ANADOLU, J. of AARI
7 (1) 1997, 9 - 20
MARA

ISOZYME VARIATION AMONG SOME EUROPEAN AND AMERICAN OAT CULTIVARS

Halil SÜREK

Thrace Agricultural Research Institute, P.O. Box 16 Edirne, TURKEY

J. VALETINE M. D. HAYWARD

Welsh Plant Breeding Station Plas Gogerddan, Aberystwyth Dyfed, SY 23, 3 EB, UK.

ABSTRACT*: The aims of this study were to investigate isozymic variation among some European and American cultivated oat (****Avena sativa*** *L.) varieties and to determine parental lines for an assessment of possible relationship between these markers and quantitative traits. Starch gel electrophoresis was used to determine isozyme variation among cultivars. The research was conducted at Welsh Plant Breeding Station in 1992. The variation was found for Esterase (EST), Leucine Aminopeptidase (LAP), Diaphorase (DIA), Alcohol Dehydrogenase (ADH), and Benzyol Arginine Aminopeptidase (BAP), Peroxidase (PER) among cultivars. The most extensive variation detected for EST. It indicated that North American originated lines were more variable than United Kingdom or Northern European lines. No variation was observed for other isozyme systems among oat cultivars in this study. Five bands were visualized for Glutamase Oxaloacetate Transaminase (GOT). Phospho Gluco-Isomerase (PGI) and Superoxidase-Oxidoreductase (SOD) had one band for all lines. Two active zones were observed in the parental lines for Malic Enzyme (ME). Four bands in the first zone from origin and two bands in the second were identified. Two bands were found in all parental lines for Phospho-Glucomutase (PGM).*

Five oat lines N327-6, N313-2, Exeter, 78-34Cn5 and Pendragon were chosen as parental cultivars for further study to examine association between isozyme and quantitative traits. These lines differed from each other for ADH, BAP, and DIA enzyme systems.

Keywords*: Electrophoresis, isozyme variation, isozyme system, oat,* ***Avena sativa*** *L.*

INTRODUCTION

The origin of oats can be traced back to about 2000 BC. Archaeological discoveries showed that oats originated in the Middle East, especially the areas surrounding the Mediterranean sea. Oats have been domesticated several millennia after wheat and barley (Ladizinsky, 1988).

Germplasm collection can be an excellent source of potential useful characters. However, a breeder can spend a long time screening useful accessions. The breeder must have a means of choosing the accessions most likely to possess the trait of interest. Breeders have used morphological and physiological traits to screen their material, but they have recently started to utilize allozyme polymorphism.

(*) : Part of M.Sc. Thesis submited by the first author to The University of Wales.

A direct extension of the use of isozyme in the measurement of genetic diversity in germplasm collections is the identification of cultivars (Weeden, 1989). It has been firmly established that genetic difference among cultivars may be reflected in variation in allozyme polymorphism, and isozyme phenotypes have been used to distinguish cultivars for nearly all the crops.

Yndgaard and Iloskuldsson (1985) identified six uses of isozyme markers in plant germplasm collection and assessment (1) description of a population or cultivars, (2) detection of genetic difference among individuals or cultivars, (3) determination of phylogenetic relationships within a species, (4) analysis of migration patterns of a species from centers of origins, (5) identification of duplicated accessions, and (6) aid in the planning of new collection expeditions.

In a survey of genetic variation in *Avena barbata* in California, Clegg and Allard (1972) found that allelic frequencies at five enzyme loci and at two loci governing morphological variants are distributed in non random patterns closely associated with environmental origin. In the semi arid summer region of California, all populations of this species were monomorphic for a specific combination of alleles, whereas most populations in the generally more Mediterranean cool-summerregion were extensively polymorphic for these loci. Hamrick and Allard (1972) studied microgeographical distributions of alleles of five enzyme loci and one morphological variant. Their results showed that the spatial distributions of alleles are non-random and highly correlated with variation in habitat in California.

Marshall and Jain (1969) examined genetic polymorphism in natural population of *Avena fatua* and *A. barbata*. They reported that *A. barbata* was more abundant in region with lower maximum temperatures, coastal fog and humidity and the longer growing season, while *A. fatua* was more abundant in the warm Mediterranean region.

Garcia *et al.* (1985) studied allelic and genotypic composition of ancestral Spanish and Colonial Californian gene pools of *Avena barbata.* Assays of the Spanish and Californian gene pools of this species for 15 isozyme loci show that the present day Spanish gene pool, particularly that of Southwestern Spain is identified or virtually identical to that of California for five loci and closely similar for nine loci. Jain and Singh (1979) examined genetic similarity among 15 *Avena* species. Variation among populations of the same species was highly significant. After pooling of results for all its populations, each species could be characterized by certain “diagnostic” sites using

presence/absence patterns. Kahler et al. (1980) investigated collection from 31 populations of *A. barbata* from diverse habitats in Israel. Many different isozyme phenotypes were found in all of the populations, also the array of isozyme phenotypes found in each population differed distinctly from that found in each other population. Temperature and moisture related variables were significantly correlated with particular isozyme phenotypes and also isozyme variation was found to correspond closely to mosaic patterns of the habitat.

Coffman *et al*. (1961) reported that the ten most important ancestral cultivars of Nort American oat varieties represented diverse geographical origins, Victoria and Santa Fe were introduced from South America, Milton and Banner from Northern Europe, Landhafer from Central Europe, Kherson and Green Russian from Eastern Europe and Red Algerian, Hajira and probably Red Rusproof originated in the Mediterranean region. Souza and Sorells (1991) studied current and historically important North American oat cultivars. They suggested that fall and spring planted oat cultivars were generally divergent groups. The fall-planted cultivars were more genetically divers than the spring-planted cultivars.

The aims of this study were to investigate, isozymic variation among some European and American oat varieties and to identify parental lines for an assessment of possible relationship between these markers and quantitative traits.

MATERIAL AND METHODS

This research was conducted in two steps at Welsh Plant Breeding Station in 1992. Twenty-two cultivars presented in table. 1, were used in this work. Twenty of these cultivars, except N313-2 and 78-34Cn5, were used to detect isozymic variation among cultivars to select parental lines for further investigation. According to the results of the first step isozyme study, lines Pendragon, N327-6 and Exeter were chosen to be used as parent in the experiment for further investigation to study association between Quantitative Trait Loci (QTL's) and isozyme loci. These lines had different band patterns for esterase. In addition to these, two lines N313-2 and 78-34Cn5 were also chosen in terms of their different characteristics.

In order to detect more isozymic variation among parental lines, second step study was carried out.

Isozyme Analysis: Starch Gel Electrophoresis

Fresh starch gels were prepared by mixing 60 g hydrolyzed potato starch and 500 ml. gel buffer (9 part of 0.066M Tris solution adjusted to pH 8.0 with 1M citric acid

and 1 part of electrode buffer) in a 1 litter conical flask. The mixture was heated until it became transparent at 75 °C. The air was then removed by vacuum pump. The mixture was then poured into a mould on a flat surface and covered with a glass plate and left overnight to cool.

Table 1. The characteristics of oat cultivars used in the isozyme study.

Cultivar	Pedigree	Origin	Winter spring	Notes
Solva	(072700n x Oyster)	UK	W	
Lustre	(6367Cn 11/8xM.Osprey)	UK	W	
Kynon	[Pendrwm x (06683Cn1/pennal)]	UK	W	
Pendragon	(06765Cn/10/4 x Bulwark)	UK	W	Naked
84-23Cn1/1	[78-1Cn(Pannal x Bulwark) x Solva]	UK	W	
Sun-II	(EAGLE x STAR)	Sweden	S	
Melys	[M. Oberon x (Margam x CC6311)] x [M. Oberon x Milo]	UK	S	
10589Cn	[(*A. barbata* x M. Tabard x Saracan] x COKER	UK, USA	S	
10017	Pc54 x Dula	Canada	S	Very resistant to mildew
OT329	-----	Win, Can	S	Very large grain
OA698-2	-----	Ott. Can	S	Large grain, early
Pa8494	-----	Pa, USA	S	Contains DW6 gene
IL82-1657	Froter x Hazel	III, USA	S	Large grain, early
IL64-1431-1	IL75-1062 x Preston	III, USA	S	Large grain
Valley	-----	USA	S	
Pol	-----	Finland	S	
Coker-H-833	-----	Tex, USA	W	
N327-6	-----	USA	S	High oil line
N313-2	-----	USA	S	High oil line
Exeter	Victory x Rusota	USA	S	Low oil line
78-34Cn5	Pioner xOyster	USA, UK	W	Medium oil line
CC6501	-----	USA	S	High protein line

From each seedling to be screened, approximately 4 cm of young leaf was collected. Each leaf was crushed with a steel rod in two drops of crushing buffer (0.1M

Tris adjusted to pH 7.2 with HCl containing ul2-mercapto ethanol per 50 ml). Rectangular Whatman paper wicks 4 mm wide were used to absorb the extracts from the crushed leaf.

The wicks were inserted into a split in the starch gel which was cut approximately 4 cm from the cathode end. A small amount of bromophenol blue was used on the wall of the split portion of the gel which acts as an indicator of the extent of migration.

Electrode buffer (0.2M boric acid adjusted to pH with 1M lithium hydroxide) was used for passing electricity through the gel. Electrophoretic migration was carried out first 20 minutes at 30 MA (150-170 Volts) and after removing the wicks at 35 MA (150-180) until the extract front had migrated 6-8 cm (about 4-5 hours). Following electrophoresis the gels were sliced horizontally into 3 slices. Each slice was used for staining.

The banding patterns were visualized using modification of staining solution described by Shaw and Prashad (1970). The gel was stained for Glutamate Oxaloacetate Transaminase (GOT), Esterase (EST), Leucine Aminopeptidase (LAP), Malic Enzyme (ME), Phospho Gluco-Isomerase (PGI), Alcohol Dehydrogenase (ADH), Benzoly Arginine Peptidase (BAP). Peroxidase (PER), Phospho Glucomutase (PGM), Superoxide-Oxidoreductase (SOD), and Diaphorase (DIA).

RESULTS AND DISCUSSION

Within cultivated crop varieties, one can use genetic markers to determine relatedness between individuals. Much of this work has employed isozymes, largely because they are simply detected at low cost, with as few as 20 markers (Soller and Backman,1983).

In this study, isozyme was used to determine variation among oat cultivars (*Avene sativa L.*). The results would be utilized to choose the parental lines. Isozyme systems assayed in the first step study were Glutamate Oxaloacetate Transamase (GOT), Esterase (EST), Leucine Aminopeptidase (LAP), Phospho Gluco-Isomerase (PGI), Superoxidase-Oxidoreductase (SOD).

Esterase (EST)

Esterase isozyme showed the most extensive differences between varieties. Generally United Kingdom originated lines, except 10589Cn and 10017Cn, had the same band pattern for EST, they had 5 bands while 10017Cn and 10589Cn had 6 different band

patterns (Fig.1) The Swedish variety Sun II had 5 bands in the same manner such as UK lines. There was a huge variation among U.S.A and Canada lines. IL 84-1431-1 and Coker-H-833 had 6 and 7 bands differed from the other U.S.A lines while Canadian line OT329, the American lines IL82-1657, and Valley possessed the similar band patterns to the British lines. Canadian OA698-2 and American Pa8494-1934 had three bands. Interestingly, Finnish cultivar Pol expressed a very different isozyme variation in terms of number of bands. The other American lines, N327-6, Exeter, and CC6501 varied each other and from other cultivars.

American originated oat lines were more variable than UK or Northern European lines. This difference might be due to the genetic bases of these lines. On the other hand, Coffman *et al*. (1961) reported a large geographical origin for the 10 most important ancestral cultivars of North American oat varieties. Similarly, Souza and Sorells (1989a) examined the pedigree of North American oat cultivars and they noted that the most important ancestral parent was the South American cultivars, “Victoria”, followed by Swedish landrace “Milton” and the *A. byzantina* landrace, “Red Rustproof”. Assuming that genetic diversity is correlated with geographical origin, they pointed out that the North American germplasm pool currently appeared to be broadly based. In contrast, Almgârd and Clapham (1975) and Singh *et al.* (1973) found variation for esterase among Swedish and American lines respectively. Nevertheless the r band pattern was found by Almgârd and Clapham for esterase using α-naphthyl acetate substrate occurred in 9 out of 18 lines and may correspond with the pattern found for European oats in this study. Singh *et al*. (1973) found many bands for esterase using the same substrate and the ten varieties that they examined, all had different patterns from each other, none of which appears to correspond with Almgârd and Clapman’s or the present study.

In the second step of this work only the British line Pendragon differed from other 4 lines for esterase (Fig.3). Three of the four lines were U.S.A lines, and the other (78-34Cn5) was developed from a cross between U.S.A and UK lines.

Leucine Aminopeptidase

In the first step isozyme study Leucine Aminopeptidase showed variation only for an American line CC6501, whereas the other lines had the same band pattern (Fig 2). But, Singh *et al*., (1973) examined 10 Californian varieties for LAP and using the variation of LAP as a criterion, they put the varieties in three groups.

According to the results of the first step isozyme study, lines Pendragon, N327-6 and Exeter were chosen to be used as parent in the experiment for further investigation to study association between Quantitative Trait Loci (QTL’s) and isozyme loci. These lines

had different band patterns for esterase. In addition these lines, two lines N313-2 and 78-34Cn5 were also chosen in terms of their different characteristics. Further, the second step of isozyme study was then conducted.

Isozyme systems which were examined in the parental lines are Esterase (EST), Malic Enzyme (ME), Phospho Gluco-mutase (PGM), Superoxidase-Oxidoreductase (SOD), Peroxidase (PER), Alcohol Dehydrogenase (ADH), Benzyol Arginine Aminopeptidase (BAP), and Diaphorase (DIA).

Alcohol Dehydrogenase (ADH)

The variation was observed among parental lines for ADH. The spring lines Exeter, N313-2 and N327-6 had different band from the winter lines, Pendragon and 78-34Cn5 (Fig.4). Two bands were observed for both types. But, their band distances were different. Similarly Almgård and Clapham (1975) reported variation among Swedish oat varieties for ADH in seed samples.

Benzoyl Arginine Amino Peptitase (BAP)

The winter and spring lines had different band pattern for this enzyme as well (Fig.4). While spring lines showed a single band, the winter types had two bands. Similar result was obtained by Morikawa and Leggett (1989), they found a single band in *A caneriensis*. Also Nahar (1991) reported single band "b" and doubled band "a"in the wild oat populations.

Diaphorase (DIA)

The winter line (78-34Cn5) possessed a different isozyme band pattern than other parental lines (Fig.4) having 5 active and one null band, while the other showed 6 active bands. This result agrees with Souza and Sorells (1989b), they noted six distinct zones of diaphorase in majority of 70 American oat cultivars. Thirteen of the 70 cultivars were found to lack activity in one zone at Rf 0.76. They also proposed gene symbol for these active zones (present) as Dia1 and non-active (null) dia1, respectively.

Peroxidase (PER)

Some variations were observed among lines for PER. but, band patterns were not clearly visualized, although the winter lines had more bands than the spring. On the other hand, Singh *et al*. (1973) and Almgård and Clapman (1975) used peroxidase to distinguish American and Swedish oat cultivars. They determined distinguishable variation among varieties that could be utilized in variety identification.

No variation was observed for other isozyme systems in this study. Five bands were visualized for GOT. PGI and SOD had one band for all lines. Two active zones were observed in the parental lines for ME (Fig.3). Four bands in the first zone from origin and two bands in the second were identified. All lines had the same band pattern without showing variation. Two bands were found in all parental lines for PGM (Fig.3) and no variation was observed among lines.

Some of isozyme variation obtained in this study were used to investigate relationship between isozyme markers and quantitative traits in another study.

Figure 1. Zymogram of Esterase for cultivars.

Figure 2. Zymogram of LAP for cultivars.

Figure 3. Zymogram of EST, ME, and PGM.

Figure 4. Zymogram of ADH, BAP, and DIA.

REFERENCES

Almgârd, G., and L. Landegren. 1975. Isoyme variation distinguishing 18 *Avena* cultivars grown in Sweden. Swedish J. Agric. 5:61-67.

Clegg, M.T., and R.W. Allard. 1972. Patterns of genetic differentiation in the slender wild species *Avena barbata.* Proc. Nat. Acad. Sci. USA 69:1820-1824.

Coffman, F.A., H.C. Murphy, and M.H. Chapman. 1961. Oat breeding. In Oats and Improvement. F.A. Coffman (Ed) American Society of Agronomy, Madison, Wisconson

Garcia, P., F.J. Vences, M. Perez de la Vega, and R.W. Allard, 1985. Allelic and genotypic composition of ancestral Spanish and colonial Californian gene pool of *Avena barbata:* evolutionary implications. Genetic 122:687-694.

Hamrick, J.L., and R.W. Allard, 1972. Microgeographical variation in allozyme frequencies in *Avena barbata.* Proc. Nat. Acad. USA. 69:2100-2104.

Jain, S.K., and R.S. Singh, 1979. Population biology of *Avena*. Allozyme variation in relation to genome analysis. Bot. Gaz. 140:356-362.

Kahler, A.L., R.W. Allard, M. Krazakowa, C.F. Wehrhal, and E. Nevo, 1980. Association between isozyme phenotype and environment in the slender wild oat (*Avena barbata*) in Israel. Theor. Appl. Genet. 56:31-47.

Ladizinsky, G., 1988. The domestication and history of oats. Third international oat conference. Lund, Sweden, July 4-8, 1988. pp 7-12.

Marhhall, D.R., and S.K. Jain, 1969. Genetic polymorphism in natural populations of *Avena fatua* and *A. barbata*. Nature, 221:276-278.

Morikowa and J.M. Leggett, 1989.Isozyme Polymorphism in natural populations of *Avena canariensis* from the Canary Island. Heredity, 64:403-411.

Nahar, K., 1991. Studies of some wild *Avena* populations. M. Sc. Thesis, University of Wales, Aberystwyth.

Shaw, C.R., and Prasad, 1970. Starch gel electrophoresis of enzymes. A compilation of reciepes. Biochemical Genetics 4:297-320.

Singh, R.S., S.K., Jain and C.O. Qualset, 1973. Protein electrophoresis as an aid to oat variety identification. Euphytica, 22:98-105.

Soller, M., and J.S. Backman, 1983. Genetic polymorphism in varietal identification and genetic improvement. Theor. Appl. Genet. 67:25-33.

Souza, E., and M.E. Sorells, 1989(a). Pedigree analysis of North American oat cultivars released from 1951 to 1985. Crop Sci. 29:595-601.

Souza, E., and M.E. Sorells, 1989(b). Inheritance and frequency of a null allele for diaphorase activity in North American oat cultivars. Jour. Hered. 80:501-503.

Souza, E., and M.E. Sorells, 1991. Relationships among 70 North American oat germplasms; II. cluster analysis using quantitative characters. Crop Sci. 31:599-605.

Weeden, N.F., 1989. Applications of isozyme in plant breeding. Plant Breeding Reviews, 6:11-54.

Yndgaard, F., and A. Iloskuldsson, 1985. Electrophoresis a tool for genebanks. Plant Gen. Res. Newsletter, 63:34-40.