Effects of Macro-components and Sucrose in the Medium on in vitro Red-color Pigmentation in *Dionaea muscipula* Ellis and *Drosera spathulata* Labill.

Sayuri ICHIISHI*, Toshiharu NAGAMITSU*, Yusuke KONDO*, Tsukasa IWASHINA**, Katsuhiko KONDO*** & Norikazu TAGASHIRA***

*Plant Bio-technology Section, Sanmei Electric Co., Ltd., 5-7, Sumiyoshi-Cho, Handa City 475-0862, Japan
**Tsukuba Botanical Garden, National Science Museum, 4-1-1, Amakubo, Tsukuba City 305-0005, Japan
***Laboratory of Plant Chromosome and Gene Stock, Faculty of Science, Hiroshima University, 1-4-3 Kagamiyama, Higashi-Hiroshima City 739-8526, Japan

E-mail:

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Abstract

Effects of the five macro-components and sucrose in half strength MS (1/2 MS) agar medium on red-color pigmentation were studied in the plant bodies of *Dionaea muscipula* and *Drosera spathulata* generated from multiple shoots in vitro. In 1/2 MS agar medium modified with 10.31 mM NH₄NO₃ and 9.40 mM KNO₃ and supplemented with 0.75 or 0% sucrose the subcultured plants continuously proliferated by multiple shoots and generated large, green-colored plants, while with dilution of those nitrogen components and increase of sucrose to 1.5% the red-color anthocyanin pigmentation spread from the glands or glandular hairs to the entire leaves and the plant sizes and dry weight decreased in inverse proportion to the depth of red color. The anthocyanin pigments of *Dionaea muscipula* consisted of delphinidin 3-0-glucoside which was new to the species and cyanidin 3-0-glucoside (chrysanthemin), and those of *Drosera spathulata*, studied here for the first time, consisted of cyanidin 3,5-di-0-glucoside (cyanin), cyanidin 3-0-galactoside (idaein), cyanidin 3-0-glucoside, pelargonidin 3-0-galactoside, and pelargonidin 3-0-glucoside (callistephin).

*Dionaea muscipula* Ellis and *Drosera spathulata* Labill. in the Droseraceae are ornamental, carnivorous plants (Kondo and Kondo, 1983). The plant bodies of the two species vary in coloration from green to red. However, plants with stable red color are horticulturally more desirable than green ones. The commercialized cultivars of ‘Red Dragon’, ‘Red Giant’, ‘Red Purple’, and ‘Royal Red’ of *Dionaea muscipula* are artificially selected to bring out a stable, deep red-color pigment in whole plant body when cultivated *in vivo*.

Although anthocyanin pigmentation in leaves of a few species of *Dionaea* and *Drosera* is a common phenomenon, it has been poorly characterized with limited references; cyanidin-3-glucoside in *Dionaea muscipula* (Di Gregorio and Di Palma, 1961; Jay and Lebreton, 1972), cyanidin-glucoside, malvidin-glycosides, pelargonidin-glucoside, quercetin-3-galactoside, and quercetin-3-digalactoside, in a few species of *Drosera* other than *D. spathulata* (Gascoigne *et al.*, 1948; Paris and Denis, 1957; Paris and Delaveau, 1959; Bienenfeld and Katzmeister, 1966; Bendz and Lindberg, 1968; Bendz and Lindberg, 1970; Ayuga *et al.*, 1985).

*Dionaea muscipula* and *Drosera spathulata*, which are autotrophic plants, commonly occupy relatively closed ecosystems where the soil is poor in nutrient substances, wet and acid. They take in and absorb nutrients directly from small animal resources by way of carnivorous leaves (Lloyd, 1942). Darwin (1875) and some other workers (Kellermann and Raumer, 1878; Thum, 1988, 1989; Gibson, 1991) stated that the plants of some *Drosera* species fed small animals artificially through their leaves increased the number of flowers, total weight of seeds and vegetative organs. In contrast, the plants of *Dionaea muscipula* and some *Drosera* species fed mineral nutrients through their underground roots *in vivo* grew poorly (Robert and Oosting, 1958; Juniper *et al.*, 1989). A tissue culture
of another carnivorous plant, *Utricularia praehonga* St. Hil., (Idei and Kondo, 1998) showed different organogenesis, micropropagation, growth forms, and so on by adjusting KNO₃ concentrations between 24.73 and 3 mM as well as BAP (N6-benzylaminopurine) concentrations in B5 (Gamborg et al., 1968) liquid medium. However, studies on the correlation between nutrients and anthocyanin pigmentation in *Dionaea muscipula* and *Drosera spatulata* as well as other carnivorous plants are very much lacking.

Seeds of *Dionaea muscipula* and *Drosera spatulata* Kanto type collected in cultivation were surface-sterilized with 0.1% (w/v) benzalkonium chloride solution for 5 min, 1% (w/v) sodium hypochlorite solution for 5 min, 70% (v/v) ethanol for 30 s and were rinsed three times with sterile, distilled water before they were sown on half strength of MS (1/2 MS; Murashige and Skoog, 1962) medium supplemented with 0.8% sucrose. They germinated 30 to 60 days after they were sown.

Individual leaves of *Dionaea muscipula* used as explants were planted on 1/2 MS supplemented with 1.5% sucrose. After 5 months they propagated an average of 6 plants per explant by adventitious buds and multiple shoots. Each plant averaged 2 cm in diameter, 0.12 ± 0.04 g fresh weight, and 0.02 ± 0.00 g dry weight was used for the present experiment.

Individual plants of *Drosera spatulata* averaging 2.5 cm in diameter each were used as explants. They were planted and subcultured at intervals of 20 days on 1/2 MS supplemented with 1.5% sucrose. After 5 months they propagated numerous plants per explant by multiple shoots. Plants averaging 2.5 cm diameter, 0.14 ± 0.03 g fresh weight, and 0.02 ± 0.00 g dry weight was used for the present experiment.

All cultures were planted on 50 ml medium supplemented with no growth regulator at pH 5.5 in cylindrical-shaped, culture vials 80 mm diameter X 129.5 mm high, 450 ml capacity, air-tight with a transparent, clear lid at 25 ºC under 3500 lux continuous illumination. 0.2 g fresh weight of plant bodies, especially leaves, per sample was utilized to extract anthocyanin pigments with 1 ml MeOH-HCl mixture (methanol:hydrochloric acid=1000:1) for 3 h to overnight and filtrated by Toyopak ODS M (Tosoh) and Maisyoridisc H-13-5 0.45 µm (Tosoh) pre-cartridge. The composition of plant extracts was determined by the methods of Iwashina (1996).

Modified 1/2 MS media with no or no macro-components and with more sucrose induced red-color pigmentation in the inner surface of the trap lobe in *Dionaea muscipula* and in the glandular hair in *Drosera spatulata* and in the whole leaves of the both species after four months culture. However, they made plant growth worse. In contrast, 1/2 MS medium with more to complete macro-components promoted deeper green color in the whole plant bodies and larger growth and more proliferation in the both species.

Moreover, the modified 1/2 MS media with no NH₄NO₃ resulted in some red colored glands, glandular tissues and sensitive hairs but green color in the other parts of the leaves in *Dionaea muscipula* after four months culture and the modified 1/2 MS medium with no NH₄NO₃ and no KNO₃ resulted red coloration in the inner surface of the trap lobe and relatively red color in the whole leaves and reduction of dry weight (Fig. 1). Thus, NH₄NO₃ among the macro-components of the 1/2 MS media could be the major nitrogen source for *Dionaea muscipula* to giving thin red-color pigmentation and more green color and to increasing plant growth. The natural habitat of *Dionaea muscipula* in North Carolina, U.S.A. has low contents of NH₄⁺ (2 mg/Kg dry weight), PO₄ (less than 2 mg/Kg), K (2 mg/Kg) and Mg (1 mg/Kg) and no NO₃⁻, Ca and Mn (Robert and Oosting, 1958).

Similarly, the lack of NH₄NO₃ and KNO₃ among the macro-components of the 1/2 MS medium deepened red-color pigmentation in glandular hairs and the other parts of the leaves and reduced plant growth and dry weight in *Drosera spatulata*. On the other hand, the lack of NH₄NO₃ and MgSO₄ · 7H₂O among the macro-components of the 1/2 MS medium exhibited healthy-looking plant bodies without any dead leaf but no plant growth perhaps due to the balanced combination of N, P, K, and Ca. In contrast, when the 1/2 MS medium lacked CaCl₂, MgSO₄ · 7H₂O and KH₂PO₄ the leaf and shoot tips died perhaps due to an unbalanced combination of less Ca against more N and K. Thus, NH₄NO₃ and KNO₃ among the macro-components of the 1/2 MS media could be the major nitrogen sources for *Drosera spatulata* creating thin red-color pigmentation and more green color and increasing plant growth and even propagation.

The anthocyanin pigments of *Dionaea muscipula* consisted of delphinidin 3'-0-glucoside and cyanidin 3-0-glucoside (chrysanthemin), and those of *Drosera spatulata* consisted of cyanidin 3,5-di-0-glucoside (cyanin), cyanidin 3-0-glucoside, pelargonidin 3-0-glucoside (callistephin). Delphinidin 3-0-glucoside was reported here in *Dionaea muscipula* for the first time, while the other one was already known in the species (Di Gregorio and Di...
Fig. 1 Effects of the macro-components (NH₄NO₃, KNO₃, CaCl₂, MgSO₄·7H₂O and KH₂PO₄) in a half strength of MS agar medium supplemented with 1.5% sucrose on growth and coloration of clonal Dionaea muscipula and Drosera spathulata in vitro after 4 months treatment. A-F: Dionaea muscipula, G-L: Drosera spathulata, A, D, G and J: Large-size plants grown in the medium with the complete macro-components, B, E, H and K: Medium-size plants grown in the medium with 9.40 mM KNO₃ and 0.75 mM MgSO₄·7H₂O, C, F, I and L: Small-size plants grown in the medium with no macro-components. Bars=1 cm.
Fig. 2 Effects of macro-components of 1/2 MS medium in combination on red color pigmentation (line graphs) and growth in dry weight (bar graphs) in Dionaea muscipula (A) and Drosera spathulata (B).  •-• = delphinidin 3-0-glucoside. ▼-▼ = cyanidin 3,5-di-0-glucoside. ★ - ★ = cyanidin 3-0-glucoside. ◆ - ◆ = pelargonidin 3-0-glucoside. Relative peak areas of the anthocyanins (absorption maxima • sec) were measured at the wavelength of 510 nm by HPLC. Macro-component a: 10.31 mM NH₄NO₃; b: 9.40 mM KNO₃; c: 1.50 mM CaCl₂; d: 0.75 mM MgSO₄·7H₂O; and e: 0.62 mM KH₂PO₄.

Palma, 1961; Jay and Lebreton, 1972). All of the anthocyanins found here in Drosera spathulata have been reported in other species of Drosera (Gascoigne et al., 1948; Paris and Denis, 1957; Paris and Delaveau, 1959; Bienenfeld and Katzmeister, 1966; Bendz and Lindberg, 1968, 1970; Ayuga et al., 1985).

Thus, dilution of NH₄NO₃ and KNO₃ and increase of sucrose up to 1.5% mainly promoted and could be in relatively inverse proportion to depths of red-color anthocyanin pigmentation spread from glands or glandular hairs to entire leaves in the both species (Fig. 2- A, B). Insects contain total nutrients of N (99–121 g/kg dry weight), P (6–14.7 g/kg), K (1.5–31.8 g/kg), Ca (22.5 g/kg) and Mg (0.94 g/kg) (Reichle et al., 1969; Dixon et al., 1980; Watson et al., 1982) that are somewhat similar to the medium requirements studied here. Prey would be more attracted to and captured by red-colored plants of Drosera species than by green-colored ones. Generally, carnivorous plants might have adaptation strategies to barren, wet and low pH soil conditions by interaction between leaf carnivory and low root consumption of nutrients (Adamec, 1997). The present study suggests that the two species would turn red color when they became deficient in nitrogen compounds to make themselves attractive to prey and would catch more prey if they had too low a root consumption of nutrients to survive, grow and propagate. The anthocyanin pigmentation in the two species may make it possible to be biosensitive to nitrogen consumption uptake.

Since this phenomenon is observed throughout our tissue culture experience in Drosera adelae F. Muell., D. anglica Huds., D. binata Labill., D. burkeana Planch., D. capensis L., D. petiolaris R. Br. ex DC., and D. rotundifolia L. in the Drosoraceae (whole plant bodies), Nepenthes mirabilis Drue in the Nepenthaceae (pitchers especially peristomes), Cephalotus follicularis Labill. in the Cephalotaceae (whole pitchers), and Sarracenia flava L. and S. purpurea L. in the Sarraceniaceae, this methodology may be generalized to the majority of the red-color pigmented carnivorous plants.

References


