Development of Genetic Stocks and Biochemical Markers to Facilitate Utilization of *Aegilops variabilis* in Wheat Improvement

M. D. H. M. William and A. Mujeeb-Kazi*

International Maize and Wheat Improvement Center (CIMMYT), Lisboa 27,
Apartado Postal 6–641 06600 México, D.F., Mexico

Accepted September 6, 1995

Aegilops variabilis ($2n=4x=28$, UUSS) accession 13E is resistant to Karnal bunt (Warham *et al.* 1986). With the objective of transferring this attribute to wheat (*Triticum aestivum* L.; AABBDD, $2n=6x=42$) we have a dual program underway to (i) produce disomic chromosome additions of *Ae. variabilis* in *T. aestivum* and (ii) manipulate homologous group introgressions from *Ae. variabilis* to wheat through the recessive *ph* locus of *T. aestivum* cv. Chinese Spring initiated at the F_1 hybrid stage, advanced by backcrosses to elite wheat cultivars and ending with genetic stability. For both situations to materialize, it is necessary to have a wide range of diagnostic markers for detecting alien chromosomal presence, establishing homoeology and eventually tracking cryptic alien genetic introgressions. Though cytological and/or morphological parameters have been used to establish homoeology (Mujeeb-Kazi and Asiedu 1990) a more effective way is by utilizing biochemical markers; of which proteins and isozymes are an option. We have conducted these biochemical analyses on the amphiploids of *T. aestivum*/*Ae. variabilis* and *T. turgidum*/*Ae. variabilis* through the application of seven markers; high molecular weight glutenin (HMW-Glu), glucose phosphate isomerase (GPI), seed esterase (EST-5), 6-phosphogluconate dehydrogenase (6-PGD), β -amylase (β -AMY), glutamate oxaloacetate transaminase (GOT) and α -amylase (α -AMY). These results form the basis of this paper.

Materials and methods

Germplasm. Seeds of *Aegilops variabilis* ($2n=4x=28$, UUSS), CIMMYT Wide Crosses accession 13E, were the source for biochemical analyses and hybridization following the prior determination of its Karnal bunt resistance status (Warham *et al.* 1986). A composite of 5 seeds were analyzed for HMW-glutenins, GPI, EST-5, 6-PGD, β -AMY and α -AMY. Leaf extracts from young seedlings were analyzed for GOT. *Triticum aestivum* cvs. Chinese Spring (CS), Alondra/Pavon "S", *T. turgidum* cv Laru "S", amphiploids of the *T. aestivum* and *T. turgidum* cultivars with *Ae. variabilis* ($2n=10x=70$ and $2n=8x=56$, AABBDDUUSS and AABBUUSS) were additionally utilized in the study. The amphiploids would determine whether the biochemical markers specific to *Ae. variabilis* were expressed in the wheat backgrounds.

Enzyme extraction. Mature seeds were used for all systems except for GOT and α -AMY that utilized young leaves from rapidly growing seedlings (GOT) or 6 day old germinating seeds (α -AMY) respectively. Individual bread wheat, durum wheat and *Ae. variabilis* seeds were ground and extracted with 200 μ l of 0.01 M Tris-HCl (pH 7.5) for GPI and 6-PGD. The extraction buffer for β -AMY was 0.01 M Tris-HCl (pH 7.5) with 0.2 M 2-mercaptoethanol and

* Corresponding author: A. Mujeeb-Kazi.

10% sucrose, of which 500 μ l was utilized to extract the mature kernel. GPI and EST-5 extractions were done at 4°C for 1 hr, while those for 6-PGD and β -AMY were overnight at 4°C. For α -AMY the 6 day old germinating seeds were extracted with 0.5 M Tris-HCl (pH 7.5) + 0.002 M CaCl_2 . The extracts were heated for 10 min at 70°C to inactivate β -AMY. Leaves from young, fast growing seedlings were macerated with sand by a mortar and pestle, extracted with 0.05 M Tris-HCl (pH 7.5) containing 10% sucrose and immediately used for GOT analysis. All samples were centrifuged for 2 min at room temperature prior to electrophoresis.

Isoelectric focusing. Polyacrylamide gel isoelectric focusing (IEF) was used for EST-5, GPI, α -AMY and β -AMY. EST-5 and GPI isozyme separations were on pH 3.5–9.5 gels while those of α -AMY and β -AMY were on pH 4.0–6.5 gels. The anode/cathode buffer solutions and the electrophoretic details were similar to those previously described (William and Mujeeb-Kazi 1993).

Native polyacrylamide gel electrophoresis. Discontinuous native polyacrylamide gel electrophoresis (native-PAGE) was used for the separation of 6-PGD, GOT and β -AMY isozymes. Running gels were 8.5% for 6-PGD and β -AMY, and 6% for GOT. A 5% stacking gel was used for all three systems. The seed or leaf extracts were mixed with 0.005% bromo-phenol blue prior to electrophoresis, of which 30 μ l were utilized for electrophoresis using gel composition and running buffer protocols similar to those of Davis (1964). Electrophoresis was conducted on 1.5 mm thick gels, dimension 180 $\times$ 160 mm, on a Hoeffer vertical electrophoresis unit at 25 mA/gel constant current with a temperature set at 4°C until the blue dye front reached the end of the gel.

SDS-PAGE. High molecular weight glutenin sub-units were separated on 8.5% polyacrylamide gels in the presence of sodium dodecyl sulfate (SDS-PAGE) with seed extraction and gel composition details similar to Payne *et al.* (1980). Three percent stacking and 8.5% running gels were used on a vertical Hoeffer electrophoresis unit with the same dimensions as for native-PAGE. The electrophoretic run was conducted at room temperature at 5 mA/gel for 2 hr followed by 10 mA/gel, until the dye front reached the end of the gel. The gels were rinsed in 30% ethanol for 30 min to remove SDS and stained overnight in a 0.1% Coomassie brilliant blue R-250 + 50% methanol + 10% acetic acid solution. Gels were destained in a 40% methanol + 10% acetic acid solution and stored in 10% acetic acid.

Enzyme staining. Staining for EST-5, GPI, α -AMY and β -AMY was according to William and Mujeeb-Kazi (1992) with the two AMY procedures being similar. The GOT isozyme was stained in 50 ml 0.01 M Tris-HCl (pH 7.4) containing 100 mg pyruvic acid, 50 mg α -keto-glutamic acid with a well mixed 5 mg of pyridoxyl-5-phosphate + 75 mg fast blue BB salt addition just prior to staining. The 6-PGD staining solution comprised of 50 ml Tris-HCl (pH 7.4) containing 10 mg 6-phosphogluconic acid, 0.5 mg NADP, 10 mg MTT and 1 mg PMS.

Results and discussion

High molecular weight glutenins (HMW-Glu). Identification and characterization of different allelic forms of high molecular weight glutenin subunits in bread wheat is important because of its implications on bread making quality. These forms are well characterized (Payne and Lawrence 1983). *Ae. variabilis* possessed two bands in the HMW-Glu region of lower mobility than the most common slow moving HMW-Glu subunits of bread wheat (Fig. 1). These two lower mobility bands were also expressed in the wheat/*Ae. variabilis* amphiploids. The HMW-Glu genes are located in the long arms of homoeologous group 1 wheat chromosomes (Beitz *et al.* 1975, Lawrence and Shepherd 1981). The two bands observed could therefore serve as markers for characterizing *Ae. variabilis* chromosomes homoeologous to

Fig. 1. Separation of high molecular weight glutenins of SDS-PAGE (8.5%) gels of *Triticum aestivum* cv. Chinese Spring (CS), cv. Alondra/Pavon (AP), *T. turgidum* cv. Laru (L), *Ae. variabilis* (Ae) and the amphiploids of CS/*Ae. variabilis* (1), AP/*Ae. variabilis* (2) and L/*Ae. variabilis* (3). Diagnostic marker bands are indicated by arrowheads.

group 1 of both bread and durum wheat.

Glucose phosphate isomerase (GPI). Glucose phosphate isomerase separation on isoelectric focusing gives 8 to 10 bands in bread wheat and 4 to 5 bands in durum cultivars (Chojecki and Gale 1982). Bread wheat cultivars have a distinctly different banding pattern than *Ae. variabilis* (Fig. 2). Both bread and durum wheat amphiploids with *Ae. variabilis* coexpress the wheat and *Ae. variabilis* bands. This marker can thus be utilized to characterize *Ae. variabilis* chromosomes and establish homoeologous relationships with wheat, since GPI genes are reported to locate on the homoeologous group 1 chromosomes of wheat (Hart 1979, Chojecki and Gale 1982).

Grain esterase (EST-5). *Ae. variabilis* expressed a multitude of bands for grain esterase (EST-5) of which several were unique for the species (Fig. 3). Two of these bands express in the basic region of the IEF gels and can potentially serve as diagnostic markers, since neither durum or bread wheats possess them. EST-5 genes are located in the long arms of homoeologous group 3 chromosomes of wheat (Ainsworth *et al.* 1984). Since the two marker bands (Fig. 3) were also expressed in the wheat/*Ae. variabilis* amphiploids, homoeology diagnosis of *Ae. variabilis* chromosomes with group 3 chromosomes of wheat is established.

6-Phosphogluconate dehydrogenase (6-PGD). Bread and durum wheat cultivars express a single 6-PGD band on native-PAGE (Fig. 4). *Ae. variabilis* possesses one overlapping band

Fig. 2. Grain GPI banding profiles on isoelectric focusing gels of *Triticum aestivum* cv. Chinese Spring (CS), cv. Alondra/Pavon (AP), *T. turgidum* cv. Laru (L), *Ae. variabilis* (Ae) and the amphiploids of CS/*Ae. variabilis* (1), AP/*Ae. variabilis* (2) and L/*Ae. variabilis* (3). Diagnostic marker bands are indicated by arrowheads.

Fig. 3. Grain esterase (EST-5) banding profiles on IEF (pH 3.5–9.5) of *Triticum aestivum* cv. Chinese Spring (CS), cv. Alondra/Pavon (AP), *T. turgidum* cv. Laru (L), *Ae. variabilis* (Ae) and the amphiploids of CS/*Ae. variabilis* (1), AP/*Ae. variabilis* (2) and L/*Ae. variabilis* (3). Diagnostic marker bands are indicated by arrowheads.

Fig. 4. Native-PAGE (8.5% acrylamide) of 6-phospho gluconate dehydrogenase isozymes of *Triticum aestivum* cv. Chinese Spring (CS), cv. Alondra/Pavon (AP), *T. turgidum* cv. Laru (L), *Ae. variabilis* (Ae) and the amphiploids of CS/*Ae. variabilis* (1), AP/*Ae. variabilis* (2) and L/*Ae. variabilis* (3). Diagnostic marker bands are indicated by arrowheads.

with wheat and two distinctive bands; one each of slower or faster mobility than wheat. These bands were also present in the amphiploids and are anticipated to serve as identifiers of the presence of *Ae. variabilis* chromosomes with homoeology to group 6 or 7 of wheat. Genes for 6-PGD are located on chromosomes 6A, 6BL and 7BL in bread wheat (Hsam and Zeller 1982).

β -Amylase (β -AMY). The *T. aestivum* cultivars Chinese Spring and Alondra/Pavon expressed a multitude of bands when β -AMY was separated on IEF, pH 4.0–6.5 (Fig. 5). There were two to three diagnostic bands for *Ae. variabilis*, that distinguished it from the respective wheat cultivars. The wheat/*Ae. variabilis* amphiploids also expressed this *Ae.*

Fig. 5. Separation of β -AMY isozymes on IEF gels (pH 4.0–6.5) of *Triticum aestivum* cv. Chinese Spring (CS), cv. Alondra/Pavon (AP), *T. turgidum* cv. Laru (L), *Ae. variabilis* (Ae) and the amphiploids of CS/*Ae. variabilis* (1), AP/*Ae. variabilis* (2) and L/*Ae. variabilis* (3). Diagnostic marker bands are indicated by arrowheads.

Fig. 6. Native-PAGE (8.5% acrylamide) glutamate oxaloacetate transaminase isozymes of *Triticum aestivum* cv. Chinese Spring (CS), cv. Alondra/Pavon (AP), *T. turgidum* cv. Laru (L), *Ae. variabilis* (Ae) and the amphiploids of CS/*Ae. variabilis* (1), AP/*Ae. variabilis* (2) and L/*Ae. variabilis* (3). Diagnostic marker bands are indicated by arrowheads.

Fig. 7. IEF (pH 4.0–6.5) separation of α -AMY isozymes of *Triticum aestivum* cv. Chinese Spring (CS), Alondra/Pavon (AP), *T. turgidum* cv. Laru (L), *Ae. variabilis* (Ae) and the amphiploids of CS/*Ae. variabilis* (1), AP/*Ae. variabilis* (2) and L/*Ae. variabilis* (3). Diagnostic marker bands are indicated by arrowheads.

variabilis specific band (Fig. 5). β -AMY genes are located on wheat chromosomes 4A β , 4DL (β -Amy 1) and 5AL (β -Amy 2), (Ainsworth *et al.* 1983). The above *Ae. variabilis* specific band marker provides us with the diagnostic tool for identifying *Ae. variabilis* chromosomes homologous to group 4 or 5 chromosomes of wheat.

Glutamate oxaloacetate transaminase (GOT). When GOT isozymes are separated on native-PAGE, bread wheats exhibit three bands in the GOT-2 region (Hart 1975). GOT-2 isozyme genes are located in homoeologous group 6 chromosomes of wheat. These three bands arise by a recombination of monomeric subunits encoded by genes present in the different group 6 chromosomes to form an active dimeric GOT enzyme (Hart 1975). *Ae. variabilis* has 3 bands in the GOT-2 region of comparable staining intensities to the GOT-2 region of bread wheat (Fig. 6) and do not provide a positive marker. Durum wheats (*T. turgidum*) however, have a single band and the durum/*Ae. variabilis* amphiploid possess three bands in the GOT-2 region that can be potential markers to diagnose the presence of *Ae. variabilis* chromosomes homoeologous to group 6 of wheat.

α -Amylase (α -AMY). There are two zones of activity when α -amylase isozymes are separated on IEF (Nishikawa and Nobuhara 1971, Gale *et al.* 1983). The banding patterns of α -AMY 2 (low pI region) showed differences between wheat and *Ae. variabilis* (Fig. 7) that further expressed in their amphiploids. α -AMY genes are located in homoeologous group 6 (α -Amy 1 locus) and group 7 chromosomes (α -Amy 2 locus) of wheat (Nishikawa and Nobuhara 1971, Gale *et al.* 1983). The α -AMY 2 isozyme differences present can thus be used to characterize *Ae. variabilis* chromosomes with homoeology to group 7 chromosomes of wheat.

Summary

Aegilops variabilis ($2n=4x=28$, UUSS) with a high level of resistance to Karnal bunt (*Tilletia indica*); an important soil-borne disease; is a potential genetic variability source for transfer to wheat (*Triticum aestivum* L.). Diagnostic markers using proteins and isozymes have hence been established to detect the presence of *Ae. variabilis* chromosomes in *Triticum* background. High molecular weight glutenins (HMW-Glu), glucose phosphate isomerase (GPI), seed esterase (EST-5), glutamate oxaloacetate transaminase (GOT), 6-phosphogluconate dehydrogenase (6-PGD), α -amylase (α -AMY) and β -amylase (β -AMY) were positive biochemical markers. These markers enable identification of the disomic chromosome additions of *Ae. variabilis* to wheat and also determine their homoeology with the homoeologous groups of wheat following evaluation of their respective amphiploids; *T. aestivum*/*Ae. variabilis* ($2n=10x=70$) and *T. turgidum*/*Ae. variabilis* ($2n=8x=56$).

Key words: Alien genetic variation, Biochemical markers, Intergeneric hybridization, Triticeae, Wheat improvement.

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