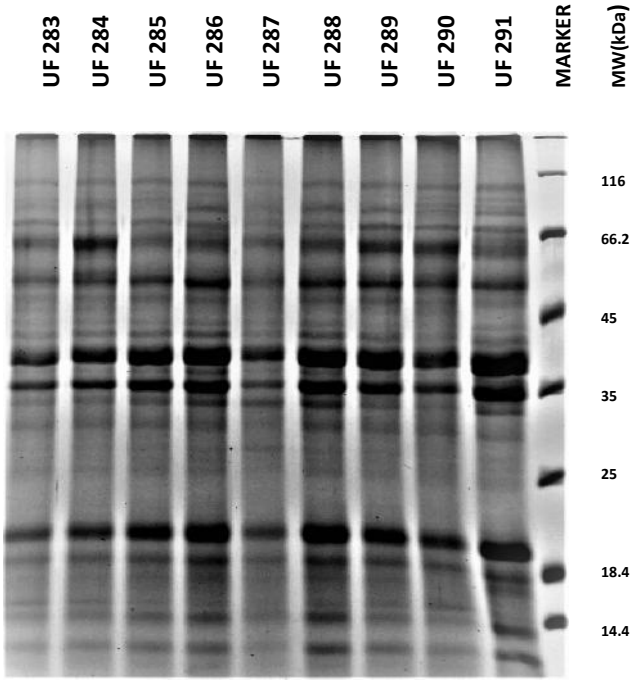

Fig. 3. SDS-PAGE Profiles of storage seed proteins of 9 different pipeline varieties of Fennel (Variety designation is indicated on the top of each lane)

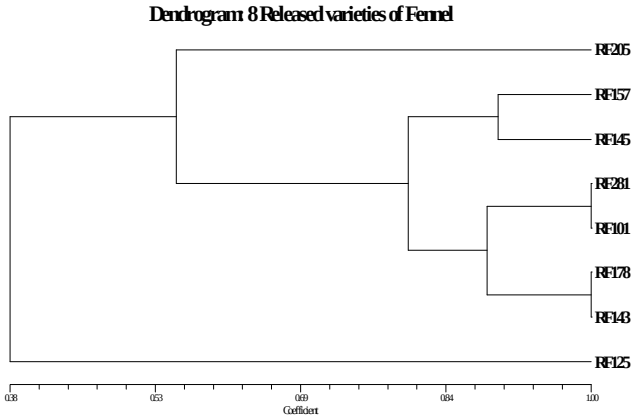

Fig. 4. Dendrogram of 8 released varieties of fennel revealed by UPGMA cluster analysis.

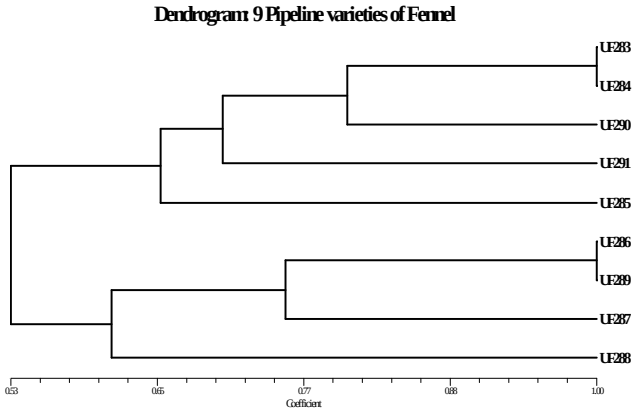

Fig. 5. Dendrogram of 9 pipeline varieties of fennel revealed by UPGMA cluster analysis.

subunit bands of region B and C were largely monomorphic. However the bands belonging to region A were relatively polymorphic. The subunit bands of pipeline varieties corresponding to 34-44 kDa were relatively thicker and intense as compared to those of released varieties although procedure of extraction of proteins was same.

With the observed subunit band polymorphism, the dendrogram for the released varieties revealed two major clusters (Fig.4). Cluster I was represented by 7 varieties where as cluster II by variety RF 125 only. It may be mentioned that variety RF125 was developed through half sib selection in an exotic collection 243380 from Italy (Singh *et al*., 2015). The 7 varieties of cluster I were indigenous and fell in two sub-clusters, Ia and Ib. Sub cluster Ia was represented by RF 205 alone whereas subcluster Ib by rest 6 varieties. Within sub-cluster Ib the dendrogram identified RF 157/ RF 1145 as identical and similarly RF 101/RF281 and RF178/RF143 as identical. It may be noted that RF 101 is the first fennel variety released for cultivation in the year 2002 for the Rajasthan state and was developed through recurrent half sib selection from a local collection of Sohela village in Tonk district (Singh *et al.,* 2015), whereas RF-281 was released in the year 2012 and developed through recurrent half sib selection from F_2 generation of cross between UF (M)-1 x JF-25. Thus identical seed storage protein profile may not necessarily reflect a common decent. Further, it is interesting to observe that both the variety RF 205 and RF 125 were diverse from each other in the present study but morphologically both are closely similar.

In case of pipeline varieties also, the dendrogram revealed two clusters (Fig.5). Cluster 1 was comprised of the lines UF-283, UF-284, UF- 290, UF-291, UF-285 whereas cluster II contained the rest 4 lines. In cluster-I, UF-283, UF-284 were identified as identical whereas in cluster-II UF-286 and UF-289 identified as identical.

The basic knowledge of genetic background of seed storage proteins comes from studies on *Arabidopsis thaliana* where the microarray analysis revealed that only 18 out of 2600 genes expressed in the development of seeds encode seed storage proteins (Mehrotra *et al*., 2008). In a recent review, Gacek *et al*., (2018) have reported that in *A. thaliana* there are 11 genes for synthesis of seed storage proteins (SSPs), 5 genes for accumulation of SSPs and 9 other genes confer transcriptional and hormonal regulations of SSPs accumulation. Thus the number of genes involved seems to be too few to generate differences among one variety to another in the same species. However, from the results of present investigation it is evident that (1) the procedure for extraction of proteins optimized in the present investigation was adequately dependable to probe out the differences between the developed varieties, (2) the selection skill and breeding methods employed in the development of varieties were effective and (3) the SDS-PAGE could be further used for characterizing a set of germplasm lines which is currently in progress in our laboratory.

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