GENETICA POLONICA
Vol. 28 1987 No. 1 - 2

APPLICATION OF ELECTROPHORETIC METHODS OF ISOZYMES SEPARATION TO GENETICAL CHARACTERIZATION OF PEA (*PISUM SATIVUM* L. s. lat.) CULTIVARS[1]

WOJCIECH K. ŚWIĘCICKI, BOGDAN WOLKO[2]

Poznań Plant Breeding, Plant Breeding Station, Wiatrowo

Summary. The range of variation for 43 enzyme systems controlled by 77 loci in *Pisum* has been described in the literature. As monogenic characters with stable phenotypic expression (recognizable in the seedling stage and in seeds) they can be used as markers for description and identification of cultivars. As an example, 56 cultivars of different types of usage and origin have been investigated. For their description the routine measurements of plant characters and observations of genotype — as for the lines in the pea gene bank at Wiatrowo — were performed. Moreover, an electrophoretic analysis (on the starch gel) was made for 3 enzyme systems (LAP, GOT, 6PGD) controlled by at least 6 loci. The obtained results showed the usefulness of the above technic for distinguishing cultivars especially when the hitherto used methods are insufficient (e.g., in the case of selected cultivars or cultivars with identical phenotype or of a similar origin).

The services of cultivar and seed testing, field qualification as well as breeders more and more often meet with difficulties with variety description and in consequence — with estimation of the distinctness, homogenity and stability (DHS).

Descriptions of varieties contain many "unprecise" features strongly influenced by the environment (the hue of green colour of field) or classes (high, medium, small — for the stem length). Also, new suggestions of the International Union for the Protection of New Varieties of Plants (UPOV) for example including the waving of vexillum or width and length of the calyx lobe of pea flowers are not too valuable regarding precision of a cultivar description. For this purpose markers for distinguishing the genotype are necessary—suitable in doubtful explanations on ununiformity and mixtures of qualified materials. The most valuable are monogenic features with stable expression and full penetrance.

For the above purpose the electrophoretic methods of isozymes separation are also suitable. This testing was applied in oat (Almgard, Clapham 1975), barley (Almgard, Landegren 1974), bean (Bassiri, Adams 1978) and grasses (Hayward, Adam 1984, Jones 1984). They were also some indications for peas (Alm-

[1] Received for publication: February 1986.

[2] First author: Doc. Dr. hab.; second author: Dr. Present address: 62-100 Wągrowiec, Poland.

gard 1970). An important contribution for classification and characterization of *Pisum* gene resources are further studies cf Przybylska on isozymes of *Pisum* taxa (Przybylska et al. 1982). But the most significant were studies of Weeden (Weeden, Marx 1984, Weeden 1985). This author has tested 43 enzyme systems. The recognized range of enzymatic variation is controlled by 77 loci (Weeden 1983a).

The main aim of our work was to study the range of enzymatic variation of pea cultivars in several enzyme systems. On the basis of the obtained results (differences between varieties) it was decided to demonstrate the usefulness of isozyme analyses for cultivar characterization.

MATERIAL AND METHODS

The investigations covered 56 lines from the *Pisum* Genebank at Wiatrowo (Święcicki et al. 1981), which presented cultivars for different types of usage (vegetable, dry seeds — for fodder and for human consumption as well as green forage), bred and cultivated in Poland, as well as originating from other countries (Table 1). In exceptional cases it was possible to distinguish one variety from the others without any doubts. Eg. only the cv. Novella was characterized by the gene combination *af, fas*. But within the investigated group, the varieties for which the hitherto known methods of description and identification appeared to be insufficient were taken into consideration:

1. Cultivars of different origin, but with identical phenotype (Neuga, Oranienkroon),
2. Cultivars selected from foreign varieties (Wonder of Kelvedon, Wonder of Amerika),
3. Cultivars with identical hybrid formula or of a similar origin (Aster and Opal bred at different stations, originated from reciprocal cross of the same parental lines; different genotypes of WTD 583 and WTD 785 were selected from WTD 3002 ×Paloma).
4. The polymorphic line Wt 4367 was chosen as an example of the occurrence of two "electrophoretic alleles" of one locus among individuals of the population.

Observations and measurements of morphological features, developmental phases and genotype for cultivar description were performed according to the list of characters and genes observed routinely in the pea genebank (Blixt, Święcicki 1980). The range of plant characters taken into consideration exceeded that of the description used by the registering institution in Poland as well as that contained in the recommendations of UPOV — especially with the regard to the features controlled by single genes.

Plants were sown in the field in 1985. For each plot (cultivar) 60 plants were measured and observed and 20 plants were electrophoretically analyzed.

Three enzyme systems were investigated: LAP (leucine aminopeptidase — 3.4.1.1.), GOT (glutamate oxaloacetate transaminase — 2.6.1.1) and 6PGD (6-phosp-

hogluconate dehydrogenase — 1.1.1.4.3). Leaves from the 3rd — 4th node were used for the analysis. The leaf tissue was crushed and the crude extract was absorbed by filter paper wicks (Whatman 31ET). Electrophoretic separation was performed in starch gel (Serva). For analysis of LAP and GOT 9 per cent gel and the TRIS — citrate/lithium borate buffer system pH 8,1 was used (Scandalios 1969). The electrophoresis was carried out at 30 V/cm² for 3 — 4 hrs at the temperature of 4°C. During the analysis of 6PGD separation was performed in 14 per cent gel using 0.5 M TRIS-versene-borate buffer, pH 8 (Shaw, Prasad 1970). The electrophoresis was carried out at 15 V/cm² for 5 hrs at the temperature of 4°C.

The staining methods for revealing the enzymatic activity of LAP, GOT and 6PGD were identical to the prescriptions of Shaw and Prasad (1970) or were slightly modified.

RESULTS AND DISCUSSION

THE RANGE OF ENZYMATIC VARIATION

The polymorphism of LAP in different tissues and phase development of pea plant was investigated by Scandalios and Espiritu (1969) as well as Almgard and Ohlund (1970). They described two zones of enzyme activity of LAP-1 and LAP-2. Further studies showed that the locus with two co-dominant alleles is responsible for the expression of the faster, polymorphic zone of LAP-1 (Scandalios, Campeau 1972). The third, rare allele of the locus *Lap-1* was described in *P. humile* by Przybylska et al. (1982). Among the investigated cultivars two alleles of the locus *Lap-1* were found in the shape of intensive bands appearing on the level A_1 or A_2 (Fig. 1). Seven polymorphic cultivars were found for the variation range of the locus *Lap-1*, 17 monomorphic for allele A_1 and 32 monomorphic for allele A_2 (Table 1).

The locus *Lap-2* was described as monomorphic by Scandalios and Espiritu (1969). The variation of this zone was reported by Przybylska et al. (1982) as four variants of single-banded, electrophoretic phenotypes, differing insignificantly by electrophoretic mobility. Like in our earlier investigations (Wolko et al. 1985)

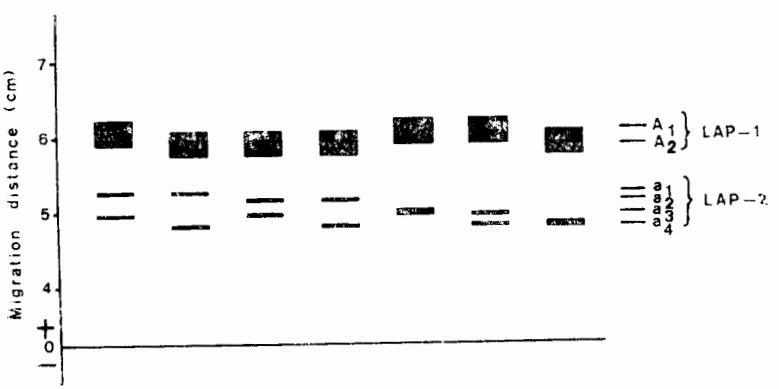

Fig. 1. The range of enzymatic variation of LAP

Table 1. The passport information and description of pea cultivars

Cultivar	Variety gene markers	Origin	Electrophoretic phenotype					
			LAP-1	LAP-2	GOT-1	GOT-2	6PGD-1	6PGD-2
Dry peas								
Aster	*a, le, I*	POL, Flavanda × Auralia	A_1	a_1a_2	B_2	C_2	D_1	E_2
Auralia	*a, Le, I*	DDR,	A_1, A_2	a_1a_3, a_2a_3	B_2	C_2	D_1	E_1
Flavanda	*a, le, I*	NLD, Cebeco	A_1	a_1a_3	B_2	C_2	D_1	E_1, E_2
Hamil	*a, le, I, af*	POL, Porta × (Wąsata × 21/2)	A_1, A_2	a_1a_4, a_3a_4	B_2	C_2	D_1, D_2	E_1
Kaliski	*a, Le, I*	POL, Kujawski Wczesny × Ceser	A_2	a_3a_4	B_2	C_2	D_1	E_1
Karat	*a, Le, I*	POL, (Buława × Iwo) × (Buława × Grestikai)	A_2	a_2a_3	B_2	C_2	D_1	E_2
Legenda	*a, le, I, af*	POL, (Usatyj 5 × Porta) × Porta	A_1	a_1a_3	B_2	C_2	D_1	E_1
Mihan	*a, le, i, af*	POL, (Wąsata × Biała) × Neugatersleben	A_1	a_3a_3	B_2	C_2	D_1	E_1
Neuga	*a, le, i*	DDR,	A_1	a_3a_4	B_2	C_2	D_1	E_1, E_2
Opal	*a, Le, I*	POL, Auralia × Flavanda	A_1	a_2a_3	B_2	C_2	D_1	E_2
Oraniencroon	*a, le, i*	ZAF,	A_2	a_3a_4	B_2	C_2	D_1	E_2
Paloma	*a, le, I*	NLD, Cebeco	A_2	a_3a_3	B_2	C_2	D_1	E_1
WTD 785	*a, le, I*	POL, WTD 3002 × Paloma	A_2	a_2a_3	B_2	C_2	D_1	E_1
Sum	*a, le, I, af*	POL, Porta × Wąsata	A_1	a_1a_4	B_2	C_2	D_1, D_2	E_1
Zefir	*a, le, I*	POL, Buława × Orlik	A_2	a_2a_4	B_2	C_2	D_2	E_2
Wt 4367	*a, Le, I*	ZMB, Local Mprocoso	A_1, A_2	a_1a_3, a_2a_3	B_1B_2	C_2	D_1D_2H	E_1, E_2
Dry peas (for fodder)								
Karo	*a, Le, I*	POL, Selected from land race	A_2	a_1a_4	B_2	C_2	D_1	E_2
Mige	*a, Le, I, Pl*	POL, (Wąsata × I.GL.79) × I.P.III	A_2	a_1a_4	B_2	C_2	D_1	E_1, E_2
Milewska	*a, Le, I, Pl*	POL, Gome × (Wąsata × Biała)	A_1, A_2	a_3a_4	B_2	C_2	D_2	E_1, E_2
Wąsata	*a, Le, af, I, Pl*	POL, Mutant *afila*	A_2	a_2a_2	B_2	C_2	D_2	E_1
Greeu fodder								
Austrian Winter	*A, Le, oh, F, I*	AUS	A_2	a_1a_4	B_1	C_2	D_1, D_2	E_1
Delfin	*A, I, Pl, oh*	NLD, Manholts	A_1	a_1a_4	B_2	C_2	D_1	E_2
Fidelia	*A, Le, I, Obs*	POL, Zeiners Kurz und Gut × R. 1002	A_1, A_2	a_1a_4, a_3a_4	B_2	C_2	D_1, D_2	E_1
Fioletowa	*A, Le, I, oh, Pl*	POL, Country pop. × English pop. × Wiktoria × Folger hybrid × Russian pea	A_1, A_2	a_2a_3	B_2	C_2	D_1, D_2	E_1, E_2
Helia	*A, Le, Pl, l, Obs*	POL, Poneka × Dorina	A_2	a_3a_4	B_2	C_2	D_1	E_2
Kosieczyńska	*A, Lo, I, oh*	POL, (*P. sativum* × *P. arvense*) . *P. quadratum*	A_2	a_2a_4	B_2	C_2	D_1	E_1
Mazurska	*A, Le, I, oh*	POL, (Pop. antocjanowa . Weibull) × × Mutant 361	A_2	a_2a_4	B_2	C_2	D_1	E_1
Mewa	*A, Le, I, Pl, oh, oli*	POL, Fioletowa × Przebędowska Oliwkowa	A_2	a_2a_3, a_1a_4, a_2a_4	B_2	C_2	D_1	E_1, E_2
Nieznanicka	*A, Le, I, oh, Pl*	POL, Selected from land race	A_2	a_2a_2	B_2	C_2	D_1	E_1
Pomorska	*A, Le, I, oh*	POL, Pop. 294 Litwa × Pop. 187 Poniewierz	A_2	a_1a_4	B_2	C_2	D_1	E_1
Rosacrone	*A, b, Le, fas, I, oh*	DEU, K. Behm	A_2	a_3a_4	B_2	C_2	D_1	E_2
WTD 3002	*A, le, oh*	POL, Delfin × Neuga	A_1, A_2	a_1a_4, a_3a_4	B_2	C_2	D_1	E_1, E_2
WTD 583	*A, le, I, Obs, oh*	POL, WTD 3002 × Paloma	A_2	a_2a_4	B_2	C_2	D_1	E_1

Garden peas								
American Wonder	*le, i, R*	USA, Selected	A_2	a_3a_3	B_1	C_2	D_2	E_2
Wonder fon Amerika	*le, i, r*	CHE, Selected	A_2	a_1a_3	B_1	C_2	D_2	E_1
Beniaminck	*Le, i, R*	POL, Bordewunder × Cud Kelvedonu	A_1	a_1a_4	B_2	C_2	D_2	E_2
Confidance	*le, n, r, i*	POL, Selected from Dutch varieties	A_1	a_3	B_1	C_2	D_1	E_1
Cud Kelvedonu	*le, i, r*	POL, Selected from Dutch varieties	A_2	a_1a_3	B_1	C_2	D_2	E_1
Wonder of Kelvedon	*le, i, r*	Selected	A_2	a_1a_3	B_1	C_2	D_2	E_1
Kelvedon Wonder	*le, i, r*	NLD, Selected	A_2	a_1a_3	B_1	C_2	D_2	E_1
Merveille den Kelvedon	*le, i, r*	Selected	A_2	a_1a_3	B_1	C_2	D_2	E_1
Delisa II	*le, i, r*	POL, Selected from *cv.* Delisa	A_1	a_3a_4	B_1	C_2	D_2	E_1
Early Snap	*le, mo, n, p, v, i, r*	USA, Marx, Geneva	A_1	a_1a_3	B_2	C_2	D_2	E_1, E_2
Iweta	*le, i, r*	POL, Delisa II × Gloriosa	A_2	a_2a_4	B_2	C_2	D_1	E_1
Jarek	*fas, le, i, r*	POL, Kronenerbse × Delisa II	A_2	a_3a_4	B_1	C_2	D_2	E_1
Konserwowy IHAR	*le, i, r*	POL, Selected from *cv.* Delikates	A_2	a_3a_1	B_2	C_2	D_1	E_2
Meteor	*le, i, r*	POL, Delisa × Eisparl	A_1	a_3a_4	B_2	C_2	D_1	E_2
Nefryt	*le, i, r*	POL, Cud Kelvedonu × Borda wundor	A_2	a_1a_3	B_1	C_2	D_2	E_1
Nike	*Le, i, r*	POL, Mutant from Delikates	A_1	a_3a_4	B_1	C_2	D_2	E_1
Nora	*le, i, r*	POL, Selected from *cv.* Delikates	A_1	a_3a_4	B_1	C_2	D_2	E_1
Novella	*le, af, fas, I, r*	USA	A_2	a_1a_4	B_1	C_2	D_1, D_2	E_2
Pegaz	*le, n, i, r*	POL, Cud Kelvedonu × Brillant	A_2	a_1a_4	B_1	C_2	D_2	E_1
Rarytas	*le, I, r*	POL, Beta × Deli	A_2	a_2a_4	B_2	C_2	D_2	E_2
Sześciotygodniowy	*Le, i, R*	POL, Selected from *cv.* Express	A_2	a_3a_4	B_2	C_2	D_2	E_2
Telefon	*Le, i, r*	POL, Selected from *cv.* Telefon	A_1	a_4	B_2	C_2	D_1	E_1
Topaz	*le, i, r*	POL, Hada × Szlachetna Perla	A_1	a_4	B_2	C_2	D_2	E_2

a very large enzymatic variation of this zone was found among the presented cultivars. The detected phenotypes are single, broad bands or a combination of two bands of a weaker expression (Fig. 1). Band combinations in individual phenotypes appear on four possible levels marked in the paper from a_1 to a_4. The character of this variation is most probably correlated with properties of the diploid leaf tissue used for the analysis. It suggests the activity of more than one enzymatic locus in the LAP-2 zone. The genetic character of this variation is now being investigated.

The enzymatic variation observed in the LAP-2 zone allowed to find 6 polymorphic cultivars. The remaining, monomorphic varieties are characterized by 7 different electrophoretic phenotypes (Table 1).

Isozymic variation for GOT in *Pisum* was described by Weeden, Gottlieb (1980) and Weeden, Marx (1984). Four isozyme loci were found. The locus *Aat-1* was monomorphic. Isozymes of three remaining, polymorphic loci appeared in different cell organelles.

Isozymes of the locus *Aat-2* appeared in chloroplasts, those of the locus *Aat-3* in mitochondrias, and isozymes of the locus *Aat-4* — in cytosol. The variation of two, polymorphic zones of enzymatic activity was found by Przybylska et. al. (1982). The faster zone was marked as GOT-1 and the slower one — as GOT-2.

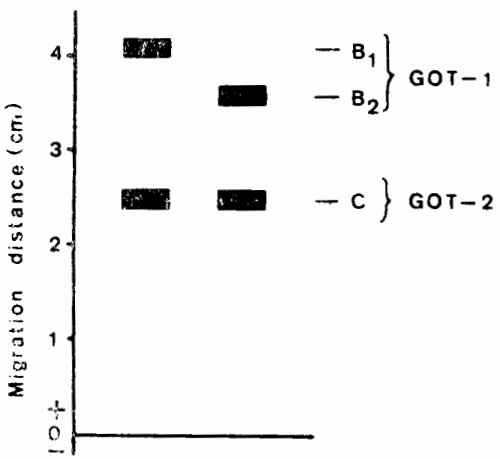

Fig. 2. The range of enzymatic variation of GOT

Among the investigated cultivars the activity of two enzymatic loci has been found. The faster one *Got-1* is adequate to Weeden's locus *Aat-2*. The slower one *Got-2* equals to the locus *Aat-4*. For the locus *Got-1* two variants of single bands on the level B_1 or B_2 have been detected. The locus *Got-2* showed no variation in either of the analysed varieties. They were characterized by a single band C (Fig. 2). Regarding the locus *Got-1* only the line Wt 4367 had a polymorphic character. The remaining cultivars were monomorphic — 15 represented the allele B_1, 40 the allele B_2 (Table 1).

The last analyzed enzyme system — 6 PGD was described by Weeden and Marx (1984) in pea. Two loci were separated: the faster one *6Pgd-1* and the slower

one *6Pgd-2*. The isozymes connected with the locus *6Pgd-1* were revealed in chloroplasts and those for the locus *6Pgd-2* — in the cytosol. The genetic analysis demonstrated that the plastid and cytosolic isozymes were specified by distinct nuclear genes which exhibited independent assortment (Weeden 1983b). In our review of pea cultivars a very broad polymorphism has been found for these loci. The enzymatic activity appeared in a form of single bands: in the zone 6 PGD-1 on the level D_1 or D_2 and in the zone 6 PGD-2 on the levels marked as E_1 or E_2 (Fig. 3). For the locus *6Pgd-1* the polymorphism was revealed in 7 varieties. Among the remaining, monomorphic cultivars 29 represented the allele D_1 and 20 the allele D_2. The analysis for the locus *6Pgd-2* showed 9 polymorphic cultivars, 30 monomorphic for the allele E_1 and 17 for E_2 (Table 1).

Fig. 3. The range of enzymatic variation of 6PGD

Within the line Wt 4367 heterozygote individuals have been found for the locus *6Pgd-1*. Their electrophoretic pattern contained three bands — two on the level of the parental bands and the third one in the middle, a stronger band being a characteristic effect of hybridization of two kinds of the enzyme subunits of the dimeric structure. It should be recognized that it is a very rare example of cross-pollination in pea. It is worth mentioning that the co-dominant type of inheritance of isozyme alleles (heterozygotes differ from all homozygote alleles) gives a possibility to verify the hypothesis of cross-pollination in a qualified material of the cultivars.

APPLICATION OF ENZYMATIC VARIATION FOR IDENTIFICATION AND CHARACTERISATION OF CULTIVARS

The enzymatic variation presented above could be a basis for the discussion on the use of electrophoretic isozyme separation to characterize pea genotypes. The variation range for 43 enzyme systems in pea controlled by 77 loci influencing monogenic characters with a stable phenotypic expression was described in the literature (Weeden 1983a). Meanwhile, our own experiments scarcely for 3 enzyme systems (controled by at least 6 loci) could be useful for additional characteristic of varieties, especially in cases, when the methods used so far seem to be insufficient.

For example, observations of a genotype as well as measurements of plant

characters could show no differences between the cultivars Neuga and Oraniencroon. But in the locus *Lap-1* the cv. Neuga has the allele A_1 and the cv. Oraniencroon — the allele A_2. Moreover, for the gene *6Pgd-2* polymorphism was found in the cv. Neuga: in the analyzed population, individuals with the allele E_1 constituted 10 per cent and those with E_2 90 per cent. Therefore, Neuga and Oraniencroon in spite of the same phenotype (according to the hitherto range of the morphological description) have different genotypes. They differ with the regard to two monogenic characters, i.e. fulfil the requirement for distinctness of cultivars.

Electrophoretic separation of isozymes can be used for a description of the selected varieties. Particularly useful could be the loci for which the polymorphism was found in initial cultivar. It is possible that the selected varieties will differ by the marker gene.

As an example 4 cultivars called Wonder of Kelvedon were analyzed — these varieties were most probably selected in different countries. Unfortunately, it appeared that they have identical electrophoretic pattern for all the investigated loci (equal alleles of the genes *Lap-1*, *Lap-2*, *Got-1*, *Got-2*, *6Pgd-1*, *6Pgd-2*). Then, looking for varietal differences it should be reasonable to analyze other enzyme systems.

On the contrary, the analysis of enzyme variation can be used for distinguishing 2 cultivars named Wonder of America. They have another electrophoretic phenotype in the zone of enzyme activity of LAP-2 and different allele of the gene *6Pgd-2*. The fact, that both varieties are monomorphic for opposed alleles suggests that they were selected from another, third cultivar — polymorphic for the gene *6Pgd-2*.

It seemed interesting to compare cultivars of close origin, especially when they were bred from the same parental forms. For example, Aster and Opal — bred at different breeding stations (Lipie and Wiatrowo) — originated from reciprocal crossing of cv. Auralia and Flavanda (Table 1). The analysis of enzyme variation of the parental varieties showed polymorphism in Auralia for *Lap-1* (allele A_1 — 63 per cent of individuals, A_2 — 37 per cent) and *Lap-2* ($a_1\,a_3$ — 47 per cent, $a_2\,a_3$ — 53 per cent). In the cv. Flavanda the phenotype $a_1\,a_3$ of *Lap-2* and the allele A_1 of the locus *Lap-1* were found. Moreover, this variety was polymorphic for the locus *6Pgd-2* (E_1 — 5 per cent: E_2 — 95 per cent). In the genotypes of the cultivars bred from the crossing of Auralia and Flavanda the allele A_1 of the gene *Lap-1* and the allele D_1 of *6Pgd-1* were detected. However, they differ in phenotypes of *Lap-2* (Opal — a_2a_3, Aster — a_1a_3). With regard to the investigated isozyme loci Aster and Opal differ from one another and from the parental varieties — at least by one allele. Moreover there is a difference in the stem length: Aster is about 60 cm long and Opal — 80 cm long.

A similar analysis can be conducted for the strains WTD 583 and WTD 785 originating from the cross combination WTD 3002 ×Paloma (Fig. 4). Both the parental forms and breeding strains differ from one another by the electrophoretic pattern in the LAP-2 zone. Moreover, they have different alleles of the gene causing anthocyanin synthesis (*A*—the presence of anthocyanin in a plant: *a*—anthocyanin inhibition).

From the view point of the analysis of enzyme variation, cultivars with polymorphism of a given isozyme locus are of interest. It should not be surprising that in pea, diploid and self-pollinating species, individuals with different alleles of one locus, exist in the population (even in the theoretically pure line). It concerns genes not considered during selection.

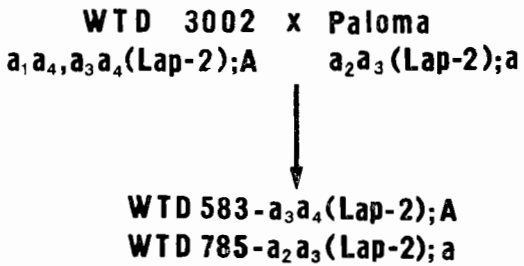

Fig. 4. The origin and the genotype of the strains WTD 583 and WTD 785

The line Wt 4367 found in the earlier studies to have polymorphism for LAP-1, LAP-2, GOT-1, 6PGD-1 and 6PGD-2 was included in the group of the investigated varieties. The analysis confirmed a stable frequency of alleles of polymorphic genes in two, consecutive years, 1984 and 1985. Polymorphism was also found for other 13 cultivars (Table 1). It means that pea varieties being pure lines with the regard to a number of characters/genes considered during breeding process can be polymorphic from the point of view of the isozyme loci. However, the polymorphism of enzyme genes is not an obstacle for variety identification. A stable per cent of alleles of a given locus in a population is also characteristic of a cultivar.

The enzyme variation observed in investigated set of cultivars for LAP, GOT and 6PGD permits to infer that not each enzyme system is equally useful for the description of a cultivar. LAP and 6PGD, contrary to GOT, are characterized by a wide range of variation. The more polymorphic is a system the more useful it seems to be for characterization and identification of cultivars.

The results of the investigations presented above indicate that it is possible to employ electrophoretic analysis for a description of cultivars. The use of an additional group of a number of monogenic characters of stable expression (recognizable in the seedling stage and in seeds) should be a routine while registering varieties and testing seeds. The presented methods should be applied supplementally when descriptions used so far for testing DHS are insufficient. This concerns cases when a distinquishing feature of a cultivar is searched for before registration it or when there are certain doubts in descriptions of homogenity of qualified fields.

REFERENCES

1. Almgard G. (1970). Cultivar distinction by means of isozyme technique in *Pisum*. Pisum Newslet., 2: 9.
2. Almgard G., Clapham D. (1975). Isozyme variation distinguishing 18 *Avena* cultivars grown in Sweden. Swedish Journal of Agricultural Research, 5: 61 - 67.

3. Almgard G., Landegren U. (1974). Isoenzymatic variation used for the identification of barley cultivars. Zeitschrift für Pflanzenzüchtung, 72: 63 - 73.
4. Almgard G., Ohlund K. (1970). Inheritance and location of a biochemical character in *Pisum*. Pisum Newslet., 2: 9.
5. Bassiri A., Adams M. W. (1978). An electrophoretic survey of seedling isozymes in several *Phaseolus* species. Euphytica, 27: 447 - 459.
6. Blixt S., Święcicki W. K. (1980). Międzynarodowy bank genów *Pisum*. Post. Nauk Rol., 6: 31 - 38.
7. Hayward M. D., McAdam N. J. (1984). Interpretation and application of isozyme techniques for variety discrimination in grasses. Proceedings of ISTA Symposium on Biochemical Tests for Cultivar Identification: 78.
8. Jones T. W. A. (1984). Optimising isozymic separation for cultivar identification in grasses. Proceedings of ISTA Symposium on Biochemical Tests for Cultivar Identification: 109.
9. Przybylska J., Blixt S., Parzysz H. and Zimniak-Przybylska Z. (1982). Isozyme variation in the genus *Pisum*. Electrophoretic patterns of several enzyme systems. Genetica Polonica, 23: 103 - 121.
10. Scandalios J. G. (1969). Genetic control of multiple forms of enzymes in plants. A review. Biochem. Genet., 3: 37 - 79.
11. Scandalios J. G., Campeau M. A. (1972). Mutant aminopeptidases in *Pisum*. II. Nuclear gene control of mitochondrial isozyme. Mutation Research, 14:397-403.
12. Scandalios J. G., Espiritu L. G. (1969). Mutant aminopeptidases of *Pisum sativum*. I. Developmental genetics and chemical characteristics. Molec. Gen. Genetics. 105:101-112.
13. Shaw Ch. R., Prasad R. (1979). Starch gel electrophoresis of enzymes a compilation of recipes. Biochemical Genetics, 4: 297 - 320.
14. Święcicki W. K., Święcicki W., Czerwińska S. (1981). The Catalogue of *Pisum* Lines, PWRiL, Poznań.
15. Weeden N. F. (1983a). Isozyme variation at selected loci in *Pisum*. Pisum Newslet., 15: 58 - 59.
16. Weeden N. F. (1983b). Location and genetics of 6 phosphogluconate dehydrogenase isozymes in *Pisum sativum*. Plant Physiology, 72, suppl. 1: 124.
17. Weeden N. F. (1985). An isozyme linkage map for *Pisum sativum*. The Pea Crop — A Basis for Improvement (Proceedings). University of Nottingham School of Agriculture.
18. Weeden N. F., Gottlieb L. D. (1980). The genetics of chloroplast enzymes. The Journal of Heredity, 71: 392 - 396.
19. Weeden N. F., Marx G. A. (1984). Chromosomal locations of twelve isozyme loci in *Pisum sativum*. The Journal of Heredity 75: 365 - 370.
20. Wolko B., Krzakowa M., Święcicki W. K. (1985). Leucine aminopeptidase (LAP-2) variability in the genus *Pisum*. Pisum Newslet., 17: 86 - 88.

ZASTOSOWANIE ELEKTROFORETYCZNYCH METOD ROZDZIAŁU IZOENZYMÓW DO GENETYCZNEJ CHARAKTERYSTYKI ODMIAN GROCHU (*PISUM SATIVUM* L. *S. LAT.*)

Streszczenie

Badania obejmowały 56 odmian grochu o różnym pochodzeniu i kierunku użytkowania. Wykonano obserwacje cech roślin i genotypów według systemu stosowanego w Banku Genów Grochu w Wiatrowie. Przeprowadzono ponadto rozdział elektroforetyczny (na żelu skrobiowym)

dla trzech systemów elektroforetycznych (LAP, GOT, 6PGD), kontrolowanych przynajmniej przez 6 loci. Uzyskane wyniki wskazują na przydatność technik elektroforetycznych dla odróżniania odmian uprawnych, szczególnie w tych przypadkach, gdy dotychczas stosowane sposoby opisu okazują się niewystarczające (np. dla odmian selekcjonowanych lub odmian o identycznym fenotypie czy też o bliskim pochodzeniu).

ИСПОЛЬЗОВАНИЕ ЭЛЕКТРОФОРЕТИЧЕСКИХ МЕТОДОВ ДЕЛЕНИЯ ИЗОЭНЗИМОВ ДЛЯ ГЕНЕТИЧЕСКОЙ ХАРАКТЕРИСТИКИ СОРТОВ ГОРОХА (*PISUM SATIVUM* L. *S. LAT.*)

Резюме

Исследования охватывали 56 сортов гороха различного происхождения и использования. Проведены наблюдения признаков растений и генотипов согласно системе, применяемой в банке генов гороха в Вятрове. Кроме того, произведён электрофоретический раздел (на крахмальном гелю) для трёх электрофоретических систем (LAP, GOT, 6 PGD), контролируемых по крайней мере 6 локусами. Полученные результаты указывают на пригодность электрофоретической техники для выделения культивируемых сортов, особенно в тех случаях, когда применяемые до сих пор способы описания оказываются недостаточными (например, для селектированных сортов или для сортов с идентичным фенотипом или близкого происхождения).