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Inter and Intra-specific Variation in SDS-PAGE Electrophoregrams of Total Seed Protein in Wheat, Barley and Their Wild Species

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Abstract: A total of 108 accessions of *Triticum*, *Aegilops* and *Hordeum* species collected from Central Asia were examined for genetic diversity in total seed protein using SOS-PAGE. Based on the electrophoretic pattern of the molecular weight range higher than 24 KD, clear inter-specific variation was observed in the genus *Aegilops* and *Hordeum.* A wide range of genetic variation was observed among cultivars of bread wheat. Intra-specific variation was also found in cultivated barley as well as in *H. Spontaneum.* It is suggested that electrophoresis of total seed protein is a useful and effective method to analyze genetic variability in plant genetic resources.

Key words: Genetic diversity, storage protein, electrophoresis, landraces, wheat and barley

Introduction

Genetic diversity in germplasm collections is fundamental to broadening the genetic base of cultivated crops. Assessment of genetic diversity in germplasm provides the basis for its utilization in crop improvement programmes. The data on agronomic, morphological and physiological plant traits are generally used to estimate the magnitude of genetic diversity present in the germplasm. However, such data may not provide an accurate indication of genetic diversity because of environmental influences upon the expression of observed traits. Moreover field evaluation of plant material is often laborious and time consuming especially, when a large number of accessions are to be analyzed. Considering these difficulties, biochemical markers such as isozyme or storage proteins have received more attention in recent years from the crop geneticists for assessment of genetic variability in germplasm collections (Nevo and Payne, 1987; Perry *et al*., 1991; Ciaffi *et al*., 1993). A large number of germplasm lines can be characterized for biochemical marker in a short period of time. In addition, the data reflect more truly the genetic variability as biochemical markers are direct product of gene and their expression is not influenced by the environments.

The analysis of storage protein variation in wheat have proved to be a useful tool not only for diversity studies but also to optimise the variation in germplasm collections and to breed cultivars with improved bread making quality (Lawrence and Shepherd, 1980; Payne *et al*., 1983; Shewry *et al*., 1989). Nevo and Payne (1987) studied diversity of HMW glutenin subunits in tetraploid wild progenitor of wheat. They observed that endosperm of wild emmer (*Triticurn dicoccoides*) contains many allelic variants of glutenin storage protein that are not present in bread wheat and could be utilized in breeding varieties with improved bread making qualities. Remarkable allelic diversity was also found in hordein storage protein of wild barley, *Hordeum spontaneum* (Nevo *et al*., 1983). Several accessions of *Aegilops squarrosa,* the progenitor of the D genome of bread wheat have been analyzed for HMW-GS based on SOS-PAGE by Lawrence and Shepherd (1980).

The objective of this investigation was to standardized the protocol of SDS-PAGE for wheat, barley and their wild species and to determine the extent of genetic diversity present in the material collected from Central Asia.

Materials and Methods

Plant material: Material used in the present study comprised of 108 accessions which includes *Triticum aestivuml* 201, *Aegilops* (50) and *Hordeum* (38) species collected from Central Asia (Fig. 1) by the joint expedition mission of National Institute of Argo-biological Resources, Tsukuba, Japan and Vavilov. All Russia Plant Production Research Institute (NIAR/VIR) during 1993. The range of altitude explored was from 100 to 2300 ma.s.l.

Protein extraction and sample preparations: Total seed protein was extracted from 10 mg of wheat flour with 0.5 ml of 0.05 M Tris-HCl buffer (pH 8.0) which has 0.2% SDS and 5 M Urea. To this samples extraction buffer, 5 μl of 2-mercaptoethanol was added, thoroughly mixed and cetrifuged for 5 minutes. Just before the electrophoresis, 10 μl of extract solution was applied into each well with a microsyringe.

Electrophoresis: The total seed protein was analyzed through the slab type SOS PAGE following the method of Laemmli (1970) using 11.5% polyacrylarnide gel. The electrophoresis for the first 30 minutes was performed at 100 V and 150 V for another 2 hours. All the gels were stained with coomasie brilliant blue (0.225%) for about 2030 minutes and then destained in a 5% methanol-20% acetic acid until the color of back ground disappeared and the electrophoretic bands are clearly visible. After destaining, gels were dried using Gel Drying Processor for about 100 minutes. The experiment was conducted at National Institute of Agro-biological Resources, Tsukuba, Japan.

Results and Discussion

Geographical distribution and habitats of the seed samples used in this experiment are given in Fig. 1, After electrophoresing all the samples, it was observed that there is a wider variation in the electrophoretic pattern of seed storage protein of wheat, barley and their wild species collected from Central Asia.

Genetic variation in wheat and their wild relatives: Proteins extracted from pulverized seeds were analyzed by

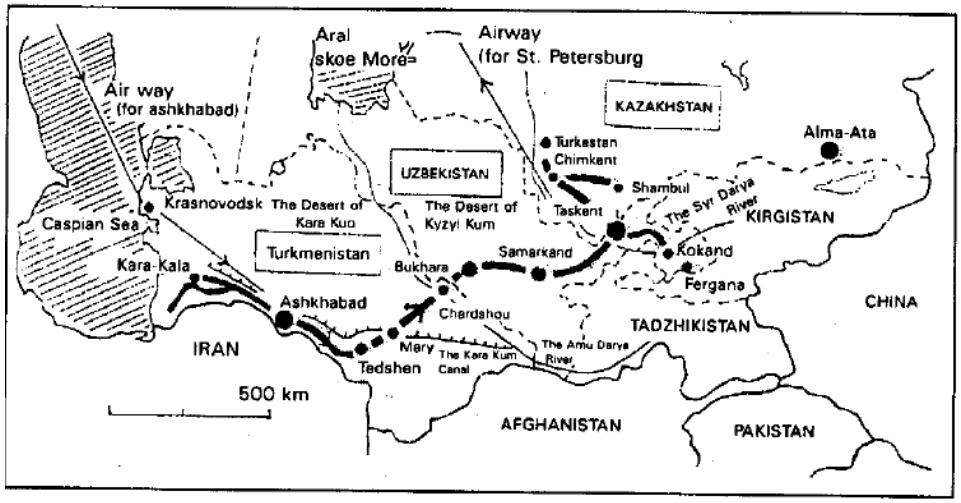

Fig. 1: A map showing exploration route in central Asia

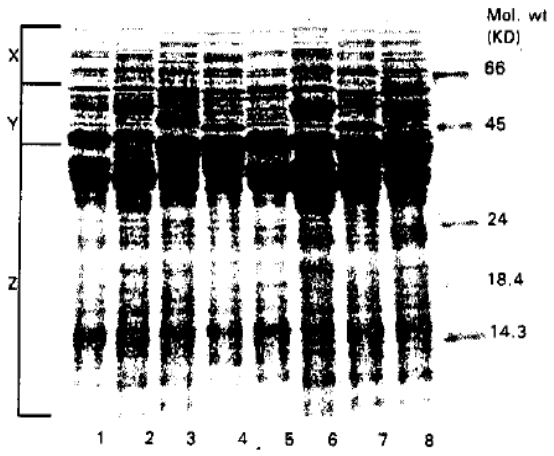

Fig. 2: Interspecific veriation in SDS-PAGE electrophoregrams of total seed protein from *Aegilops* species. Left to right *Ae. squarrosa* (1, 5); *Ae. triunclelis* (2, 6); *Ae. crassa* (3, 7); *Ae. juvenalis* (4, B)

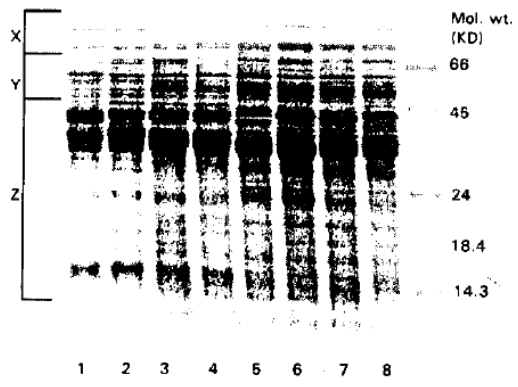

Fig. 3: Interspecific veriation in SDS-PAGE electrophoregrams of total seed protein from *Aegilops* species. Left to right *Ae. squarrosa* (1, 4); *Ae. triunclelis* (5-8)

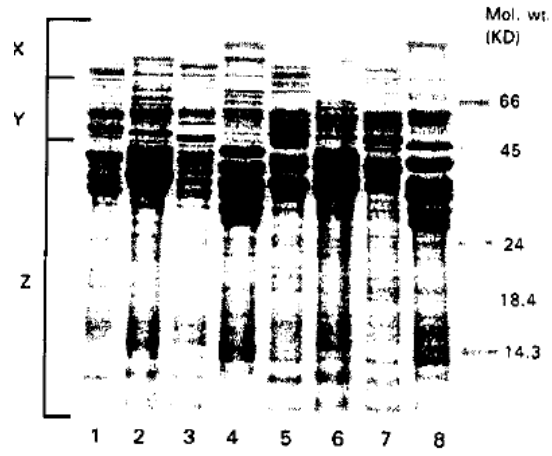

Fig. 4: Interspecific veriation in SDS-PAGE electrophoregrams of total seed protein from *Hordeum* species. Left to right: *H. spontaneum* (1, 5); *H. bulbsoum* (2, 6); *H. murinum* (3, 7); *H. murinum* (4, 8)

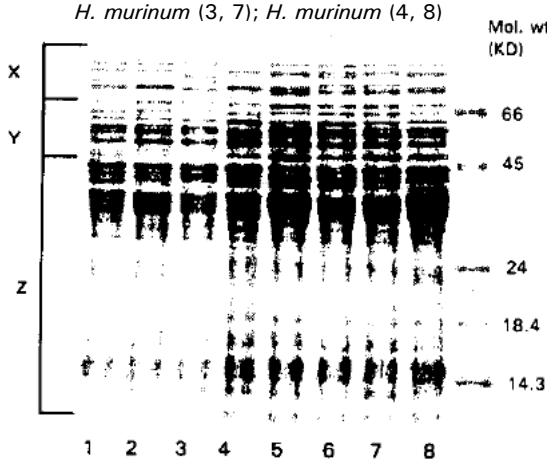

Fig. 5: SDS-PAGE variation of total seed protein from eight accessions of hexaploid wheat *Triticum aestivum*

SDS-PAGE after Laemmli (1970). The electrophoregrams were divided into three regions X, Y and Z. Both X and Y regions represent variation in high and low molecular weight subunits of glutenin, respectively. As the banding was apt to be ambiguous in the Z region of molecular weight lower than 24 KD, we investigated the intra-and inter-specific variation based on the electrophoretic pattern of the molecular weight range higher than 24 KD. Clear inter-specific variation was observed in *the genus Aegilops* (Fig. 2). Whereas intra-specific variation was also found in A*egilops squarrosa* and *Aegilops triuncialis* (Fig. 3). Diversity within species is the main concern of genetic resources programs and key to the survival of species in nature in the long term. A wide range of genetic variation were also recognized among cultivars of wheat (Fig. 5). By the application of high resolution SDS-PAGE considerably more variation was observed in the HMW subunits by Galili and Feldman (1985). Lawrence and Shepherd (1980) evaluated several diploid species and observed much wider genetic variation of HMW glutenin in *Aegilops squarrosa* which is well known as the D genome donor to hexaploid wheat. The diversity present in *Aegilops squarrosa* will be of particular interest for the breeders to develop varieties with high quality protein and resistance against biotic and abiotic stresses.

Genetic variation in barley and its wild relatives: The HMW prolamines are called D hordein in barley. SOS-PAGE electrophoregrams were also divided into three regions X. Y and Z. Both X and Y region exhibited a wide range of variation between *Hordeum* species. The variation found in the Z region was quite ambiguous like in wheat, therefore, we were mainly concerned with the variation observed in molecular range higher than 24 KD. Bands with different mobilities and intensities were observed (Fig. 4).

The intra-specific genetic variation was observed in *H. spontaneum* as well as in cultivated barley. *Hordeum spontaneum* is recognized an immediate progenitor of cultivated barley and is fully compatible with *Hordeum vulgate.* The genetic variation present in *Hordeum spontaneum* will be of great importance for the breeders to develop barley cultivars with desirable characteristics. Nevo *et al*. (1983) found large variation in the hordein subunits similar to that of gluten storage proteins in wild wheat (*Triticum dicoccoidesl.*

Overall, our results shows that wheat, barley and their wild relatives in the Central Asian region explored harbor a broad range of genetic variation in terms of their total seed protein. This area is closed to the Southwest Asia, the primary centre of diversification for wheat/barley and coincides with the route whereby these crops migrated to the East. *Aegiolps squarrosa,* one of the ancentral species of wheat, *Hordeum spontaneum* and original type of barley also distribute in the region. Diversity present in this material can be exploited to broaden the genetic base of wheat'and barley cultivars.

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