Theor. Appl. Genet. 63, 283–287 (1982)

THEORETICAL AND APPLIED GENETICS

Location of Structural Genes for Glucose Phosphate Isomerase and for Leucyl Aminopeptidase on Chromosome VII of *Petunia*

H. J. W. Wijsman and B. M. van den Berg
Institute of Genetics, University of Amsterdam, Amsterdam (The Netherlands)

Summary. A gene termed *gpiB*, coding for one of the two isoenzyme zones of glucose phosphate isomerase in *Petunia*, has been mapped to a locus on chromosome VII by means of linkage to the marker *An4*, and by an allelic dosage effect on enzyme activity in trisomics. The high degree of linkage of electrophoretic alleles of *gpiB* to the pollen colour allele pair *An4/an4*, as demonstrated in the ancestral species, *P. axillaris* s.l. and *P. integrifolia* s.l., has been conserved in all cultivars of *P. hybrida* investigated. Another gene, coding for the enzyme leucyl-aminopeptidase could also be mapped to chromosome VII and the gene order *An4* – *lapB* – *gpiB* determined. Apparently, distribution of *lapB* alleles is not related to the hybrid descent of *P. hybrida*.

Key words: *Petunia hybrida* – Glucose phosphate isomerase – Leucyl-amino peptidase – Structural gene – Linkage

Introduction

Isoenzymes of *Petunia* have been studied and some structural genes encoding them have been mapped to one of the seven chromosomes (review, Wijsman, in press). Isozyme markers are free of phenotypic interaction and as such practical for genetics. In *Petunia*, classical markers are known on all chromosomes (Maizonnier and Moessner 1979). However, the "linkage group" on chromosome VII comprises the gene *An4* as nearly the only gene suitable to simple genetic analysis and even so only in a particular genetic background (i.e., when the factor *Hfl* is present, Wiering et al. 1979). The present paper reports on two isozyme structural genes on chromosome VII.

Glucose phosphate isomerase is an enzyme of prime importance in glucose metabolism. The present paper describes the existence in *Petunia* of two alleles of one of the gene loci coding for the enzyme and location of the gene on the genetic map. In *Clarkia* (Gottlieb 1977), *Lolium* (Hayward and McAdams 1977), *Pinus* (Adams and Joly 1980), and tomato (Tanksley 1980) the symbol *pgi* has been used for the gene; in mouse (Peterson and Wong 1978) the symbol is *gpi*. We decided to follow the latter convention, in accordance with the recommendations of IUPAC.

Leucyl-aminopeptidase was one of the first enzymes for which a structural gene could be characterized (in maize: Beckman et al. 1964).

Materials and Methods

To study the migrational variation in electrophoretic bands of glucose phosphate isomerase, several inbred lines were used of the *Petunia* collection of the Institute of Genetics, University of Amsterdam. As parents for crosses the following lines have been used: V23 (cultivar "Blauzwerg"), R51 (from cultivar "Royal Ruby"), S6 (*Petunia inflata* from Dr. K. C. Sink); and A4, Vu 6, V35 (recombinant lines). For *gpi* and *lap* genotypes, see Table II; in addition: A4: *gpiB2B2*, Vu 6: *B2B2*, V35 *gpiB2B2*. Most lines investigated have *lapB1B1* genotype; the exceptions are mentioned in the text.

Trisomics VII were found among the collection of *Petunia* trisomics formed by the late Dr. F. J. Smith; in different genetic backgrounds they were the following clones: PX3018J14 (*gpiB1B2B2*); PY3010-64 (*gpiB1B1B2*); PT7601A2 (*gpiB1B1B2*). Furthermore, a complete series of seven trisomics from the Station d'Amélioration des Plantes, Dijon (Dr. D. Maizonnier), twice backcrossed to the doubled haploid and inbred line TLh1, was used.

Horizontal starch gel electrophoresis was carried out with the TVB buffer system and staining for glucose phosphate isomerase activity, both as described by Siciliano and Shaw (1976), but with the following modifications: concentration fructose-6-phosphate at 200 mg per 100 ml; nitro blue tetrazolium instead of dimethyl-thiazolyl-diphenyl tetrazolium bromide (MTT); fixation in 50% methanol.

Staining for leucyl aminopeptidase activity was according to Beckman et al. (1964). The gel, however, was preincubated during 15 min in a 0.5 M borate (+0.005 M MgCl 2) buffer, pH 4.2. Staining was in 0.1 M phosphate buffer (pH 6.1) after 45 min incubation with substrate at 37 °C.

0040-5752/82/0063/0283/$ 01.00

Leaves or pieces of stems were crushed in a buffer solution (0.1 M Tris-HCl, pH 7.5) with dithioerythritol (0.02 M) added, as well as insoluble polyvinyl pyrrolidone concentration (Polyclar AT, BDH Chemicals Ltd).

As to the nomenclature for loci governing isoenzymes, we follow a recent proposal for *Petunia* (de Vlaming et al. 1980). Classes of genes are indicated by three-letter, lower case symbols; genes by capitals; alleles by a serial number; enzymes corresponding to the gene by upper case symbols; e.g., the *GPIa* enzyme as specified by the *gpiA* gene.

Results

1 Electrophoretic Variation

In *Petunia* leaves, stems, roots, and flowers, glucose phosphate isomerase (GPI) activity can electrophoretically be separated into two zones, A and B, as is the case in several other plants (*Clarkia:* Gottlieb 1977; loblolly pine: Adams and Joly 1980; tomato: Tanksley 1980; *Silene:* Verkley 1980; *Stellaria:* Verkley et al. 1980; pea: Weeden and Gottlieb 1981); (see Figs. 1, 2). From gels as the one in Fig. 2 it appears that glucose phosphate isomerase does not form interzone heterodimers. However, often, as in Fig. 1, there is a zone of staining probably indicating native glucose-6-phosphate dehydrogenase activity about halfway between the GPI zones.

Variation in electrophoretic mobility has only been found for the *gpiB* product. Two allelomorphs can be distinguished, and the responsible alleles have been designated *gpiB1* and *gpiB2,* coding for the faster and slower band, respectively.

Staining for leucyl aminopeptidase (LAP) activity reveals one major activity zone accompanied by some minor bands (Fig. 3). The major band seemed invariant until in one inbred line (Vu 6) a slower variant was found. The standard allele was designated *lapB1*; the allele coding for the slower variant *lapB2*. Later, another variant has been discovered (see discussion).

2 Crosses (Diploids)

The GPIb as well as the LAPb allozymes behave codominantly in a heterozygous plant and seem to have a monomeric character (Figs. 1–3).

The inbred lines V23 and R51 were crossed and a backcross to R51 was analyzed. The parents differ in several flower colour markers (Wiering et al. 1979), at least one marker gene on each chromosome. High linkage of 95% between gpiB and *An4* could be demonstrated; the linkage was confirmed by data from the F2 generation (unpublished results). From its linkage to An4 follows assignment of the gene *gpiB* to chromosome VII (Maizonnier and Moessner 1979).

In a backcross to line Vu 6 of the F1 of inbred lines V23 and Vu 6, linkage between *An4* and lapB was found (85%). From the B1 a recombinant plant was crossed to inbred line V35. From the three-point cross the gene order *An4-lapB-gpiB* can be deduced (Table 1), with distances *An4*-lapB = 2 cM ± 1.3, and *An4*-gpiB = 8 cM ± 2.6.

3 Trisomic Dosage Effect

Amongst a collection of random trisomics those with the phenotype of trisomy VII as described by Maizonnier (1976) were preselected; the additional chromosome was identified as chromosome VII, the smallest chromosome of *Petunia* with a median centromere. Next, several clones trisomic for chromosome VII were investigated to demonstrate the presence of a third allele of gpiB; moreover, a largely isogenic series of trisomics obtained from Dr. M. Maizonnier could be investigated. Normally the slow allele, *gpiB2,* gives a darker band than *gpiB1.* In some heterozygous trisomics, to which we ascribe the genotype *gpiB1B1B2,* the difference is much less extreme; in others, by contrast, it is more extreme and we assume these to have a

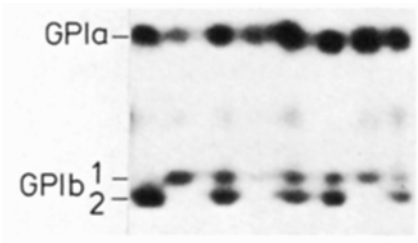

Fig. 1

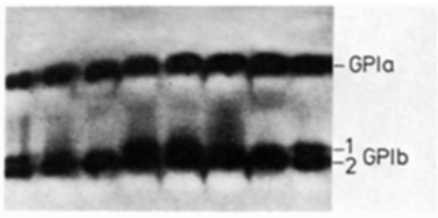

Fig. 2

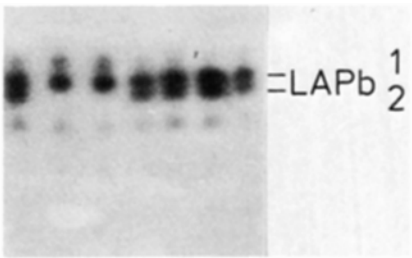

Fig. 3

Fig. 1. Gel showing genetic segregation for *gpiB* in a backcross. Parents feature on the left. Upper zone: *GPIa;* lower zone: *GPIb;* the faster allozyme is *GPIb1,* the slower *GPIb2*

Fig. 2. Gel showing genetic segregation when a gpiB1B1B2 trisomic (far right) has been crossed to the diploid tester, line A4 (second from right). Furthermore, from right to left, progeny of the cross with different contributions of the B1 and B2 electromorphs. Densitometric tracings of the parents and of two progeny plants can be found in Fig. 4b, c

Fig. 3. Gel showing genetic segregation for *lapB1* and *lapB2.* The *lapB1* parent was line V23; in this particular line there is an additional fast band (LAPa) that seems to be linked to the presence of *LAPb1*

Table 1. Three-point cross involving *An4*, lapB, and gpiB

Parental lines:	V23	*An4An4*	*lapB1B1*	*gpiB2B2*
	Vu 6	*an4an4*	*lapB2B2*	*gpiB1B1*
	V35	*an4an4*	*lapB1B1*	*gpiB1B1*

An isolated plant from the backcross (V23 × Vu 6) × Vu 6 of putative genotype *An4an4 lapB1B2 gpiB1B2* was crossed to V35

Genotype			Number		Number of cross-overs required if gene order *An4 – lapB – gpiB*	
An4an4	*lapB1B1*	*gpiB1B2*	47	101	0	
an4an4	*lapB1B2*	*gpiB1B1*	54			
An4an4	*lapB1B2*	*gpiB1B1*	1	2	1	
an4an4	*lapB1B1*	*gpiB1B2*	1			
An4an4	*lapB1B1*	*gpiB1B1*	2	7	1	
an4an4	*lapB1B2*	*gpiB1B2*	5			
An4an4	*lapB1B2*	*gpiB1B2*	0	0	2	
an4an4	*lapB1B1*	*gpiB1B1*	0			

Table 2. Genotype for the linked genes *An4* and gpiB in inbred lines derived from different cultivars of *Petunia hybrida*, as well as for wild species from different sources

Inbred line	Cultivar/species	*An4*	Genotype	
			gpiB	*lapB*
R1	Fire Chief	*An4*	2	1
R51	Royal Ruby	*an4*	1	1
M1	Rose of Heaven	*An4*	2	1
M3	Radiance	*an4*	1	1
H11	Silvery Blue	*An4*	2	1
V2	Admiral	*An4*	2	3
V7	Gottfried	*An4*	2	1
V21	Michaelis Dark Purple	*an4*	1	1
V23	Blauzwerg	*An4*	2	1
V30	Dwarf Velvet Ball	*An4*	2	1
W4	Pendula Cyanea	*an4*	1	3
W28	Roter Vogel	*an4*	1	1
S1, S2	*P. axillaris* s. s.	*an4*	1	1
S7	*P. axillaris ssp. parodii*	*an4*	1	1
S6, S9	*P. integrifolia ssp. inflata*	*An4*	2	1
S 12	*P. integrifolia* s. s.	*An4*	2	1

gpiB1B2B2 genotype (Figs. 2, 4). The expression of B1 relative to B2 is somehow variable from assay to assay, but an additional gene dose as illustrated by relative activities, can reproducibly be demonstrated.

To confirm the assignment, trisomic progeny of the cross PY3010-64 (*gpiB1B1B2*) × A4 (*gpiB2B2*) was preselected in view of the low transmission rate of trisomy VII. As in all trisomics in *Petunia*, trisomic VII seed germinates distinctly later than diploids, and is slower

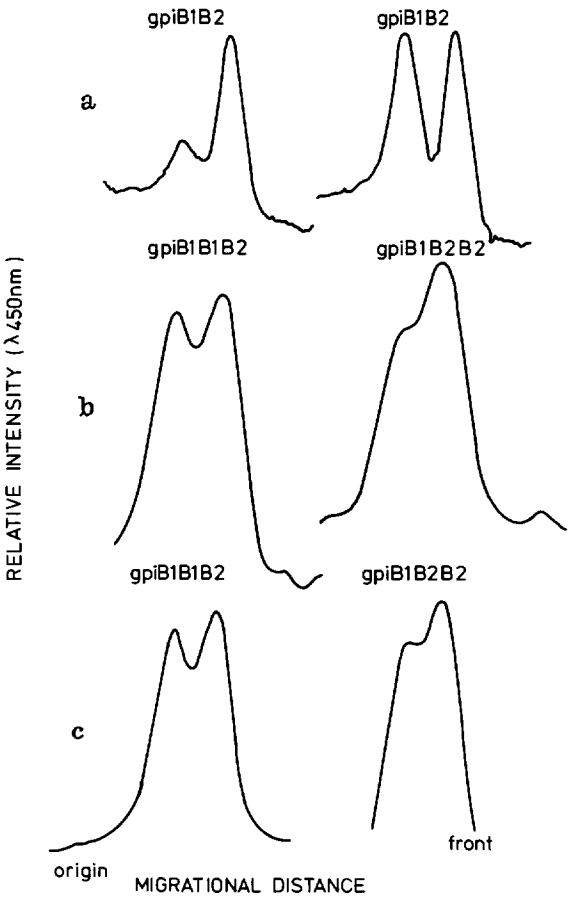

Fig. 4a–c. Densitrometric scans of the genotypes indicated. **a** Largely isogenic trisomics of the Dijon collection; left, trisomic I; right trisomic VII, with an extra dose of gpiBl on the additional chromosome. **b** Trisomics VII from the Amsterdam collection, to show the differential effect ascribed to extra doses of gpiBl and B2, respectively. **c** Trisomics from the progeny when the trisomic *gpiB1B1B2* (left plant of b) was backcrossed to diploid *gpiB2B2*. Both types expected could be found. B and C are from the same gel, A is from a different gel

in development. By selection of late germinators 8 trisomics could be recovered from the progeny and divided into two classes, one representing *gpiB1B1B2* and the other *gpiB1B2B2* (Figure 4c). All trisomics had one of the two phenotypes; in view of the low number recovered we must assume that the numbers found (4 for each of the two classes) fit the numerical 1 : 2 relationship expected. As to *lap*, such a trisomic analysis has not been carried out.

4 Distribution of the Alleles Among Cultivars

Petunia hybrida does not occur in nature and is a diploid of hybrid descent. The high linkage of *gpiB* and *An4* apparently is a feature inherited from the ancestral species, *P. axillaris* s.l. and *P. integrifolia* s.l. (see

Wijsman 1982). In our material all *P. axillaris* (ssp. *axillaris* and ssp. *parodii*) has yellow pollen (*an4an4*) combined with gpiB1, and all *P. integrifolia* (ssp. *integrifolia* and ssp. *inflata*) blue pollen (*An4An4*) combined with *gpiB2*. We investigated inbred lines representing 12 cultivars. Complete conservation of the linkage of *An4* to gpiB2 and of *an4* to gpiB1 was found (Table 2). Note that *An4An4* in a *hf1hf1* background also leads to yellow (or white) pollen (Wiering et al. 1979).

On the other hand, the distribution of *lapB* alleles among cultivars and inbred lines as well as wild species appears to be independent of the hybrid descent and could either find its origin in polymorphism in both wild species or in mutations during cultivation.

Discussion

We consider the combination of high linkage of gpi to *An4* as well as the association of a dosage effect with trisomy VII sufficient evidence for the location of *gpiB*. *An4* must be located at one of the ends of the metacentric chromosome (Maizonnier & Moessner 1979), so *gpiB* is likely to be situated not far removed from the same end. Other markers on chromosome VII are scarce. Fortunately, the lap B gene is also highly linked to *An4*.

The dosage effect found is further evidence for situating *An4*, *lapB*, and *gpiB* on chromosome VII. A change in the relative contribution of allelic products, as revealed by a zymogram, seems to us an elegant demonstration of the presence of a gene, if it is only assumed that the color intensity of a band is strongly correlated with the number of primary gene products formed. Expressing the total activity per μg protein is not necessarily the proper measurement, on the one hand because we have been working with crude extracts, on the other because of the possible influence of the trisomy involved on protein content. We believe determination of a gene dosage effect in absolute terms is dependent on expressing the one enzyme activity relative to that of another enzyme activity, with both enzymes residing in the same subcellular fraction (organelle, cytosol); and then only when the relevant structural genes are not situated on the additional chromosome concerned. We are the more reluctant to take our data as an absolute assay because of the (minor) variation from gel to gel in relative intensity between the two allele products, for which we cannot yet suggest a plausible cause.

As to the formation of dimers, there is a marked difference between our data and the apparent homodimerization between allelic products of the slower GPI enzyme as found in tomato, *Clarkia*, *Pinus*, *Lolium*, and pea (for references see above). If GPIb is indeed homologous to the enzyme for the chloroplastic, dimeric glucose phosphate isomerase, as in *Clarkia* and pea (Weeden and Gottlieb 1980), possibly our way of preparing the extract may have created the absence of a homodimer as an artifact.

Since telotrisomics have been isolated in the progeny of several trisomics VII and diploid testers, we can now look for the presence of gpiB to distinguish between the two possible telotrisomics of equal length. Sometimes in the progeny of trisomics aberrant types can be found that carry another surplus chromosome than the parental one; the same occurs in *Petunia* (unpublished observations). Therefore, absence of a GPI dosage effect need not necessarily identify the other possible telotrisomic VII; careful cytological measurements are necessary, the more so since no marker has as yet been found on chromosome VII located to the other side of the centromere, relative to *An4*. *An4* is not a very practical standard marker since it only expresses itself in plants carrying the Hf1 factor (Wiering et al. 1979), absent from many cultivars.

Apparently, the hybrid descent of *Petunia hybrida* is at the root of the allelic variation found. Amongst a limited number of plants of *P. integrifolia* (*spp. integrifolia* and *ssp. inflata*) grown from seeds collected in the wild, no other allelomorphs were discovered. In all cultivars investigated the arrangement of *An4* and *gpiB* was the ancestral one. By contrast, in the case of leucyl aminopeptidase, *lapB1* is the predominating allele, with *lapB2* (slow) present in a few inbred lines only (viz., Vu 6 and the line R96 (=Sh2), inbred in Dijon and of doubled haploid descent). However, in addition a third allele, *lapB3*, faster than lapB1, was discovered in some of our lines (V2, R74, W4, and W115), of which the latter two may share a *P. axillaris* ancestor. During cultivation of *P. hybrida* no new alleles of gpi seem to have been created by mutation, as apparently has been the case with leucyl aminopeptidase or shikimate dehydrogenase (unpublished observations), though it can never be excluded that such "third" alleles are derived from local populations. Table 2 clearly demonstrates contribution of both ancestral species to the present day *P. hybrida* gene pool.

Literature

Adams, W.T.; Joly, R.J. (1980): Genetics of allozyme variants in loblolly pine. J. Hered. **71**, 33–40

Beckman, L.; Scandalios, J.G.; Brewbaker, J.L. (1964): Genetics of leucine aminopeptidase isozymes in maize. Genetics **50**, 899–904

Fobes, J.F. (1980): Trisomic analysis of isozymic loci in tomato species: segregation and dosage effect. Bioch. Genet. **18**, 401–421

Gottlieb, L.D. (1977): Evidence for duplication and divergence of the structural gene for phosphoglucoisomerase in diploid species of *Clarkia*. Genetics **83,** 289–307

Hayward, M.D.; McAdams, N.J. (1977): Isozyme polymorphism as a measure of distinctiveness and stability in cultivars of *Lolium perenne*. Z. Pflanzenzücht. **79,** 59–68

Khush, G.S. (1973): Cytogenetics of aneuploids. New York, London: Acad. Press

Maizonnier, D. (1976): Etude cytogenetique de variations chromosomiques naturelles ou induites chez *Petunia hybrida* Hort. Thèse, Université de Dijon

Maizonnier, D.; Moessner, A. (1979): Localization of the linkage groups on the seven chromosomes of the *Petunia hybrida* genome. Genetica **51,** 143–148

Peterson, A.C.; Wong, G.G. (1978): Genetic regulation of glucose phosphate isomerase in mouse oocytes. Nature **276,** 267–269

Siciliano, M.J.; Shaw, C.R. (1976): Separation and visualization of enzymes on gels. In: Chromatographic and electrophoretic techniques, **2.** Zone electrophoresis (ed. Smith, I.), pp. 185–209. London: Heinemann Medical Books

Tanksley, S.D. (1980): PGI-1, a single gene in tomato responsible for a variable number of isozymes. Can. J. Genet. Cytol. **22,** 271–278

Verkley, J.A.C. (1980): Isoenzyme variation in pioneer species from ecologically different habitats. PhD Thesis, Free University, Amsterdam

Verkley, J.A.C.; Boer, A.M. de; Lugtemborg, T.F. (1980): On the ecogenetics of *Stellaria media* (L.) Vill. and *Stellaria pallida* (Dum.) Piré from abandoned arable fields. Oecologia **46,** 354–359

Vlaming, P. de; Wiering, H.; Maizonnier, D.; Cornu, A. (1980): Proposal for a uniform genetic nomenclature in *Petunia hybrida*. Plant Mol. Biol. Newslett. **1,** 45–47

Weeden, N.F.; Gottlieb, L.D. (1980): The genetics of chloroplast enzymes. J. Hered. **71,** 392–396

Wiering, H.; Vlaming, P. de; Cornu, A.; Maizonnier, D. (1979): *Petunia* genetics. I. List of genes. Ann. Amél. Plant. **29,** 611–622

Wijsman, H.J.W. (1982): On the interrelationships of certain species of *Petunia*. 1: Taxonomic notes on the parental species of *Petunia hybrida*. Acta Bot. Neerl. **31,**

Wijsman, H.J.W.: Current status of isozyme research in *Petunia*. In: Isozymes in plant genetics and breeding (ed. Tanksley, S.D.; Orton, T.J.). Amsterdam: Elsevier (In press)

Received July 16, 1982
Communicated by H. F. Linskens

Dr. H. J. W. Wijsman
Drs. B. M. van den Berg
Institute of Genetics
Kruislaan 318
NL-1098 SM Amsterdam (The Netherlands)