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Short communication

Germination of *Spirodela polyrhiza* turions: the role of culture conditions during turion development

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The rapidly germinating "old" turions of *Spirodela polyrhiza* were shown to derive mainly from the slowly germinating "young" turions. This modification to "old" turions could occur even in isolated "young" turions, and was accelerated by sucrose. It is suggested that this modification is a form of turion senescence and that turion initiation and maturation are strongly influenced by exogenous carbon and nitrogen sources.

Key words: Ageing — Duckweed — Germination — *Spirodela polyrhiza* — Sucrose — Turion.

In a previous communication (*11*), the turions of *Spirodela polyrhiza* were classified into young (Y) type (green turions, germinating slowly only in light, producing a frond during germination) and old (O) type turions (light brown turions, rapidly germinating in the light or dark, producing a new frond following root formation). In this report, we attempt to determine whether the age of the parent fronds or "ageing" of the turions themselves was responsible for the development of O turions. By collecting the turions at various times and by manipulating the culture conditions during turion development we show that Y turions develop (age) into O turions.

The duckweed *Spirodela polyrhiza* (L.) Schleiden, strain O-381 was cultured in Hoagland's solution containing 1% (w/v) sucrose as described previously (*11*). For optimal turion induction, nitrate concentration in the medium was decreased to 1/20 of the control, i.e. the levels of KNO_3 and $Ca(NO_3)_2$ were decreased from 5 mM to 0.25 mM and supplemented with KCl and $CaCl_2$, each at 4.75 mM to maintain a constant cation level. All cultures were kept at 26 ± 1°C, in high relative humidity and under continuous white fluorescent light (4 klux). Growth was determined by counting the numbers of fronds in each flask.

In both, complete and 1/20 nitrate Hoagland's media, sucrose accelerated frond growth. In the nitrate deficient medium, vegetative growth ceased on day 9 in the presence of sucrose and on day 12 in the absence of sucrose. Turions began to appear at about these times. Turion production ceased by day 34 (about 100 turions per flask) in the nitrate deficient cultures with sucrose. Turion production

Abbreviations: Y turions, "young" turions; O turions, "old" turions.

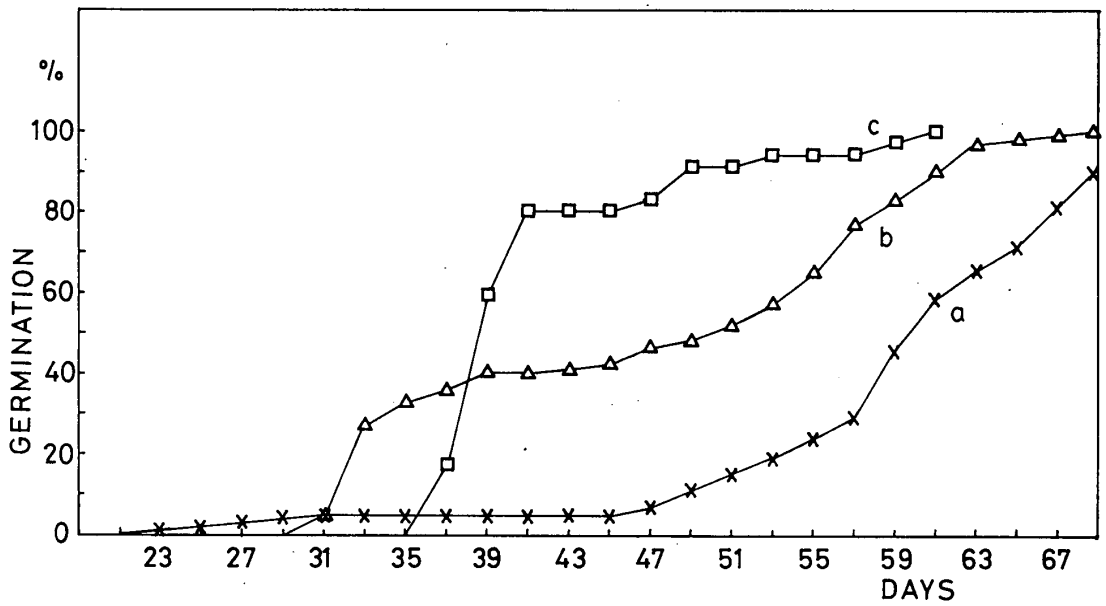

Fig. 1. *Germination of turions sampled from the same culture flask at 3–4 day intervals.* Culture medium: 1/20 nitrate Hoagland's solution, 1% sucrose. Turions were sampled during: a) days 14–18, b) days 24–28, c) days 31–34. Germination tests were started immediately after collection on; a) day 18, b) day 28 and c) day 34. Data for the samples collected between days 18–21, 21–24 and 28–31 are not shown.

was slower in cultures lacking sucrose and maximum turion number was not reached by day 40.

For the germination tests, the turions were collected using a sieve (mesh #4, 4.76 mm) which effectively retained parent colonies. About 25 washed turions were spread on four layers of filter paper moistened with 5 ml of 1 mM $Ca(NO_3)_2$ in a 6 cm glass Petri dish. Since long periods were needed for complete germination, distilled water was added periodically to compensate for evaporation loss. Germination was at 26 ± 1°C in 4 klux light. Appearance of a frond or a root was taken as a criterion of germination and the germinated turions were removed from the dish. The starting points of germination tests relative to the total culture period are indicated in the respective figure legends.

Germination tests using whole populations of turions formed in the presence of sucrose confirmed the previously reported patterns of Y and O turions. However, by examining the germination of turions sampled at various times after the start of nitrate deficient culture (Fig. 1), we are able to show that turions produced early in the culture period were Y turions and those produced later, i.e. between days 31 to 34, germinated rapidly as O turions even though they had been recently formed and released. Thus the terminology of "young" and "old" refers to the time of turion collection and not to the length of development of turions and the time of discharge from the parent fronds.

A question then arises: Do the parent fronds exert any influence over the formation of O turions from already released Y turions? As shown in Fig. 2, Y turions isolated from the parent fronds at various times and maintained submerged in the medium until day 34 germinate as typical O turions. This indicates that parent

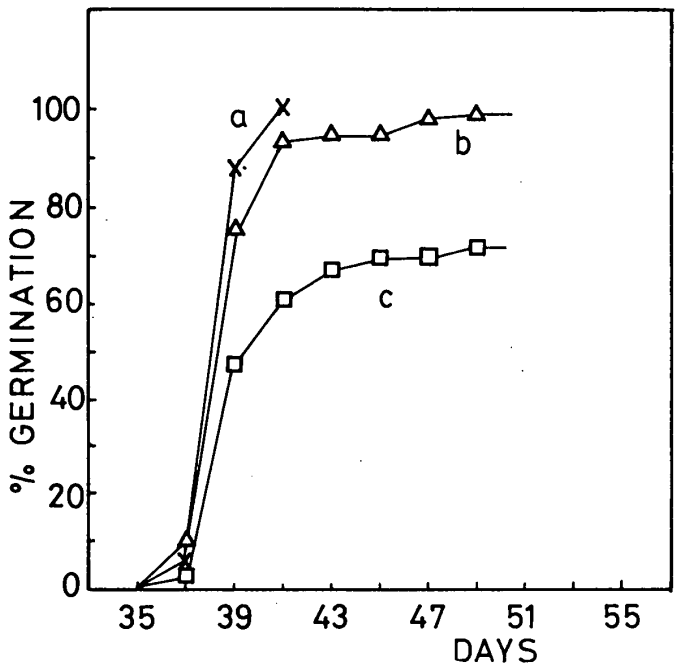

Fig. 2. *Germination of turions left in the medium for various periods after the removal of parent fronds.* Culture medium: 1/20 nitrate Hoagland's solution, 1% sucrose. Parent plants were removed on; a) day 18, b) day 28 and c) day 34. The germination test was started on day 34.

fronds play only a minor role, if any, in the Y to O transformation. The possibility that semi-anaerobic conditions at the bottom of the medium led to this transformation is unlikely, since Y turions submerged in sterile water were not converted to O turions.

The transformation may be due to some component(s) of the culture medium. An observation that in cultures grown in 1/20 nitrate medium lacking sucrose, turions were modified into O turions only slowly (turions collected on day 41 were still Y type and on day 63 were Y-O intermediate) led us to investigate the role of sucrose in the formation of O turions. As shown in Fig. 3, the turions collected as early as on day 18 develop into O turions in about 10 days of contact with fresh sterile 1% sucrose. Thus sucrose appears to greatly accelerate the Y to O transformation. The transformation may be considered senescence of the turions, since it is associated with loss of chlorophyll and also of viability of turions (note the decrease in total germination of turions collected after day 30; curve *c* of Fig. 2 and curve *d* of Fig. 3).

Deleterious effects of exogenously applied metabolisable sugars have been noted in other chlorophyllous organisms grown in light (*2*, *12*), particularly where nitrogen levels in the medium are low (*6*). Added glucose or sucrose modifies various metabolic reactions (*3–5*, *7*, *9*, *10*). Though physiological significance of these metabolic shifts and their relevance to turion senescence are difficult to assess at the present time, the general indication is that, under low nitrogen levels, added metabolisable sugars cause a metabolic shift toward degradative reactions. Similar conclusions, linking sucrose and stimulation of senescence were reached by others (*1*, *12*).

As shown by Sibasaki and Oda (*11*) and Newton et al. (*8*), turion induction in *Spirodela* can be achieved by manipulating the levels of nitrogen or carbon sources

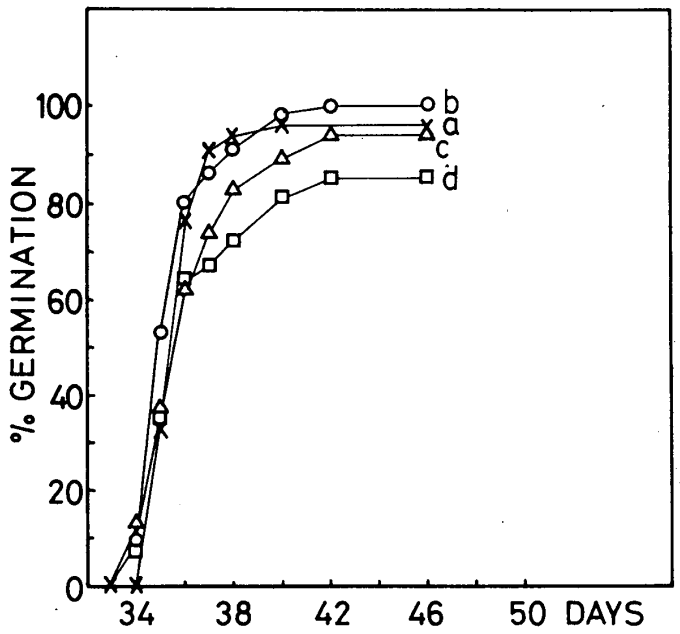

Fig. 3. *Germination of turions transferred into fresh sterile 1% sucrose solutions.* Culture medium: 1/20 nitrate Hoagland's solution, 1% sucrose. Turions were isolated and transferred to fresh 1% sucrose solution on; a) day 18, b) day 25, c) day 28 and d) day 31. Germination test was started on day 31; turions in d), therefore, were not exposed to fresh sucrose solution.

in the medium. As shown above, also development of turions is strongly affected by the medium composition. In oversimplified terms, it appears that the C/N ratio in the medium plays an important role in both developmental processes. Since amino acids are an early product of carbon and nitrogen assimilation, our future investigations will center on their role in turion initiation and development.

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