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BRIEF COMMUNICATION

The relationships between fertility and contents of gibberellic acid, sugars and dry mass in apical parts of *Chara vulgaris* thalli

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Abstract

The contents of endogenous gibberellic acid (GA_3), sugars, and dry mass in apical parts of fertile and sterile thalli of *Chara vulgaris* were estimated. The GA_3 concentration in the first node of fertile thallus, determined by capillary electrophoresis, was about 70.0 mg kg^{-1} of fresh mass (f.m.). *Pisum sativum*-bioassay showed GA_3 concentration of 80.0 mg kg^{-1}(f.m.) which was about 3 times higher than in the first node of sterile thallus. The higher amount of GA_3, glucose, and the lower starch content and dry mass in fertile plants than in sterile ones suggest the interdependence between fertility and contents of studied components.

Additional key words: capillary zone electrophoresis, fertile thallus, glucose, starch, sterile thallus.

Gibberellins (GAs) control germination, hypocotyl and epicotyl elongation and flower and fruit development. GAs induce growth of vegetative tissues by promoting the longitudinal expansion and inhibit the lateral expansion of cells in various plants (Huttly and Phillips 1995). GA plays essential role in regulation of internode elongation of *Pisum sativum* (Yang *et al.* 1996). Some dwarf mutants of petunia with disturbance in the GA biosynthetic pathway show abnormal flower, fruit, and seed development, and development of normal flowers can be rescued by application of GA (Huttly and Phillips 1995). Application of GA_3 induces parthenocarpy in many species. In ferns, differentiation of gametophytes and formation of generative organs is regulated by gibberellins and antheridiogens, *i.e.*, gibberellin-like substances (Schraudolf 1962, Banks 1997). In *Chara vulgaris*, exogenously applied GA_3 inhibits the elongation of internodal cells of the main axis and pleuridia (Kwiatkowska and Godlewski 1980) and accelerates the development of generative organs (Kwiatkowska *et al.* 1991).

To gain information about relationships between endogenous GA_3, content of sugars and dry mass and fertility of *C. vulgaris*, fertile and sterile thalli were examined.

Apical parts of generatively matured (fertile) and generatively unmatured (sterile) *Chara vulgaris* L. thalli were collected from the pond in Łódź Botanical Garden. The thalli were washed with distilled water and separated into particular nodes, the first node being the highest and the fourth being the lowest of the apical part. Gibberellic acid was isolated according to the method of Fujioka *et al.* (1988) modified by Kaźmierczak (1999). Determination of GA_3 in purified sample of algal extract was done on the basis of results obtained from standard detection of GA_3 performed by *BioFocus 3000* capillary electrophoresis system *(BioRad Laboratories,* Munich, Germany; Kaźmierczak 1999). Bioassay was carried out according to Crozier *et al.* (1970) on *Pisum sativum* L. cv. Ilówiecki (3-d-old seedlings) using 5 mm-long epicotyls (Kaźmierczak 1999). The extraction and analysis of sugars were done according to Antikainen and Pihakaski (1994). Dry mass samples were examined after 24 h of drying at 80 °C when no further mass loss occurred.

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

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In sterile *C. vulgaris* thallus, the concentration of GA_3 was lowest in the first node and in the next three nodes it gradually increased up to 150 mg kg^{-1}(f.m.) in the fourth node. In fertile thallus of *C. vulgaris* an inverse gradient of GA_3 concentration was observed. Amount of GA_3 gradually decreased from the first node (with young antheridia and oogonia) up to the fourth node (Fig. 1*A,B*). The similar pattern of GA_3 concentrations was revealed by CE and bioassay test. The decreased gradient of GA_3 concentration could be explained by preparation of sterile plants (during vegetative growth and elongation of thallus) to development of gametangia. On the other hand, the highest GA_3 concentration in the first nodes of fertile plants was connected with high content of GA_3 which was found in antheridia at cell division stage (leading to formation of spermatids) of antheridial filaments [560.0 mg kg^{-1}(f.m.)]. This amount is 5.3 times greater than in antheridia at a differentiation stage of spermatozoids, spermiogenesis [36.0 mg kg^{-1}(f.m.), Kaźmierczak *et al.* 1999]. Likewise, the first nodes of male and female *C. tomentosa* thalli contained ten times more GA_3 than next three analysed nodes, and the concentration was 4 times higher in the first male node as compared to female one (Kaźmierczak and Rosiak 2000). The development of antheridia during spermatogenesis depended on drastically reduced GA_3 import from thallus (Kwiatkowska 1988) which has been decreased by spontaneous or induced symplasmic isolation in antheridia (Kwiatkowska 1995). The present results confirmed those obtained with generatively matured thallus of *C. vulgaris* and exogenously applied GA_3 indicating that the development of antheridia at the proliferation of antheridial filament cells stage require high content of GA_3 and elongation and apical dominance of thalli is the best at low content of GA_3 (Godlewski and Kwiatkowska 1980, Kwiatkowska and Godlewski 1980).

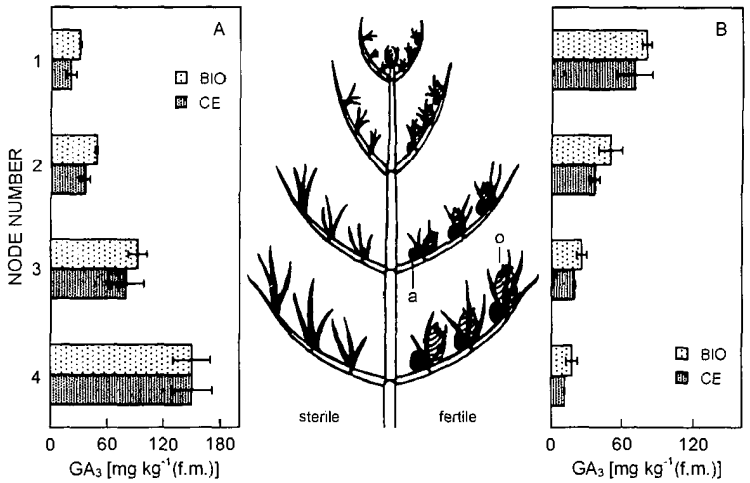

Fig. 1. Gibberellic acid content determined by capillary zone electrophoresis (CE) and activity of gibberellin expressed in mg of gibberellic acid equivalent determined using the bioassay (BIO) in four apical part of sterile (*A*) and fertile (*B*) *Chara vulgaris* thallus; a - antheridium, o - oogonium.

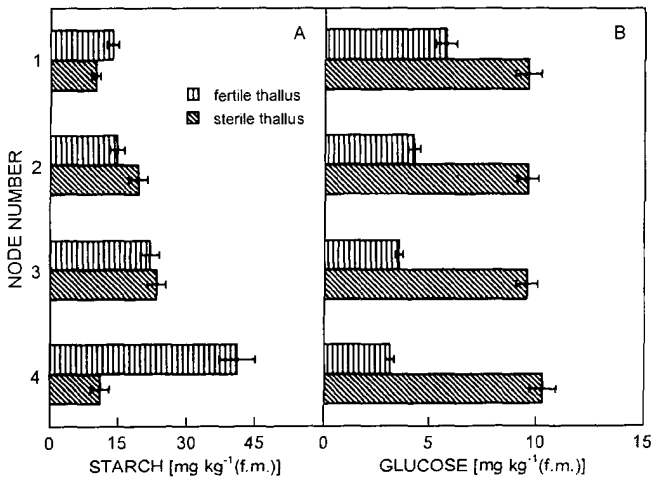

Fig. 2. Contents of starch (*A*) and glucose (*B*) in apical part of sterile and fertile *Chara vulgaris* thallus.

Starch was accumulated during development of both thalli of *C. vulgaris* but especially during maturation of fertile thallus. The content of starch increased from the first node to the third and then decreased to the first node level in sterile plants but increased up to 41 mg kg^{-1}(f.m.) in fertile ones (Fig. 2*A*). The sterile plants contained more glucose than the fertile ones where its amount decreased from the first to the fourth node (Fig. 2*B*). Dry mass of sterile *C. vulgaris* thallus in four nodes was similar (6 %) while dry mass of fertile thallus was about two times higher and increased from 10 % in the first and second and 11 % in the third to 14 % in the fourth node.

The present results revealed that the appearance of antheridia and oogonia on the *Chara* thallus is connected with change of relations between the concentrations of sugars and GA_3. In apical part of fertile thallus the higher content of dry mass and starch was accompanied by lower contents of GA_3 and glucose. Increasing evidence shows that glucose and GA_3 signalling interact in the regulation of plant metabolism and development (*e.g.*, GA_3-induced gene expression in barley grains; Perata *et al.* 1997). The changes between proportion of free and storage sugars may be the signal for changes of GA_3 concentration and commonly could act as interdependent signals in regulation of generative and vegetative development of *C. vulgaris* thallus.

References

Antikainen, M., Pihakaski, S.: Eearly development in RNA, protein, and sugar levels during cold stress in winter rye (*Secale cereale*) leaves. - Ann. Bot. **74**: 335-341, 1994.

Banks, J.A.: Sex determination in the fern *Ceratopteris*. - Trends Plant Sci. **2**: 175-180, 1997.

Crozier, A., Kuo, C.C., Durley, R.C., Pharis, R.P.: The biological activities of gibberellins in nine plant bioassays. - Can. J. Bot. **48**: 867-877, 1970.

Fujioka, S., Yamane, H., Spray, C.R., Gaskin, P., MacMillan, J., Phinney, B.O., Takahashi, N.: Qualitative and quantitative analyses of gibberellins in vegetative shoots of normal, dwarf-1, dwarf-2, dwarf-3, and dwarf-5 seedlings of *Zea mays* L. - Plant Physiol. **88**: 1367-1372, 1988.

Godlewski, M., Kwiatkowska, M.: Effect of gibberellic acid on the formation and development of antheridia and oogonia in *Chara vulgaris* L. - Acta Soc. Bot. Pol. **49**: 459- 469, 1980.

Huttly, A.K., Phillips, A.L.: Gibberellin-regulated plant genes. - Physiol. Plant. **95**: 310-317, 1995.

Kaźmierczak, A.: Determination of GA_3 in *Chara vulgaris* by capillary electrophoresis system. - Acta Physiol. Plant. **21**: 344-348, 1999.

Kaźmierczak, A., Kwiatkowska, M., Popłońska, K.: GA_3 content in antheridia of *Chara vulgaris* at the proliferative stage and in spermiogenesis estimated by capillary electrophoresis. - Folia histochem. cytobiol. **37**: 49-52, 1999.

Kaźmierczak, A. Rosiak, M.: 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. - Biol. Plant. **43**: 369-372, 2000.

Kwiatkowska, M.: Symplastic isolation of *Chara vulgaris* antheridium and mechanisms regulating the process of spermatogenesis. - Protoplasma **142**: 137-146, 1988.

Kwiatkowska, M.: Effect of symplasmic isolation and antigibberellin treatment on morphogenesis in *Chara*. - Folia histochem. cytobiol. **33**: 133-137, 1995.

Kwiatkowska, M.: Godlewski, M.: Effect of gibberellic acid and AMO-1618 on the development of vegetative systems in generatively matured thalli of *Chara vulgaris* L. - Acta Soc. Bot. Pol. **49**: 445- 458, 1980.

Kwiatkowska, M., Gosek, A., Godlewski, M.: Effect of GA_3, IAA and their mixtures on the formation and development of cell systems in the vegetative and generative thallus of *Chara vulgaris* L. - Acta Soc. Bot. Pol. **60**: 313-326, 1991.

Perata, P., Matsukura, C., Vernieri, P., Yamaguchi, J.: Sugar repression of gibberellin-dependent signaling pathway in barley embryos. - Plant Cell **9**: 2197-2208, 1997.

Schraudolf, H.: Die Wirkung von Phytohormonen auf Keimung und Entwicklung von Farnprothallien 1. Auslösung der Antheridienbildung und Dunkelkeimung bei Schizaeaceen durch Gibberellinsäure. - Biol. Zentralbl. **81**: 731-740, 1962.

Yang, T., Davies, P.J., Reid, J.B.: Genetic dissection of the relative roles of auxin and gibberellin in the regulation of stem elongation in intact light-grown peas. - Plant Physiol. **110**: 1029-1034, 1996.