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GENETIC DIVERSITY IN COMMON OAK (*QUERCUS*) SPECIES USING SDS-PAGE

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ABSTRACT

Five Oak species found in Pakistan are *Q. baloot, Q. dilatata, Q. incana, Q. glauca* and *Q. semecarpifolia*. The seed storage proteins in these five species of Oak found in Pakistan were analyzed. Proteins were extracted at room temperature for about 20 minutes in extraction buffer containing 0.05 M Tris, 2.0 % SDS, 5 M Urea, and 1-2% beta mercaptoethanol. Gels were prepared in 30% Acrylamide solution having Acrylamide: bisacrylamide ratio of 30:0.8. One sample of *Quercus incana* collected from Shawer; Swat was identified where a protein band was missing indicating possibilities of previously miss identification of the species. More research work is needed for better understanding of the genetic structure of the species.

Key words: Oak, *Quercus*, Fagaceae, Seed storage Protein, SDS-PAGE, *Quercus incana*, Shawer Swat, Pakistan

INTRODUCTION

Oak belongs to family Fagaceae and genus *Quercus*. Fagaceae is comparatively small family comprising 8 genera and 900 species. The most important genus is *Quercus* which is represented by 450 diverse species (Nasir, 1976). Common Oak comprises of monoecious evergreen or deciduas trees, rarely shrubs. The wood of plant is tough and durable. It is generally used as fuel, making agriculture tools and/or medicinal purposes (Kamalak *et al*; 2004). The oak can take some 60 years to mature and produce its first full crop of fruit. Depending on seasonal conditions, tufts of pale green leave appear on short stalks (Knowles, 2002; Hussain *et al*., 2006). Locally species are called Tor banj or Serai, spin banj, Tor banj, kaner or mer and Rei, respectively (Shinwari *et al*., 2006; Khan *et al.*, 2007). Different species are differentiated by its fruits which consist of a nut encircled or enclosed in a hared woody involucres of many often spine tipped bracts with small swelling fruit oblong or ellipsoidal nut. The nut are 2-3 cm long depend on different species (Anonymous, 2007). Perianth is 3-5, locular. Fruits are partially enclosed in a capsule which is formed from hard scale. Acorns of the species are solitary or in pairs and grow to lengths of 3 inches (Gillis, 1971; Khosravi and Atoosa, 2006).

Most of the species of *Quercus* generally intercross resulting in a variety of intermediates. It is due to this genetic and species diversity that oaks have got a wide range of ecological adaptation (Ahmad, 1999; Bruce *et al.*, 1974). They can be found from the far North into the Western tropical parts of South America. Some species are native to the temperate and tropical Europe and Asia, and others are found in North and South Africa (Bhopal and Chaudhri, 1977). Like any other plants, in oak prior knowledge of existing genetic variability is of prime importance for improving and conservation. Characterization of genetic diversity in plant species has long been based mainly on morphological traits. However, morphological variation is often found to be of limited use because of (i) limited number of morphological characters and (ii) expression of morphological traits may be affected by environmental conditions, thereby constraining the analysis of genetic variation (Shah, 2002). Later biochemical and cytological markers were utilized for identification of existing genetic variability in plants of commercial importance (Islam and Shepherd, 1991; Pavlic *et al*., 1991).

These biochemical and cytological markers though successful in tagging of many useful genes and/or characterization of germplasm were not considered suitable for large scale screening (for example screening of segregating populations) mainly because of limited number of such markers in species of commercial importance (Bretting and Widrlechner, 1995; Collada, 1988).

During recent years, molecular biology has emerged as more powerful tool and complementary strategy to traditional approaches in the management of plant genetic resources and improvement (Ayad *et al*., 1997; Rao *et al.*, 1992; Das and Mukharjee, 1995). Seed storage proteins profile using Sodium Dodycyle Sulphate Poly Acrylamide Gel Electrophoresis is commonly used to study structure in many species including wheat, legumes and trees (Soria *et al*., 2004; Lioi *et al*., 1999; Ferreira *et al*., 2000).present work is first documented attempt to analyze seed storage protein profile in Oak species commonly found in Pakistan (Sultan-ud-din, 2007).

MATERIALS AND METHODS

Specimens from five oak (*Quercus*) species viz. *Quercus baloot*, *Quercus dilatata*, *Quercus incana*, *Quercus glauca* and *Quercus semecarpifolia* were collected from Northern areas of Pakistan and AJK. Mature seeds were collected from each species and were used to extract seed storage protein. For Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis, single seed from each species were taken and seed coat or testa from each seed was removed. The seeds were placed in oven over night at 37^0 C to remove the water content of the seed. The Dry seeds of each genotype were ground to a powder with the help of mortar and pestle. Four hundred µl of protein extraction buffer (0.05 M Tris, 2.0 % SDS, 5 M Urea, 1-2% beta mercaptoethanol) was added to 0.01g of seed flour and vortexed thoroughly to homogenize. The proteins were extracted at room temperature for 20 minutes. In order to purify, the homogenate samples were centrifuged at 12,000 rpm for 10 minutes at room temperature. The extracted crude proteins were recovered as clear supernatant and were transferred to a new 1.5 ml Eppendorf tubes and stored at 4^oC until they were run on the Poly acryl amide gel. In an attempt to optimize the Sodium Dodyecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) protocol suitable for Oak seed, different protocols were tested. For example the protocols used previously by (Payne, 1987) for protein analysis of wheat and (Lioi *et al*., 1999) for chickpea (Cicer) were used using modifications. In total 13 different Gels were run, using different modifications of the protocols. The protocol that yielded best results is summarized as; the electrophoretic procedure was carried out using slab type SDS-PAGE Model: MGV-202, with 12.5% polyacrylamide gel. A 12.5% resolving gel (3.0M Tris-HCL (Sigma) pH 9, 0.4% SDS (Wako) and 4.5% stacking gel (0.4M Tris-HCL pH 7.0, 0.4% SDS) polymerized chemically by addition of 17 µl of N, N', N', N' tetramethylene diamine and 10% Ammonium persulphate. Then electrode buffer solution (0.025 M Tris, 1.29 M Glycine (Sigma), 0.125% SDS) was added to the top pool of the apparatus.12-15µl of the extracted protein were loaded with the micropipette into the wells of the gels. The apparatus was connected with constant electric supply and electric current of 70V was used. The gels were run till the tracking dye "bromophenol blue" (BPB) reaches the bottom of the gel (Makkar *et al*., 2004). Gels were stained using 0.2% (W/V) Coomassie Brilliant Blue-R 250 dissolved in 10% (V/V) acetic acid, 40% (V/V) methanol and water in the ratio of 10:40:50 (V/V) for about an hour at room temperature. Gels were distained in a solution containing 5 % (V/V) acetic acid and 20% (V/V) methanol. After de-staining the gels were photographed using "Uvitec" gel documentation system. (Sultan-ud-din, 2007).

RESULTS AND DISCUSSION

Analysis of total seed storage proteins has been used since few decades for better understanding and ultimately utilization of useful genes in crops of commercial importance, including wheat, Brassica, maize etc. Similar techniques have also been used in various plant species of economic importance. Unfortunately such kinds of studies have been limited in Pakistan. For example Oaks are very important plants of Northern areas of Pakistan and a number of species have been cultivated in these areas since centuries. But no report has yet been documented where seed storage protein profiles in oak have been analyzed. Present study is the first documented attempt to study variation in total seed proteins of oak.

Because no previous report has been found in literature regarding extraction and separation of seed storage proteins in oak in Pakistan, initial attempts were made to optimize protein extraction and gel electrophoresis (protein separation) procedures suitable for indigenous species of oak. In the literature, many procedures for extraction of total seed storage proteins have been described. Two main procedures utilized during present study included (1) seed protein extraction procedure described by Sambrook *et al*., (1989) for general purpose and (2) procedure described by Payne (1987) and Soria *et al*., (2004)) for isolating High Molecular Weight Glutenin subunits of wheat.

It was found that when using protein extraction procedure developed by Payne (1987) and Soria *et al*. (2004), Jorge *et al. (*2004). No protein was extracted from oak seeds (because no protein bands were visible after SDS-PAGE. After failure of extracting the seed storage proteins using Payne and Soria procedure, procedure described by Sambrook *et al*. (1989) and Laemmli (1970) was employed to extract total proteins from oak seeds. After extraction, the proteins were separated on 12.5% Polyacrylamide gels. Initially a single protein band (of very low mobility) per sample was observed. Same gel was repeated for a shorter time (gel running continued at constant voltage of 70 V for 1 hour only as compared to previous gel (which was run at constant voltage of 70 V for 3 hours). The modification was made with an assumption that there might be some bands of low (or very low) molecular weight which might have migrated out of the gel during longer running. Similar results were obtained, only a single band was observed. In a further attempt to resolve the problem, extraction protocol was modified once again. Samples were treated at 100^O C for 10 minutes after extraction of proteins from oak seeds. Rest of the conditions for electrophoresis was kept same as described. But heat treatment of the protein extracts did not produce any useful

polymorphism. A further improvement /alteration in the protocol were made by using higher concentration of bis-acrylamide (10X) was used in stacking and separating gels.

Several modifications were made in extracting seed storage proteins, gel compositions and/or gel running conditions to obtain useful polymorphism in total seed storage protein profile of locally grown oak species. Finally modified protocol was optimized giving high resolution, reproducible and useful polymorphism (Fig.1) "Proteins were extracted at room temperature for about 20 minutes in extraction buffer containing 0.05 M Tris, 2.0 % SDS, 5 M Urea, 1-2% beta mercaptoethanol. Gels were prepared in 30% Acrylamide solution having Acrylamide: bisacrylamide ratio of 30 : 0.8. Gel running conditions were: constants voltage of 70-V for approximately 3 hours (or until tracking dye Bromophenol blue) reached the bottom of the gel. The described procedure yielded uniform, high resolution useful protein banding pattern. A Polymorphic band (indicated by arrow in Fig. 1) for *Quercus incana* collected from Shawer, Swat was observed. This band was found missing in *Quercus incana* collected from Shawer, Swat but present in *Quercus incana* collected from Derai, Swat and all the other *Quercus* species collected from Swat and Muzzafarabad (*Quercus baloot*, *Quercus dilatata*, *Quercus semecarpifolia*).To make sure about the findings, the gel was run with minor modifications and modified gels also confirmed the finding. It is inferred that it may be because of inter specific hybridization of various species or it may be the species has been miss identified previously. More research work is needed for better understanding of the finding(Sultan-ud-din; 2007).

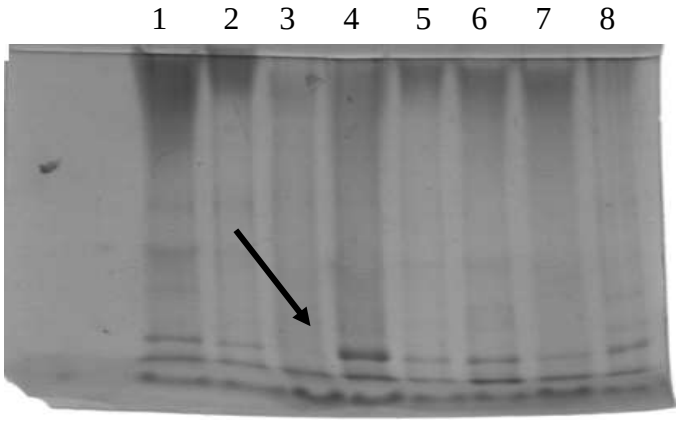

Fig.1. SDS-PAGE of total seed storage proteins from Oak seeds using optimized protocols. A Polymorphic band for *Quercus incana* collected from Shawer, Swat is indicated by arrow. 8= *Quercus baloot* (Marghuzar, Swat), 7= *Quercus baloot* (Kabal, Swat), 6= *Quercus dilatata* (Onara Derai, Swat), 5= *Quercus dilatata* (Shawer, Swat), 4= *Quercus incana* (Miandam, Swat), 3= *Quercus incana* (Shawer, Swat), 2= *Quercus incana* (Onara Derai, Swat), 1= *Quercus semecarpifolia* (Dandosare Doph, Swat).

REFERENCE

Ahmad, H. (1999). Oak species and their Distribution in Pakistan. Environmental protection society (EPS). *Udyana Today* 6: 5-7.

Anonymous. (2007). Oak and their distribution. Wikipedia.org/wiki/oak.

Ayad, W. G., T. Hodgkin, A. Jaradat and V. R. Rao (1997). Molecular genetic techniques for plant genetic resources. Proceeding of IPGRI workshop, International Plant Genetic Resources Institute, Rome: 9-11.

Bhopal, F.G., and M.N.Chaudhri. (1977). Flora of Pothohar and the adjoining Area. Pakistan systematic.2: 18-22.

Bretting, P.K. and M.P. Widrlechner. 1995. Genetic markers and plant genetic resources. Plant Breeding Reviews. 13: 11-86.

Bruce, L., R. D. Anderson, V. W. Pieper, and J. Howard (1974). Growth Response and Deer Utilization of Fertilized Brows. *The Journal of Wildlife Management*. 38:525-530.

Collada, C., R. G. Caballero, R. Casado and C. Aragoncillo (1988). Different Types of Major Storage Seed Proteins in Fagaceae Species. Journal of Experimental Botany. Oxford Journals *Oxford University Press*: 1751-1758.

Das, S. and K.K. Mukharjee (1995). Comparative study of seed proteins of *Ipomoea*. Seed Science and Technology. 23: 501-509.

Ferreira, J.J., E A. Lvarrez, M.A. Roca and R. Giraldez (2000). Determination of the out crossing rate of *Phaseolus vulgaris* L. using seed protein markers. *Euphytica*, 113: 259-263.

Gillis, W. T. (1971). *The Systematic and Ecology of Poison Ivy and the Poison Oak.* Rhodora.Houghton Mifflin, Boston : 419.

Hussain, F., M. Islam, and A. Zaman (2006). Ethnobotanical profile of plants of Shawer Valley, District Swat, Pakistan. *International Journal of Biology and Biotechnology,* 3: 301-307.

Islam A.K.M.R. and K.W. Shepherd (1991). Alien genetic variation in wheat improvement. In: *Chromosome engineering in plant: Genetics, Breeding and Evolution*. Vol. A. (Gupta, P.K. Tsuchiya, T. eds). Elsevier Science Publisher, Amsterdam: 291-312.

Jorge, I., R. M. Navarro, C. Lenz, D. Ariza, C. Porras and J. Jorrín (2004). The Holm Oak leaf proteome: Analytical and biological variability in the protein expression level assessed by 2-DE and protein identification tandem mass spectrometry *de novo* sequencing and sequence similarity searching. *Wiley inter science,* 5: 222 – 234.

Kamalak,A., O. Canbolat, O. Ozay and S. Aktas (2004). Nutritive value of Oak *(Quercus* species) leaves. *Small Ruminant Research,* 53: 161-165.

Khan, S.M., H.Ahmad, M. Ramazan and M. M. Jan (2007). Ethno medicinal plant resources of Shawer Valley *Pakistan Journal of Biological Sciences,* 10: 1743-1746.

Khosravi, A. D. and Atoosa (2006). Evaluation of the antibacterial activity of the seed hull of *Quercus* brantii on some gram-negative bacteria. *Pakistan Journal of Medicinal Science*, 22: 429-432.

Knowles, G. (2002). *In Worship of Trees. The Oak tree.* Controverscial.com/oak.htm.

Laemmli, U.K. 1970. Cleavage of structural protein during the assembly of the head of bacteriophage. T4. *Nature,* (London). 227: 680-685.

Lioi, L., F. Sparvoli and R. Bollini (1999). Variation and genomic polymorphism of lectin-related protein in Lima Bean (*Phaseolus lunatus* L). *Seed Genetic Resources and Crops Evaluation*, 46:157-182.

Makkar, H. P. S., L. Niemeyer, G. L. Henson, G. Ansong, R. Forkner and A. E. Hagerman (2004). A modified method for determining protein-binding capacity of plant polyphenolics using radiolabels protein. Department of Chemistry and Biochemistry, Miami University, Oxford, OH 45056, USA. *Photochemical Analysis*, 15:159-163.

Nasir, Y. (1976). *Family Fagaceae. Flora of West Pakistan*. N. H. Agri. Res. Council, Rawalpindi.104: 1-9.

Pavlic, B. M., P. C. Muick, S. G. Johnson, and M. Popper (1991). *Oak of California*. Cachuma Press: 10-12.

Payne, P.I. (1987). Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. *Ann. Rev*., 38: 141-153.

Rao R., M. Del Vaglio, M.D.U. Paino and L. Monti (1992). Identification of *Vigna* species. through specific seed storage polypeptides. *Euphytica*, 62: 39-43.

Sambrook, J., E. F. Fritsch and T. Maniatis (1989). *Molecular cloning: A laboratory manual*. 2nd ed. Cold. Spring. Harbours. Lab. Press, New York.

Shah, S. T. (2002). *Taxonomic details of the Quercus species. Palynological investigation of some Angiosperms of Pakistan*. M.Sc thesis. Dept of Bot. Govt PostGraduat Jehanzeb College. Swat: 45-48.

Shinwari, Z. K., T. Watanabe, M. Rehman and T. Yoshikawa (2006). *Family Fagaceae. A pictorial Guide to medicinal plants of Pakistan*. KUST, ISBN: 351-352.

Soria M. A., I. A. Khan, J. A. Anderson, B. Guedira, K. G. Campbell, A. K. Fritz, B. S. Gill, K. S. Gill, S. Haley, S. F. Kianian, K. Kidwell, N. L. Lapitan, H. VOhm, J. Sherman, M. E. D. Sorrells, E. Souza, Talbert and Dubcovsky (2004). The MAS wheat project: Bringing genomics to the wheat fields. Proceedings of the Plant and Animal Genome XII Conference, January 2004, San Diego, CA., USA, Poster. 410:173

Sultan-ud-din. (2007). *Studies on Genetic Diversity in common Oak species using SDS-PAGE*. M. Phil thesis. Department of Botany, Hazara University, Mansehra.54

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