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In-vitro induction of aerial leaves and of precocious flowering in submerged shoots of *Limnophila indica* by abscisic acid

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Abstract. Nodal explants of submerged shoots of *Limnophila indica* (L.) Druce were cultured in Nitsch's liquid medium containing abscisic acid (ABA, 10^{-9}–10^{-6} M). At 10^{-7} and 10^{-6} M, ABA induced typical aerial leaves (entire, ovate, opposite-decussately arranged) even under submerged conditions and completely suppressed the development of water leaves (pinnately dissected and whorled). Flowers that invariably arise from aerial shoots were induced precociously by ABA even on submerged nodes.

Key words: Abscisic acid – Aquatic plant – Flowering – Growth phase change – Heterophylly – *Limnophila*.

Introduction

Heterophylly is a characteristic feature of several amphibious plants (for review, see e.g. Sculthorpe 1967). *Limnophila indica* (L.) Druce bears pinnately-dissected leaves, arranged in whorls, under water (Fig. 1 C) and entire, ovate and opposite-decussately arranged leaves on the aerial shoots (Fig. 1 D). The water leaves are thin, have a simple internal structure, and are devoid of stomata. The aerial leaves are similar to the leaves of mesomorphic terrestrial plants. There is a short transitional zone of three or four nodes bearing intermediate leaf types. The flowers of the plant are not adapted for hydrophily and are normally borne only on the aerial shoots.

Heterophylly has been variously attributed to a variety of internal causes – genetic variability, age and nutritional status of the plant; as well as to environmental factors – moisture conditions, osmotic stress, light intensity, photoperiod and temperature (for reviews see Sculthorpe 1967, pp. 217–247; Hutchinson 1975, pp. 157–196). There are very few studies on the role of natural plant growth regulators on heterophylly, not-withstanding their involvement in foliar morphogenesis (Wallenstein and Albert 1963; McComb 1965). Recently, Anderson (1978) reported the formation of floating leaves under submerged conditions in sprouting tubers of *Potamogeton nodosus* treated with abscisic acid (ABA). In the present communication we report the induction of aerial leaves and of precocious flowering on submerged shoots of *Limnophila* in response to exogenous ABA.

Abbreviation: ABA = abscisic acid

Material and methods

Plants of *L. indica* were raised aseptically from surface-sterilised seeds collected near Jaipur, Rajasthan State, India (see Rao and Mohan Ram 1981). The seeds were sterilised with nascent chlorine water for approx. 3–5 min, rinsed in sterile distilled water, dipped quickly in 90% ethanol, and rinsed again in sterile distilled water with several changes. Surface moisture was removed with a sterilised blotting paper and 30–50 seeds were planted in tubes containing nutrient medium under aseptic conditions. After germination they were transferred individually into tubes containing liquid medium to serve as stocks for all experiments. These stocks were maintained by clonal propagation in Nitsch's basal liquid medium (with salts and vitamins as recommended by Nitsch 1969; 2% sucrose; pH adjusted to 5.6 with 0.1 N NaOH before autoclaving).

Explants consisting of a node bearing a whorl of dissected water leaves and 0.5-cm portions of internode above and below were obtained from six-week-old stock plants, taking care that no visible lateral buds or roots were present. The explants were individually transferred to culture tubes (Corning glass; 25 mm diameter, 150 mm long) containing 20 ml of basal medium, or basal medium supplemented with ABA (obtained from Fluka, Buchs, Switzerland) at concentrations of 10^{-9}, 10^{-8}, 10^{-7} and 10^{-6} M. The cultures were maintained at a temperature of $25 \pm 3°$ C, under continuous illumination provided by fluorescent lights (daylight tubes; Philips India Ltd. Bombay, India) supplying 4,500–6,000 lx at the level of the cultures.

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Twelve cultures were raised for each treatment and each experiment was done thrice. The cultures were examined every week to record the extent of growth and quantitative data were obtained on various parameters at the end of six weeks from eight cultures for each treatment. The significance of the differences between treatments was determined using Student's t test for the difference of means at the 5% level (Spiegel 1972, p. 188).

Results

All explants cultured in control medium regenerated shoots and roots. The explants floated just below the surface of the medium and one or occasionally two lateral shoot buds grew into submerged shoots bearing water leaves. After three or four weeks the shoot emerged above the surface of the medium but continued to produce water leaves, perhaps as a result of high humidity in the culture tubes. (In Nature shoots bearing water leaves remain just below the water surface. When they emerge above the water they bear aerial leaves.) About 20–30 adventitious roots were initiated (Table 1) from the basal nodes of the shoots and from the original explant (from below the node or at the cut end). Lateral branches were formed at the basal nodes of the shoots and they also bore only water leaves (Fig. 1 A). After about six weeks, one or two branches produced aerial leaves and flowers in approx. 10% of the cultures.

Regeneration of shoots and roots was also noted in all ABA-treated plants. At 10^{-9} M, ABA had little effect on the growth of the plants except in terms of fresh and dry weight (Table 1). Growth was retarded at all higher concentrations, number of branches and roots, and fresh and dry weights decreasing with increasing ABA concentration (Table 1). At 10^{-6} M the length of the main shoot was reduced on account of shortening of the sub-

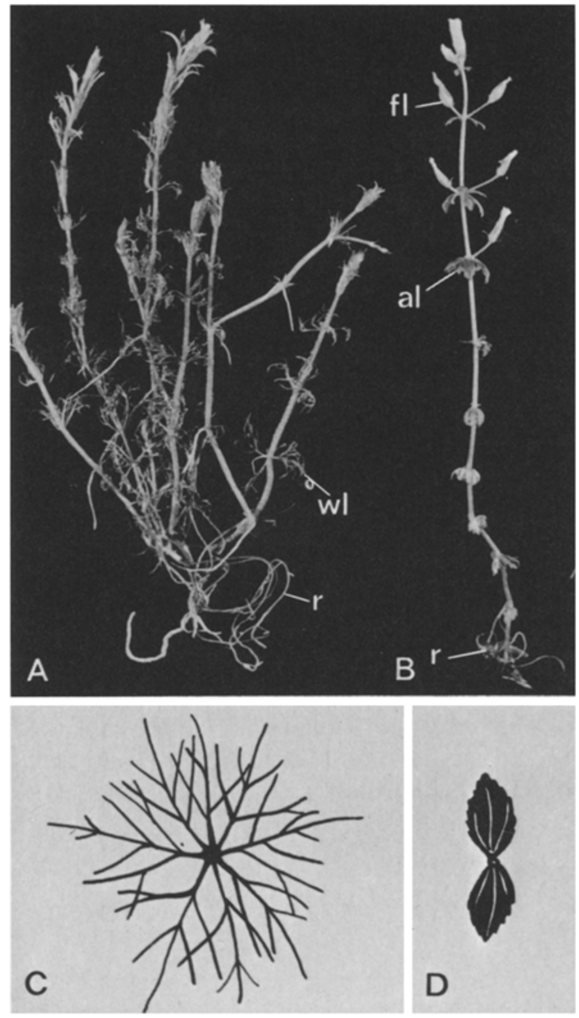

Fig. 1 A–D. Plants of *Limnophila indica* grown in vitro for six weeks. *al*, aerial leaves; *fl*, flower; *r*, root; *wl*, water leaves. **A** Control plant grown in basal medium, bearing water leaves. ×0.5. **B** Plant grown in Nitsch's basal liquid medium $+10^{-7}$ M ABA bearing aerial leaves at all the nodes. ×0.5. **C, D** Silhouettes of nodes bearing whorled water leaves and opposite aerial leaves, respectively. ×4

Table 1. Effect of ABA on the growth of nodal explants. Data recorded after six weeks from eight cultures per treatment (mean ±SD)

Concentration of ABA	Main shoot				Roots	Flowers	Weight (g)	
	No. nodes	Nodes bearing aerial leaves (%)	Length (cm)	No. lateral branches	(No. per culture)	(No. per culture)	Fresh	Dry
0 (control)	16.3±1.6	–	11.4±2.5	4.5±2.1	23.0±5.3	0.4±1.1	1.00±0.34	0.11±0.03
10^{-9} M	17.8±3.4	36.5	12.1±2.1	8.4±1.9	17.3±3.2[a]	0.4±0.5	0.64±0.13[a]	0.08±0.01[a]
10^{-8} M	18.4±3.1	46.7	12.5±2.1	2.1±1.5[a]	13.5±2.3[a]	2.0±3.7	0.53±0.09[a]	0.06±0.01[a]
10^{-7} M	12.9±2.1[a]	100.0	13.1±0.8	0.8±1.4[a]	11.0±1.8[a]	10.0±2.9[a]	0.23±0.04[a]	0.02±0.00[a]
10^{-6} M	11.7±1.1[a]	100.0	9.9±2.4	3.3±1.8	16.0±3.1[a]	12.3±3.5[a]	0.52±0.19[a]	0.04±0.01[a]

[a] Significantly different from control at $P \lesssim 0.05$

merged internodes. Remarkably, shoots developing from explants grown in 10^{-7} and 10^{-6} M ABA produced aerial leaves from the very first node in spite of being submerged, the formation of water leaves being totally suppressed (Fig. 1 B). The aerial leaves on the submerged stems were borne in whorls of six, were dark green, small, and epinastic. However, as the shoots emerged from the liquid, the number of leaves in the newly formed nodes was decreased to two, with an opposite-decussate phyllotaxy. The leaves expanded and resembled the aerial leaves formed on control plants. In plants treated with 10^{-9} and 10^{-8} M ABA, nodes bearing aerial leaves were observed on the main shoot (Table 1) whereas in the control plants nodes with such leaves were present only on lateral branches.

In Nature, the flowers of *Limnophila* are borne about 10 cm above the water level in the axils of aerial leaves. In response to 10^{-7} M ABA flowers were, however, initiated even on submerged nodes; their number increased as the shoots emerged above the medium (Fig. 1 B). Flowers were initiated in all cultures within two weeks in response to 10^{-7} and 10^{-6} M ABA, in contrast to the control plants in which flowers were formed in only 10% of the cultures and only after six weeks. Thus, ABA stimulated precocious and uniform flowering.

Discussion

In the present work ABA induced the formation of aerial leaves in *L. indica* under totally submerged conditions. In addition, precocious and increased flowering was induced, an effect of ABA not previously noticed in an amphibious, heterophyllous angiosperm. In *Potamogeton nodosus* floating leaves were induced in sprouting tubers treated with ABA, but the plants failed to flower although they were maintained for four months (Anderson 1978).

The effects caused by ABA may be interpreted as responses to induced stress. Under natural conditions, aerial leaves are produced by *L. indica* when the water recedes in the temporary pools. As the shoots emerge above water they are subjected to desiccation. Formation of aerial leaves on submerged stems cultivated in sea water has been reported in *Ranunculus baudotii* (Gessner 1940), and *Callitriche intermedia* (Jones 1955). In the water ferns *Marsilea drummondii* and *Regnellidium diphyllum,* aerial leaves were induced in media containing high concentrations of sugars or mannitol (Allsopp 1953, 1955; Rao 1966).

Heterophylly in hydrophytes has also been interpreted as a manifestation of heteroblastic development (see Allsopp 1965); the submerged shoots bearing water leaves are considered juvenile and the aerial shoots, which are almost invariably associated with flowering, as representing the adult phase. Abscisic acid is known to stabilise the adult phase in *Hedera helix* (Frydman and Wareing 1974; Rogler and Hackett 1975). The complete suppression of the water leaves in ABA-treated plants of *L. indica* and the initiation of flowers on the shoots bearing aerial leaves indicate the operation of a phase change in this plant.

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