SEED STORAGE PROTEINS PROFILING OF PAKISTANI DATE PALM (*PHOENIX DACTYLIFERA* L.) CULTIVARS FOR ESTIMATION OF GENETIC DIVERSITY THROUGH SDS-PAGE

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Abstract. Date palm (2n=36) is a dioecious monocotyledonous plant belonging to the highly heterogeneous and variable Arecaceae family. In irrigable desert areas date palm cannot be replaced due to its capability to withstand the unfavorable conditions where other fruit crops cannot survive. For date palm breeding, cultivar identification and diversity assesment are important factors. Genetic variations of 15 cultivars were assayed by SDS-PAGE from three different areas of Pakistan. It was credible to check the genetic variability and taxonomic convolution using seed protein profiling. Performance of seed storage protein revealed that Dedi (De) and Dhaki (Dh) were more divergent for exploring genetic diversity comparatively to other date palm cultivars. Begum jangi (Bj) and Khudrawi (Khu) were found different but Aseel and Toto were very closely related. Dendrogram based on electrophoretic analysis clusterd the date palm genotypes into 4 groups. The lowest similarity coefficient was among Assel, Muzawati and Haleeni (0.15) that revealed a distant relationship among these cultivars while the highest similarity was observed among Zaidi, Shamran and Eaden Shah (0.85). All cultivars were grouped on the basis of level of similarity and relatedness.
Keywords: *date palm, SDS-PAGE, polymorphism, identification, genetic variations, cluster analysis*

Introduction

Date palm (*Phoenix dactylifera* L.) is a major fruit crop of culticvated in arid and semi-arid regions, having critical economic significance for many countries including Pakistan.Date palm is a dioecious perennial, monocotyledon fruit tree and its heterogeneous genetic form makes its progeny strongly heterogeneous and variable (Ali et al., 2024; Ma et al., 2024). Renouned for human and animal nutrition, cultivated not only for its fruit but also tree parts are used in various industrial process i.e. packing

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material, timber, rope and furniture (Elhoumaizi et al., 2002; Wang et al., 2024). Pakistan is leading date palm producing country due to diverse cultivars adopted in unique agro-climatic conditions (Kelany and Yemiş, 2022). However, diversitry among these adopted cultivars in relations to origen of germplasm in still unexplored, very limited efforts has be in this regard (Li et al., 2024). There many techniques to assess the diversity among the availabe germpalsm, their utilization depends on availabe resources and experties, mostly based on structural protien (Zhang et al., 2024).

Generally variatal indification is carried out on the basis of morphological and molicular markers, but the results are not repoducable as infulences by enviomental conditions and cultural practices (Masmoudi-Allouche et al., 2009). However biochmeical analysis are considered more reliable and reproducable as compeared to morpholocial can molecular markers (Han et al., 2024; Niu et al., 2025). Similaerly, Yang et al. (2019) also found that biochemical markers particulalerly protien profiling is more reliable protocole to assess the genetice diversity among the cultivars. Furthermore, Biochemical markers (proteins and isozymes) have been effectively used for varietal characterization and identification (Yang et al., 2021; Kim et al., 2022). Genetic markers have been proven efficient and authentic for screening and detection of germplasm with limited time and labor (Capraro et al., 2008; Ahmad and Anjum, 2018). In order to find out the evolutionary and taxonomic relationship and genetic homology at molecular level, seed protein pattern attained from electrophoresis have been magnificently used. Patterns of seed storage proteins can be used effectively for cultivar identification and description for particular crop (Emre, 2009). They can also be used for distinguishing cultivars of particular crop species (Emre, 2009).

The most significant and economic fruit plant in Iraq is date palm; to assess genetic diversity among date palm existing germplasm molecular markers are used. The need for DNA extraction is not an observation but is a prerequisite, but in case of date palm high content of polyphenol and polysaccharides obstruct DNA extraction process which leads the researchers to find alternate and efficient methods (Hayee et al., 2020; Hzaa and Al-Amiry, 2021). Among the biochemical techniques, SDS-PAGE of seed storage proteins is extensively used due to its simplicity and effectiveness for describing the genetic structure of crop germplasm. SDS-PAGE has been measured to be realistic and consistent method due to independence of seed storage proteins on environmental fluctuations (Takáč et al., 2011). Among biochemical tools, SDS-PAGE is being extensively used to assess genetic diversity of available germplasm as it is a simple and effective tool. There three major areas where seed storage protein is being used i.e. (a) assessment of biodiversity among accessions of crop, (b) genomic relationship among accessions of specific crop and (c) germplasm conservation and parent selection for breeding programs. Seed proteins are independent of environmental fluxes which strengthen the applicability and reliability of SDS-PAGE (Stoyanova et al., 2011). Due to these advantages seed storage proteins will be used for the estimation of genome analysis and genetic diversity within and between the accessions.

Materials and methods

Fifteen date palm cultivar seeds were collected from Bahawalpur, Khairpur, Turbat and D.I.Khan as mentioned in *Table 1*. The collection cites are also mentioned in *Fig. 1*.

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Table 1. *Date palm cultivars along their collection sites*

Bahawalpur	Khairpur	Turbat	D.I.Khan
Dedi (De) Zari (Za) Shamran (Sh) Eaden shah (Es) Toto (To) Hillawi (Hl)	Pinari (Pin) Dhaki (Dh) Katch makran (Km) Aseel (Ase)	Haleeni (Hal) Mazawati (Maz) Begum jhangi (Bj)	Zaidi (Zai) Khudrawi (Khu)

Figure 1. *Map of sample collection sites in Pakistan*

Collected seeds were stored in refrigerator at 8°C until extraction. Ten seeds were individually analyzed by SDS-PAGE independently just to access the homogeneity of each accession (Ehsanpour et al., 2010).

The homogeneity of each accession was determined by using 10 seeds at the level of individual seed by SDS-PAGE independently (Ehsanpour et al., 2010; Liu et al., 2024). For each cultivar assessment was performed after ensuring the homogeneity of protein patterns. Seeds were properly cleaned and washed and for extraction of seed storage protein 15 seeds from each cultivar were grinded properly. After grinding into fine powder it was washed with 10x n-Butyl alcohol and vacuum dried. In 1.5 ml plastic eppendorf 100 mg seed powder from each bulk was added along with 1 ml 0.22 mol/L Tris-Hcl buffer having a pH of 6.8, mixed well and refrigerate at 4°C overnight (Alwhibi, 2017). This mixture was centrifuged for 10 minutes at 15000 rpm and supernatant was collected in another eppendorf, 200 µl supernatant was mixed with 800 µl (1:4 v/v) SDS-PAGE disruption mixture (2.5% β-mercaptoethanol, 5% glycerol, 125 mmol/L Tris HCl with pH 6.8, 1% SDS and bromophenol dye) (Laemmli, 1970). This sample mixture was heated at 95°C for 5 minutes than centrifuged 15000 rpm for 5 minutes and sample solution (concentration: 1.85-2 mg/ml) of storage protein was prepared for analysis with SDSS-PAGE (Howland, 1996). The concentrations of resolving and stacking gels were

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sustained at 5 and 4% to 11% gradient, respectively. The electrophoresis was performed at a constant voltage i.e. 50 V at beginning and followed by 25 V of 50 mA in protein gel apparatus under submerged mode of a tank buffer with the composition of 0.025 M Tris with pH 8.3, 0.1% SDS and 0.192 Glycine. 50 µl sample was loaded in each well and gel was fixed with TCA solution (10% w/v), after fixation gel was stained using 0.25% (w/v) coomasive brilliant blue and then 15 ml of 15% ethanol was added to de-stained the gel. In the last step 175 ml (5%) acetic acid and 60 ml distilled water was added to clear the background and finally it will become transparent. Before making the working solutions all the stock solutions were prepared. Gel was placed on transilluminator system and photographed using the DSLR cannon camera (EOS 1500D) with white transparent screen and during all this procedure gel is kept wet with the application of acetic acid (5%) to avoid the drying and air bubble trapping.

Statistical analysis

Each cultivar was analyzed by the presence (1) and absence (0) of band. The binary data matrix was used to calculate principle component analysis for accessing genetic diversity using PAST (Hammer and Khoshbakht, 2005) and Cluster analysis (Dendrogram) to compute genetic correlation and association between genotypes through PopGEN (Yeh, 1999).

Results and discussion

For analysis of seed storage proteins, bandding patttern resolved in 5 to 11 detectable peptides on SDS-PAGE. Performance of seed storage protein revealed that Dedi (De), Dhaki (Dh) and Khudrawi (Khu) were more divergent for exploring genetic diversity comparitivley to other date palm cultivars. Katch Makran (Km) and Mazawati were found different but Zaidi and Eaden shah (Es) were very closely related (*Fig. 2*).

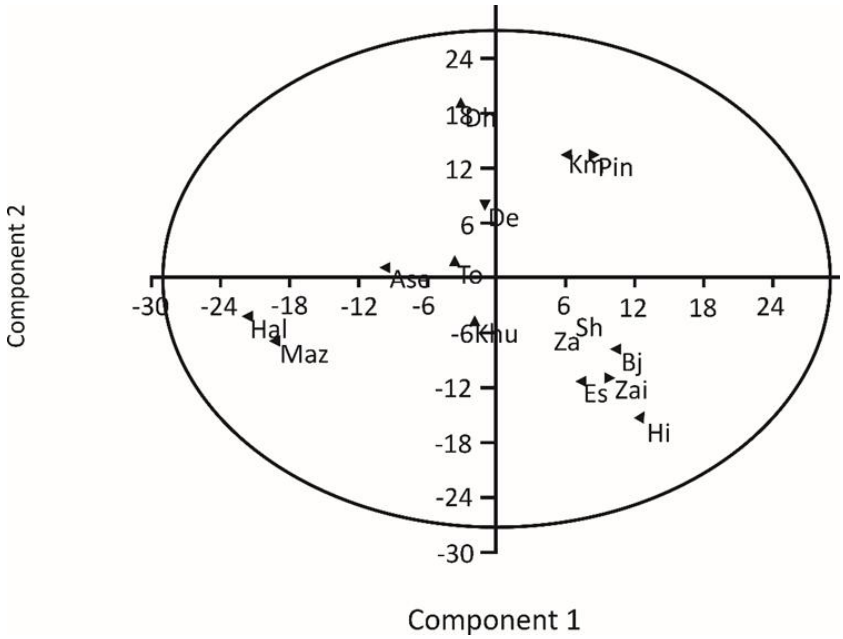

Figure 2. *Two dimensional PCA plot for 15 date palm cultivars*

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Dendrogram based on celectrophoretic analysis clusterd the date palm genotypes into 4 groups (*Fig. 3*). Genotype Dede (De), Kach makran (Km) and Pinari (Pin) showd close homology as grouped togather but genotype Dede (de) was different within this group. However, in second group Toto (To), Aseel (Ass) and Dhaki (Dh) genotypes were clusterd togather but Toto (To) and Aseel (Ass) showed narrow gene pool while Dhaki (Dh) had different genetic make up within this cluster. Three genotypes clusterd in group three i.e. Khudrawi (khu), Haleeni and Mazawati (Maz) however, Haleeni and Mazawati (Maz) were closely related. Group four comprised of six genotypes including Begum jangi (Bj), Zari (Za), Eden shah (Es), Zaidi (Zai), Shamran (Sh) and Hillawi (HI) where Eden shah (Es) and Zaidi (Zai) shared common gene pool, similarly Shamran (Sh) and Hillawi (HI) were closely associated while Begum jangi was different within this group.

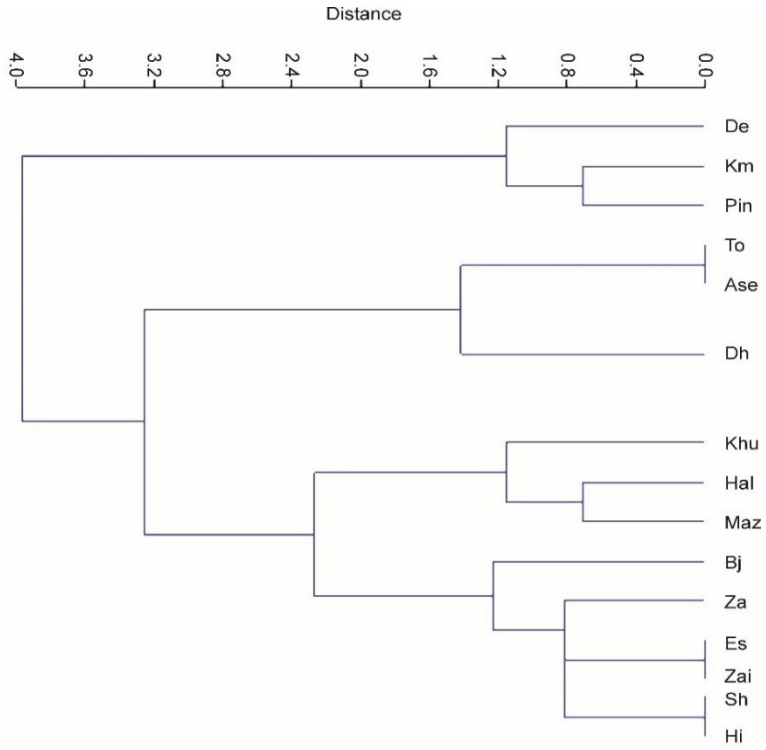

Figure 3. *Date palm genotypes clustered based on polymorphism in seed storage proteins*

According to the statistical analysis on the date palm in term of presence (1) and absence (0) of each band the similarity index between each genotype was evaluated. It varied from 1.00 to 0.154 (*Table 2*). Least value for similarity cofficient was among Assel, Muzawati and Haleeni (0.15) that revealed how far the relationship among these cultivars while highest similariy was observed among Zaidi, Shamran and Eaden Shah (0.85). All cultivars showed different levels of similarity but still were grouped with each other.

Protein electrophoresis is an influential tool for genetic analysis (Parker et al., 1998). As environmental fluctuations do not influenced the storage protein so SDS-PAGE technology is predominantly considered as most consistent and reliable tool for germplasm characterization (Javaid et al., 2004; Iqbal et al., 2005).

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Table 2. *Genetic similarity matrix among 15 date palm cultivars with Nei's and Lei's similarity indices using popgen (ver 1.44)*

	De	**Za**	**Sh**	**Es**	**Zai**	**BJ**	**HI**	**Pin**	**Dh**	**Km**	**To**	**Khu**	**Ase**	**Hal**	**Maz**
De	****	0.4286	0.8571	0.8571	0.5714	1.0000	0.7143	0.5714	0.7143	0.8571	0.4286	0.7143	0.8571	0.7143	0.7143
Za	0.8473	****	0.5714	0.5714	0.5714		0.7143	0.8571	0.7143	0.5714	0.7143	0.4286	0.5714	0.7143	0.4286
Sh	0.1542	0.5596	****	0.7143	0.4286	0.8571	0.5714	0.7143	0.5714	1.0000	0.5714	0.5714	0.7143	0.8571	0.5714
Es	0.1542	0.5596	0.3365	****	0.7143	0.8571	0.8571	0.7143	0.8571	0.7143	0.5714	0.5714	1.0000	0.8571	0.8571
Zai	0.5596	0.5596	0.8473	0.3365	****	0.5714	0.5714	0.4286	0.8571	0.4286	0.5714	0.8571	0.7143	0.5714	0.8571
BJ	0.0000	0.8473	0.1542	0.1542	0.5596	****	0.7143	0.5714	0.7143	0.8571	0.4286	0.7143	0.8571	0.7143	0.7143
HI	0.3365	0.3365	0.5596	0.1542	0.5596	0.3365	****	0.8571	0.7143	0.5714	0.7143	0.4286	0.8571	0.7143	0.7143
Pin	0.5596	0.1542	0.3365	0.3365	0.8473	0.5596	0.1542	****	0.5714	0.7143	0.8571	0.2857	0.7143	0.8571	0.5714
Dh	0.3365	0.3365	0.5596	0.1542	0.1542	0.3365	0.3365	0.5596	****	0.5714	0.4286	0.7143	0.8571	0.7143	0.7143
Km	0.1542	0.5596	0.0000	0.3365	0.8473	0.1542	0.5596	0.3365	0.5596	****	0.5714	0.5714	0.7143	0.8571	0.5714
To	0.8473	0.3365	0.5596	0.5596	0.5596	0.8473	0.3365	0.1542	0.8473	0.5596	****	0.4286	0.5714	0.7143	0.7143
Khu	0.3365	0.8473	0.5596	0.5596	0.1542	0.3365	0.8473	1.2528	0.3365	0.5596	0.8473	****	0.5714	0.4286	0.7143
Ase	0.1542	0.5596	0.3365	0.0000	0.3365	0.1542	0.1542	0.3365	0.1542	0.3365	0.5596	0.5596	****	0.8571	0.8571
Hal	0.3365	0.3365	0.1542	0.1542	0.5596	0.3365	0.3365	0.1542	0.3365	0.1542	0.3365	0.8473	0.1542	****	0.7143
Maz	0.3365	0.8473	0.5596	0.1542	0.1542	0.3365	0.3365	0.5596	0.3365	0.5596	0.3365	0.3365	0.1542	0.3365	****

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Estimation of genetic variations have always been an important tool for assessment and identification of gene pool, as a guide for genetic breeding and germplasm collection. For more comprehensive studies of cultivars its essential to identify the similarities and variances of cultivars by using different methods such as morphological characters, biochemical and cytological properties or protein and DNA markers (Mohamedahmed et al., 2024), so in this study we tried to characterize some Pakistani date palm genotypes more efficiently. Khoshroo et al. (2011) concluded that different cultivars exhibit the 'extreme' divergence in their genetic pool when examined through seed storage proteins. Similar trend was observed in 15 date palm genotypes evaluated using the seed storage proteins as different grouping and divergence was observed showing the variations among the genotypes.

Cluster analysis showed low level of inter-specific diversity without the clear differentiation for origin or agronomic characteristics as different clusters were observed with mixed genotypes from different origins (Ahmad and Slinkard, 1992; Ghafoor, 2002; Li et al., 2023) but different trend was observed in present study as clear difference was made for genotype origin on the basis of seed storage proteins. Seed storage protein can also be used for cultivars differentiation of particular species (Ladizinsky, 1979; Maqbool et al., 2021; Mohamedahmed et al., 2024).

Conclusion

It is concluded that electrophoresis (SDS-PAGE) of seed storage proteins is economically efficient for diversity estimation and germplasm differentiation. In the present study different cultivars exhibit the extreme divergence in their genetic pool when examined through seed storage proteins.

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