Response of Isozymes in *Allium* to Thermal and Aerobic Stress

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ABSTRACT

Screening for tolerance to stress can be an expensive and time consuming process, and can be difficult to accurately assess. If molecular markers could be identified that are linked to tolerance to a specific stress, selection would be more exact. We tested two species of onion using 18 enzyme systems to ascertain utility of the isozymes as markers for stress tolerance. Only alcohol dehydrogenase exhibited promise of differential expression under different stress conditions. Inducibility, however, was not consistent.

KEY WORDS: breeding selection, marker selection, onion

As plant breeders, we are interested in identifying molecular markers to assist in selection of onion plants tolerant to stress. Isozymes are preferred markers for us because we use isozymes routinely in our breeding program, and they have been used to detect changes in hardiness in other plant species (Faw and Jung, 1972, cited by Kang and Titus, 1987). Isozymes are easily extracted and electrophoresed, many plants can be assayed in a single day, relatively little plant material is required, and they usually demonstrate repeatable and consistent banding patterns. Among enzymes which have exhibited variant alleles in response to stress in other systems are alcohol dehydrogenase (Benfey and Chua, 1989), glucose-6-phosphate dehydrogenase (Sadakane and Hatano, 1982), glutathione reductase (Guy and Carter, 1984), and malate dehydrogenase (DeJong, 1973). Benfy and Chua (1989) report that changes in gene expression accompanying oxygen deprivation are similar to those of thermal stress and that ADH is an inducible enzyme. Thus, the conditions for our study were cold and oxygen deprivation. Our study was conducted to ascertain if enzyme systems could be identified in *Allium* in which novel isoforms would be exhibited consistently and with sufficient resolution when plants were exposed to stress conditions.

Publication number T-4-512 from the College of Agricultural Sciences and Natural Resources.
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MATERIALS AND METHODS

Leaf and root material of *Allium cepa* cv 'Temprana.' *A. fistulosum* var 'Heshiko,' and interspecific (Allium *fistulosum* x A. *cepa)* hybrids were extracted in either glutathion buffer [1.5% (w/v) glutathion in 0.1M Tris buffer pH 3.5 with a final pH 7.0-7.5] or dithiothreitol (DDT) buffer (10mM DTT in 75mM Tris-HCl pH 8.5). Extracts were electrophoresed on horizontal starch or polyacrylamide gel systems. Horizontal starch: 12% starch of Tris-Citrate and Histidine buffer systems (Vallejos, 1983), or 13% starch of F system for maize (Stuber et al, 1988). Polyacrylamide: protocol followed Hanson and Jacobsen (1984) with these modifications: 20% (w/v) sucrose was added to the running gel; gels were run with 0.12 M Tris-0.96 M glycine buffer (pH 8.5) in both electrode tanks (Laemmli, 1970) pulsed 3 h at 10 mamps and run 16 h at 15 mamps at 40°C.

Eighteen enzymes were studied. Those stained after Vallejos (1983) were: alcohol dehydrogenase (ADH; E.C.1.1.1.1), diaphorase (DIA; E.C.1.6.-.-), glucose phosphoisomerase (GPI; E.C.5.3.1.9), glutamate oxaloacetate transaminase (GOT; E.C.2.6.1.1), isocitrate dehydrogenase (IDH; E.C.1.1.1.42), lactate dehydrogenase (LDH; E.C.1.1.1.27), malate dehydrogenase (MDH; E.C.1.1.1.37), malic enzyme (ME; E.C.1.1.1.40), peroxidase (PRX; E.C.1.11.1.7), phosphoglucomutase (PGM; E.C.2.7.5.1), 6-phosphoglucanate dehydrogenase (6-PGDH; E.C.1.1.1.44), shikimate dehydrogenase (SKDH; E.C.1.1.1.25), superoxide dismutase (SOD; E.C.1.15.1.1), triosephosphate isomerase (TPI; E.C.5.3.1.1); and after Acquaah (1992): amylase (AMY; E.C.3.2.1.-), glutathion reductase (GR; E.C.2.7.5.1), succinate dehydrogenase (SUD; E.C.1.3.99.1), xanthine dehydrogenase (XDH; E.C. 1.2.1.37).

Plants of each genotype were grown at the TTU Greenhouse in 3" pots for 12 weeks under 25°C ambient conditions and then subjected to one of two stress treatments. Following stress treatments, plants were returned to the greenhouse. Plants either were held in a growth chamber at 5°C for seven days, tissue was electrophoresed on days 3 and 7 during treatment and on days 3 and 7 after treatment under greenhouse conditions; pots were held at ambient greenhouse conditions, 25°C, but immersed in water for seven days with samples electrophoresed on days 3 and 7 during treatment and days 3, 7, and 14 after plants were no longer flooded. Control plants were maintained under ambient greenhouse conditions in the greenhouse.

Cloned 'Heshiko' individuals were collected after plants multiplied as tillers. Individuals heterozygous for ADH-1, $Adh\text{-}1^3/Adh\text{-}1^6$, were selfed and genotypes $Adh\text{-}1^3/Adh\text{-}1^6$ and $Adh\text{-}1^6/Adh\text{-}1^6$ selected for further analyses and were also crossed with genotypes $Adh\text{-}1^4/Adh\text{-}1^4$ in order to recover $Adh\text{-}1^4/Adh\text{-}1^6$ individuals for comparison studies of expression of allele 6 (Mangum and Peffley, 1994). Treatments were conducted on clones of Heshiko that expressed $Adh\text{-}1^3/Adh\text{-}1^3$ (3/3), $Adh\text{-}1^3/Adh\text{-}1^4$ (3/4), $Adh\text{-}1^3/Adh\text{-}1^6$ (3/6), and $Adh\text{-}1^4/Adh\text{-}1^6$ (4/6). One individual was grown under ambient greenhouse conditions as an experimental control, while a sister clone was placed under one of each stresses. Tissue of control plant was extracted prior to stress and electrophoresed.

RESULTS AND DISCUSSION

Enzyme resolution appears in Table 1. Polymorphism between species was observed for ADH, DIA, GPI, IDH, LDH, MDH, ME, 6-PGDH, PGM, PRX, SKDH, and TPI. AMY and GR did not resolve on any of the gel systems used. ADH was the only enzyme exhibiting an altered banding pattern following either stress treatment; subsequent investigations focused on expression of ADH.

Table 1. List of enzymes exhibiting resolution.

	polymorphism		Plant	Gel		polymorphism		Plant	Gel
LOCUS	Ac	Af	material	system	LOCUS	Ac	Af	material	system
Adh-1	f	s[1]	rts	TC,P	6-Pgdh-2f	s	rts,lvs	F	
Dia	f	s	rts,lvs	F	Prx	f	s	rts,lvs	TC
Got			rts,lvs	P	Pgm	f	s[1]	rts,lvs	TC,P
Gpi-1	f	s[1]	rts,lvs	His	Sod			rts	TCP
ldh-1	f	s[1]	rts	His	Skdh-1	f	s	rts,lvs	TC,F,P
Ldh	f	s	rts	His	Sud			rts,lvs	TC,F,P
Mdh	f	s	rts	TC,F,P	Tpi	f	s	rts,lvS	TC,F,P
ME	f	s	rts	TC	Xdh			rts	F,P
6-Pgdh-1	s	f[2]	rts,lvs	F					

Ac = *Allium cepa;* Af = *A. fistulosum*
TC = Tris-Citrate, His = Histidine, F = F system for maize, P = Polyacrylamide
1 = described in Peffley et al., 1985.
2 = described in Mangum and Peffley, 1994.

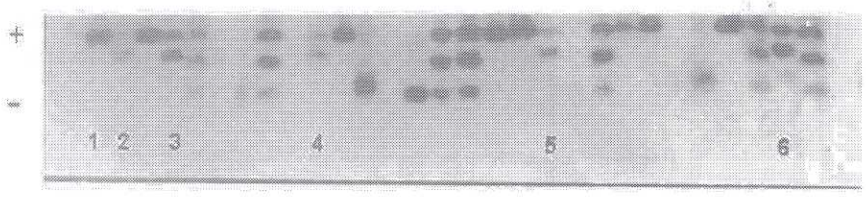
Heshiko plants with *Adh-1^3/Adh-1^6* genotype when not stressed exhibited the expected banding pattern for a dimeric enzyme, i.e., with a faster migrating band *Adh-1^3*, a slower migrating band *Adh-1^6*, and a band of intermediate mobility, the heterodimer. Lanes exhibiting all three bands were scored as *Adh-1^3/Adh-1^{6s}*, the 's' designating the presence of a 6/6 homoallele (Fig. 1, lanes 3 and 6). Allele 6 always disappeared after *Adh-1^3/Adh-1^{6s}* plants were exposed to stress, but the heterodimer, 3/6, and the 3/3 bands remained, leaving a pattern with two bands (Fig. 1, lanes 2, 4, and 5). Throughout this study, any individual expressing only the two faster, cathodal bands was scored as *Adh-1^3/Adh-1^6*.

Adh-1^3/Adh-1^{6s} plants grown under ambient greenhouse conditions and expressing the 6/6 band were placed into the growth chamber at 5°C for seven days. The 6/6 homoallele disappeared in all plants after three days of cold treatrnent. After seven days of stress, plants were returned to greenhouse conditions and usually the 6/6 reappeared after three days. In many individuals, the 6/6 homoallele did not reappear even after seven days. *Adh-1^3/Adh-1^{6s}* plants exposed to anaerobic stress also lost activity of the slow band after three days, the slow allele returned in all plants only after the plants had been in greenhouse conditions for 14 days.

When the *Adh-1^6* protomers bind other allele 6 protomers, making a functional 6/6 enzyme, a slow band appears. Even when the slow band appears, however, it stains less

intensely than the expected 1:2:1 ratio exhibited when all subunits reassociate in ratios expected for a dimeric enzyme. The allele 6 protomers apparently are still translated under stress conditions, else the heterodimer would not be present. However, the binding of 6/6 homoprotomers is either not functional, loosely-associated in its quanternary structure or the binding is very sensitive to stress conditions. An aberrance perhaps related to the fitness of plants with the 6/6 genotype has been observed in an independent study where controlled crosses yielded fewer than the expected number of 6/6 progeny (Mangum and Peffley, 1994).

We could induce the loss of the 6/6 band, but we could not always induce the reappearance of the 6/6 band. This pattern was observed over a two-year period after repeated runs of Heshiko material. The qualifications for a molecular marker are that they are predictable and reliable. After eight repeated tries with starch and polyacrylamide gels, we could resolve the 6/6 homoallele in only 19 of 109 plants, thus disqualifying its use as a marker for stress.



Photograph of *Allium* electrophoretic gel of ADH-1. Lane 1 *Adh-1*^{3}/*Adh-1*^{3}; lanes 2, 4, and 5 *Adh-1*^{3}/*Adh-1*^{6}; lanes 3 and 6 *Adh-1*^{3}/*Adh-1*^{6e}.

This work was supported by USDA specific Cooperative Agreement #58-7 MN 1-9-948 to J. B. and E. P. We thank Anatoly Pashkar, Qing Hong, and David Young for their technical contributions.

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