Sour Cherry (*Prunus cerasus* L.) Production Towards the Utilization for a New Century

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Keywords: Anthocyanins, callus cell culture, flavonoids, fruit, growth curves

Abstract

In the last years many studies on anthocyanins, the flavonoids responsible for the orange, red and blue colors in different plant organs, have revealed their antioxidant activity and a possible use as chemotherapeutics. The finding that tart cherries (*Prunus cerasus* L.) contain a great amount of anthocyanins, constituents that possess strong antioxidant and anti-inflammatory activities, have attracted much attention. Here, we report the preliminary results of the induction of anthocyanin biosynthesis in sour cherry callus cell cultures and the characterization of those callus cultures for cell growth and anthocyanin production. Callus cultures have been obtained from the leaf of in vitro grown shoots of two sour cherry cultivars, Amarena Mattarello (A.M.) and Stevnsbaer (S.). Several in vitro culture conditions have been tested to induce the anthocyanin biosynthesis using different media with reduced nitrogen and elevated sucrose contents in order to stimulate anthocyanin accumulation. No stimulatory affect from elevated sucrose concentration was observed, while reduced nitrogen and light seems to be the triggering factors. The pigment production is absent during the first days of growth at the light; afterwards, the pigment accumulation is stimulated by elicitors as light and nutritional factors, and reach a maximum nearly the 20th day of culture, during the exponential phase of growth. The in vitro pigment production system described in cherry callus cultures has given these preliminary but encouraging results, making the plant material very suitable and plastic to the in vitro manipulations.

INTRODUCTION

Anthocyanins, one of the major group of pigments, belonging to the secondary metabolite group of flavonoids, are responsible for the orange, red and blue colors in fruits, vegetables, flowers and other storage tissues in plants. The best known property of flavonoids in general is their strong antioxidant activity in metabolic reactions due to their ability to scavenge oxygen radicals and other reactive species, implicated in ageing process, cancer incidence and oxidative stress. Since it has been reported that anthocyanins inhibit the growth of cancer cells (Kamei et al., 1993) and act as chemotherapeutics for numerous diseases (Bomser et al., 1996), anthocyanins have become an important substance not only as food additive but also for medicine. The finding that tart cherries (*Prunus cerasus* L.) contain a great amount of anthocyanin (Wang et al., 1997) has attracted much attention to this species. Although cherry biomass is used as meat additive, the use of purified anthocyanins extracted either from fruit biomasses or from cherry cells cultured in vitro is a world novelty. Anthocyanins from cherry have been demonstrated to possess strong antioxidant and anti-inflammatory activities (Wang et al., 1999). Moreover, cyanidin, the anthocyanin aglycone, showed more efficient anti-inflammatory activity than aspirin. Thus, the production of a “natural aspirin” will represent an alternative pharmaceutical source for people with digestive tract ulcer or allergic to aspirin and to non-steroidal anti-inflammatory compounds. The production of anthocyanins from cherry fruits is restricted to the seasonal production.

Proc. XXVI IHC – Future for Medicinal and Aromatic Plants
Eds. L.E. Craker et al.
Acta Hort. 629, ISHS 2004
Publication supported by Can. Int. Dev. Agency (CIDA)

Plant cells/tissue cultures represent a continuous source of plant metabolites. Moreover, plant cell culture is an attractive production source, since it lends itself to scaling up or down according to the needs.

Here we report the preliminary results of the induction of anthocyanin bio-synthesis in sour cherry (*Prunus cerasus* L.) callus cell cultures and the characterization of those callus cultures in term of cell growth and anthocyanin production.

MATERIALS AND METHODS

Plant Material and Callus Induction

Callus cultures were inducted from the leaf segments of *Prunus cerasus* L. 'Amarena Mattarello' and 'Stevnsbaer' on a Callus Induction Medium (CIM) (Table 1). Leaf explants were taken from plants grown in vitro on a Plant Multiplication Medium (PMM) (Table 1). The explants were then incubated in a growth chamber at 25 ± 2 °C at the dark. Callus cultures were maintained on the same "Callus Induction Medium" (CIM) at the dark and transferred to fresh CIM medium every 3 weeks.

Biosynthesis Induction of Anthocyanins In Vitro

Callus cultures of both cultivar, at the final growth cycle, were transferred on different "Anthocyanin Induction Media" (AIM) (Table 1) and then incubated under the light (Philips TLD/83, 125 $\mu mol \cdot m^{-2} \cdot sec^{-1}$), with a 16 h-light photoperiod. Callus growth on different media, at the light or at the dark, has been reported for 40 days as fresh and dry weight. At the same time, anthocyanins from pigmented calli have been extracted, following the methodology reported by Francis (1982).

RESULTS AND DISCUSSION

Callus production from explants of both cultivar was different. The cv. Amarena Mattarello (A.M.) produced in a short time (20 days) an abundant and friable callus from nearly 100 % of the explants put on CIM, instead the cv. Stevnsbaer (St.) produced less abundant callus from nearly 40 % of the explants. The time course of A.M. callus growth, as fresh and dry weight, follows the typical curves of cell cultures and calli in vitro (Fig. 1). On AIM3, at the light, the increasing of FW was 2300 % and 2000 % for DW, instead on CIM, at the dark, was 2000 % for FW and 1500% for DW.

We tested different media with reduced nitrogen and elevated sucrose contents to stimulate anthocyanin accumulation, according to the experiment of Decendit and Mérillon (1996) on *Vitis* cell cultures, and Mori and Sakurai on strawberry suspended cells (1996). In media with elevated sucrose concentration (AIM1 and AIM2) we did not observe an increased anthocyanin accumulation in respect to the control (AIM0), but rather a slightly reduction in growth (data not shown). In media with a changed ammonium:nitrate ratio (AIM3-AIM8) we observed a slight increase in the anthocyanin accumulation. The time course of anthocyanin accumulation in A.M. callus cultures grown on AIM3 is reported in Fig 2. The pigment production is absent during the first days of growth at the light; afterwards, the pigment accumulation is stimulated by elicitors as light and nutritional factors, and reach a maximum nearly the 20^{th} day of culture, during the exponential phase of growth.

Even if these experimental data are preliminary and need to be confirmed by additional experiments, studies to deepen our understanding the aspects of anthocyanin biosynthesis in sour cherry cell cultures is worth investigating. On the other hand, the in vitro pigment production system from cherry callus has given these preliminary but encouraging results, making the plant material very suitable and plastic to in vitro manipulations. Previous studies on in vitro sour cherry propagation (Leva et al., 1981, Blando, 2001), protoplasts production and adventitious regeneration (Kondakova and Druart, 1997; Blando, in prep.) emphasized this aspect.

In conclusion, studies on triggering factors for anthocyanin biosynthesis, pigment identification and characterisation of key enzymes in the flavonoid pathway are the future research we intend to approach.

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Tables

Table 1. Composition of the media used in the experiments.

	Mineral salts and vitamins	Sucrose (g/L)	NAA (mg/L)	IBA (mg/L)	BAP (mg/L)
PMM	MS	30		0.1	0.5
CIM	MS	30	1		0.1
AIM0 (control)	MS	30	1		0.1
AIM1	MS	50	1		0.1
AIM2	MS	70	1		0.1
AIM3	MS (NH_4NO_3 free)	30	1		0.1
AIM4	MS (NH_4NO_3 free)	50	1		0.1
AIM5	MS (NH_4NO_3 free)	70	1		0.1
AIM6	MS (NH_4NO_3 & KNO_3 ½ concentration)	30	1		0.1
AIM7	MS (NH_4NO_3 & KNO_3 ½ concentration)	50	1		0.1
AIM8	Ms (NH_4NO_3 & KNO_3 ½ concentration)	70	1		0.1

Figures

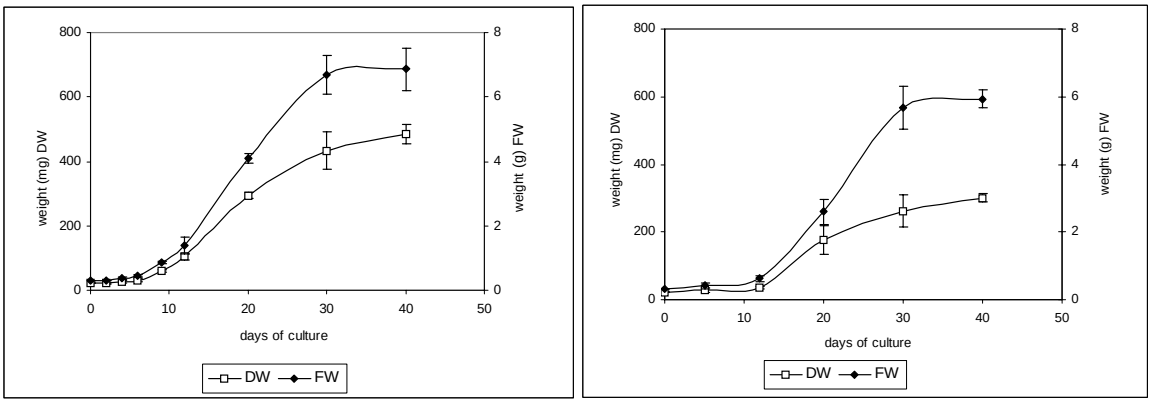

Fig. 1. Growth curves of A.M. callus on AIM3 medium (for anthocyanin biosynthesis induction) (on the left) and on CIM medium (for callus growing at the dark) (on the right); FW= fresh weight, DW= dry weight. Vertical bars represent standard deviation (n=3).

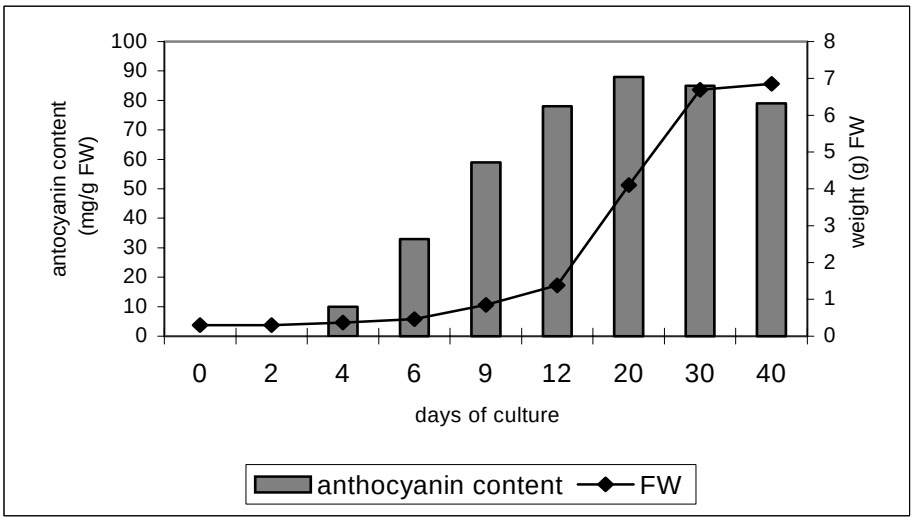

Fig. 2. Anthocyanin content of A.M. calli grown on AIM3 medium during the growth curve as fresh weight (FW).