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Research article

Signal transduction in artichoke [*Cynara cardunculus* L. subsp. *scolymus* (L.) Hayek] callus and cell suspension cultures under nutritional stress

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ABSTRACT

Stimulated production of secondary phenolic metabolites and proline was studied by using cell cultures of artichoke [*Cynara cardunculus* L. subsp. *scolymus* (L.) Hayek] submitted to nutritional stress. Artichoke cell cultures accumulated phenolic secondary metabolites in a pattern similar to that seen in artichoke leaves and heads (capitula). This paper shows that both callus and cell suspension cultures under nutritional stress accumulated phenolic compounds and proline, at the same time their biomass production was negatively affected by nutrient deficiency. The results obtained strongly suggest that plant tissues respond to nutrient deprivation by a defensive costly mechanism, which determines the establishment of a mechanism of trade-off between growth and adaptive response. Furthermore, the results of this research suggest that perception of abiotic stress and increased phenolic metabolites are linked by a sequence of biochemical processes that also involves the intracellular free proline and the oxidative pentose phosphate pathway. The main conclusion of this paper is that, once calli and cell suspension cultures respond to nutrient deficiency, in acclimated cells the establishment of a negative correlation between primary metabolism (growth) and secondary metabolism (defence compounds) is observed.

1. Introduction

Plants require adequate energy, water, and mineral nutrient supply to maintain optimal growth. Indeed, these resources to be allocated for growth, reproduction, and defence are limited. Therefore, according to first law of thermodynamics (the total amount of energy in a system remains constant), plants have to choose how distribute these limited resources amongst different physiological functions. This decision implies the existence of a trade-off among plant various functions (Bazzaz et al., 1987; Chapin et al., 1987; Caretto et al., 2015). In this connection, Herms and Mattson (1992) in a review dealing with ecological constraints on the induction of plant defence against herbivores say that "*plants are continuously facing the dilemma: to grow or defend*". This query was addressed to first land plants when they, moving from water to land, have found a habitat characterized by stressful conditions, such as UV radiations. Their successful adaptation to land was accomplished by producing UV-absorptive phenolic compounds derived from phenylalanine (Swain, 1975, 1981; Lowry et al., 1980, 1983).

Plants, being sessile, constantly synchronize their growth to their changing habitat that convey information to plants, which, in turn, adapt their physiology, morphology, and development. To survive under adverse environmental conditions plants must efficiently balance growth, reproduction and defence. This balance is achieved by means of dynamic responses, which involve a complex system for signal recognition and transduction integrating both endogenous and exogenous signals (Lattanzio et al., 2009; Krasensky and Jonak, 2012; Prasch and Sonnewald, 2015). How plants distribute resources among competitive functions under stress conditions is a subject that intrigues plant physiologists and biochemists. Several hypotheses have been proposed as context for investigating such a strategy (Stamp, 2003). An adequate understanding of how plants allocate their resources to various functions could allow to make predictions about their ability to adapt to the environment. In this connection, amount, classes, and biological activity of natural products have been considered as a part of this adaptive strategy (Fraenkel, 1959; Lattanzio et al., 2008).

During their evolutionary process, plants have demonstrated the ability to produce a large number of natural products, such as phenolic compounds, that are essential for their adaptation to the environment. Phenolic compounds in plant tissues act as defence (against pests and fungal pathogens) and signal compounds (to attract pollinating insects),

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as well as protecting the plant from harmful UV radiations. Plant productivity is strongly conditioned by these biotic and abiotic constraints. These conditions divert carbon skeletons from primary to secondary metabolism for the production of defence-related compounds and this, in turn, is reflected in decreases in the biomass production (Glynn et al., 2007; Cheynier et al., 2013). Biosynthesis of phenolics, in fact, uses as precursors some primary metabolites [erythrose-4-phosphate provided by oxidative pentose phosphate pathway (OPPP) and Calvin cycle, and phosphoenolpyruvate produced by glycolysis]. In addition, OPPP is the source of NADPH for phenolic compound biosynthesis. Therefore, OPPP activity is increased under conditions of increased carbon flux towards phenylpropanoid biosynthesis (Coley, 1988; Fahrendorf et al., 1995; Hare et al., 1999; Leone et al., 2014).

Metabolic adjustments in response to environmental stresses, such as nutrient deficiency, often include free proline accumulation. This increase has been related to the capacity of proline to promote osmotic adjustment, to protect enzymes and cell membranes, and to preserve $NAD(P)^+$/NAD(P)H ratio at physiological level (Hare et al., 1998; Kavi Kishor et al., 2005; Krasensky and Jonak, 2012).

The expression of plant responses to environmental stresses is thought to be restricted by multiple trade-offs. If resources are limited, plants that synthesize high levels of secondary metabolites should allocate less resources to growth, with consequent energy drain from the primary to secondary metabolism. This energy drain determines an adaptation to stressful habitat. Plant responses, at cellular and molecular level, to environmental stresses include perception of external signals and transmission of these signals to cellular machinery that, in turn, trigger adaptive responses. Accumulation of phenolics in plant tissues, as well as the increased activity of enzymes of phenolic metabolism, is a peculiar plant response to environmental stress (Coley et al., 1985; Van der Plas et al., 1995; Brown, 2002; Messina et al., 2002; Cipollini et al., 2003; Walters and Heil, 2007; Lattanzio et al., 2012; Matyssek et al., 2012).

The present research was aimed to study changes in growth, proline, and phenolic production in cell cultures of artichoke [*Cynara cardunculus* L. subsp. *scolymus* (L.) Hayek] under nutritional stress. In these conditions a growth-defence trade-off may enables plants to manage energy fluxes between competing physiological functions, thus negatively affecting plant growth when carbon skeletons are allocated to adaptive responses in stressed plant tissues. In addition, the paper also suggests a transduction pathway, between environmental stress and plant tissue response, involving changes in phenolic and proline metabolism.

2. Materials and methods

2.1. Plant materials and growth conditions

Callus and cell suspension cultures were established according to Caretto et al. (2011) using head and stem fragments of artichoke (var. Romanesco) plants. For callus induction, axenic fragments were incubated on agarized Gamborg B5 medium (Gamborg et al., 1968), integrated with 30 g/L sucrose, 0.2 g/L peptone from casein, 0.2 mg/L 6-benzylaminopurine (BAP) and 1.3 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), pH 5.7, in continuous light conditions (125 μmol photons/m^2·s) in a growth chamber. Once established, callus cultures were subcultivated every 30 days on agarized medium with the same composition as for induction. Suspension cultures were initiated as described by Caretto et al. (2011) by transferring pieces of calli into Erlenmeyer flasks containing Gamborg B5 liquid medium. Once established, cell suspension cultures were subcultivated every 30 days into Erlenmeyer flasks by transferring the 30-day-old suspension into fresh medium. Growth of artichoke cell cultures was evaluated by quantifying dry weight increase during the culture cycle. Lyophilized cell cultures were used for chemical analyses.

2.2. Stress conditions

In order to evaluate the effects of nutrient deficiency on growth rate and secondary metabolite production in plant tissues, artichoke callus and cell suspension cultures were subcultivated for 30 days in half-strength Gamborg B5 medium supplemented as described above, but using a half concentration. At the end of the treatment, suspension cell samples were filtered, frozen, and lyophilized overnight. Callus samples were directly frozen and lyophilized. Before metabolite extraction dried samples were weighed for evaluating growth rate. Again, lyophilized cell cultures were used for chemical analyses.

2.3. Phenolic metabolite extraction and analysis

Phenolic compounds were extracted and analysed according to Lattanzio and Van Sumere (1987). HPLC analyses were performed with a Hewlett Packard liquid chromatograph equipped with a spectrophotometric photodiode array detector, and a column thermostat set at 45 °C according to Lattanzio et al. (2009). The solvent system and the elution profile was as reported by Lattanzio and Van Sumere (1987).

2.4. Total phenolics evaluation

Total phenolics in plant cell extracts were assayed using a spectrophotometric method, which selectively detect ortho-dihydroxyphenolic compounds (Arnow, 1937). One mL of extract sample was placed in a test tube and 1 mL 0.5 N HCL was added. Tube was well mixed and then 1 mL Arnow's reagent was added. A pink colour was developed on adding 1 mL N NaOH. Absorbance was read at 515 nm. Total ortho-dihydroxyphenolic content was expressed as 5-*O*-caffeoylquinic acid (chlorogenic acid).

2.5. Determination of free proline

Free proline was assayed using ninhydrin method of Bates et al. (1973). Lyophilized plant material was homogenized in aqueous sulfosalicylic acid, centrifuged, then supernatant was placed in a reaction test tube. The sample reacted with 1 mL of acid-ninhydrin. The reaction mixture was extracted with 2 mL toluene. The absorbance of the resulting organic layer was measured at 520 nm. The proline concentration was expressed as μg/mg of lyophilized plant material.

2.6. Enzyme extraction and activity assay of glucose 6-phosphate dehydrogenase

Extraction of glucose 6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) activity was performed according to Tato et al. (2013) in a buffer solution pH 7.5 in the presence of 20% (w/w) poly(vinylpolypyrrolidone) (PVPP). G6PDH activity was measured as described by Rabotti et al. (1995) by monitoring $NADP^+$ reduction at 340 nm at 26 °C. G6PDH activity was calculated in nmol NADPH min^{-1} mg^{-1} protein.

2.7. Protein determination

Protein content in the extracts was determined by the Bradford (1976) procedure using bovine serum albumin as a standard.

2.8. Statistical analysis

Experiments were made in three replicate. Results are presented as mean ± standard error (SE). The statistical significance of data was evaluated through one-way analysis of variance (ANOVA). Statistical significance was defined as $p < 0.05$ (Tukey's test). Statistical comparisons were carry out using SigmaStat version 11.0 software.