

A Comparative Study of Isoenzyme Patterns, Morphology and Chromosome Number of *Hydrilla verticillata* (L.f.) Royle in Africa

ARNOLD H. PIETERSE¹, JOS A. C. VERKLEIJ² AND HAN P. M. STAPHORST²

ABSTRACT

Although *Hydrilla verticillata* (L.f.) Royle is becoming a major aquatic weed in various tropical and sub-tropical areas, such as Southeast Asia, Australia, the Panama canal and the warmer regions of the United States, it is only of local occurrence in Africa. Isoenzyme patterns, morphology and chromosome number of specimens from Uganda, Rwanda and Burundi, as well as the relationship of these plants to previously studied *Hydrilla* plants from other continents, were compared. The African plants showed very similar isoenzyme patterns, which in comparison to *Hydrilla* from other continents, were not very exceptional. The Uganda and Burundi plants differed considerably in their morphology from the Rwanda plants as well as from *Hydrilla* elsewhere in the world.

Key words: Genetic variability, isoenzyme patterns, leaf form, leaf size, leaves per whorl, clones.

INTRODUCTION

Hydrilla verticillata (L.f.) Royle (Fam: Hydrocharitaceae) is becoming a major aquatic weed in various tropical and sub-tropical areas (1, 2, 5, 6, 10, 14). It is causing considerable problems with usage of the affected waterbodies in the Panama canal and the warmer regions of the United States, where the plant recently was introduced, and in Southeast Asia and Australia. On the other hand, in Europe and Africa it is only of local occurrence.

Comparison of morphology and isoenzyme patterns of various *Hydrilla* specimens collected from different parts of the world, cultivated under similar conditions in the laboratory, indicates that large genetic differences occur within the species (7, 12, 13). In particular, plants from Europe showed strongly deviating isoenzyme patterns, a characteristic which may be related to an adaption to specific environmental conditions as well as genetic drift. African plants were not included in the previous studies. However, the fact that the plants in Africa do not actively extend their growth area, i.e. they mainly occur in and around the central lakes, and do not create serious weed problems could imply that they represent a different race.

In order to estimate the genetic relationship between African *Hydrilla* and *Hydrilla* from other parts of the world, isoenzyme patterns, morphology and chromosome numbers were examined in eight strains collected from Uganda, Rwanda and Burundi, after cultivation under similar conditions in the laboratory.

MATERIALS AND METHODS

Eight strains of *Hydrilla verticillata* (L.f.) Royle were collected in Uganda, Rwanda and Burundi by Dr. Markham of the Kenya Station of the Commonwealth Institute of Biological Control and kindly sent to Amsterdam. In Figure 1 the location of the collection sites of these strains is shown, and in Table 1, data are presented on their provenance. A clone of each collection was grown in the laboratory in an aquarium (50 x 50 x 30 cm) which was filled with rain water. These aquaria contained a layer of clay (2 cm) on the bottom, covered by a similar layer of superficially washed sand. The aquaria were placed in a growth chamber which was kept at 25 ± 2 C. The plants were exposed to an illumination of $1200 \mu J cm^{-2} sec^{-1}$ and the light regime was 8 h light and 16 h darkness. Five clones collected in Asia, Australia and North America were grown under the same conditions for comparison to the African plants (Kashmir (India), New Delhi (India), the northern island of New Zealand, Florida (USA) and Washington, D.C (USA)). These were clones 2,3,19,23 and 26, respectively, in a previous publication (12).

The homogenization of the plant material for isoenzyme analysis as well as electrophoresis was performed according to Verkleij et al. (12) after the plants had been grown during a period of two months in the aquaria. Eight enzymes, which in previous studies proved to be polymorphic in *Hydrilla* (12, 13), were tested, i.e. phosphoglucomutase (PGM, EC 2.7.5.1), peroxidase (PO, EC 1.11.1.7), shikimate dehydrogenase (SDH, EC 1.1.1.25), glutamate-oxaloacetate transaminase (GOT, EC 2.6.1.1), super-oxide dismutase (SOD, EC 1.15.1.1), NADH-dehydrogenase (NADH.DH, EC 1.6.99.3), malic enzyme (ME, EC 1.1.1.40) and alcohol dehydrogenase (ADH, EC 1.1.1.1). Staining and fixation techniques for PO, SOD and ME were according to Verkleij et al. (11), PGM and ADH according to Smith (9), SDH according to Rothe (8), NADH.DH according to Menken (4) and GOT according to Khavkin & Sukhorzhevskaja (3). Apart from the African plants, GOT was also analyzed in the control plants from New Zealand and Washington, D.C.

¹Royal Tropical Institute, Department of Agricultural Research, Mauritskade 63, 1092 AD Amsterdam, The Netherlands.

²Biological Laboratory, Free University, P.O. Box 7167, 1077 MC Amsterdam, The Netherlands.

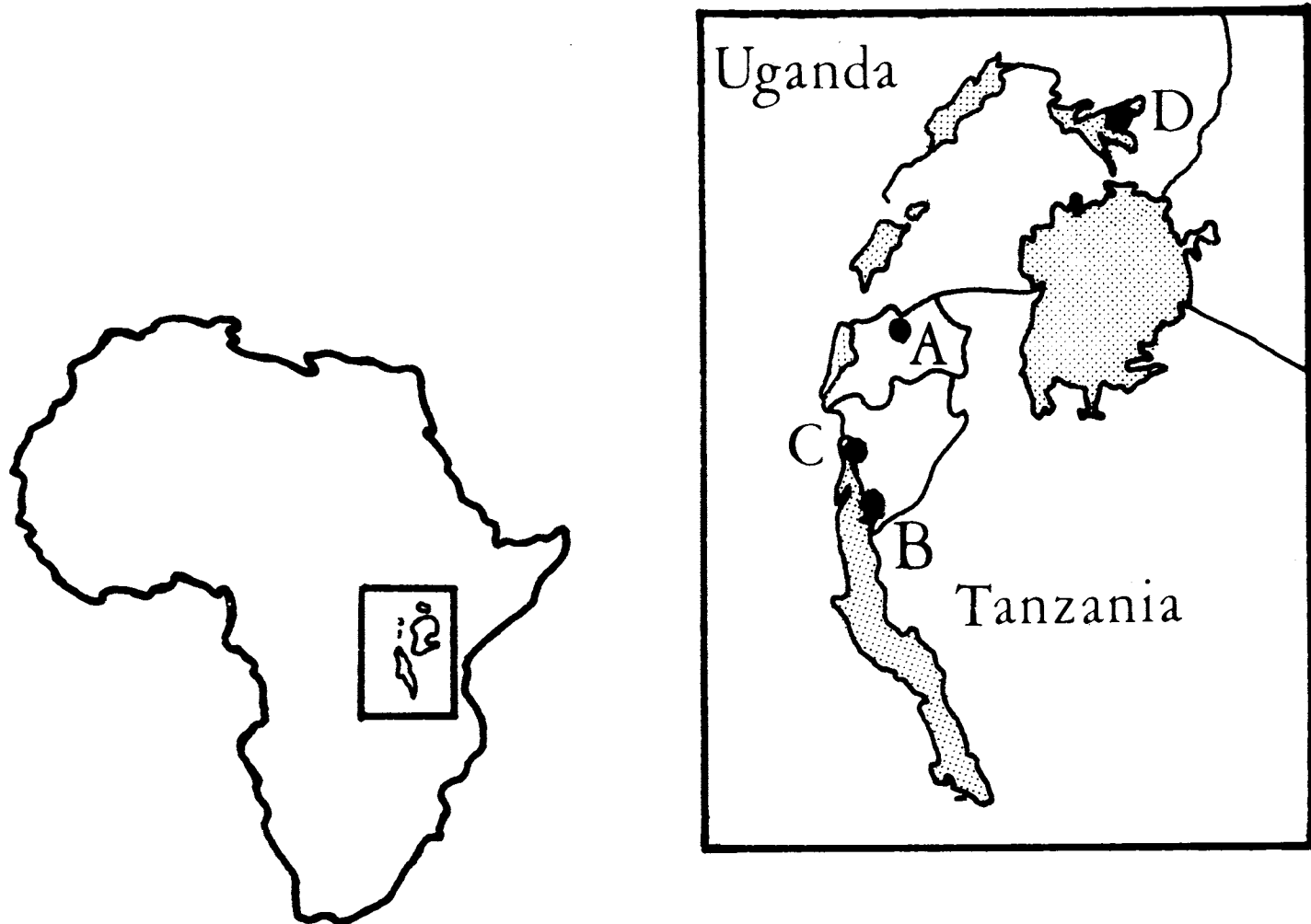


Figure 1. Location of the collection sites of the various clones A = clone no.II and no.III; B = clone no.VIII; C = clone no.IV-no.VII; D = clone no.I (scale detailed map 1 : 25.000.000).

TABLE 1. DATA ON THE PROVENANCE OF THE VARIOUS HYDRILLA CLONES.

Clone No.	Place of origin	Country of origin	Date of collection
I	Lake Kioga, near Bukungu	Uganda	8-3-1983
II	Lake Bulera, near Kagogo	Rwanda	8-6-1983
III	River below dam, exit from Lake Ruhondo	Rwanda	11-6-1983
IV	Lake Tanganyika, near Bujumbura	Burundi	9-2-1983
V	Lake Tanganyika, near Bujumbura	Burundi	15-6-1983
VI	Lagoon adjacent to lake Tanganyika, 12 km from Bujumbura	Burundi	14-6-1983
VII	Lagoon adjacent to lake Tanganyika, 12 km from Bujumbura	Burundi	16-6-1983
VIII	Lake Tanganyika, near Rumonge	Burundi	17-6-1983

For recording morphological data, young apices were used which had developed in the aquaria (5 shoot tips per plant). These apical portions were harvested two months after planting the starting material. Ten internodia of an actively growing stem apex, counted from the second clearly visible internodium below the apex, were examined. The data which were registered included:

- leaf surface, expressed as the product of length and width;
- the number of leaves in a whorl;
- leaf form, expressed as the quotient of length and width; and
- the number of teeth per cm along the margin (of one side) of a chosen leaf in each whorl.

Morphological studies were conducted with the African plants and also on control plants from Kashmir, New Delhi and Florida. Chromosome numbers were determined according to the method previously described (12).

TABLE 2. DISTRIBUTION OF THE DIFFERENT ISOENZYME PHENOTYPES OF PGM, PO, SDH, GOT, SOD, NADH·DH, ME, AND ADH OVER THE VARIOUS CLONES OF HYDRILLA FROM AFRICA AS WELL AS THE CLONES FROM NEW ZEALAND AND WASHINGTON, D.C.

No.	Place of origin	PGM	PO	SDH	GOT	SOD	NADH·DH	ME	ADH
I	Uganda, Lake Kioga	1	1	2	2C	1	2	2	3
II	Rwanda, Lake Bulera	1	1	2	2A	1	2	2	3
III	Rwanda, Lake Ruhondo	1	1	2	2B	1	2	2	3
IV	Burundi, Lake Tanganyika	1	1	2	2C	1	2	2	3
V	Burundi, Lake Tanganyika	1	1	2	2C	1	2	2	3
VI	Burundi, Lake Tanganyika	1	1	2	2C	1	2	2	3
VII	Burundi, Lake Tanganyika	1	1	2	2C	1	2	2	3
VIII	Burundi, Lake Tanganyika	1	1	2	2C	1	2	2	3
19	New Zealand	2 ^x	6 ^x	2 ^x	2D	3 ^x	3 ^x		
26	USA, Washington, D.C.	4 ^x	5 ^x	2 ^x	2D	1 ^x	2 ^x	1 ^x	4 ^x

^x = analysed by Verkleij *et al.* 1983a and b.

RESULTS

The distribution of the different isoenzyme patterns for the various *Hydrilla* plants from Africa is presented in Table 2. Only one of the eight enzymes tested, GOT, showed variation in isoenzyme phenotypes. There were three different patterns which occurred respectively in clone no.II from Rwanda, clone no.III from Rwanda, and clones no.I and no.IV-VIII from Uganda and Burundi (Figure 2). These differences are based upon isoenzyme bands which had not been detected in previous studies (7, 12, 13). These additional bands, which are characterized by a relatively high electrophoretic mobility, could be demonstrated by means of difference in staining technique, based mainly on a different colouring substance (3). If only the bands which have been described in the previous papers are taken into consideration, the isoenzymes of GOT

would be the same in all African plants. As this phenotype would be identical to GOT phenotype 2 of Verkleij *et al.* (12), the isoenzyme phenotypes of the African plants were designated 2A, 2B and 2C for clone no.II, clone no.III and clones no.I/no.IV-VIII, respectively. When GOT was analyzed in the control plants from New Zealand and Washington, D.C., which previously had shown phenotype 2, there were also additional bands (Figure 2). This pattern, which was the same in the two control plants, was different from the pattern found in the African plants (it was designated 2D).

The isoenzyme phenotypes of the other enzymes tested, i.e. PGM, PO, SDH, SOD, NADH, ME and ADH, were identical to the formerly described phenotypes 1,1,2,1,2,2 and 3, respectively (12, 13).

The morphology of the Rwanda clones (no.II and no.III) was very different from that of the Uganda and Burundi clones. This difference is mainly due to the form of the leaves which were ovate to widely ovate in the plants from Uganda and Burundi and elongate in the plants from Rwanda (Figure 3 and Table 3). Other differences between these two types were the number of leaves in a whorl and the number of teeth along the margin of a leaf (Table 3). Although the two Rwanda plants were very similar, the plants from lake Bulera (no.II) were more robust, which is reflected in the larger leaf surface (Table 3). In general it can be concluded that the plants from Asia, Australia and North America were morphologically more similar to the plants from Rwanda than to the plants from Uganda/Burundi. This does not hold for the number of leaves in a whorl in the plants from New Delhi, which was relatively small.

The chromosome number of all African plants tested was 16.

DISCUSSION

According to the isoenzyme patterns it may be concluded that the African plants are closely related to each other. However, this seems to be in contradiction with the marked differences in morphology between the plants from Uganda and Burundi on the one hand and the plants from Rwanda on the other hand. These differences, which are apparently genetically based as they persist when the plants are grown under identical circumstances in the lab-



Figure 2. Electrophoretic patterns of the enzyme GOT, analyzed in shoot extracts of various clones of *Hydrilla*. Isoenzyme bands are illustrated according to their electrophoretic mobilities in the gel from cathode to anode (as indicated by arrow).

TABLE 3. AVERAGE LEAF SURFACES, EXPRESSED AS LENGTH (mm) X WIDTH (mm), AVERAGE NUMBER OF LEAVES IN A WHORL, AVERAGE LEAF FORM EXPRESSED AS LENGTH (mm)/WIDTH (mm) AND AVERAGE NUMBER OF TEETH ALONG THE MARGIN OF A LEAF PER CM OF THE VARIOUS CLONES OF HYDRILLA.¹

Clone No.	Origin	Leaf surface	No leaves	Leaf form	No. teeth
I	Uganda, Lake Kioga	19.9 c	5.0 a	1.8 f	23.1 c
II	Rwanda, Lake Bulera	36.1 b	3.3 d	4.8 bc	14.0 ef
III	Rwanda, Lake Ruhundo	19.6 c	4.0 bcd	6.0 a	11.3 f
IV	Burundi, Lake Tanganyika	23.2 c	4.3 abc	2.2 f	38.5 a
V	Burundi, Lake Tanganyika	17.1 c	4.4 ab	3.0 e	32.4 b
VI	Burundi, Lake Tanganyika	18.7 c	4.2 abc	3.5 de	31.5 b
VII	Burundi, Lake Tanganyika	22.0 c	4.3 abc	3.3 de	30.6 b
VIII	Burundi, Lake Tanganyika	15.6 c	4.2 abc	3.4 de	32.0 b
2 ²	India, Kashmir	23.9 c	3.5 cd	6.1 a	16.1 d
3	India, New Delhi	34.1 b	4.3 abc	5.2 ab	17.4 d
23	USA, Florida	52.0 a	3.8 bcd	4.2 cd	12.1 f

¹The data were statistically analyzed with the Student-Newman-Keuls test. The letters indicate plants which do not differ significantly ($\alpha = 0.01$). Each value is based upon 50 individual measurements (10 measurements in 5 equal portions).

²Clone numbers in arabic are taken from Verkleij *et al.* (1983a).

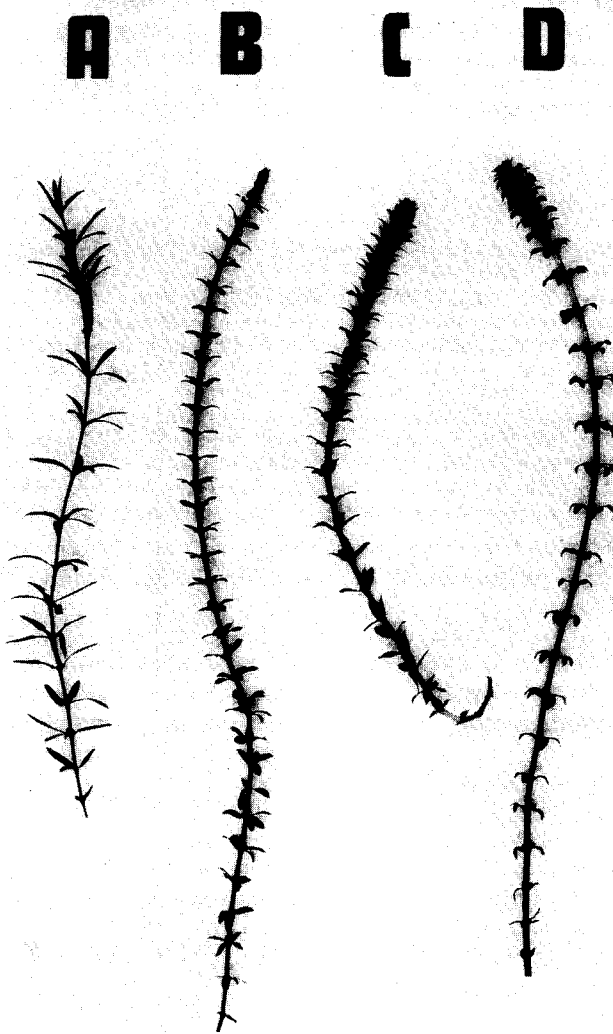


Figure 3. Shoots of various *Hydrilla* clones from Africa after cultivation under similar conditions in the laboratory. A = no.II from Rwanda; B = VIII from Burundi; C = no.IV from Burundi, D = no.I from Uganda.

oratory, are reflected only in the phenotype of the additional GOT bands. It seems, therefore, in accordance with previous results (12) obtained with *Hydrilla* plants from other continents that the variabilities in morphology and isoenzyme patterns of the enzymes tested, are not correlated. This assumption is corroborated by the fact that there are also differences between the GOT patterns of the two clones from Rwanda, which, in spite of the differences in leaf surface, are morphologically very similar.

The identical isoenzyme patterns in the plants from Uganda and Burundi, as well as the uniformity in their morphology, could imply that they belong to the same widespread clone. This is not completely unexpected for the Burundi plants as all specimens were collected in or near lake Tanganyika. On the other hand, the distance between the collection site of the clone in Uganda (in lake Kioga) and the sites in lake Tanganyika (about 750 km) is relatively large, especially in connection with the collection site of the Rwanda plants, which is about 200 km from lake Tanganyika (Figure 1). Moreover, the site in Uganda is situated in a different watershed, that of the river Nile, which flows through lake Kioga (lake Tanganyika drains off via the Zaire river) and as a consequence transport by water can be excluded.

On a world-wide basis the isoenzyme patterns of the African *Hydrilla* plants are not very distinctive as they also occur in plants from other continents (see 12,13). The additional bands of GOT cannot be taken into consideration in this context as they have only been tested in two non-African clones. With regard to the multi-isoenzyme phenotype, which is obtained when the different isoenzyme phenotypes are pooled together, the African plants most closely resemble the following previously studied specimens (12,13): "USA hydrilla I" (which refers to *Hydrilla* plants from the Gulf states and California, including no.20 to 25), no.2 from Kashmir (India) and no.18 from Rawa Pening (Indonesia). In "USA hydrilla I", six out of eight isoenzyme phenotypes are identical to the isoenzyme phenotypes found in the African plants. All the isoenzyme patterns in the *Hydrilla*'s from Kashmir and Rawa Pening are identical, however in these plants ME and ADH have

not been analyzed and as a consequence the multi-isoenzyme phenotypes are only based on six enzymes.

Hydrilla plants from Europe are genetically the most distant from the African plants (7,12). A clone from Poland (no.28) showed only one identical isoenzyme pattern and a clone from Ireland only two identical patterns of the eight enzymes tested.

The morphology of the plants from Uganda and Burundi is much different than plants from other continents. This is due mainly to the shape of the leaves and the number of teeth alongside the leaf margins (see control plants in the present study and Verkleij et al. 12). Cook & Lüönd (1) have presented a drawing of a plant from lake Tanganyika in Burundi (collected by Symoens, no.14760), which closely resembles the plants from Uganda and Burundi in the present investigation. These authors suggest that the plant is an example of a sterile phenodeme which is found in alkaline lakes. Whether the Uganda/Burundi plants are sterile remains to be investigated, up to now they have not flowered in the laboratory in Amsterdam. Their uniformity could be an indication that generative multiplication is lacking.

In all probability the African plants have not recently been introduced from other continents. This could also be concluded from the fact that *Hydrilla* plants in Africa were already collected by Speke and Grant during their 1860-1863 expedition to the sources of the Nile (1). It is likely that the identical multi-isoenzyme phenotype found in the plants from Kashmir and Rawa Pening is a coincidence. As far as "USA hydrilla I" is concerned, which is probably introduced to the American continent in the late 1950's, the similarity of the isoenzyme patterns to that of the African plants is remarkable. An African origin of these plants, however, is not very probable in connection with the chromosome number, which is 24 in "USA hydrilla I" and 16 in plants from Africa. Whether the African plants are potential aquatic weeds remains to be investigated.

ACKNOWLEDGMENTS

The authors are grateful to Mr. J. T. Jong for advising on statistical analysis of the data obtained and Mrs. G. J.

T. Horneman for art work and typing the manuscript. Thanks are also due to Professor Dr. W. H. O. Ernst for critically reading the manuscript.

LITERATURE CITED

1. Cook, C. D. K. and R. Lüönd. 1982. A revision of the genus *Hydrilla* (Hydrocharitaceae). *Aquat. Bot.* 13:485-504.
2. Haller, W. T. 1976. *Hydrilla*: a new and rapidly spreading aquatic weed problem. University of Florida, Gainesville. Circ. S-245, 13 pp.
3. Khavkin, E. E. and T. B. Sukhorzevskaia. 1979. Maintenance of isoenzyme spectra in callus and suspensor cultures derived from internodes of maize (*Zea mays* L.). *Biochem. Physiol. Pfl.* 174:431-437.
4. Menken, S. B. J. 1980. Allozyme polymorphism and the speciation process in small hermine moths (Lepidoptera, Yponomeutidae). *Studies in Yponomeuta* 2. Ph.D. Thesis, University of Leiden, The Netherlands.
5. Pieterse, A. H. 1977. Control of tropical aquatic weeds. Bull. Dept. Agric. Res. no.300. Royal Tropical Institute, Amsterdam.
6. Pieterse, A. H. 1981. *Hydrilla verticillata* a review. *Abstracts Trop. Agric.* 7(6):9-34.
7. Pieterse, A. H., A. E. H. Ebbers and J. A. C. Verkleij. 1984. A comparative study on isoenzyme patterns in *Hydrilla verticillata* (L.f.) Royle from Ireland and North Eastern Poland. *Aquat. Bot.* 18:299-303.
8. Rothe, G. 1973. Shikimisäure-Dehydrogenase (EC 1.1.1.25) in keimenden Erbsen. *Biochem. Biophys. Pfl.* 164:475-486.
9. Smith, J. 1976. Chromatographic and electrophoretic techniques. V II. Zone electrophoresis. London: William Heineman Medical Book Ltd.
10. Steward, K. K., T. K. Van, V. Carter, and A. H. Pieterse. 1984. *Hydrilla* invades Washington, D.C. and the Potomac. *Amer. J. Bot.* 71:162-163.
11. Verkleij, J. A. C., A. M. de Boer, and T. F. Lugtenborg. 1980. On the ecogenetics of *Stellaria media* (L.) Vill. and *Stellaria pallida* (Dum.) Piré from abandoned arable field. *Oecologia* 46:354-359.
12. Verkleij, J. A. C., A. H. Pieterse, G. J. T. Horneman, and M. Torenbeek. 1983. A comparative study of the morphology and isoenzyme patterns of *Hydrilla verticillata* (L.f.) Royle. *Aquat. Bot.* 17:43-59.
13. Verkleij, J. A. C., A. H. Pieterse, H. P. M. Staphorst, and K. K. Steward. 1983. Identification of two different genotypes of *Hydrilla verticillata* (L.f.) Royle in the USA by means of iso-enzyme studies. *Proc. Int. Symp. Aquat. Macrophytes*, Nijmegen, 18-23 September, 1983, pp. 251-261.
14. Yeo, R. R., H. Falk, and J. R. Thurston. 1984. The morphology of *Hydrilla* (*Hydrilla verticillata* (L.f.) Royle) *J. Aquat. Plant Manage.* 22:1-17.