Effect of Chelating Agents and Metal Ions on Gametangial Formation in the Moss *Bryum argenteum* Hedw.

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**ABSTRACT**

Chelating agents such as EDTA and EDDHA markedly stimulate the formation of gametangia in the dioecious moss *Bryum argenteum*, and the effect is more pronounced on male than on female clones. EDTA-stimulated gametangial formation is associated with significant increases in endogenous iron and copper. Ferric citrate also enhances gametangial formation, but copper sulphate is inhibitory. The present findings are discussed in the context of earlier investigations on other plants in an attempt to explain the possible involvement of chelating agents and metal ions in stimulating the onset of the reproductive phase in this moss.

Key words: *Bryum argenteum*, gametangial formation, chelating agents, metal ions.

**INTRODUCTION**

Morphogenetical changes in bryophytes are regulated by a variety of factors. Chelating agents such as ethylenediaminetetraacetic acid (EDTA) and ethylenediamine di-(o-hydroxyphenylacetic acid) (EDDHA) affect processes such as vegetative growth (Sood, 1974), bud induction (Chopra and Rashid, 1969) and gametangial formation (Sood, 1974; Chopra and Bhatla, 1983). Recently, salicylic acid (well known to chelate iron and copper in animal systems) has also been observed to induce buds in *Anoectangium thomsonii* Mitt. under otherwise non-inductive conditions (Saxena and Rashid, 1980).

It is generally supposed that EDTA and EDDHA act by regulating the availability of metals such as iron and copper. There are reports of altered endogeneous levels of iron and copper in *Wolffia microscopica* Griff. and *Lemna gibba* L. in response to EDDHA treatment, accompanying its effect on growth and flowering (Seth, Venkataraman and Maheshwari, 1970; Pieterse, 1975). A definite correlation, however, between the morphogenetic effect and the chelating property of these agents (as indicated by changes in the endogenous levels of iron and copper) has yet to be demonstrated. Against this background endogenous levels of iron and copper were determined in plants of *Bryum argenteum* treated with the chelating agents. In the light of the results, an attempt is made to specify the possible role of these chelates in plants.

**MATERIAL AND METHODS**

*Cultures*

Full details of the culturing procedures of *B. argenteum* are given elsewhere (Chopra and Bhatla, 1981). Ten-day-old, bud-free protonemata, growing on semi-solid medium were sub-cultured in liquid medium containing 1 per cent sucrose. The protonemata floated on the surface of the medium, and normal gametophores appeared within 20 d of sub-culturing. The 20-d-old, vegetative gametophytes were then subjected to different treatments by transferring them to fresh media containing chelating agents.
EDTA and EDDHA, in concentrations ranging from $10^{-8}$ to $10^{-4}$ M, were autoclaved with the medium.

Estimation of iron and copper

The iron and copper content of plants was estimated according to Allen et al. (1974) using an Atomic Absorption Spectrophotometer (Perkin-Elmer-306). Male and female clones were separated and washed thoroughly with glass-distilled water. Fresh weight was taken after soaking off extra moisture with Whatman no. 1 filter paper.

The material was dried in an oven at temperature gradually brought to 80 °C. Dry weight was recorded every 24 h until it became constant. The dried material was digested in Kjeldahl flasks of 30 ml capacity, in a mixture of 60 per cent perchloric acid (1 ml), nitric acid (5 ml) and sulphuric acid (0.5 ml), using a Microkjeldahl digestion unit. The colourless to slightly pink digest was filtered through Whatman no. 44 filter paper, and final volume of the filtrate was made up to 20 ml.

To estimate iron and copper each 20 ml sample was aspirated at 248.3 and 324.7 nm wavelength respectively, using air–acetylene mixture as fuel. Standard solutions of iron and copper were prepared using ferric citrate and copper sulphate. The calibration curve prepared by using these standard solutions was employed to obtain the content of iron and copper (ppm) in the sample solutions.

If $C$ = ppm iron or copper obtained from the graph, then for the plant material

$$\text{iron or copper (per cent)} = \frac{C \times \text{solution volume (ml)}}{10^4 \times \text{sample weight (g)}}.$$

The effect of chelating agents on gametangial formation was observed at 5-d intervals, till 20 days after the treatments started.

RESULTS

EDTA

EDTA did not affect the time of induction of gametangia which appeared 3 d after transferring the gametophytes to various treatments, as well as in control. At $10^{-8}$ M EDTA the percentage of fertile gametophores was not markedly affected. The response was significant and optimum at $10^{-7}$ (167 per cent of control) and $10^{-6}$ M (129 per cent of control), in male and female clones respectively (Fig. 1). The number of gametophores per culture also increased gradually with increase in EDTA concentration up to $10^{-5}$ M. The extent of optimum stimulation of gametophore formation was almost identical in both clones (the number of gametophores per culture being 157 and 156 per cent of control, in male and female clones respectively).

The effect of EDTA on gametangial formation was more pronounced in male than in female clone. Moreover, it elicited optimum response at a lower concentration ($10^{-7}$ M) in male than in female clones ($10^{-6}$ M). The concentration of EDTA optimum for gametophore formation was even higher ($10^{-5}$ M).

EDDHA

EDDHA also significantly enhanced the formation of gametangia in both clones, without affecting the time of their initiation. The response per culture was more pronounced in male than in female clones (Fig. 2). In male clones the response increased sharply up to $10^{-7}$ M (164 per cent of control); at $10^{-6}$ M the effect was not appreciable; at still higher concentrations ($10^{-5}$ and $10^{-4}$ M) there was inhibition. In female clones gametangial formation was stimulated at all concentrations, and the response was optimum at $10^{-6}$ M EDDHA (155 per cent of control).

EDDHA treatment also resulted in an increase in the number of gametophores. In
male clones the concentration optimum for gametophore formation was the same as that for gametangial formation (10⁻³ M). In female clones the average number of gametophores per culture was the same at 10⁻⁶ and 10⁻⁵ M, whereas the maximum number of gametangia were observed at 10⁻⁵ M. At optimum concentration of EDDHA, stimulation of gametangial formation was more than that of gametophore formation in male clones, whereas in female clones it affected both processes to the same extent.
Changes in endogenous iron and copper contents

Since the effect of EDTA and EDDHA on gametangial formation was similar, endogenous levels of iron and copper were determined only in plants treated with the former.

Accumulation of iron and copper at the concentration of EDTA optimal for gametangial production was greater in male than in female clones as was the stimulation of gametangial formation. In male clones iron and copper content was optimum at $10^{-7}$ M (165 and 178 per cent of that in the controls), which is also the optimum concentration for gametangial formation. There is an excessive accumulation of iron (0.055 per cent) in male clones at $10^{-4}$ M EDTA, accompanying inhibition of gametangial formation. In the female clone, on the other hand, iron content gradually increased with increasing concentration of EDTA (Fig. 3), irrespective of the gametangial response evoked. Copper content, however, slightly increased at $10^{-7}$ M EDTA, and decreased at higher levels.

**Fig. 3.** Effect of EDTA on the iron (○) and copper (●) content of male (A) and female (B) clones of *Bryum argenteum*. Each datum indicates the mean of three replicates. Data from 40-d-old cultures.

Effect of ferric citrate and copper sulphate

Ferric citrate markedly stimulated gametangial formation without affecting the time of their initiation; the response per culture being maximum at $3 \times 10^{-6}$ M in both clones (Fig. 4). The effect on the number of gametophores was less marked. When cultures were transferred to fresh basal media containing different concentrations of copper sulphate (0.012, 0.025, 0.05, 0.1 and 0.2 ppm), gametangia appeared in 3 d in all the treatments. In male clones the response per culture was not appreciably affected by copper sulphate up to 0.1 ppm, but was slightly inhibited at 0.2 ppm (it being 73 per cent of control). In these clones the number of gametophores was slightly lowered at all levels of copper sulphate, and at 0.2 ppm it was 75 per cent of that in control. In female clones the inhibitory effect on gametangial formation was evident at 0.05 ppm and was more pronounced than in male clones. At 0.2 ppm the gametangial induction response was 51 per cent of that in the absence of copper sulphate, without any significant effect on the number of gametophores (Fig. 5).
Fig. 4. Effect of ferric citrate on gametophore number and gametangial formation in male (■) and female (□) clones of Bryum argenteum. Other details are as in Fig. 1.

Fig. 5. Effect of copper sulphate on gametophore number and gametangial formation in male (■) and female (□) clones of Bryum argenteum. Other details are as in Fig. 1.
DISCUSSION

EDTA and EDDHA enhance flowering in *Lemna gibba* L. (Hillman, 1961) and *Wolffia microscopica* Griff. (Seth et al., 1970). EDDHA has also been reported to induce flowering in *L. gibba* L. (Pieterse, Bhalla and Sabharwal, 1970) and *Pistia stratiotes* L. (Pieterse, 1978) under non-inductive conditions. Among bryophytes, iron salts of EDTA and EDDHA considerably enhance archegonial production in *Riccia crystallina* L., and antheridial formation is marginally stimulated (Sood, 1974). Contrary to these observations, in *B. argenteum* stimulation of gametangial formation is significantly higher in male than in female clones. EDTA and EDDHA also markedly increase gametangial production in *Bartramidula bartramioides* Schimp. (Rahbar, 1981) and formation of antheridia in male clones of *Barbula gregaria* (Mitt.) Jaeg. and *Bryum coronatum* Schwaegr. (Kumra, 1981).

Measurements of endogenous levels of iron and copper in the EDTA-treated plants of *B. argenteum* reveal that male and female clones exhibit variable trends in the content of these ions, despite the similarity in their morphogenetic response. This might perhaps be due to differential ionic requirements for the onset of reproductive phase in the two clones. Nevertheless, the content of iron in both clones is significantly more at the EDTA concentration optimal for gametangial formation than in the control cultures. Copper content, on the other hand, increases only slightly in male clones, and does not vary in female clones. Since exogenously supplied ferric citrate also stimulates gametangial induction in both clones, it is possible that iron is involved in the promotion of gametangial formation.

The role of iron in flowering has also been pointed out earlier by Smith, McIlrath and Bogord (1957) in *Xanthium pennsylvanicum* L. Seth et al. (1970) reported that EDDHA promotes flowering in *W. microscopica* Griff. and this is accompanied by a several-fold increase in the endogenous iron content. However, the observation that in *L. gibba* L., EDDHA enhances gibbosity (and flowering) irrespective of the presence of iron in the nutrient medium (Pieterse, 1975) casts doubt on the involvement of iron chelates in morphogenetic response. Thus even though the chelates enhance iron uptake in plants, iron and chelating agents appear to affect the morphogenetic processes through independent pathways. Chelation of iron is probably one of the many effects of EDTA and EDDHA.

Recent studies on higher plants have indicated that chelating agents might be affecting plant processes in some ways other than chelation of iron and copper. Oota and Tsudzuki (1971) observed that there is no essential difference between flowering response of *L. gibba* L. to growth substances, and to agents chelating cupric and ferric ions. A comparison of the effect of chelates on gametangial formation in *Bryum* with that of IAA and GA₃ (Bhatla and Chopra, 1981) indicates some similarity in their dose–response pattern. It has also been suggested that the chelating agents are somehow connected with membrane properties (Pieterse and Muller, 1977). Possibility of the involvement of other ions such as calcium is also not ruled out. The variable trends in the iron and copper contents of EDTA-treated male and female clones of *Bryum* recorded here indicate that although one particular metal ion may play a dominating role in the onset of reproductive phase, it is ultimately a balance of certain ions, which regulates the morphogenetic responses.

It is thus evident that the stimulatory effect of EDTA on gametangial formation in *B. argenteum* is accompanied by greater bioavailability of metal ions, especially iron. However, further experiments are needed to confirm the involvement of iron in the morphogenetic processes. It would be useful to know whether or not iron-containing components such as cytochromes, are influenced by these chelators.
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LITERATURE CITED


