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Content of gibberellic acid in apical parts of male and female thalli of *Chara tomentosa* in relation to the content of sugars and dry mass

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Abstract

The contents of gibberellic acid (GA_3), glucose and starch, and dry mass in four nodes of apical parts of male and female thalli of *Chara tomentosa* were measured. The GA_3 concentration was about 4 times higher in male than in female thallus and about 10 times higher in the first node than in the next three nodes of both thalli. The apical part of male thallus contains 2.5 times less glucose and 1.7 times less starch than female one. The average amount of dry mass of male and female thallus was similar: 9.9 and 9.8 % of fresh mass. The findings of this investigation demonstrate the interdependence of gibberellic acid and sugars in development of male and female *Chara tomentosa* thalli.

Additional key words: algae, capillary zone electrophoresis, glucose, starch.

Introduction

Plant hormones affect sex expression in many monoecious and dioecious species and the effect of a specific hormone varies between species. Gibberellins seem to be important for male fertility in most dicotyledonous and monocotyledonous species (Lebel-Hardenack and Grant 1997). Gibberellin-deficient mutants in *Arabidopsis* are male-sterile and application of GA_3 to cucumber and hemp is masculinizing rather than feminizing. Application of GA_3 to female cucumber flowers is masculinizing, whereas application of gibberellic acid to male flowers of maize can be feminizing (Lebel-Hardenack and Grant 1997). The studies with an inhibitor of gibberellin synthesis (paclobutrazol) showed that GA_3 had dual effect on sex expression - promoting maleness and inhibiting femaleness (Yin and Quinn 1995). The examples of sex reversion by hormone application suggest that in many species unisexual floral meristems are sexually bipotent, and sex determination genes may trigger the switch between alternative developmental programs by changing the levels or ratios of endogenous hormones (Yin and Quinn 1995, Lebel-Hardenack and Grant 1997).

The endogenous GAs play a key role in the control of sexuality. In maize, the content of gibberellin-like substances was much lower in tassels than in ears. In developing apical meristems of maize, the level of acidic, ethyl acetate soluble gibberellin-like substances increased in tissues at inflorescence initiation, and then fell rapidly. At anthesis, only a trace of GA-like activity remained in the apical (male) inflorescence, whereas moderate activity, mostly of a nonpolar nature, was present in lateral female inflorescence (Rood *et al.* 1980).

In few species of ferns, the pheromone with gibberellin-like structure, named antheridiogen, is the primary sex determinant (Näf 1975, Banks 1997). Antheridiogen or gibberellin induces formation of antheridia and inhibits formation of archegonia (Schraudolf 1962, Takeno and Furuya 1977). In the presence of antheridiogen or gibberellin the fern gametophytes develop almost exclusively as males (Schraudolf 1962, Yamane *et al.* 1987, Kaźmierczak and Maszewski 1997, Kaźmierczak 1998), whereas in the absence of these factors, gametophytes develop exclusively as hermaphrodites (Schraudolf 1962, Näf 1975, Takeno *et al.* 1987, Banks 1997).

The fact that high concentration of exogenous GA_3

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Abbreviations: CZE - capillary zone electrophoresis; d.m. - dry mass; f.m. - fresh mass; GA_3 - gibberellic acid.

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stimulates the development of antheridia (Kwiatkowska and Godlewski 1980, 1988, Kwiatkowska *et al.* 1991) and hastens the maturation of oogonia (Godlewski and Kwiatkowska 1980) and the possibility to measure gibberellic acid in extracts of thallus (Kaźmierczak 1999) and in male sex organs (Kaźmierczak *et al.* 1999) of *Chara vulgaris* by capillary zone electrophoresis, stimulated present investigations aimed at determination and finding out the differences in endogenous GA_3 content in apical parts of male and female *Chara tomentosa* thalli. Moreover, additional analysis was undertaken in order to get a closer insight into the correlation between the content of gibberellin acid and sugar as well as dry mass.

Materials and methods

Plants of *Chara tomentosa* L. f. *vulgaris* Mig., collected from the lake Szarcz (north-west Lake District of Poland) were cultivated in the laboratory for few days at temperature about 18 °C under fluorescent tubes (14-h photoperiod, irradiance of 5 $W \cdot m^{-2}$) and then selected for studies.

The apical parts of thalli were washed with distilled water and separated into particular nodes and used for preparation. The first node (I) is the highest and fourth (IV) is the lowest of the apical part. The isolation of gibberellic acid was carried out according to the method described by Fujioka *et al.* (1988) and modified by Kaźmierczak (1999) using methanol and acidic ethyl acetate. GA_3 content was measured by capillary zone electrophoresis using *BioFocus 3000 capillary electrophoresis system* (*BioRad Laboratories,* Munich, Germany), equipped with a UV scanning detector and fused-silica capillary of 25 μm I.D. of the total length of 17 cm. Electrophoreograms were recorded and analysed with *BioFocus Integration* software 5.0, based on Microsoft Windows, at a detector rise time setting of 1 s and data sampling rate of 5 Hz. Samples were injected at high pressure (138 kPa) and electrophoreograms were analysed with scanning detector (200 - 320 nm). The separations were carried out in sodium borate running buffer (30 mM, pH 8.5) with sodium borate sample buffer (3 mM at pH 8.5) at 10 kV at the polarity, - to +. Migration time of gibberellic acid was determined to be 1.86 min and the UV maximum absorption was at 258 nm (Kaźmierczak 1999).

For determination of GAs activity bioassay was carried out according to Crozier *et al.* (1970) on *Pisum sativum* L. cv. Iłowiecki (3-d-old seedlings) using the 5 mm-long epicotyl. Samples were diluted in 3 mM borate buffer with 5 mm^3 of ethanol (50 %) and 5 mm^3 of Triton X-100 in one cm^3. After application of a standard (GA_3) or extracts (10 mm^3), seedlings were planted at above mentioned conditions. Three days later, the length of epicotyl and internode was measured. Experiments were made in three replicates.

The extraction and analysis of sugars were done in four nodes of apical part of generatively matured male and female thallus according to Antikainen and Pihakaski (1994). Glucose or starch solutions were used as standards. Dry mass (DM) was examined after 24 h of drying at 80 °C when no further mass loss occurred.

Results and discussion

Gibberellic acid content in the *Chara tomentosa*: The formation of male and female reproductive organs in plants, in many cases, occurs under the control of exogenous or endogenous gibberellins (Rood *et al.* 1980, Malepszy and Niemirowicz-Szczyt 1991, Lebel-Hardenack and Grant 1997). Analysis of purified extracts from apical parts of both male and female thalli of *Chara tomentosa* revealed the differences in relative amount of GA_3. The apical part of male thallus, bearing youngest antheridia, contained aproximately about four times higher concentration of gibberellic acid [14.6 mg kg^{-1}(f.m.) determined by CZE and 16.6 mg kg^{-1}(f.m.) determined by biotest], than an analogous part of female one, bearing the youngest oogonia [3.9 mg kg^{-1}(f.m.) determined by CZE and 4.0 mg kg^{-1}(f.m.) determined by biotest], respectively. In the first node of male or female thallus GA_3 concentration was 10 times higher, on a fresh matter basis, then in next nodes (II, III and IV), which showed only small differences (Fig. 1*A,B*). These data correlate with the results obtained with exogenously applied GA_3 and AMO-1618 (an inhibitor of GA_3 synthesis) which lowers the content of endogenous gibberellins in generatively matured thallus of *Chara vulgaris*. It has been found that high concentration of GA_3 sufficient or suboptimal for development of generative organs, is supraoptimal for the growth of vegetative part of thallus (Kwiatkowska and Godlewski 1980). GA_3 caused a significant, proportional to concentration, reduction in growth of thallus internodes as well as elongation of multinuclear polyploidal cells of pleuridium internodes which bear generative organs (Kwiatkowska and Godlewski 1980, Kwiatkowska *et al.* 1991). Moreover, gibberellic acid increased the number of antheridial filaments formed in antheridia and increased the number of spermatids within a single

filament. The inhibitor (AMO-1618) evokes opposite effect (Kwiatkowska and Godlewski 1988). Autoradiographic and cytophotometric studies have shown that generative organs import gibberellins from pleuridia by plasmodesmal connection (Kwiatkowska 1988, Kwiatkowska 1995) and the spontaneous or induced symplasmic isolation decreased content of gibberellins in antheridia (Kwiatkowska 1988, Kwiatkowska 1991, Kwiatkowska 1995). Indeed, in the extracts from antheridia at the stage of proliferation of antheridial filament cells, the high content of GA_3 was detected by capillary electrophoresis and bioassay. They contain 5.3 times more GA_3 than antheridia at stage of sperm cells maturation - spermiogenesis (Kaźmierczak *et al.* 1999). The symplasmic isolation decreased the level of gibberellins in antheridia and spermiogenesis is not regulated by gibberellins (Godlewski and Kwiatkowska 1980, Kwiatkowska 1991) Therefore, the lowered amount of GA_3 results from the fact that analysed nodes (especially III and IV) of apical part of male thallus bear antheridia at the last stage of maturing spermatids.

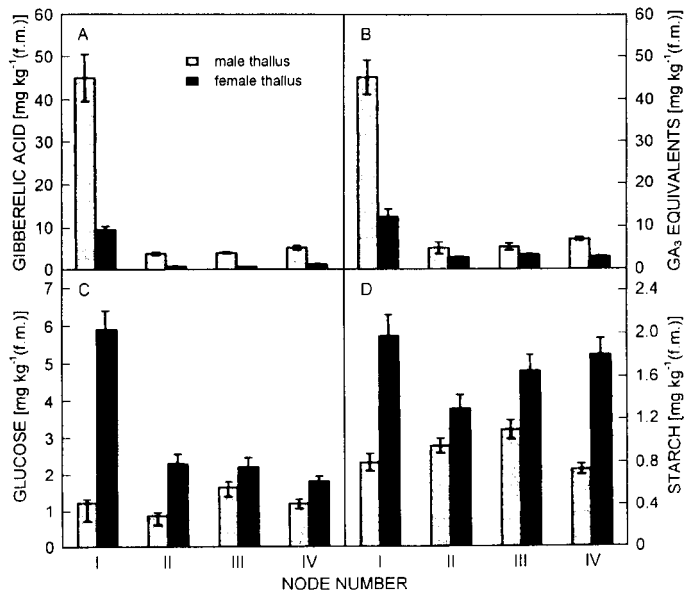

Fig. 1. Gibberellic acid content determined by capillary zone electrophoresis (*A*), activity of gibberellin expressed in mg of gibberellic acid equivalent) determined using the bioassay (*B*), contents of glucose (*C*), and starch (*D*) in apical part of male and female thallus of *Chara tomenosa*.

Sugar content: The female thallus contained more sugars than male one. The amount of glucose was 2.5 times higher and starch content was about 2 times higher. The male thallus revealed maximum glucose and starch contents in the third nodes and the female thallus in the first nodes (Fig. 1*C,D*). These results indicate that average sugar content is negatively correlated with the amount of GA_3. In *Chara vulgaris* starch is accumulated in oogonia after the symplasmic transport of gibberellins from the thallus to the antheridium is decreased (Kwiatkowska *et al.* 1997). Moreover, starch in antheridia is accumulated in the last stages of spermatozoid maturation (Kwiatkowska 1996). Hence, it is possible that in female thallus, lower content of GA_3 is connected with higher content of sugars. During fructification, charophytes store huge amounts of starch in their oogonia. Developing oogonia and antheridia are important sinks for transported sugars (Schulte *et al.* 1994). In fertile *Chara vulgaris* plants sucrose is identified as the main transported sugar. The accumulation of sucrose coincides with sexual reproduction and it is assumed to be the prerequisite for reproduction (Schulte *et al.* 1994). On the contrary, in the dioecious species *C. aspera*, only minor differences in sucrose concentration were observed between sterile and fertile plants during the reproductive period and none at all between the sexes (Winter and Kirst 1992). In GA_3-induced male organs of gametophytes of *Anemia phyllitidis* the contents of glucose and starch are increased. The increase in sugar content in GA_3-treated gametophytes of *A. phyllitidis* was connected with antheridia formation (Kaźmierczak 1998).

Dry mass: Dry mass content of apical thallus of *C. tomentosa* slightly increased from the first to the fourth nodes of male and female thalli (Table). Endogenous GA_3 concentration was not directly involved in the regulation of shoot dry matter of *Brassica napus* (Potter *et al.* 1999), whereas exogenously applied GA_3 on gametophytes of *A. phyllitidis* increases DM by 50 % (Kaźmierczak 1998).

In conclusion, the results of this study demonstrate interdependence of gibberellic acid content, sugars content and sex of developing thalli in *Chara tomentosa*.

Higher gibberellic acid content is connected with male thallus and developing of young (first) nodes of male and female thalli, whereas higher average sugars content is associated with growth of female thallus. The dry mass is not correlated with content of GA_3, sugars and sex of *C. tomentosa* thallus.

Table 1. Dry matter [% f.m.] of four nodes of apical part of male and female *Chara tomentosa* thallus. Means ± SE, $n = 3$.

Node number	I	II	III	IV	Average
Male thallus	8.6 ± 0.7	8.9 ± 0.7	10.1 ± 0.9	11.7 ± 0.1	9.8 ± 0.7
Female thalus	8.9 ± 0.7	8.8 ± 0.8	9.8 ± 0.8	12.8 ± 0.1	9.9 ± 1.3

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