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Inheritance of ADH, 6-PGDH, PGM, and SKDH in *Allium fistulosum* L.

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Abstract. **Horizontal starch gel electrophoresis was used to study the inheritance of isozyme phenotypes of four enzyme systems [alcohol dehydrogenase (ADH), 6-phosphogluconate dehydrogenase (6-PGDH), phosphoglucomutase (PGM), and shikimate dehydrogenase (SKDH)] in *Allium fistulosum* L. by monitoring segregations in backcross and F_2 progeny. Segregation for most of the polymorphisms fit the expected Mendelian ratios as tested by the chi-square statistic. Three new isozyme loci were defined for onion. Two loci were found for 6-PGDH. Locus one was dimeric with two alleles, and locus two was monomorphic. SKDH was monomeric with two alleles.**

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Allium are outcrossing species; therefore, considerable plant-to-plant variation exists within populations. The genetic control of several qualitative and quantitative characteristics in *A. cepa* has recently been reviewed by Rabinowitch and Brewster (1990). Genetic studies in *A. cepa* are slow because of the long generation time (generally 2 years). *Allium fistulosum* L., a species closely related to *A. cepa*, has been investigated in our program because it has several characteristics that make it more amenable to genetic studies than *A. cepa*.

1) *Allium fistulosum* is perennial and bolts without vernalization under greenhouse conditions in Lubbock, Texas.

2) Each individual frequently produces several scapes over several months, an activity that allows the use of the same individual for several crosses in the same season.

3) Cytogenetically, *A. fistulosum* is very similar to *A. cepa*: both are diploid (x = 8) with similar chromosome morphology (de Vries, 1990), although the *A. fistulosum* chromosomes are considerably smaller (Jones and Rees, 1968). In situ hybridization investigations have revealed ribosomal DNA coding genes to be on two *A. cepa* and *A. fistulosum* homoeologues (Ricroch et al., 1992). The species cross-fertilize readily, yielding interspecific F_1 hybrids and hybrid derivatives (Peffley, 1992). Thus, characterizing *A. fistulosum* genes would enhance genetic understanding of the common bulb onion, *A. cepa*. In this report, we describe the various types of alcohol dehydrogenase (ADH)-1, 6-phosphogluconate dehydrogenase (6-PGDH)-1, and shikimate dehydrogenase (SKDH)-1 banding patterns found in *A. fistulosum*. The banding pattern for phosphoglucomutase (PGM)-1 has been described previously (Peffley et al., 1985). We present genetic analyses and the inheritance for each of these isozymes.

Materials and Methods

Plant materials. Plants were selected from among the *A. fistulosum* plant introductions (PIs) 418953, 461395, 433630, 461401, or 433629 (U.S. Dept. of Agriculture, Northeast Regional Plant Introduction Station, Geneva, N.Y.) or clonally propagated from the *A. fistulosum* cultivar Heshiko (seed supplied by C. Briggs, Nickerson Zwaan Seed Co.). Some crosses were made with selfed 'Heshiko' individuals ('Heshiko' S_1) in Texas Tech Univ. greenhouses. Individual plants used in crosses were selected for their isozyme phenotype.

Pollen evaluation. Stainability of pollen collected from 'Heshiko' genotypes *Adh-1*^{3}/*Adh-1*^{6} and *Adh-1*^{6}/*Adh-1*^{6} and from PI433629 genotype *Adh-1*^{4}/*Adh-1*^{4} was recorded at pollination. Pollen from the anthers of each of three plants of each genotype was placed in a microfuge tube to which two drops of 1% acetocarmine were added. Vibrating with a mini-vortex dispersed the pollen. The solution was micropipetted onto a slide for viewing. At least 100 pollen grains were observed per sample.

Electrophoresis. Roots from seedlings or seed of progeny were harvested, and samples for electrophoresis were macerated in extraction buffer (0.05 mM glutathion in tris-buffer, pH 7.0) and separated on starch gels. Enzymes assayed included ADH (E.C.1.1.1.1), 6-PGDH (E.C.1.1.1.44), PGM (E.C.2.7.5.1), and SKDH (E.C.1.1.1.25). ADH and PGM were evaluated after electrophoresis with tris-citrate buffer system, pH 7.8 (Vallejos, 1983), and 6-PGDH and SKDH were evaluated with buffer system F for maize (Stuber et al., 1988). All were stained according to Vallejos (1983).

Individual loci were discerned by segregating the bands on the gels (Fig. 1). In systems with multiple loci, the locus with the greatest anodal migration was designated as number 1 and slower migrating loci were designated as number 2. Putative alleles at each locus were named in the order in which they were found within populations; i.e., the first allele identified was designated as number 1, the second, number 2. In most cases the allele designated as number 1 is the most cathodal.

Crosses. Controlled crosses were made among individuals of each isozyme phenotype. Plants for crossing were kept in single crossing cages. Florets were emasculated daily before anther dehiscence. Pollen was collected and applied to receptive stigmas with camel-hair brushes. Plants flowered from January through June 1988–92. Seeds were harvested when mature and either electrophoresed after 30 min inbibition in extraction buffer or planted into flats in the greenhouse. Allelic constitution for each of the scored loci was recorded separately for each plant and tested for Mendelian inheritance with a chi-square heterogeneity test. Seed set, germination, and establishment percentages were recorded for some crosses when segregation ratios deviated from expected ratios. Nonrandom joint segregation of pairs of loci (linkage) was

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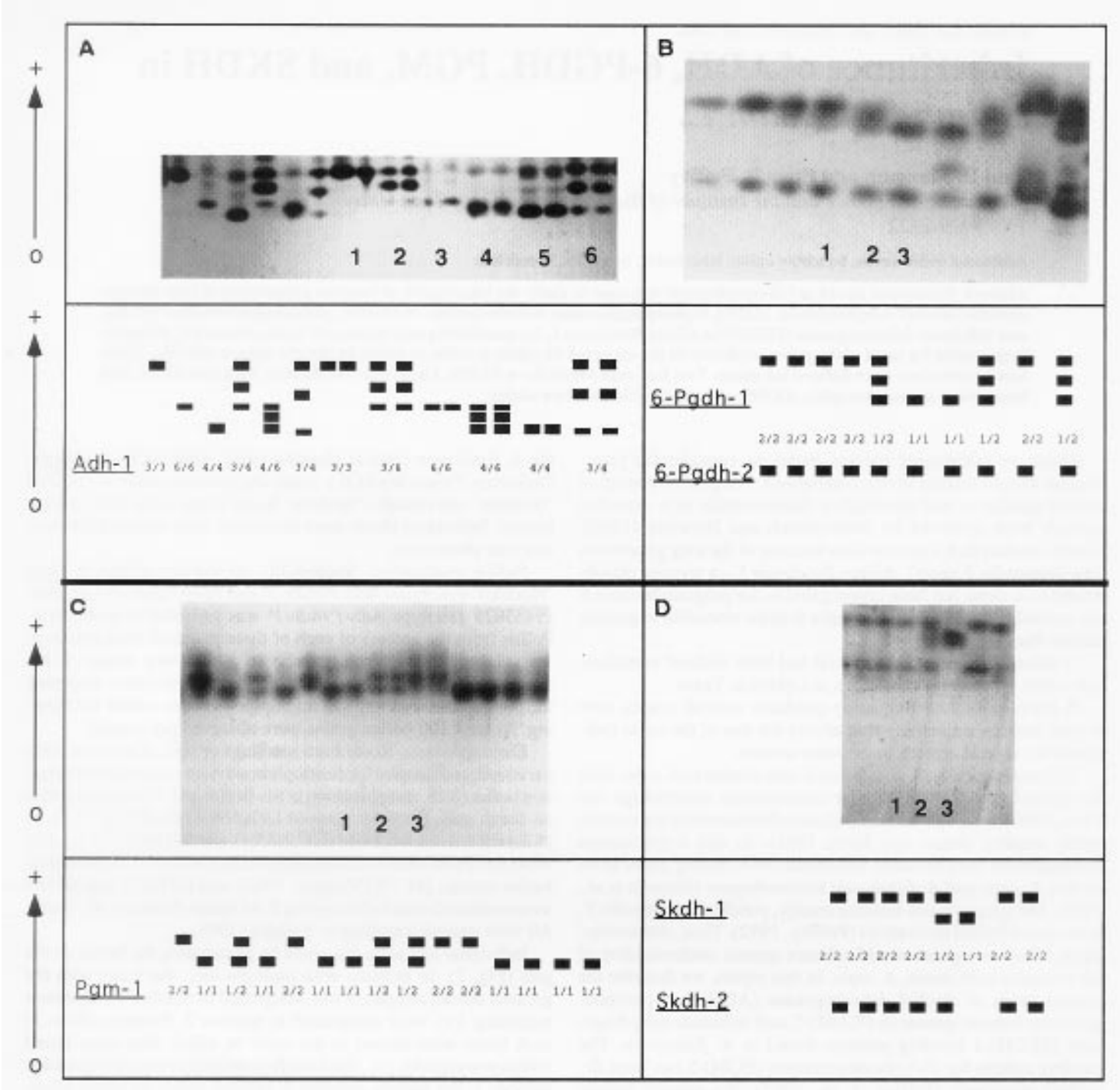

Fig. 1. Photographs of *Allium* electrophoretic gels illustrating genotypes. Schematic of isozyme phenotypes for each of the four enzyme systems investigated appear below. (**A**) ADH profile: 1) *Adh-1*^{3}/*Adh-1*^{3}, 2) *Adh-1*^{3}/*Adh-1*^{6}, 3) *Adh-1*^{6}/*Adh-1*^{6}, 4) *Adh-1*^{4}/*Adh-1*^{6}, 5) *Adh-1*^{4}/*Adh-1*^{4}, 6) *Adh-1*^{3}/*Adh-1*^{4}. (B) 6-PGDH profile: 1) *6-Pdgh-1*^{2}/*6-Pdgh-1*^{2}, 2) *6-Pgdh-1*^{1}/*6-Pgdh-1*^{2}, 3) *6-Pgdh-1*^{1}/*6-Pgdh-1*^{1}. (C) PGM profile: 1) *Pgm-1*^{1}/*Pgm-1*^{1}, 2) *Pgm-1*^{1}/*Pgm-1*^{2}, 3) *Pgm-1*^{2}/*Pgm-1*^{2}. (D) SKDH profile: 1) *Skdh-1*^{2}/*Skdh-1*^{2}, 2) *Skdh-1*^{1}/*Skdh-1*^{2}, 3) *Skdh-1*^{1}/*Skdh-1*^{1}. The anode is at the top of the figure.

analyzed for independent segregation using the chi-square statistic.

Results and Discussion

Gene symbols. Symbols for several putative genes coding for isozymes in the bunching onion are accompanied with corroborating segregation data (Table 1). Figure 1 displays photographs of electrophoretic gels of ADH-1, 6-PGDH-1, 6-PGDH-2, PGM-1, and SKDH-1. A brief description of each enzyme follows.

ADH. Three phenotypes were investigated for ADH: *Adh-1*^{6}, and two previously reported alleles, *Adh-1*^{3} and *Adh-1*^{4} (Peffley and Orozco, 1987) (Fig. 1A). Results of crosses between plants heterozygous at ADH-1 for *Adh-1*^{3}, *Adh-1*^{4}, and *Adh-1*^{6} and several test crosses are shown in Table 1. When the population for a cross resulted from individuals with the same genotype, chi-square heterogeneity analyses were done to test for pooling. In each case, population pooling was not done. The segregation ratio for the *Adh-1*^{3}/*Adh-1*^{4} (3/4 x 3/4) and *Adh-1*^{3}/*Adh-1*^{6} (3/6 x 3/6) crosses were ≈1:2:1 when tested for Mendelian ratios with the chi-square statistic. Segregation ratios for progeny of the *Adh-1*^{4}/*Adh-1*^{6} (4/6, selfed) deviated from the expected 1:2:1 ratio. The homoallele 6/

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6 is the deviant class, with fewer numbers than expected recovered. The 4/6 selfed progeny were then tested for the 1:2:0 expected ratio with a lethal allele and for dominance effects with 3:1 (4/4:4/6, 6/6) and 1:3 (4/4, 4/6:6/6) ratios. All the tests were rejected. Deviation of *Adh-1* in the 4/6 family may be due to linkage of allele 6 to a semilethal allele in this population. Aberrances in pollination or fertilization may account for the fewer numbers of individuals homozygous for allozyme *Adh-1*^{6}. Viability of *Adh-1*^{6} pollen seems to be normal, with pollen stainabilities from 3/6, 4/6, and 6/6 individuals of 97.1%, 90.3%, and 97.6%, respectively, suggesting normal microsporogenesis. Pollen competition favoring *Adh-1*^{4} may be occurring, or *Adh-1*^{6} pollen may compete less favorably under these conditions. Pollen-tube growth was not tested in this material. In these crosses, the 4/6 parent had a 4.75% seed set. Occasionally, empty seed coats were found among seeds recovered from the 4/6 parent. This, however, was not enough to account for the missing 6/6 individuals. Seed germination was 96%, and all seeds established into healthy seedlings. These results would suggest that the 6/6 homozygotes are lost at some point between pollination and seed maturation. Whatever is causing the low fertility is affecting the frequency of the expected genotypes. To add to the dilemma, not all crosses with individuals possessing the

Table 1. Segregation and χ^2 statistic for inheritance of alcohol dehydrogenase (ADH)-1, 6-phosphogluconate dehydrogenase (6-PGDH)-1, phosphoglucomutase (PGM)-1, and shikimate dehydrogenase (SKDH)-1 in *Allium fistulosum*.

Cross	Progeny: Segregation classes				Test ratio	χ^2	*P* (%)
			ADH-1				
3/4 x 3/4	3/3	3/4	4/4		1:2:1		
PI433630, self-pollinated	17	28	8			3.22	20
PI433629, self-pollinated	8	17	6			0.55	70–90
Heshiko x Heshiko	19	28	22			2.71	20–30
3/4 x 4/4					1:1		
PI461401 x PI433629	6	24	31			1.39	20–30
4/4 x 3/4					1:1		
PI433629 x PI461401	0	5	8			0.69	30–50
3/4 x 3/3					1:1		
PI433630 x PI433630	34	40	1			0.47	50
3/3 x 3/4					1:1		
PI433630 x PI433630	61	49	0			1.30	20–30
3/6 x 3/6	3/3	3/6	6/6		1:2:1		
Heshiko x Heshiko	115	202	99			1.58	30–50
Heshiko x Heshiko	21	38	0			19.85	0.0001
3/4 x 3/6	3/3	3/4	3/6	4/6	1:1:1:1		
PI461401 x Heshiko S_1	40	43	39	28		3.44	30–50
4/6 x 4/6	4/4	4/6	6/6		1:2:1		
PI433629, F_1 self-pollinated	79	124	21			32.62	<1.0
PI433629, F_1 self-pollinated	28	66	16			6.60	<5.0
			6-PGDH-1				
1/2 x 1/2	1/1	1/2	2/2		1:2:1		
Heshiko x Heshiko	128	228	127			1.51	30–50
Heshiko x PI433629	13	47	16			4.49	10
1/1 x 1/2					1:1		
Heshiko x Heshiko S_1	11	12	0			0.04	70–90
			PGM-1				
1/2 x 1/2	1/1	1/2	2/2		1:2:1		
Heshiko, self-pollinated	13	21	12			0.38	80–95
Heshiko x Heshiko	33	44	20			4.30	10–20
Heshiko x PI418953	1	3	0			1.00	80
PI418953 x PI418953	3	15	4			2.99	20–50
PI461395 x PI461395	21	25	13			3.52	20–50
PI433630 x PI433630	11	13	4			3.63	20–50
1/1 x 1/2					1:1		
Heshiko x Heshiko	125	122	0			0.04	70–90
Heshiko x Heshiko S_1	15	13	0			0.14	70–90
			SKDH-1				
1/2 x 1/2	1/1	1/2	2/2		1:2:1		
PI433629, self-pollinated	56	118	54			0.32	80–95
Heshiko x Heshiko	40	76	25			4.05	20–30
Heshiko, self-pollinated	12	17	10			0.85	50–70

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Adh-1^{6} allele yield skewed ratios, since 6/6 individuals are recovered in expected ratios in sibbed 3/6 x 3/6 crosses. It may be that allele 6 competes more favorably with allele 3 than allele 4. However, when the cross 3/4 x 3/6 was made, each class was recovered fitting a 1:1:1:1 ratio.

6-*PGDH*. Gels stained for 6-PGDH had two zones of activity (Fig. 1B). Three phenotypes were observed in the anodal zone 1: two single-banded phenotypes (*6-Pgdh-1*^{2} and *6-Pgdh-1*^{1}) and one triple-banded phenotype with a band of intermediate mobility (*6-Pgdh-1*$^{1/2}$). The segregation ratios for the phenotypes were ≈1:2:1, 1/1:1/2:2/2 (Table 1), a result suggesting the enzyme is a dimer. There was no deviation from the expected segregation ratio of 1:2:1 in 559 progeny when tested by the chi-square analysis. The more cathodal zone (zone 2) was invariant with a single-banded phenotype. From these results we infer that the three phenotypes in zone 1 and the single band in zone 2 are controlled by two separate loci: *6-Pgdh-1* (more anodal) and *6-Pgdh-2* (more cathodal), respectively. The more cathodal allele has been designated as *6-Pgdh-1*^{1} and the more anodal allele has been designated as *6-Pgdh-1*^{2}. Segregation ratios of *6-Pgdh-1*^{2}/*6-Pgdh-1*^{2} (2/2) backcrossed to *6-Pgdh-1*^{1}/*6-Pgdh-1*^{2} (1/2) demonstrated no deviation from Mendelian expectations of a 1:1 segregation ratio (Table 1). These patterns of variation are consistent with a model for two loci controlling 6-PGDH, with the anodal locus coding for a functionally dimeric protein. No conclusions can be reached on the protomers encoding 6-PGDH-2.

PGM. Three phenotypes appear on gels stained for PGM (Fig. 1C). These previously have been designated as PGM-1 (Peffley et al., 1985): the more anodal allele is designated as *Pgm-1*^{2} and the slower migrating allele is designated as *Pgm-1*^{1}. Heterozygous individuals, *Pgm-1*^{1}/*Pgm-1*^{2}, display two bands, a result suggesting that the enzyme is a monomer. Segregation ratios for progeny recovered from 1/2 x 1/2 crosses were ≈1:2:1 when tested with the chi-square goodness of fit (Table 1). Segregation for progeny of the 1/1 x 1/2 and reciprocal crosses fit the expected 1:1 ratios. The reciprocal cross 1/2 x 1/1 was done but, because of the recovery of an unexpected class of *Pgm-1*$^{2/2}$ indicating selfs, the data is not included in Table 1.

SKDH. Two zones of activity were found on gels stained for SKDH (Fig. 1D). The more-anodal migrating zone 1 had three phenotypes: *Skdh-1*^{2}, *Skdh-1*^{1}, and *Skdh-1*$^{1/2}$. Heterozygous individuals expressed two bands, a result suggesting that the enzyme is a monomer. The slower-migrating zone 2 was found only in *A. cepa* individuals. SKDH-1 was assigned to the more-anodal locus. *Skdh-1*^{1} has been assigned to the more-cathodal allele and *Skdh-1*^{2} to the more-anodal allele. The segregation ratios were ≈1:2:1, 1/1:1/2:2/2, and all families showed no deviation from the expected segregation ratio (Table 1).

Except for the ADH 4/6 selfed, all isozymes investigated in this study were inherited in a Mendelian fashion and, thus, are suitable as molecular markers to tag genes of interest. Tests for independent segregation ratios fit the expected chi-square statistic, a result indicating no linkage between the isozyme loci.

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