Euphytica 32 (1983) 877–883

A RAPID METHOD FOR THE EVALUATION OF VARIATION IN GERMPLASM COLLECTIONS OF CEREALS USING POLYACRYLAMIDE GEL ELECTROPHORESIS

A. B. DAMANIA[1], E. PORCEDDU[1] and M. T. JACKSON[2]

[1] Germplasm Institute, Bari, Italy
[2] Department of Plant Biology, University of Birmingham, England

Received 28 January 1983

INDEX WORDS

Triticum aestivum, bread wheat, *Triticum turgidum*, *Hordeum vulgare*, barley, chemotypes, electrophoresis variation, prolamines, gliadins, hordeins, electrophoregram, genetic resources.

SUMMARY

Polyacrylamide gel electrophoresis (PAGE) of storage proteins (prolamines) was used to screen 64 landraces of wheat and barley from Nepal and the YemenArab Republic and two cultivars for comparison. Altogether 3168 single seeds were examined and the advantages gained by using the vertical slab gel method were recognised. The extent of variation present within populations of landraces could be assessed easily and rapidly using the methods described. Differences in ploidy levels of wheats were detected by PAGE and investigated. Suggestions are made for improvements in sampling strategies in hilly terrain.

INTRODUCTION

Since the formation of the International Board for Plant Genetic Resources (IBPGR) in 1974, germplasm collecting activities have been intensified in the centres of diversity of major crops as well as in peripheral areas. Although most of these collections have been lodged safely in various gene banks around the world for long-term storage, little evaluation has been undertaken to determine their worth as future sources of valuable genes.

Landraces are a useful source of genetic variation, and the greater the variation, the greater the chances of a landrace possessing genes or gene combinations of interest to plant breeders. Various sampling strategies have been proposed for capturing the maximum amount of variation for the minimum time and effort (BENNETT, 1970; MARSHALL & BROWN, 1975). During the first visit to a region it is often not possible to survey thoroughly all areas, and it may be necessary to return in future years to some areas of special interest where the variation is more intense. Evaluations of germplasm collections may take over three years. With the rapid genetic erosion occurring in many parts of the world, precious germplasm may be lost before additional collections can be made.

Various measures of genetic diversity of populations may be used (MARSHALL &

BROWN, 1975), but of increasing importance is the study of isozyme systems, especially if they can be related directly to allelic frequencies (SECOND, 1982). Polyacrylamide gel electrophoresis (PAGE) of some cereal storage proteins (prolamines) is a valuable tool for gauging variation in populations of landraces and cultivars, and hordeins and gliadins from barley and wheat respectively, appear to be as good marker systems as allozymes for this purpose (DOLL & BROWN, 1979; LEE & RONALDS, 1967). It has been shown that environmental factors and year of growth have no effect on the electrophoregram of these proteins (AUTRAN et al., 1979; LEE & RONALDS, 1967; MARCHYLO & LABERGE, 1980; SHEWRY et al., 1978; ZILLMAN & BUSHUK, 1979). For example, the wheat cultivar Neepawa was grown at ten locations in Canada but still produced exactly the same banding pattern when single seeds from these diverse locations were subjected to electrophoresis using the horizontal polyacrylamide gel method (ZILLMAN & BUSHUK, 1979). The results obtained from these and other studies confirm that the storage protein electrophoregram is independent of the environment.

The application of PAGE of cereal prolamines thus permits a rapid screening of population variation without growing material in the field, in order to identify areas for additional intensive germplasm sampling. In this paper we present the results of an electrophoretic evaluation of wheats and barleys collected in Nepal and the Yemen Arab Republic.

The material from the Yemen is specially valuable now, from the point of view of genetic resources, because in December 1982 a devastating earthquake destroyed large areas of the farming communities in the regions of Dhamar and Sana'a from where nearly half of the material used in this study originates.

MATERIALS AND METHODS

The plant material selected for this study were wheat and barley populations collected by IBPGR missions to Nepal (ERSKINE & BOURGOIS, 1979) and the Yemen Arab Republic (AYAD et al., 1980). Each crop was represented by sixteen populations from four regions in each country. In the majority of cases, one wheat and one barley population came from the same collection site.

All populations were multiplied in southern Italy at the Germplasm Institute's experimental plot at Gaudiano, 41°06′N, 15°52′E, about 110 km northwest of Bari, at an altitude of approximately 150 m, during 1980–81. Fifty spikes were collected at random from each population, and single seeds from forty-eight of these were used for the electrophoretic analysis of storage proteins. Gliadins were extracted from the seeds with the method of TKACHUK & METLISH (1980), but a different solvent, isopropanol 55%, was used for hordeins (MARCHYLO & LABERGE, 1980). They were separated electrophoretically with an aluminium-lactate buffer, according to the methods of BUSHUK & ZILLMAN (1978). Separation of gliadins took 2½ hours in the vertical system. In the horizontal apparatus the separations of hordeins took 4½ hours. Gels were stained with Coomassie Brilliant Blue R-250 overnight, and were ready for analysis the following day. In the vertical thin gel, the bands were visible after only one hour in the stain at room temperature.

Samples of the Italian barley cultivar Micuccio and the tetraploid durum wheat cultivar Karel, also grown at Gaudiano, were included for comparison. For calculating

Table 1. Chemotype variation in wheat and barley populations from Nepal and Yemen Arab Republic.

Region	Village	Altitude (M)	Chemotypes	
			wheat	barley
Nepal				
Bheri	Kalagaon	780	32*	9
	Dailekh	1410	31	20
	Channa	1960	28	14
	Bhavassaini	2400	20	13
Karnali	Tulkana	1710	42	21
	Chirana	1950	31	
	Rachuli	1960		13
	Bahivanka	2145	15	
	Dillikot	2245	30*	17
	Dhaulapani	2250		13
Dhaulagiri	Armadi	800		5
	Beni	980	26	
	Taka	1680	18	13
	Malkabang	2000	16*	16
	Lomsom	2170	20**	7
Gandak-Bagmati	Jitpur	1200	35*	8
	Sunakothi	1300		7
	Thecholele	1350	34*	15
	Godavri	1400	30*	10
	Kakani	2200	19*	
Yemen				
Al Baida	Al Souma'a	1950	22	18
	Al Mashair	2600	20	
	Maswakin	2600		22
	Al Okla	2700		15
	Jubair	2700	35	18
	Al Barria	2700	15***	
Ibb	Jabal Al Khadra	2100	26	11
	Irian	2150		13
	Robat Al Gala'a	2400	38	
	Bait Yehia Obad	2500	29	14
	Al Dubarin	2550	35	8
Dhamar	25 km N. of Dhamar	2200	31	
	Dhamar	2300	38	18
	Koman	2350	11*	11
	Qarn Dhamar	2350	37	17
	Yerim	2500		18
Sana'a	Shibam Al Kharas	2200	19	
	Wadi Dhar	2400		14
	Rabo'o	2400	37	14
	Bani Mattar	2500	36	14
	Bait Mahdam	2800	37	21

* Populations with mixtures of seeds showing the 'tetraploid and hexaploid type' banding patterns.
** Population with 'tetraploid type' banding pattern in all but two of the forty-eight seeds tested.
*** This population may be a mixture of a hexaploid cultivar and a tetraploid landrace.
**** This is hexaploid cultivar.

the relative mobilities of gliadin bands the Canadian hexaploid cultivar Marquis served as a reference in all gels, and for hordeins, Micuccio was used.

RESULTS AND DICUSSION

A total of 3168 single seed analyses was made to identify variation in this material. Each population was represented by several chemotypes, and these are indicated in Table 1. Each seed having a distinct electrophoretic profile with respect to position of bands and their intensities is recognised as a different chemotype. We prefer using the term chemotype to biotype, which was previously used by several workers, because it reflects more precisely the chemical composition of a seed (plant), in this case prolamines, rather than any other biological feature. The clarity of the electrophoretic profiles is shown in Fig. 1.

The Italian wheat and barley cultivars showed two and four electrophoretic profiles respectively. This is in agreement with the findings of other workers also experimenting on electrophoretic characterisation of cultivars (KONAREV et al., 1976; KONAREV et al., 1979; WRIGLEY, 1980).

In contrast to these bred varieties, the landraces collected in Nepal and the Yemen Arab Republic were highly variable with many chemotypes in each population. Morphological analysis of the populations showed that all barleys from Nepal were 6-rowed, and those from the Yemen Arab Republic were 2-rowed; all Nepalese wheats were hexaploid *Triticum aestivum* whereas the Yemeni wheats were tetraploid *T. turgidum*, with the exception of one population which was hexaploid.

In the case of the Nepalese barley, there was no relationship between the number of chemotypes and altitude. The regions in Nepal are river valleys, and these results indicate that samples collected from lower altitudes in the valleys are as variable for hordein content as those collected at higher altitudes. The number of chemotypes found in populations decreased from west to east i.e. from the hills of western Nepal towards regions around the capital city of Kathmandu in the river valleys of the Gandak and Bagmati. In contrast, the Yemeni barleys showed a correlation between altitude and the number of electrophoretic profiles ($r = 0.43$, $p = 5$–10%), indicating a trend for increase in the number of electrophoretic profiles with altitude.

The wheats from Nepal were negatively correlated with altitude ($r = -0.52$, $p = 1$–5%) with a tendency for populations to comprise fewer chemotypes with increasing altitude. In Yemen there was no relationship between the number of chemotypes and altitude.

In Yemen, the population from Koman (Dhamar region) was the only *T. aestivum* variety, the other material being *T. turgidum*. The low number of profiles, 11, and the relative uniformity in morphological characteristics of this sample seen in the field (DAMANIA et al., 1983) makes us conclude that it is a cultivar and not a landrace as supposed by the collectors (AYAD et al., 1980). The sample from Al Souma'a (Al Baida region) had some hexaploid chemotypes (see Fig. 1), and it is presumed that they may have come from the cultivar found in Koman.

Even though there appeared to be little or no relationship between population variation, as demonstrated by the number of chemotypes, and altitude or location within

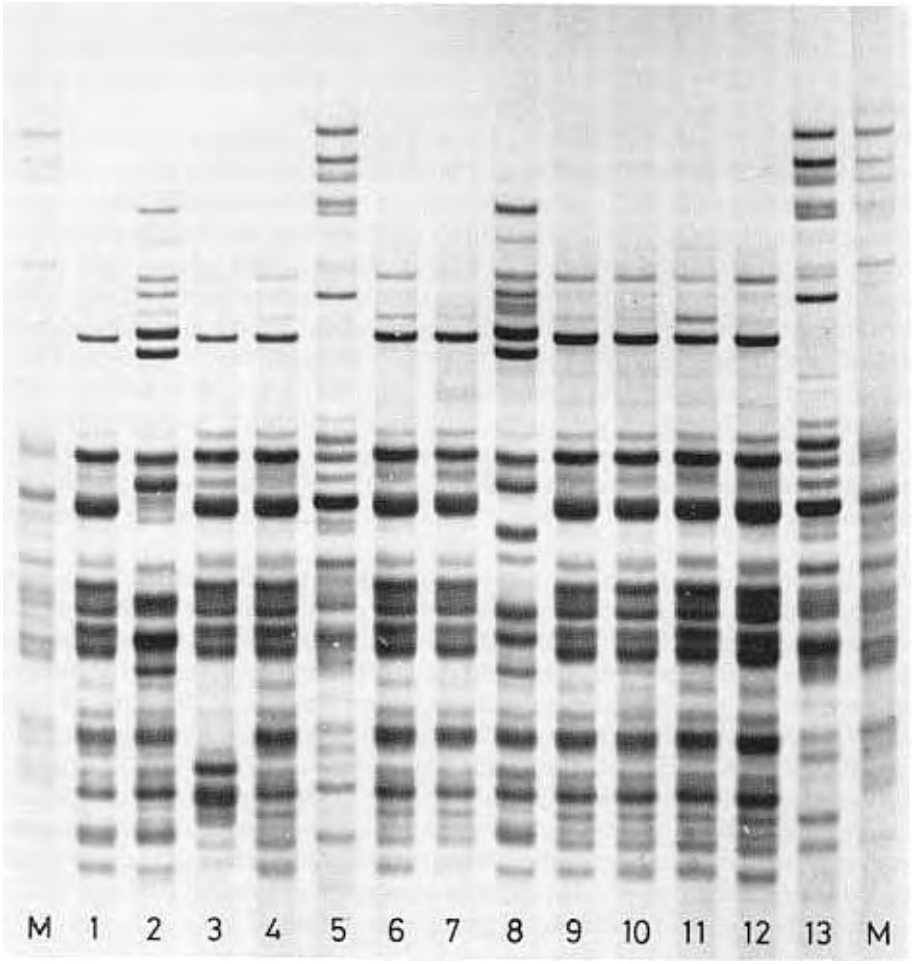

Fig. 1. Gel showing electrophoretic profiles of 13 single seeds of a landrace from the village of Al Souma'a (Al Baida region), Yemen Arab Republic. *T. aestivum* cultivar Marquis is loaded on either side of the gel as a reference. Notice the presence of two to three slowest moving bands of high molecular weight gliadins in the omega region in profiles 5 and 13, which clearly shows that these extracts came from hexaploid seeds.

a country, there was a negative correlation ($r = -0.58$, $p = 1\text{–}5\%$) between number of chemotypes of landraces and their morphological homogeneity based on field observations over two growing seasons (DAMANIA et al., 1983). Thus we feel confident that the use of polyacrylamide gel electrophoresis of cereal storage proteins is a valuable tool for assessing variation in germplasm samples.

The variation in storage protein banding patterns revealed by PAGE is considerable in the samples analysed. Other workers have suggested that 30 seeds selected at random from a variety sample should be analysed in order to survey 90% of the variation in storage proteins (WRIGLEY & MCCAUSLAND, 1977). In the present study, over 3000

single seed electrophoretic analyses were carried out using 48 seeds per population. Fifteen of the wheat populations had more than 30 chemotypes, and we believe that a sample larger than 30 seeds per landrace population is necessary to identify areas, which would require further visits for germplasm collecting.

PAGE can also be used to determine the ploidy status of wheat landraces. Most hexaploid wheats could be easily identified in an aluminium-lactate polyacrylamide gel by the presence in the profile of two or more of the slowest moving bands in the omega region which contains the gliadins with a relatively high molecular weight (BUSHUK & ZILLMAN, 1978; KASARDA et al., 1976). In the tetraploid wheats these bands are absent because they are coded by genes on the 1D chromosome (WRIGLEY, 1980; ZEHATSCHEK et al., 1981). Seven of the wheat populations from Nepal showed both 'tetraploid' and 'hexaploid' chemotypes. This result contradicted the reports of previous collectors that all Nepalese wheats were hexaploid (ERSKINE & BOURGOIS, 1979; WITCOMBE, 1975). Did this result suggest that Nepalese farmers had germplasm of tetraploid wheats which had not been collected previously? In this instance their identity was clarified using sodium dodecylsulphate (SDS) gel electrophoresis, which revealed proteins of high molecular weight, the glutenins, also coded by the D genome (CUBADDA, 1973; LAWRENCE & SHEPHERD, 1980).

All seven populations showed the presence of glutenins, indicating that they were hexaploids, and this was confirmed by chromosome counts of root-tips. Similar tests have been carried out at the Plant Breeding Institute, Cambridge (PAYNE, personal communication), which also confirmed these results. These anomalous electrophoretic patterns may be due to chromosome deletion, or suppression of the genes (KIM & MOSSÉ, 1979; LAWRENCE & SHEPHERD, 1980). Nevertheless, where discrepancies occur between PAGE profiles and collection data, the application of SDS-PAGE can help to resolve such doubts. In addition, vertical slab gel electrophoresis has several advantages over the horizontal system, including the rapidity with which material can be analysed in terms of time taken for the electrophoresis, the number of extracts which can be loaded for each run, and the superior resolution of banding patterns, and is recommended for the rapid evaluation of germplasm variation.

ACKNOWLEDGMENTS

The authors wish to record the assistance of D. Lafiandra and G. Colaprico in carrying out this work. The facilities provided by the National Research Council's Germplasm Institute, Bari, Italy are gratefully acknowledged. The study was financed through the Mediterranean Programme for Genetic Resources of the International Board for Plant Genetic Resources.

REFERENCES

AUTRAN, J. C., W. BUSHUK, C. W. WRIGLEY & R. R. ZILLMAN, 1979. Wheat cultivar identification by gliadin electrophoregrams. IV. Comparison of international methods. Cereal Foods World 24: 471–475.

AYAD, W. G., R. P. CROSTON & M. A. A. KHAWLANI, 1980. Collecting in the YAR II. Plant Genet. Res. Newsl. 43: 25–27.

BENNETT, E., 1970. Tactics in plant exploration. In: O. H. FRANKEL & E. BENNETT (Eds), Genetic resources in plants – their exploration and conservation. Blackwell, Oxford and Edinburgh.

BUSHUK, W. & R. R. ZILLMAN, 1978. Wheat cultivar identification by gliadin electrophoregrams. I. Apparatus, method and nomenclature. Can. J. Plant Science 58: 505–515.

CUBADDA, R., 1973. Chromosomal location of genes controlling the synthesis of some soluble proteins in *T. durum* and *T. aestivum*. Proc. Symp. Genet. Breeding of Durum Wheat, Bari, Italy. 653–659.

DAMANIA, A. B., M. T. JACKSON & E. PORCEDDU, 1983. Morphological variation in wheat and barley from Nepal and Yemen Arab Republic. (In preparation).

DOLL, H. & A. H. D. BROWN, 1979. Hordein variations in wild (*Hordeum spontaneum*) and cultivated (*H. vulgare*) barley. Can. J. Genet. Cytol. 21: 391–404.

ERSKINE, W. & J. J. BOURGOIS, 1979. Wheat and barley in Nepal. Plant Genet. Res. Newsl. 39: 19–22.

KASARDA, D. D., J. E. BERNARDIN & C. C. NIMMO, 1976. Wheat proteins. In: Y. POMERANZ (Ed.), Advances in cereal science and technology, Vol. 1 (4) pp. 158–236. American Association of Cereal Chemists, St. Paul, Minn.

KIM, S. I. & J. MOSSÉ, 1979. Electrophoretic patterns of oat prolamines and species relationships in *Avena*. Can. J. Genet. Cytol. 21: 309–318.

KONAREV, V. G., I. P. GAVRILYUK, T. I. PENEVA, A. V. KONAREV, A. G. KHAKIMOVA & E. F. MIGUSHOVA, 1976. Nature and origin of wheat genomes, based on data from the biochemistry and immunochemistry of wheat proteins. Agric. Biol. 11: 656–665.

KONAREV, V. G., I. P. GAVRILYUK, N. K. GUBAREVA & T. I. PENEVA, 1979. Seed proteins in genome analysis, cultivar identification and documentation of cereal genetic resources: A review. Cereal Chem. 56: 272–278.

LAWRENCE, G. J. & K. W. SHEPHERD, 1980. Variation in gluten protein sub-units of wheat. Austr. J. Biol. Sci. 33: 221–233.

LEE, J. W. & J. A. RONALDS, 1967. Effect of environment on wheat gliadin. Nature 213: 844–846.

MARCHYLO, B. A. & D. E. LABERGE, 1980. Barley cultivar identification by electrophoretic analysis of hordein proteins. I. Extraction and separation of hordein proteins and environmental effects on hordein electrophoregram. Can. J. Plant Sci. 60: 1343–1350.

MARSHALL, D. R. & A. H. D. BROWN, 1975. Optimum sampling strategies in genetic conservation. In: O. H. FRANKEL & J. G. HAWKES (Eds), Crop genetic resources for today and tomorrow. Cambridge University Press, Cambridge.

QUALSET, C. O. & C. W. WRIGLEY, 1979. Electrophoresis and electrofocussing identify wheat varieties. Calif. Agric. 33: 10–12.

SECOND, G. 1982. Origin of the genic diversity of cultivated rice (*Oryza* spp.): Study of the polymorphism scored at 40 isozyme loci. Japanese J. Genet. 57: 25–57.

SHEWRY, P. R., H. M. PRATT & B. J. MIFLIN, 1978. Varietal identification of single seeds of barley by analysis of hordein polypeptides. J. Sci. Fd. Agric. 29: 587–596.

TKACHUK, R. & V. J. METLISH, 1980. Wheat cultivar identification by high voltage gel electrophoresis. Ann. Technol. Agric. 29: 207–212.

WITCOMBE, J. R., 1975. Wheat and barley from two Himalayan regions. Euphytica 24: 431–434.

WRIGLEY, C. W., 1980. The genetic and chemical significance of varietal differences in gluten composition. Ann. Technol. Agric. 29: 213–227.

WRIGLEY, C. W. & J. MCCAUSLAND, 1977. Variety identification by laboratory methods: Instruction manual for barley, wheat and other cereals. CSIRO Wheat Research Unit, Technical Publication No. 4.

ZEHATSCHEK, W., G. GUNZEL & G. FISCHBECK, 1981. Electrophoretic analysis of gliadins in wheat, *Triticum aestivum* L., for determining their inheritance and chromosomal localisation. Z. Pflanzenzüchtg 87: 45–57.

ZILLMAN, R. R. & W. BUSHUK, 1979. Wheat cultivar identification by gliadin electrophoregrams. II. Effects of environment and experimental factors on gliadin electrophoregram. Can. J. Plant Sci. 59: 281–286.