CHEMICAL INDUCTION OF TURIONS IN WOLFFIELLA FLORIDANA (J. D. SMITH) THOMPSON

A. H. PIETERSE, P. R. BHALLA and P. S. SABHARWAL

Department of Botany, University of Kentucky, Lexington, Kentucky, 40506, U.S.A.

SUMMARY

Floating plants of *Wolffiella floridana* produced turions in modified Hutner's medium containing 3% sucrose. This is the first report on the induction of turions in the genus *Wolffiella*.

1. INTRODUCTION

During the winter season many species of Lemnaceae produce special overwintering forms called turions. These structures are modified fronds which sink to the bottom of the pond and remain submerged during the period of dormancy. The turions of *Spirodela polyrrhiza* have been thoroughly investigated (see HILLMAN 1961). These are dark green or purple, and smaller and thicker than the normal fronds. The air spaces in the turions are reduced or absent and the cells are heavily loaded with starch grains (HEGELMAIER 1868, GUPPY 1895, JACOBS 1947). Some species of *Lemna* produce similar turions which are less modified as compared to those of *Spirodela polyrrhiza* (THOMPSON 1898, HICKS 1937, LANDOLT 1957). *Wolffia* turions are usually similar to normal fronds but contain a large amount of starch (HEGELMAIER 1868, LANDOLT 1957).

In the genus *Wolffiella* turion formation has never been observed so far. DAUBS (1965) suggested that this might be one of the possible reasons for its limited distribution. Unlike *Spirodela*, *Lemna* and *Wolffia* which are cosmopolitan, *Wolffiella* is mostly restricted to the tropical and subtropical parts of America and Africa. However, one species, *Wolffiella floridana*, occurs in the temperate zones of the United States. Flowering and seed formation is extremely rare and it is not known how this plant survives through the winter season. We have recently observed that *Wolffiella floridana* produces turions when cultivated in modified Hutner's medium containing 3% sucrose.

2. MATERIAL AND METHODS

Floating fronds of *Wolffiella floridana* were collected in the Pine Hill Swamp in Union County, Illinois. The plants were sterilized by 0.5% sodium hypochlorite solution for 10 seconds and washed with autoclaved double distilled water before inoculation. The survival rate was very low and only a few plants could be maintained in culture condition.

A clone was propagated from one of these sterilized fronds in 125 ml Erlen-

meyer flasks. Each flask contained about 60 ml of 1/3 strength Hutner's medium (HUTNER 1953) containing the following quantities in mg per 1000 ml of the nutrient solution: NH_4NO_3 67, $Ca(NO_3)_2 \cdot 4H_2O$ 50, $MgSO_4 \cdot 7H_2O$ 167, $ZnSO_4 \cdot 7H_2O$ 22, $MnSO_4 \cdot H_2O$ 5, $CoCl_2 \cdot 6H_2O$ 0.3, $CuSO_4 \cdot 5H_2O$ 1.3, $Na_2MoO_4 \cdot 2H_2O$ 8.4, H_3BO_3 4.7, NaEDTA 167, KH_2PO_4 133, $FeSO_4 \cdot 7H_2O$ 8.3. The medium was supplemented with 1% sucrose and adjusted to a pH of 5.5 with 1N NaOH. The cultures were maintained at 25 ± 2°C under continuous illumination from cool white fluorescent tubes of 225–250 foot candle intensity.

The amount of starch in the fronds was estimated by iodine test. The plants were decolorized by boiling in 95% alcohol for 5–8 minutes, treated with iodine and observed under the microscope.

3. RESULTS

The influence of various concentrations of sucrose on turion formation in *Wolffiella floridana* was studied in modified Hutner's medium. At a low concentration of sucrose (1%) the plants remained floating. However, when the amount of sucrose was raised to 3% the production of turions was observed 7–8 days after inoculation. They sank to the bottom of the flasks and remained submerged. When the sucrose concentration was 2%, a mixture of floating plants and turions was observed while at a higher concentration (5%) the plants became yellow and the growth was adversely affected.

When the turions were transferred to the medium containing 1% sucrose, they produced normal daughter fronds after 6–7 days. These newly formed fronds floated, while the turions remained submerged. *Fig. 1* shows floating plants in the medium containing 1% sucrose, while *fig. 2* shows the turions in the medium supplemented with 3% sucrose.

The turions were shorter and wider than the normal vegetative fronds. Floating plants were about 3–6 mm long and 0.4–0.7 mm wide (*fig. 3*), while the turions were 2–4 mm long and 0.6–1.4 mm wide (*fig. 4*). When examined anatomically, the turions showed reduced air chambers and an increased amount of starch in the cells. *Figs. 5* and *6* illustrate the surface views of a typical vegetative frond and a turion after staining with iodine.

The effect of low temperature was studied on the floating fronds and the turions. In general, floating plants were less resistant than turions to a low temperature treatment. When cultures were exposed to 5°C for 6 weeks, the floating plants became yellow and eventually died while the turions remained green and healthy. Growth and multiplication were resumed when the turion cultures were transferred to the normal temperature of 25°C. Numerous daughter turions were observed within two weeks.

Since abscisic acid is shown to induce turion formation in *Spirodela polyrrhiza* (PERRY & BYRNE 1969, STEWART 1969), an experiment was conducted to test its effect on *Wolffiella floridana*. In the presence of 0.01–0.1 ppm of this growth regulator the daughter fronds became smaller but no turions were observed. At a relatively higher concentration (1 ppm) the growth was completely inhibited.

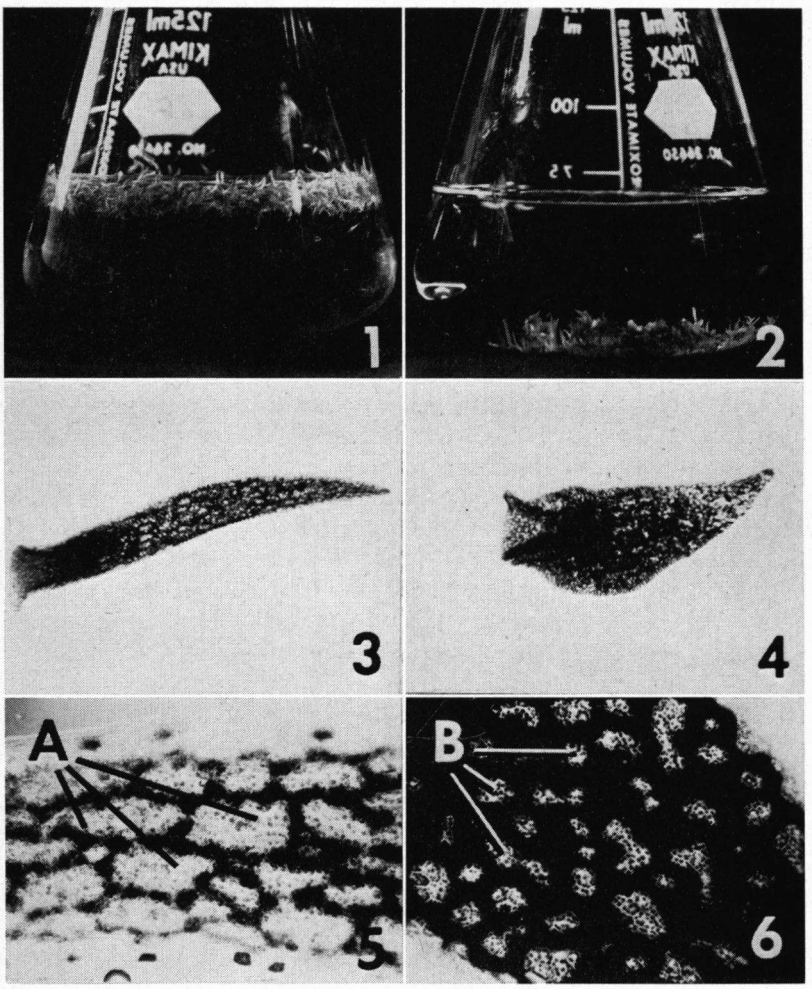

Fig. 1. Floating plants of *Wolffiella floridana* in modified Hutner's medium supplemented with 1 % sucrose. (× 0.8).

Fig. 2. Turions of *W. floridana* induced in modified Hutner's medium containing 3 % sucrose. (× 0.8).

Fig. 3. A floating frond of *W. floridana*. (× 10).

Fig. 4. A turion of *W. floridana*. Note that the turion is shorter but wider than a floating frond. (× 10).

Fig. 5. Surface view of a portion of a floating frond. Note the large air chambers (A). (× 60).

Fig. 6. Surface view of a portion of a turion showing smaller air chambers (B) and larger amount of starch than in the floating frond. (× 60).

4. DISCUSSION

In the light of the present investigation, turion formation in Lemnaceae is not restricted to the genera *Spirodela*, *Lemna* and *Wolffia*, but also occurs in *Wolffiella*. Probably *Wolffiella floridana* is able to survive the winter season in temperate zones of the United States as a result of the production of this special overwintering form which has never been discovered in nature so far.

The in vitro induced turions of *Wolffiella floridana* multiply rapidly by producing new turions. These observations are in conformity with the earlier work on *Lemna minor* and *Wolffia* (LANDOLT 1957). However, in *Spirodela polyrrhiza* turions never reproduce turions (JACOBS 1947).

Detailed observations by JACOBS (1947) on the germination of turions of *Spirodela polyrrhiza* revealed that these produce a bubble of gas at the end of the dormancy period. This increases their buoyancy and makes them rise back to the surface of the water. Subsequently, the floating turions produce normal vegetative fronds. On the contrary, the present work with *Wolffiella floridana* demonstrates the production of new floating plants by submerged turions. The germination mechanism of *Lemna* and *Wolffia* has not been worked out in detail.

The effect of sucrose on turion formation was observed in *Spirodela polyrrhiza* by CZOPEK (1963). Maximum number of turions was induced in a medium fortified with 2% sucrose. A higher sucrose concentration (5%) in the nutrient medium caused abnormal growth and resulted in frond deformation. Our results with *Wolffiella floridana* are essentially similar to the observations of CZOPEK (1963). Three percent sucrose proved to be the most effective in the present investigation.

PERRY & BYRNE (1969) and STEWART (1969) were able to induce turions in *Spirodela polyrrhiza* by incorporating abscisic acid in the nutrient medium. Our results show that abscisic acid had no influence on turion formation in *Wolffiella floridana*. On the other hand, it caused an inhibition of growth and decreased the size of the fronds. These observations are in agreement with the work of VAN OVERBEEK & MASON (1969) on *Lemna minor* where abscisic acid has growth retarding effect.

REFERENCES

CZOPEK, M. (1963): Studies on the external factors inducing the formation of turions in Spirodela polyrrhiza (L.) Schleiden. *Acta Soc. Bot. Polon.* **32**: 199–211.

DAUBS, E. H. (1965): A monograph of the Lemnaceae, *Illinois Biol. Monogr.* **34**.

GUPPY, H. P. (1895): On the habits of Lemna minor, L. gibba and L. polyrrhiza. *Jour. Linn. Soc. Bot.* **30**: 323–330.

HEGELMAIER, F. (1868): *Die Lemnaceen. Eine monographische Untersuchung*. Wilhelm Engelmann, Leipzig.

HICKS, L. E. (1937): The Lemnaceae of Indiana. *Amer. Midl. Nat.* **18**: 774–789.

HILLMAN, W. S. (1961): The Lemnaceae, or duckweeds. *Bot. Rev.* **27**: 222–287.

HUTNER, S. H. (1953): Comparative physiology of heterotrophic growth in plants. *In: Growth and differentiation in plants*. Edited by W. E. Loomis. Iowa State College Press, Ames, Iowa.

LANDOLT, E. (1957): Physiologische und ökologische Untersuchungen an Lemnaceen. *Ber. Schweiz. Bot. Ges.* **67**: 271–410.

OVERBEEK, J. VAN, M. IONA & R. MASON (1968): Dormin and cytokinin: growth regulation of Lemna. *Acta Bot. Neerl.* **17**: 441–444.

PERRY, T. O. & O. R. BYRNE (1969): Turion induction in Spirodela polyrrhiza by abscisic acid. *Plant Physiol.* **44**: 784–785.

STEWART, G. R. (1969): Abscisic acid and morphogenesis in Lemna polyrrhiza L. *Nature (Lond.)* **221**: 61–62.

THOMPSON, C. H. (1898): A revision of the American Lemnaceae occurring north of Mexico. *Mo. Bot. Gard., Rep.* **9**: 21–42.