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Genetical control and linkage relationships of Isozyme markers in sugar beet (*B. vulgaris* L.). 2. NADP- and NAD-specific malate dehydrogenases, 6-P-gluconate dehydrogenase, shikimate dehydrogenase, diaphorase and aconitase

J. P. C. Van Geyt [1,*], E. Smed [2] and M. Oléo [3]

[1] ICI Seeds-SES, Industriepark 15, B-3300 Tienen, Belgium
[2] Maribo Seed, Danisco A/S, DK-4960 Holeby, Denmark
[3] Instituut voor Moleculaire Biologie, Vrije Universiteit Brussel, Dienst Plantengenetika, B-1640 St-Genesius Rode, Belgium

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Summary. The NADP-specific malate dehydrogenase isozymes were controlled by multiple gene systems. Three genes coding for dimeric enzymes segregated in a dependent fashion (NADP-Mdh_1, NADP-Mdh_2, NADP-Mdh_3). A fourth gene (NADP-Mdh_4), also coded for dimers, but was not polymorphic in *B. vulgaris*. A fifth gene (NADP-Me_1) coded for enzymes active as monomers. Two genes were found to control the main zone of NAD-specific malate dehydrogenase: one coded for dimers (Mdh_1), while a second (Mdh_2) was not polymorphic in the assessions studied. 6-P-Gluconate dehydrogenase was not polymorphic in *B. vulgaris*; the two types detected on SGE_1 electrophoresis were due to developmental expression of the different systems. No genetical segregations could be detected in progeny of crosses of the distinct phenotypes. A shikimate dehydrogenase gene ($Skdh_1$) that coded for monomers was identified. The diaphorase system was rather complex, but one gene (Dia_1) coding for monomeric enzymes could be identified. Aconitase was found to be controlled by two independent genes (Aco_1, Aco_2), both polymorphic and coding for proteins active as monomers.

Tight linkage was found between the genes NADP-Mdh_1, NADP-Mdh_2 and NADP-Mdh_3. Linkage was also found between a pollen fertility restorer (Z) and the Mdh_1 gene. The identification of linkage with Aco_1 needs further investigation. R segregated independently from Mdh_1, Aco_1 and Dia_1. Independent segregations were scored for isozyme genes Pgm_2, Icd_1, Ak_1, Gpi_1, Aco_1 and Dia_1.

Key words: *Beta vulgaris* – Sugar beet – Isozymes – Genetics – Linkage – Pollen fertility restorer

Introduction

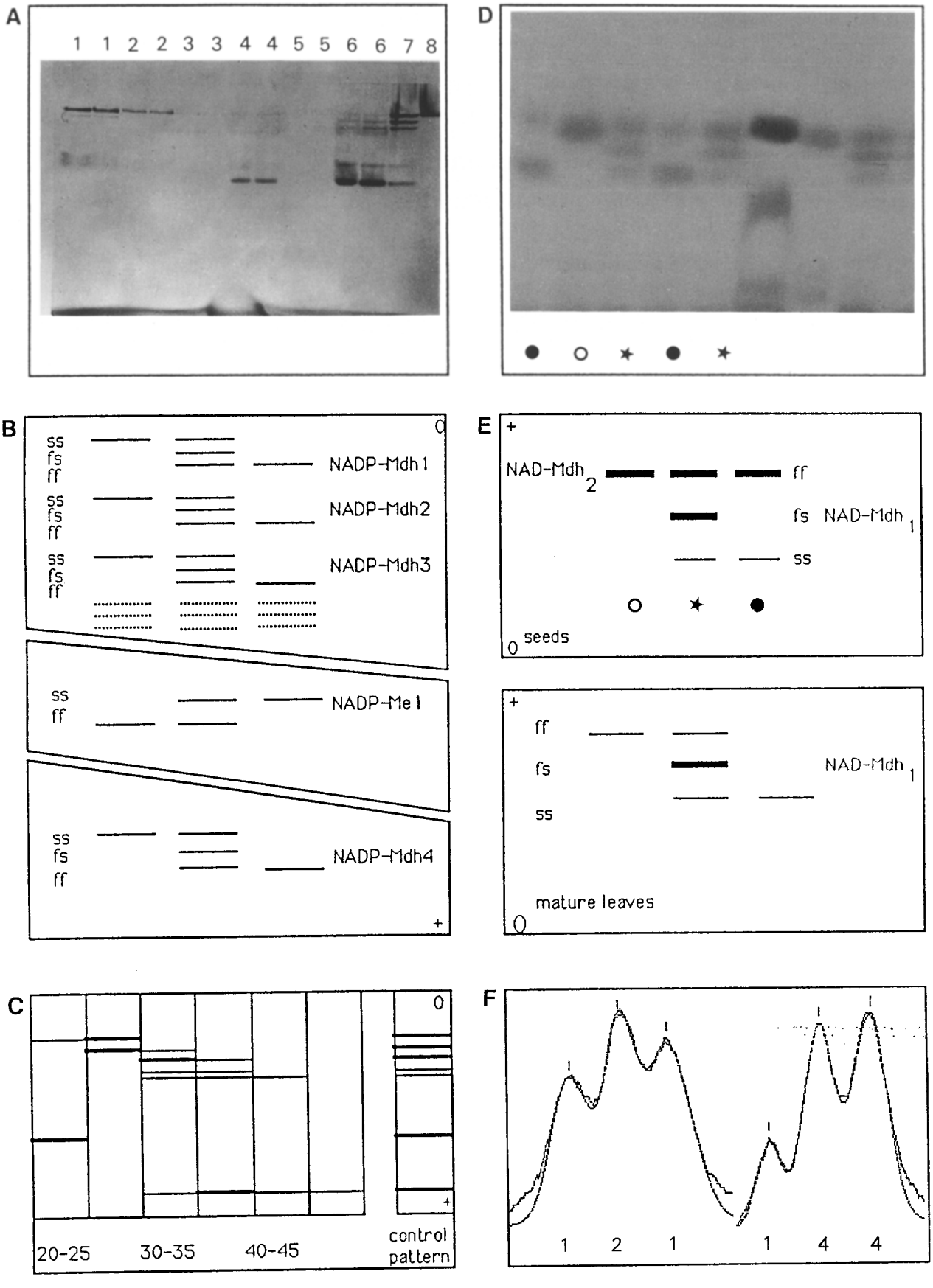
The number of identified genetic markers currently available in sugarbeet is limited. Smith (1980) listed 44 genetically characterized genes, however only hypocotyl color (R and Y), monogerm versus multigerm seed (M) and annual growth habit (B) are generally used as markers. Other markers, such as most of the morphological ones (e.g., chlorophyll mutants, root and leaf characters), can cause an unwanted decrease in the economical value of the line or are too highly affected by environmental conditions, and characters such as sterility, restorer and (in)compatibility genes are not easily screenable and often require at least one backcross in order to identify all genotypes.

Recently, several isozyme markers were characterized genetically, and in some cases linkage relationships were scored (Levites 1979; Levites et al. 1980; Maletsky and Konovalov 1985; Abe et al. 1987; Abe and Tsuda 1987, 1988; Van Geyt 1986; Van Geyt and Jacobs 1986; Smed et al. 1989). Various applications have been reported, including chromosome identification (Jung et al. 1986, Van Geyt et al. 1988), enzyme expression in artificial allopolyploids (Oléo et al. 1986), identification of regenerated haploids (Van Geyt et al. 1987) and cultivar identification (Itenov and Kristensen 1985; Lesnevitch and Borisjuk 1987; Levites and Garifullina 1987; Oléo and Van Geyt 1987, Van Geyt and Oléo 1987).

This article reports the characterization of a new series of isozyme systems. Their possible use as marker systems is discussed, and linkage tests between these

* To whom correspondence should be addressed

Abbreviations: Tris-HCl, Tris (hydroxymethyl) aminomethane-HCl; *NADP*, nicotinamide adenine dinucleotide phosphate; *NBT*, nitro-blue tetrazolium chloride monohydrate; *PMS*, phenazine methosulphate


A
1 1 2 2 3 3 4 4 5 5 6 6 7 8
D
B
0
ss
fs
ff
NADP-Mdh1
NADP-Mdh2
NADP-Mdh3
NADP-Me1
NADP-Mdh4
+
E
+
NAD-Mdh 2
ff
fs
NAD-Mdh 1
ss
0 seeds
mature leaves
C
20-25
30-35
40-45
control pattern
F
1 2 1 1 4 4

isozyme systems and with the red hypocotyl factor R and the pollen fertility restorer Z are reported.

Material and methods

Extraction and gel electrophoresis was performed as described previously (Van Geyt and Smed 1984; Smed et al. 1989). The NADP-specific malate-processing enzymes were studied on $PAGE_1$. Although activity was detected after separation of SGE buffer systems, hybrid patterns could not be resolved due to the very slight differences in migration distances. The $PAGE_1$ system when used with a decreased gel concentration (for example 6% acrylamide) increased the resolution sufficiently to obtain reliable reference values. Prior to DEAE chromatography of NADP-specific malate dehydrogenases, crude leaf homogenate was filtered through three layers of Miracloth and desalted on Sephadex G-25. The DEAE Cellulose (Sigma) column (25 × 2.5 cm) was saturated with filtrate, washed with 0.05 *M* NaCl and eluted in 3-ml fractions with a NaCl gradient (0.05 *M* – 0.2 *M*). Five fractions were pooled, concentrated ten-fold with Immersible CX-30 ultrafilters (Millipore) and separated on $PAGE_1$.

Buffer system SGE_1 was used for the separation of NAD-specific malate dehydrogenases. The buffer systems SGE_3 and SGE_4 also gave good separations. While intense background staining disturbed the pattern on the PAGE electrophoresis, a good pattern on PAGE could be obtained by pressing out the leaf juice instead of crushing the material with mortar and pestle (G. J. Speckmann, personal communication).

Shikimate dehydrogenase was studied on the $PAGE_1$ buffer system; the SGE_1 system also allowed good separation. The patterns were very clear when seed or seedling extracts were used. Leaf samples showed higher activity, but streaking occurred frequently.

Diaphorase and aconitase could be separated using SGE_1 as well as $PAGE_1$.

The staining recipes for NADP-MDH (stained as ME), ICD, GPI and PGM have been described in Van Geyt and Smed (1984). Additional isozyme systems were revealed in reaction mixtures containing, respectively: (a) 6-P-gluconate dehydrogenase: 100 ml 10 m*M* *Tris*-HCl, pH 7.5, 20 mg 6-P-gluconate, 10 m*M* $MgCl_2$ and 15 mg NADP; (b) NAD-specific malate dehydrogenase: 100 ml 10 m*M* *Tris*-HCl, pH 7.5, 1 g *DL*-sodium malate and 30 mg NAD; (c) shikimate dehydrogenase: 100 ml 10 m*M* *Tris*-HCl, pH 7.5, 100 mg shikimic acid and 15 mg NADP; (d) Aconitase: 100 ml 10 m*M* *Tris*-HCl, pH 8.0, 150 mg *cis*-aconitic acid, 30 m*M* $MgCl_2$, 1 ml isocitrate dehydrogenase (20 U) and 15 mg NADP. In all cases 2 mg PMS and 20 mg NBT were supplemented to the reaction mixtures prior to use. Diaphorase was stained in 100 ml 10 m*M* *Tris*-HCl, pH 8.5, 2 mg 2,6-dichlorophenol-indophenol, 20 mg NADH and 20 mg NBT.

The genetical analysis was performed as described previously (Smed et al. 1989). Gene symbols are written in small caps (e.g. Icd_1), gene products in capitals (e.g. ICD_1^F).

Results

Multiple genes coding for malate-converting isozyme systems could be characterized. These will be described in two groups, namely NAD- and NADP-specific enzymes, according to the appearance of the patterns after tetrazolium staining when one of the cofactors mentioned above was used in combination with *DL*-malate as substrate. Figure 1 A shows the developmental expression of the different zones of activity of NADP-specific enzymes. In seeds and young seedlings only the slowest zone of the NADP-specific enzymes was fully expressed; the slowest but one was moderately expressed, whereas the fastest zone was barely visible on the gel. In seedlings and in cell cultures we always found that the slowest migrating bands had a higher activity. In older cotyledons the fastest migrating zone was induced, while the complete pattern of the enzyme system was only expressed in mature leaves. In roots the activities were very low, and only the slowest migrating zone could be visualized in older roots. Figure 1 C shows the pattern of the different fractions eluted from a DEAE-column. The different bands eluted in different fractions, indicating that no modifications were induced during the electrophoresis technique. The slowest zone was characterized by a high level of polymorphism, which is in agreement with results reported earlier (Van Geyt and Smed 1984): three bands or nine bands were generally found. Table 1 A summarizes the progeny array of crossing experiments involving the three slowest migrating zones; Fig. 1 B shows a schematic representation of the patterns studied. The NADP-Mdh_1^{FF}, NADP-Mdh_2^{FF} and NADP-Mdh_3^{FF} bands correspond to bands 12, 9, 6, respectively, and the NADP-Mdh_1^{SS}, and NADP-Mdh_2^{SS} and NADP-Mdh_3^{SS} bands correspond to bands 14, 11 and 8, respectively of Van Geyt and Smed (1984). The progeny of crosses between genotypes showing one similar band at each zone showed the parental type. Crosses of individuals with distinct single bands at each zone revealed zymograms with three bands for each zone in any individual. Crosses of plants with three identical bands per zone segregated in a 1 : 2 : 1 ratio for the NADP-$Mdh_{1,2,3}^{FF}$: NADP-$Mdh_{1,2,3}^{FS}$: NADP-$Mdh_{1,2,3}^{SS}$ patterns and in a 1 : 1 ratio (NADP-$Mdh_{1,2,3}^{FS}$: NADP-$Mdh_{1,2,3}^{SS}$) for crosses with phenotypes with single bands in common.

No polymorphism was found in the second set.

Fig. 1 A–F. Malate-processing enzymes. **A** Ontogenic series of the NADP-dependent enzymes of cv 'Diana'. *1* Dry seed; *2–3* cotyledon and root extract, respectively of a young seedling (5 days old); *4–5* cotyledon and root extract, respectively of an older seedling (14 days old); *6* young leaf; *7* mature leaf; *8* mature root. **B** Schematic representation of the patterns studied genetically. The allelic forms of NADP-Mdh_1, NADP-Mdh_2, NADP-Mdh_3, NADP-Mdh_4 and NADP-Me_1 are represented in homozygous and heterozygous form. **C** The pattern of the different fractions eluted from a DEAE column after pooling. **D** Ontogenic series of the NAD-dependent enzymes of the cv 'Diana'. (legend as in A). **E** Schematic representation of the patterns of NAD-Mdh_1 and NAD-MdH_2 as studied in seeds and mature leaves. **F** Densitometric scanning of a heterozygous pattern of NAD-MDH in seeds and in mature leaves

Table 1. Survey of the different crossing experiments (receptor × pollinator). Plants marked with an asterisk were male sterile

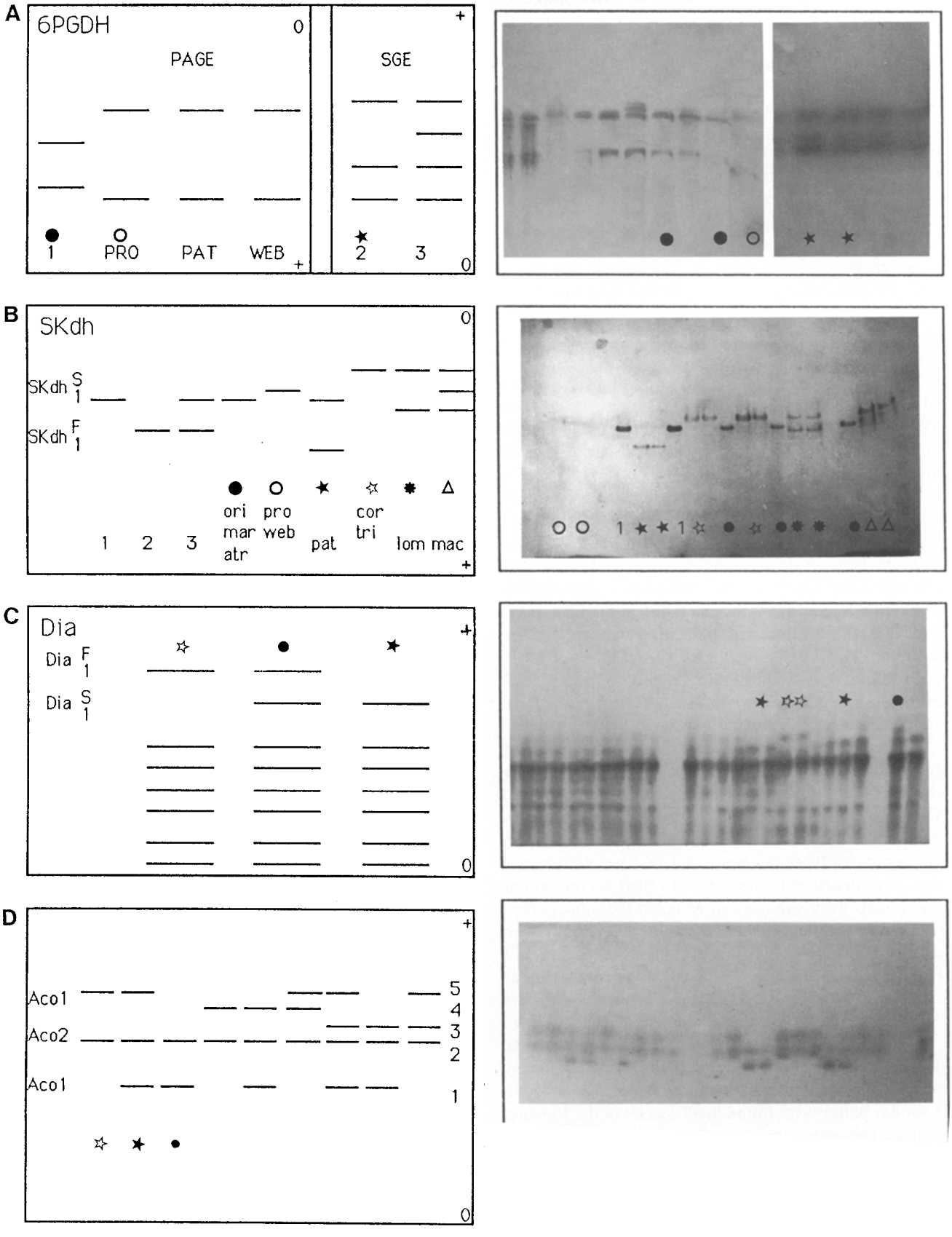
Crosses	Phenotype			Total
NADP-Mdh_1, Mdh_2, Mdh_3	$F_1F_1F_2F_2F_3F_3$	$S_1S_1S_2S_2S_3S_3$	$F_1S_1F_2S_2F_3S_3$	
$F_1F_1F_2F_2F_3F_3 \times F_1F_1F_2F_2F_3F_3$	41	0	0	41
$S_1S_1S_2S_2S_3S_3 \times S_1S_1S_2S_2S_3S_3$	0	45	0	45
$F_1F_1F_2F_2F_3F_3 \times F_1S_1F_2S_2F_3S_3$	54	0	46	100
$F_1F_1F_2F_2F_3F_3 \times S_1S_1S_2S_2S_3S_3$	0	0	28	28
$F_1S_1F_2S_2F_3S_3 \times F_1S_1F_2S_2F_3S_3$	38	28	74	140

Crosses	Phenotype			Total	Probability	
NADP-ME_1	F	FS	S		χ^2	*P*
F × F	23	0	0	23	–	–
S × S	0	0	18	18	–	–
F × S	0	27	0	27	–	–
FS × S	0	25	19	44	0.82	0.37
Mdh_1	A	C	B		χ^2	*P*
A* × A	130	0	0	130	–	–
B × B	0	0	97	97	–	–
A × B	0	64	0	64	–	–
C* × B	0	88	83	171	0.07	0.08
C × A	103	96	0	199	0.25	0.62
C* × C	75	150	80	305	0.24	0.88
$Skdh_1$	F	FS	S		χ^2	*P*
S* × S	0	0	44	44	–	–
F × F	27	0	0	27	–	–
S* × F	0	133	0	133	–	–
FS* × S	0	61	66	127	0.20	0.65
FS* × F	53	54	0	107	0.01	0.92
FS* × FS	54	107	61	222	0.73	0.70
Dia_1	F	FS	S		χ^2	*P*
S* × S	0	0	301	301	–	–
S* × F	0	154	0	154	–	–
S* × FS	0	72	88	160	1.60	0.20

Band 2 and 3 (not classified as a set in Van Geyt and Smed 1984) could be studied genetically. When the plants were grown under normal conditions, the system was not useful as a marker system due to a highly developmental and environmental dependent expression. The very faint bands only appeared after staining overnight. However, in a highly virus-infected progeny (probably BMV), the pattern showed an increased activity, which allowed us to study some genetic segregations (Table 1 B). Crosses of F types with S types resulted completely in FS progeny. The backcross of a FS genotype with a S-genotype segregated in a 1 : 1 ratio. Crosses of plants with the same one-band type were true breeding.

The fastest zone was characterized by a single band that showed no polymorphism in *B. vulgaris*. In a seed sample of *B. maritima*, however, two additional types were found: a faster migrating band and one type with three bands. Two bands migrated at positions corresponding to those of the bands mentioned above, while a third band migrated at an intermediate position. No de-

Fig. 2. Schematic representation and photographic example of the zymograms of **a** G6PDH, **b** Skdh, **c** Dia and **d** Aco. The *numbers* represent the different types of zymograms observed in *B. vulgaris* (arbitrary numbering). *vul B. vulgaris* (sugarbeet and fodderbeet), *mar B. maritima*, *atr B. atriplicifolia*, *ori B. orientalis*, *pro B. procumbens*, *web B. webbiana*, *pat B. patellaris*, *cor B. corolliflora*, *tri B. trigyna*, *lom B. lomatogona*, *mac B. macrorhiza*


A
6PGDH
0
PAGE
SGE
+
1
PRO
PAT
WEB
2
3
+
0
B
SKdh
0
SKdh S 1
SKdh F 1
ori
mar
atr
pro
web
pat
cor
tri
lom
mac
1
2
3
+
C
Dia
+
Dia F 1
Dia S 1
0
D
+
Aco1
Aco2
Aco1
5
4
3
2
1
0

tailed segregations are as yet available, but preliminary crossing data confirmed the dimeric structure of the active enzyme. The gene will be called NADP-Mdh_4.

In seeds, the main zone of NAD-specific malate dehydrogenases expressed one, two or three bands (Fig. 1 D, E). On the zymogram with three bands the fastest migrating band showed much higher activity than the two other bands. In the phenotype with two bands, the activity of the fastest migrating band decreased gradually with germination and disappeared in mature tissue. Densitometric scanning of a zymogram from mature tissue, however, approximated a 1 : 2 : 1 relationship between band intensities in diploids and a 4 : 4 : 1 ratio in triploids (Fig. 1 F). The crossing experiments with the different phenotypes of the main zone are described in Table 1 C. The same type was regained by crossing A (A′) types or B (B′) types. Crossings of A (A′) types with B (B′) generated progeny of the C (C′) types. The fastest migrating band was always present in seeds and seedlings of all segregations. The segregation of a cross of two C (C′) types fits in with a 1 : 2 : 1 ratio for types A (A′) : C (C′) : B (B′). The patterns of haploid plantlets regenerated from gynogenesis (Van Geyt et al. 1987) were compared with the patterns of their corresponding mother plants. Genotypes with one band in the maternal plants revealed the same genotype back. On the other hand, when a triple-banded genotype was used as the mother plant, both of the single-banded phenotypes were regained in the haploid regenerants.

Additional slower migrating bands appeared with overstaining. As their analysis is not yet completed, they are not discussed in this report.

When separated on PAGE G-P-gluconate dehydrogenase showed two bands, while staining after starch gel electrophoresis revealed three or four bands (Fig. 2 A). The occurrence of the fourth band was dependent on the growth and developmental stage of the plant. Crosses of the two phenotypes did not result in a genetic segregation. A comparison of diploids with their corresponding regenerants from gynogenesis revealed the same pattern for all individuals. Different migration positions, however, could be scored in plants belonging to the section *Patellares* (Fig. 2 A). While 6PGDH was expressed in all stages of development, its activity was very low in root tissue, especially in the slowest migrating zone.

Shikimate dehydrogenase was expressed in all stages of development. Figure 2 B summarizes the different bands detected in seeds of different species of genus *Beta*. A similar pattern was found in all species of the *Vulgares* section. The pattern found in *B. procumbens* and *B. webbiana* was characterized by one single band that migrated more slowly than the *B. vulgaris* band. In the *Corollinae* section several other allelic forms were found. Phenotypes with one, two and three bands could be visualized (Fig. 2 B). At present, three different types of zymograms have been found in *B. vulgaris* (Fig. 2 B). In most of the assessed genotypes only one band was expressed, which was designated S (slow). In one population two additional variants could be detected: a faster migrating (F) type and a phenotype with both bands. Table 1 D summarizes the crossing experiments performed with the different polymorphic variants. Sib-mating of cloned plants of genotypes showing a single band revealed the same phenotype as the parent (F or S). Backcrossing double-banded types (FS) with phenotypes with one band (F or S) produced progeny with a one to one ratio of the respective types. Finally, crosses of both double-banded types segregated in a 1 : 2 : 1 mode for the F : FS : S types.

The zymograms of diaphorase were complex and characterized by a low level of polymorphism. The pattern showed up to eight sharp bands of activity and some minor bands (Figure 2 C). Some of the bands were developmentally regulated. The fastest migrating zone could be investigated genetically and was observed to be characterized by one or two bands. The progeny array is summarized in Table 1 E. Crossed individuals with the same bands were true breeding. Crosses of phenotypes with distinct single-banded types generated progeny of phenotypes with two bands. Backcrosses segregated in a 1 : 1 fashion (FS and S).

The zymograms of aconitase showed two to five bands (in Fig. 2 D numbered one to five in order of increasing mobility). The migration distance of some of the bands was so close that with lower quality runs scoring was impossible. Nevertheless, we were able to discover that bands 2 and 3 behave genetically independent of bands 1, 4 and 5. Crossing data of types characterized with the expression of bands 1 and/or 5 were investigated in a crossing scheme (Table 1 F). Crosses of single-banded types were true breeding irrespective of the pattern of bands 2 and 3. Crosses of individuals with distinct bands expressed both bands in their progeny. Backcrosses segregated in an equal ratio of single-versus double-banded patterns. F_2 segregation showed a 1 : 2 : 1 ratio (band 1, 1 and 5, 5). Segregations of the types characterized by the bands 4 and 5 or 2 and 3 were impossible to analyze as the gel resolution was too low for objective scoring of the different types.

Linkage analysis

Linkage relationships were investigated by examining pairs of loci in backcrosses and F_2 segregations. Table 2 A summarizes the segregations of the color marker R, the pollen fertility restorer Z and isozyme loci Pgm_1, Gpi_2, Icd_1, Ak_1, Aco_1 and Dia_1. In most cases the joint segregations did not differ significantly from random assortment of the different alleles of both loci, indicating independent segregation. In the case of R versus Z, the probability for independent segregation was very low.

Table 2 A and B. A. Chi square tests for linkage and deviation in Mendelian ratio of isozyme markers, the color marker R, r and the pollen fertility restorer Z

Genes (A, B)	Progeny									Total	χ^2_X	χ^2_Y	χ^2_L	P_X	P_Y	P_L	Recombination (%)
	1,1	2,2	1,2	2,1	1,X	2,X	X,2	X,1	X,X								
Rr-Zz		12				11	20		26	69	7.67	0.36	8.74	0.005	0.55	0.03	–
Rr-Mdh_1^{FS}			23		30		25		14	92	2.13	0.17	5.82	0.14	0.68	0.12	–
				28		38		29	33	128	0.13	1.53	1.94	0.75	0.22	0.58	–
		47				52	40		42	181	1.60	0.27	1.92	0.20	0.58	0.58	–
	52			16	61	20				149	0.07	1.13	1.21	0.80	0.29	0.75	–
	33	15	38	12	70	22				190	0.06	0.86	1.33	0.80	0.65	0.93	–
Rr-Aco_1^{FS}			16		30		28		21	95	0.10	0.52	5.25	0.75	0.47	0.16	–
Rr-Dia_1^{FS}		4	8		5	7	12		11	47	0.19	0.02	1.76	0.90	0.89	0.97	–
Zz-Mdh_1^{FS}				17		27		36	15	95	0.52	1.27	11.90	0.48	0.25	0.007	33.7
Zz-Aco_1^{FS}		17				8	5		4	34	7.35	2.94	12.35	0.006	0.14	0.006	38.2
Zz-Dia_1^{FS}		11				13	14		4	42	0.86	1.52	5.81	0.36	0.22	0.12	–

B. Chi-square tests for independent segregation of various isozyme genes

Genes (I_1, I_2)	Allelic forms			Progeny (I_1, I_2)						Total	χ^2_{I1}	χ^2_{I2}	χ^2_L	P_{I1}	P_{I2}	P_L
	F,F	S,S	F,S	S,F	F,H	H,F	H,S	S,H	H,H							
Mdh_1-Aco_1		25					32	38	38	133	2.71	0.37	3.46	0.10	0.53	0.33
Pgm_1-Aco_1			11		13		9		7	40	0.0	1.60	2.00	1.00	0.20	0.57
Gpi_1-Aco_1			21		10		6		3	40	4.90	12.10	18.60	0.03	0.00	0.03
Icd_1-Aco_1			24		30		38		33	125	0.01	2.31	3.29	0.92	0.13	0.35
Ak_1-Aco_1		18					17	21	17	73	0.12	0.34	0.59	0.73	0.55	0.90
Pgm_1-Mdh_1			39		33		32		34	138	0.11	0.26	0.85	0.75	0.62	0.83
Pgm_1-Mdh_1	17		22		33	23	12		39	146	0.52	0.03	4.75	0.77	0.86	0.44
Gpi_1-Mdh_1				20			28	21	23	92	0.18	1.09	1.65	0.65	0.30	0.64
Icd_1-Mdh_1			47		43		43		49	182	0.02	0.02	0.60	0.89	0.89	0.90
Icd_1-Mdh_1	21		19		38	15	16		42	151	0.54	0.17	1.98	0.77	0.65	0.84
Ak_1-Mdh_1		40					47	54	43	184	0.54	0.09	2.39	0.46	0.76	0.50
Pgm_1-Dia_1			13		11		11		12	47	0.02	0.02	0.24	0.89	0.89	0.97
Aco_1-Dia_1		53					62	34	44	193	7.09	1.87	8.97	0.01	0.18	0.03
Mdh_1-Dia_1		33					38	27	29	127	1.77	0.39	2.23	0.18	0.53	0.53
Gpi_1-Dia_1		7	7		6		9	3	14	46	0.39	0.00	2.96	0.67	1.00	0.70
Ak_1-Dia_1		6	6		10		12	5	7	46	1.78	0.04	5.04	0.40	0.84	0.41

Taking into account that the red-green segregation shows, for unknown reason, a surplus of red individuals, we concluded that both loci most probably segregate independently from each other. The case of Aco_1 and Z needs further examination; this cross may show 38.2% recombination. Chi^2 analysis showed only a 0.006 chance for independent assortment; the separate segregations of both loci also were of low probability. A linkage relationship could be found in the case of Mdh_1 and Z: 33.7% recombinant genotypes were recovered. The probability of the loci treated separately is high, while the chance for independent assortment is only 0.007.

Table 2 B summarizes the segregation data between isozyme loci. In all cases the independence of the loci was scored.

From the data in Table 1 A, it was concluded that NADP-Mdh_1, NADP-Mdh_2 and NADP-Mdh_3 showed tight linkage. Their linkage relationship with the other markers has not yet determined.

Discussion

The NADP-specific isozymes of Set I were controlled by three genes whose gene products were active as dimeric enzymes and inherited in a simple Mendelian way. The genes will be named NADP-Mdh_1, NADP-Mdh_2, NADP-Mdh_3 in order of the increasing mobility of their protein bands. Our data confirms the hypothesis of Van Geyt et al. (1987) based on the comparison of the pattern of haploid regenerants and their corresponding mother plants. No recombinants could be found in the F_2 segregation. This could be explained by assuming tight linkage between the three genes. A double gene duplication could be an explanation for the phenomena. The subunits produced by the three genes, however, did not form mutual heterodimers. Another possible explanation could be interference by a modifier gene that is able to produce three different forms of the enzyme. This modifying capacity of the gene should be developmentally and environmentally

regulated as differences in intensity of the three systems were detected during the life cycle. The existence of the enzymes as independently synthesized forms was further confirmed by DEAE chromatography. The different isozymes eluted in different fractions.

A gene coding for monomeric enzymes (formally indicated as band 2 and 3) could be identified. A monomeric structure is not common for malate dehydrogenases. The possibility exists that we are dealing with malic enzyme. Specific staining for pyruvate or oxaloacetate (Vallejos 1983) did not reveal any bands, possibly due to the low activity of the enzyme (results not shown). The gene will be called NADP-Me_1.

As some of the genes were highly polymorphic and, except for some minor bands, easily detectable in leaves, this system could be used as a marker. Its developmental- and organ-specific expression, however, limits its use.

The main zone of NAD-specific malate dehydrogenases was found to be controlled by two independent genes. Two allelic forms (F and S) were found, with the F allele being most commonly found in strains of sugar and fodder beet. NAD-Mdh_1 gene is expressed at all stages of development. The other gene, NAD-Mdh_2, was active in seeds and is repressed during germination. The gene was not polymorphic in the assessed genotypes, but preliminary screening in wild species revealed polymorphic variants (results not shown). The system is very useful as a marker although confusion can arise while analyzing seeds or juvenile material, as the NAD-MDH_1 and NAD-MDH_2^{FF} bands overlap.

The different zymograms of 6-P gluconate dehydrogenase can be explained by changes in the developmental expression of two or more loci. At present no polymorphic variant could be scored within sugar of fodder beet. The use of the system will be limited to the analysis of wild species and interspecific hybridizations (Van Geyt et al. 1988).

The data demonstrate that shikimate dehydrogenase activity is controlled in a simple Mendelian way, the enzymes being active as monomers. The gene was designated as $Skdh_1$. Although a rather limited number of polymorphic variants could be detected, the enzyme system is a suitable marker system. Due to the important differences in the wild species, the isozyme system will be especially interesting for the study of interspecific crosses or fusions.

The diaphorase pattern was complex and highly influenced by the stage of development and environment. At present one activity zone proved to be inherited in a simple Mendelian way. The gene, which will be called Dia_1, occurred as two allelic forms active as monomers.

Aconitase isozymes were controlled by two independent genes. One gene could be investigated genetically and was called Aco_1; its allelic forms were designated Aco_1^A, Aco_1^B, Aco_1^C in order of increasing mobility. The gene codes for enzymes that are active as monomers. The second gene was called Aco_2. Although no segregations could be tested due to scoring problems caused by a lack of resolution, the patterns give a picture of a monomeric enzyme. The Aco_1 system was suited to be marker system, although confusion arose in the interpretation of allelic forms Aco_1^B and Aco_1^C. Although gene Aco_2 showed polymorphism, the same remark can be made for its use as a marker. Additional separation systems have to be tested in order to improve the resolution of the different forms.

No recombinants could be found in segregating progeny of NADP-Mdh_1, NADP-Mdh_2 and NADP-Mdh_3, indicating that the three genes are tightly linked. Independant segregations were scored between most isozyme loci and with R and Z. A putative linkage relationship was scored for Z versus Aco_1 and Z vs Mdh_1, but confirmation of this data is necessary, especially for the first combination.

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