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Non-genetic Origin of Isoenzymic Variability in Subterranean Turions of Monoecious and Dioecious Hydrilla

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ABSTRACT

Isoenzymic variability was investigated in subterranean turions of monoecious hydrilla collected in North Carolina and dioecious hydrilla collected in Texas and California. Two variants of each biotype were found in the field samples. One, termed Type A, had patterns of alcohol dehydrogenase and aspartate aminotransferase after gel electrophoresis that were identical to those previously reported for turions grown in the laboratory. Dioecious plants in laboratory culture were originally from Imperial Irrigation District in California, while monoecious plants were from the Washington, D.C., area. The second variant, termed Type B, had more rapidly moving electromorphs of both enzymes. The ratio of the two variants was different at each site in the field collections. Plants from monoecious turions known to be Type B produced only turions of Type A under laboratory conditions. Plants grown from dioecious plants or turions from sites in Texas that had high populations of Type B produced turions only of Type A. The presence of Type B for either biotype was not due to genetic variants within the populations, but might be due to environmental factors or ageing of the turions.

Key words: alcohol dehydrogenase, aspartate aminotransferase, biotypes, gel electrophoresis, *Hydrilla verticillata*, isoenzymes, turions.

INTRODUCTION

Isoenzymic analysis has been used to study the population characteristics of some aquatic macrophytes (Sharitz et al. 1980; McMillan and Phillips 1981; Hettiarachchi and Triest 1986; Verkleij and Pieterse 1986; Triest 1988; van Wijk et al. 1988). Only for hydrilla (*Hydrilla verticillata* (L.f.) Royle) have isoenzymes in the organs of perennation been investigated (Ryan 1988, 1989). Subterranean turions,

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sometimes called tubers, were used since it was felt that their isoenzymes would be less subject to wounding and stress effects than the relatively delicate leaf and stem tissue of aquatic macrophytes (Ryan 1988).

Two biotypes of hydrilla presently exist in the United States (Verkleij and Pieterse 1986). A dioecious female plant is established in the southern U.S. and as far west as California, while a monoecious plant is found near Washington, D.C., and in other areas in the south. Isoenzymic analysis of laboratory-grown vegetative plant material gave no indication of genetic variability in either population (Verkleij and Pieterse 1986). Recently, work with subterranean turions of monoecious hydrilla from an infestation in North Carolina indicated the existence of variability in some isoenzymes (Ryan 1989). One isoenzyme pattern was identical to that earlier described for monoecious hydrilla in the Washington, D.C. area (Verkleij and Pieterse 1986), and noted in subterranean turions of laboratory-grown monoecious hydrilla (Ryan 1988). This pattern was designated Type A. A variant form, called Type B, had more rapidly migrating isoenzymic bands for alcohol dehydrogenase (ADH), aspartate aminotransferase (AAT), and NADP-malic enzyme (NADP-ME). In this collection, the proportion of Type A to Type B was different at each of the four sites.

Monoecious hydrilla in the U.S. has been reported to set viable seed (Conant et al. 1984; Langeland and Smith 1984) but it is not known whether sexual reproduction is common or widespread. The dioecious plant in the U.S. is female and the male counterpart is not known to exist here. The presence of variants in the populations must be explained in some way other than through sexual reproduction. Variants might be found in a clonally reproducing population due to somatic mutation (Silander 1985) or through multiple introductions. Alternately, environmental factors or ageing might influence the behavior of the proteins in the storage organs, leading to the appearance of genetic variation. In the experiments described in this paper, subterranean turions were allowed to develop from turions of known variants for the monoecious biotype, and the isoenzymes of the new turions were monitored. Additionally, a similar pattern of variation in isoenzymes for two field collections of turions of dioecious plants is described: the isoenzymic pattern of the turions from these plants is described as well. Since turion production is clonal, a comparison of isoenzymic patterns of these turions provides an explicit test to distinguish genotypic and phenotypic variation.

MATERIALS AND METHODS

Subterranean turions of the monoecious biotype were collected in July, 1988, from sites in North Carolina with the assistance of Dr. David DeMont, Aquatic Weed Control Program, Department of Natural Resources and Community Development, State of North Carolina. Collections were made at approximately the same sites as for the previous report (Ryan 1989) at Lake Anne and Rodgers Lake. Collection was made on the east side of Page Lake, a new infestation since the previous collection (D. DeMont, personal communication).

Subterranean turions or segments were ground by hand in 1.5 ml polypropylene centrifuge tubes in a solution containing 50 mM Tris HCl, pH 7.8, 10% (v/v) glycerol, 1 mM phenylmethylsulfonyl fluoride and 0.34 M 2-mercaptoethanol. Suspensions were centrifuged at 7000 x g for 2 minutes and supernatants used for analysis of isoenzymes. Proteins and enzyme activities were visualized after nondenaturing electrophoresis on polyacrylamide gels as described previously (Siciliano and Shaw 1976; Ryan, 1989). For monoecious turions, a 50 mg piece was taken from the base for analysis and the remainder was planted in a container filled with 10% peat, 90% sand supplemented with inorganic nutrients (Spencer and Anderson 1986). Containers were held in 1000 L concrete vaults outdoors at the U.S.D.A. Aquatic Weed Laboratory, and maintained with weekly flushings of well water. Plantings were started in mid-July, 1988, and turions were harvested in January and February, 1989. Only subterranean turions were used for these experiments. The biotype of the subsequent subterranean turions was determined as described above.

Turions of the dioecious biotype were collected in January, 1989, from the reservoir in McLaren Park, San Francisco, CA. Turions were separated into three groups based on appearance. Turions of the first group, "cream", were uniformly beige; those in the second group, "tabby", had black leaf scales. Those in the third group, "red", had a granular red material on their surfaces. Twelve turions from each group were analyzed for AAT and ADH (Ryan 1989). A 50 mg piece was cut from the base of each turion and used for analysis of isoenzymes, as above. The remainder of the tuber was placed in 15 ml of water in a test tube and placed in a growth chamber with a 12 h photoperiod and day and night temperatures of 20 C and 16 C. Sprouting was scored after two weeks by the criterion of emergence of the plant, with discernible leaflets, from the turion.

Subterranean turions of plants of the dioecious biotype were collected from lakes in central and southern Texas. Initial collections were made in June and September, 1988, in Lake Texana. A subsequent collection was made in December 1988 in Lakes Texana, Long, Fayette, and Bastrop. Some of these turions were shipped to Davis, CA, for isoenzymic analysis. Others were sent to the growth facilities of the U.S. Bureau of Reclamation, Denver, CO for experiments on turion production (Thullen 1990). Plants were grown in a greenhouse in 95 l plastic barrels containing a Colorado loam soil to a depth of approximately 10 cm. Photoperiod ranged from 9.75 to 15 h of daylight and temperature fluctuated from 32 to 10 C, with a maximum daily range of 10 C, according to prevailing climatic conditions. Barrels were flushed three times per week with fresh water filtered through a charcoal dechlorinating system. Turions were harvested after at least three months of growth and shipped to Davis for analysis.

RESULTS AND DISCUSSION

Turions collected from Rodgers Lake (N = 19) and Lake Anne (N = 31) were Type B, while turions from the Page Lake site (N = 7) were Type A. To determine the biotype of turions grown from these plants in the labora-

TABLE 1. FIELD COLLECTED TURIONS OF THE DIOECIOUS BIOTYPE FROM TEXAS SHOW BOTH ISOENZYMIC VARIANTS, WHILE LABORATORY CULTURED MATERIAL IS ONLY TYPE A.

Lake of origin		Date of Collection	Culture	*Distribution* Type A	Type B
Texana	(N = 9)	06-08-88	field	0.56	0.44
Texana	(N = 25)	12-15-88	field	0.32	0.68
Texana[1]	(N = 22)	12-15-88	field	1.00	0.00
Texana-Simon	(N = 38)	12-16-88	field	0.84	0.08[2]
Long	(N = 17)	12-13-88	field	0.06	0.88[2]
Bastrop	(N = 18)	12-13-77	field	0.94	0.06
Fayette	(N = 31)	12-14-88	field	0.94	0.06
Texana	(N = 10)	07-05-89	greenhouse	1.00	0.00
Texana	(N = 18)	09-06-88	growth chamber	1.00	0.00
Texas-Simon	(N = 10)	07-05-89	greenhouse	1.00	0.00
Long	(N = 10)	07-05-89	greenhouse	1.00	0.00
Bastrop	(N = 10)	07-05-89	greenhouse	1.00	0.00
Fayette	(N = 10)	07-05-89	greenhouse	1.00	0.00

[1]This collection was from a second site on the lake at the same date.
[2]There were several turions in the collection that had no discernible enzymic activities in the extract: these were presumably non-viable. The sum of the fractions is thus less than 1.00.

tory, AAT and ADH were determined for 12 turions from each of four plants originally from each site. The laboratory-grown turions from the Page Lake site were Type A. All laboratory grown turions from plants originating at Rodgers Lake or Lake Anne were Type A.

The isoenzymic patterns of turions from McLaren reservoir indicated that the plants were of the dioecious strain previously characterized in the U.S. (Verkleij and Pieterse 1986; Ryan 1988). Variants of ADH, AAT, and NADP-ME were noted, however, which were qualitatively similar to those of the monoecious turions, in that variants had bands of activity for ADH and AAT that moved more rapidly than the usual isoenzymes. For this reason, Type A is used to designate the previously characterized patterns and Type B is used for the variant. Field collected turions (N = 503) were divided into three classes based on appearance. The so-called cream and tabby turions comprised 51 and 36% of the total and had mean weights of 473 ± 219 mg and 514 ± 233 mg (± standard deviation), respectively. Turions of these two classes were uniformly Type B. The red turions comprised 13% of this population with mean weight ± standard deviation of 464 ± 202 mg, with 82% of these Type A and 12% Type B. There were no significant differences among mean weights for the three classes, thus the appearance of the turion, and its biotype, was not related to size. Turions of the three classes were all viable, as determined by their ability to sprout within a two week period. Two replicate experiments using twelve turions of each class gave the following average values for percentage of turions sprouting after two weeks: 71% of the cream, 25% of the tabby, and 54% of the red. In one replicate, the tabby turions were overgrown with a fungus and there were no plants present after two weeks so the average value for this class is low.

Subterranean turions of the dioecious plant collected in the field in south-central Texas also showed the isoenzymic variant Type B. The ratio of Type A to Type B varied among sites (Table 1). When vegetative material from these sites was grown to maturity under laboratory conditions, the subsequent turions were all Type A (Table 1).

It seems unlikely that isoenzymic Types A and B of either monoecious or dioecious hydrilla represent true genetic variants. Turions grown in the laboratory from monoecious plants known to be Type B were Type A, suggesting that the differences in isoenzymic patterns are the consequence of the interaction of the turions with the environment. Type A is the form seen in newly developed laboratory cultures of the momoecious and dioecious biotypes of the plant and in recent infestations of the plant in the field, such as the Page Lake site.

Isoenzymes of turions can be used for the determination of biotype of hydrilla, as suggested earlier (Ryan 1988), however, the presence of variant isoenzymic forms in field collections must be recognized. The present work strongly suggests that determinations of biotypes from turions for new infestations or for previously uncharacterized varieties of hydrilla be accompanied by isoenzymic profiles of newly-developed turions grown under laboratory conditions. Factors such as ageing of the turion, the soil type, particular redox chemistry in the vicinity of the turion, and the presence of pathogens may play a role in promoting appearance of Type B. Further work is needed to ascertain the molecular origin of the differences between Type A and B, and to determine the conditions that promote the transition from one form to another. If this transition is due to ageing, the ratio of the two forms in a population may prove a useful measure of the age of an infestation.

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