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A COMPARATIVE STUDY OF THE MORPHOLOGY OF LEMNA GIBBA L. AND LEMNA MINOR L.

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SUMMARY

Fifteen strains of subgenus *Lemna*, collected in ponds and ditches in the western part of The Netherlands, strain G3 (previously described as *Lemna gibba*) and strains 6573 and F (previously described as *Lemna minor*) were aseptically cultured on M-medium in the presence and absence of EDDHA. When cultivated on EDDHA medium the strains showed a marked variation in the degree of gibbosity, whereas in the absence of the chelate all strains were more or less flat. The strains could be divided into two groups as far as the degree of gibbosity in the presence of EDDHA was concerned. There were no consistent differences in morphology between the two groups if cultured on the nutrient medium devoid of EDDHA. In the light of the present investigation distinction between *Lemna gibba* and *Lemna minor* seems not always possible.

1. INTRODUCTION

Lemna gibba was originally described by LINNAEUS (1753) as hemispherical at the lower side. The name *gibba* referred to the gibbous character of the fronds. HEGELMAIER (1868), however, reported that also flat forms of *L. gibba* occur. These flat forms were described as being very similar to *L. minor* but distinguishable by a more coarsely cavernous structure of the fronds. Additional morphological differences reported by Hegelmaier could not or only rarely be confirmed by DE LANGE & SEGAL (1968).

Whether the flat forms of *L. gibba* represent a distinct genetical race or a modification of the gibbous form has been disputed (see, e.g., GUPPY 1895; MASON 1957; DAUBS 1965; DE LANGE & SEGAL 1968; DEN HARTOG 1968; BHALLA et al. 1973). Recently it was found that flat plants of strain G3, determined as *L. gibba*, become gibbous in the presence of the chelate EDDHA (PIETERSE et al. 1970a,b; PIETERSE 1972). This clearly demonstrates that, at least as far as strain G3 is concerned, flat and gibbous forms belong to the same taxon and merely represent morphological modifications of the same genotype.

Nevertheless, pursuant to an earlier suggestion of DEN HARTOG (1968), the question arises whether all flat forms of *L. gibba* are modifications of gibbous plants. It is furthermore questionable whether there is always a clear difference in vegetative morphology between *L. minor* and flat forms of *L. gibba*.

2. MATERIALS AND METHODS

Fifteen flat and gibbous strains of subgenus *Lemna* (sensu DEN HARTOG & VAN DER PLAS 1970), representing either *L. gibba* or *L. minor*, were collected from ponds and ditches in the western part of The Netherlands during the period August-October 1972. None of the plants collected were flowering. In addition, strain G3 and strains 6573 and F (determined as *L. gibba* and *L. minor*, respectively) were obtained, which had been aseptically cultured in vitro for many years and used as experimental material for physiological studies. Data on the provenance of the newly collected strains and on the dimensions of the plants of these strains when collected are presented in *table 1*. The field strains were sterilized by 1% sodium hypochlorite solution for 30 seconds and subsequently washed with autoclaved distilled water before inoculation. A clone was propagated from one sterilized frond from each strain. Cultures were maintained on M-medium (HILLMAN 1961) supplemented with 1% sucrose and 10 ppm of EDDHA (ethylenediamine-di-o-hydroxyphenylacetic acid) and on M-medium supplemented with 1% sucrose but devoid of EDDHA. Every two weeks the plants were transferred to fresh media. The temperature was kept at 28° ± 2°C and the plants were exposed to a continuous illumination of 6000 lux from Gro-Lux fluorescent tubes supplemented with 600 lux from 25W incandescent light bulbs. The data shown are mean values of at least 4 replicate cultures. Observations were made at least one month after cultivation on the nutrient media.

Table 1. Details of origin and dimensions of the field strains at the day of collection.
a – minimum value
b – maximum value
c – mean value

Strain (No.)	Collected at	Date of collection	length (mm)			width (mm)			gibbosity (mm)		
			a	b	c	a	b	c	a	b	c
1	Naardermeer	23.08.1972	1.9	3.1	2.3	1.3	2.1	1.5	0.2	0.3	0.2
2	Hilversum	23.08.1972	3.8	5.7	4.7	2.7	4.2	3.4	0.7	2.3	1.5
3	Hilversum	23.08.1972	2.6	5.1	4.0	2.5	4.0	3.0	0.4	1.0	0.6
4	Krieleroord	23.08.1972	4.1	5.4	4.8	3.1	3.9	3.5	2.0	2.9	2.4
5	Spaarndam	23.08.1972	5.1	6.8	5.9	3.6	5.6	4.4	2.6	5.3	3.1
6	Hilversum	23.08.1972	4.2	4.8	4.5	3.1	3.5	3.3	1.1	1.9	1.5
7	Aerdenhout (Leijduin)	23.08.1972	3.4	4.0	3.6	2.0	3.0	2.7	0.7	1.0	0.8
8	Aerdenhout	24.08.1972	1.9	2.8	2.5	1.8	2.5	2.1	0.1	0.4	0.3
9	Amstelveen	12.09.1972	5.9	7.0	6.7	5.0	5.5	5.2	2.3	3.2	2.9
10	Amstelveen	12.09.1972	6.8	7.0	6.9	5.2	5.6	5.5	2.8	3.7	3.4
11	Bennebroek	25.09.1972	2.5	3.4	3.0	2.0	3.6	2.4	0.2	0.5	0.4
12	Westzijderveld	9.10.1972	4.6	5.7	4.8	3.0	3.5	3.2	0.3	0.4	0.3
13	Aerdenhout	9.10.1972	4.0	4.9	4.5	3.0	3.7	3.3	0.4	0.7	0.6
14	Harmelen	9.10.1972	3.0	3.5	3.0	2.0	2.5	2.2	0.3	0.6	0.5
15	Woerden	9.10.1972	3.0	3.7	3.2	2.0	2.3	2.2	0.4	0.7	0.6

From each culture a sample of 10 fronds was used for recording morphological data, all visible fronds being taken into consideration for the numerical evaluation of flowering. Measuring was performed with a sliding gauge with nonius (accuracy 0.1 mm). Counting of the air chambers was done at a magnification of × 50 in a zone along the greatest width of the fronds on the ventral side.

3. RESULTS

After cultivation on the medium supplemented with EDDHA the strains showed a marked variation in the degree of gibbosity, whereas on the medium without the chelate all strains were more or less flat (*fig. 1*). Strains 1 and 6573 did not appear to be affected as far as gibbosity was concerned, but the other strains were clearly thicker after having grown in the presence of EDDHA. Strains 2, 4, 5, 6, 9, 15, and G3 even became conspicuously inflated on EDDHA medium (up to 4.8 mm in strains 2 and 9). In the absence of EDDHA the thickness of the fronds varied only slightly from strain to strain (from 0.3 mm in strains 1, 8, 6573, and F to 1.0 mm in strain G3.

Data obtained on length and width of the fronds, number of air chambers and flowering are presented in *table 2*. Length and width of the fronds increased in most strains under the influence of EDDHA. Only in strains 1 and 6573 the frond area remained about the same on both media. The average number of air chambers along the greatest width of the ventral side of the fronds seemed hardly affected by EDDHA. In general, the average number per strain varied from 14 to 18. Only in strains 1, 9 and G3 fewer than 14 air chambers were counted. There was no flowering in the absence of EDDHA, but in the presence of the

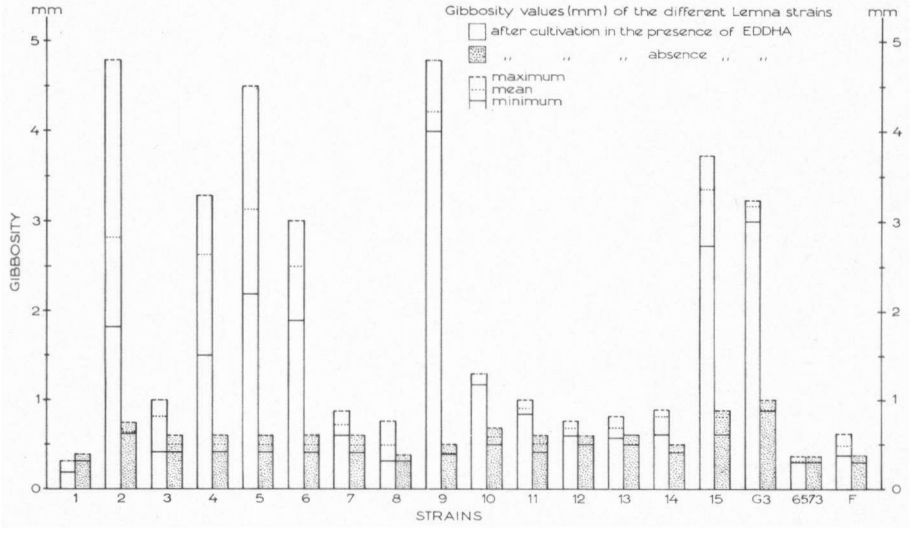

Fig. 1. Gobbosity vallues of the different *Lemna* strains.

Table 2. Data on length, width, number of air chambers at the greatest width, mean air chamber width and flowering of the different *Lemna* strains after cultivation in the presence and absence of EDDHA. a – minimum value; b – maximum value; c – mean value.

	+ EDDHA							− EDDHA						
	length (mm)			width (mm)			no. of air chambers			mean width (mm) of air chambers	FL (%)										
	a	b	c	a	b	c	a	b	c			a	b	c	a	b	c	a	b	c		
1	2.0	2.5	2.1	1.4	1.7	1.5	10	14	11	0.14	0	2.0	2.2	2.1	1.4	1.6	1.5	9	14	11	0.14	0
2	4.8	6.1	5.6	3.0	5.1	4.4	12	16	14	0.31	0	3.3	3.6	3.5	2.4	2.6	2.5	13	18	16	0.16	0
3	3.0	4.3	3.9	2.5	3.5	3.0	14	18	17	0.18	8	3.2	3.6	3.4	2.4	2.7	2.5	16	20	18	0.14	0
4	4.9	6.6	5.8	3.1	5.2	4.3	14	18	16	0.27	0	3.2	3.8	3.4	2.4	2.9	2.6	16	20	18	0.14	0
5	5.1	7.4	6.4	4.1	5.6	5.1	14	16	15	0.34	3	3.0	3.3	3.2	2.4	2.8	2.5	14	20	17	0.15	0
6	4.6	5.8	5.1	3.0	4.6	4.0	12	15	13	0.31	3	3.2	3.7	3.4	2.5	2.6	2.6	12	16	15	0.17	0
7	3.4	4.9	4.0	2.6	3.9	3.1	13	18	15	0.21	0	3.0	3.5	3.3	2.3	2.5	2.5	14	16	15	0.17	0
8	3.3	4.5	4.2	2.2	3.5	3.1	14	17	15	0.21	10	3.3	3.5	3.4	2.4	2.6	2.5	13	18	15	0.17	0
9	4.8	6.2	5.6	4.0	5.0	1.3	10	14	13	0.33	1	2.5	2.7	2.6	2.4	2.6	2.5	11	14	12	0.21	0
10	3.6	4.0	3.8	2.4	2.6	2.5	11	16	14	0.18	2	2.5	2.7	2.6	2.2	2.5	2.4	12	17	15	0.17	0
11	3.5	4.4	4.0	2.2	3.2	2.9	12	21	15	0.19	3	2.6	3.0	2.8	2.0	2.6	2.3	15	18	16	0.15	0
12	3.6	4.1	3.9	2.5	3.3	2.9	14	19	16	0.18	15	3.0	3.3	3.1	2.4	2.7	2.5	16	19	18	0.14	0
13	3.1	3.5	3.3	2.2	2.7	2.4	13	16	15	0.16	0	2.5	2.8	2.7	2.0	2.3	2.1	12	16	14	0.15	0
14	3.5	4.3	4.0	3.0	3.5	3.2	13	18	15	0.21	10	3.0	3.3	3.1	2.0	2.3	2.2	13	17	15	0.15	0
15	5.5	6.6	6.1	3.9	5.3	4.9	13	16	15	0.33	1	3.9	4.1	4.0	3.1	3.6	3.4	15	18	17	0.20	0
G 3	5.0	5.4	5.2	4.0	4.2	4.1	8	11	10	0.41	16	3.8	4.5	4.0	3.0	3.2	3.1	8	12	10	0.31	0
6573	2.0	2.2	2.1	1.0	1.4	1.3	13	17	15	0.09	0	2.0	2.3	2.1	1.5	1.7	1.6	15	18	16	0.10	0
F	3.4	3.8	3.6	2.4	2.7	2.5	15	18	16	0.16	0	2.5	2.7	2.6	2.0	2.2	2.1	15	18	16	0.13	0

chelate flowering was induced in most strains. The percentages of flowering fronds were small, however. The highest percentage (16%) was observed in strain G3.

As far as the air chamber pattern is concerned, it was noted that on a single frond all chambers were about equal in size with the exception of some extremely gibbous forms in which the air chambers in the middle of the fronds were slightly wider than those situated at the periphery. The approximate width of the air chambers in the different strains was calculated from the mean data on greatest width of the fronds and mean number of air chambers along the greatest width (*table 2*). In general, these values seemed more or less proportional to the frond width. The largest air chambers were observed in strain G3.

Strains 2 and 3, collected from the same pond, differed considerably in morphology. Moreover, on EDDHA medium flowering was observed in strain 3 but not in strain 2.

4. DISCUSSION

The strains may be divided into two groups as far as the degree of gibbosity in the presence of EDDHA is concerned. The conspicuously gibbous forms (strains 2, 4, 5, 6, 9, 15, and G3) undoubtedly correspond with *L. gibba* as originally described by Linnaeus. The question arises, however, whether the strains which remained relatively flat in the presence of EDDHA represent *L. minor*. According to VAN OOSTSTROOM & REICHGELT (1964) this would certainly hold for strains 1 and 6753 as these authors reported a minimum adult frond length for *L. gibba* of 2.5 mm and for *L. minor* of 2.0 mm. The fact that the thickness of the fronds of these strains seemed totally unaffected by EDDHA may support this assumption. Interestingly, strains 1 and 6753 were the only strains in which neither the frond area nor the gibbosity changed in the presence of EDDHA.

In the past the distinction between *L. minor* and flat forms of *L. gibba* has been mainly based upon the visible air chamber pattern at the ventral side of the fronds. Hegelmaier described the structure of the fronds of flat *L. gibba* as more coarsely cavernous than those of *L. minor*. According to DE SLOOVER (1966) the number of air chambers along the greatest width of the ventral side of the fronds is 7–8 in flat forms of *L. gibba* and 13–15 in *L. minor*. DE LANGE & SEGAL (1968) described a difference in the visibility of the air chambers: in contrast to the situation in *L. minor*, the air chambers in flat forms of *L. gibba* are clearly discernible if the plants are held up against the light.

Contrary to the statement of Hegelmaier, variations in air chamber pattern did not appear to be very obvious. Although the air chambers were relatively large in flat modifications of strain G3, it was certainly not always possible to identify a flat plant as potentially gibbous by the width of its air chambers. Differences in the number of air chambers along the greatest width of the fronds between flat modifications of conspicuously gibbous strains and strains which were not or only slightly affected by the chelate as far as gibbosity was concerned, did not prove to be consistent. Judging by the criteria mentioned by De

Sloover, only certain specimens of strain G3 would represent *L. gibba* (viz. the plants with only 8 air chambers across the broadest part of the frond). All other strains, except strains 1 and 9, but including the potentially inflated strains 2, 4, 5, 6, 9, and 15, could be partially referred to *L. minor* (viz. the plants with 15–18 air chambers). When examined through a microscope at a moderately high magnification the air chambers were visible in all eighteen clones. However, the present authors observed that the air chambers are not always very distinct in thin fronds, possibly due to the shortness of the vertical partition walls. If, as now transpires, De Lange & Segal only classified the forms with the thinnest fronds as *L. minor*, one must take into account that the thickness of the strains in the absence of EDDHA was not correlated with the degree of gibbosity of the same strains in the presence of the chelate. TÓTH (1962) reported that outdoor samples of *Wolffia arrhiza* contain smaller quantities of chlorophyll than plants cultured under laboratory conditions. This could explain the impression of the present authors that in some *Lemna* strains the visibility of the air chambers does indeed become greater after cultivation in vitro. Possibly, the chlorophyll content and the visibility of the air chambers are correlated.

In the light of the results of the present study it seems extremely difficult to define characteristic differences between *L. minor* and *L. gibba* which are valid under all circumstances. A possible criterium might be the potentiality to become markedly gibbous in the presence of EDDHA. However, this definition is not exact, and, moreover, of no use in the field. Another possibility would be to classify only full-grown flat plants with a length not exceeding 2.5 mm as *L. minor*, but it remains to be seen whether all such small forms, judging by the behaviour of strains 1 and 6573, are truly incapable of turning gibbous.

In the literature on the subject, differences in vegetative morphology between *L. minor* and flat forms of *L. gibba* other than air chamber pattern and frond length have been described. These include visibility of the peripheral side-nerves, the shape of the root cap, the size and colour of the fronds, and the length of the root (HEGELMAIER 1868; PASCHER 1936; DE SLOOVER 1966; DE LANGE & SEGAL 1968). However, these differences could only rarely be observed by DE LANGE & SEGAL (1968). MCCLURE & ALSTON (1966) reported differences in the flavonoid composition, BLAZEY & MCCLURE (1968) differences in the aldehydic derivatives, and BLACKBURN (1933) differences in the chromosome number, but, with regard to classifying *Lemna* strains in the field, these characteristics, even if consistent, are of no use. Disparities in the generative morphology reported by SCHLEIDEN (1839), HEGELMAIER (1868) and DAUBS (1965) need further investigation. It is interesting to note that HEGELMAIER (1868, p. 118) expressed some doubt as regards the significance of the generative characters in the separation of the two species in question.

As things stand at present a consistent distinction between *L. gibba* and *L. minor* is not possible. Variations in frond size and frond gibbosity may be either genetically or environmentally induced. Perhaps it would be better to combine both taxa in a species complex. Undoubtedly, within this complex several more or less distinct races would have to be distinguished, as is suggested by the

differences in morphology and flowering exhibited by the strains used in the present study after cultivation under similar experimental conditions.

GUPPY (1895) and SCHULZ (1962) suggested that the gibbous plants are aestival forms. DE LANGE & SEGAL (1968) proposed that gibbosity is induced under optimal circumstances. Future investigations into a possible relation between physico-chemical characteristics of the environment and gibbosity would certainly contribute to the understanding of the induction of gibbosity in potentially inflated strains in nature. One has to take into account, however, that genetically distinct strains often occur in a mixed vegetation as is illustrated by strains 2 and 3 which were collected from the same pond.

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