Comparison of Micro-Array Profiling in Senescing Iris and Carnation Flowers

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Abstract

Gene expression profiles of cut Iris and carnation flowers were studied using cDNA microarrays. The cDNA libraries were enriched for flower-specific genes by subtraction with cDNA from subtending growing tissue. This strategy is meant to eliminate most household genes and numerous genes that are not specific for petals and senescence. In Iris, we spotted about 1400 clones and in carnation about 2000, of which 220 and 90 clones respectively were (partially) sequenced.

Unexpectedly, during Iris senescence up-regulation was observed for many genes that previously had been characterized as being defence-related. Although such genes were also found in carnation, their relative contribution to the changes in expression seemed less pronounced. Another remarkable result was the limited number of known ethylene-related genes in carnation that were detected. Among those found was ACO1. Other ethylene-related genes may have been lost in the subtraction; and ACO1 seems specific for the ethylene climacteric. No ethylene-related genes were found in Iris. Since ethylene does not regulate petal senescence in Iris this is no surprise.

Some similarities were found between Iris and carnation. In both species a considerable proportion of the up-regulated genes encode enzymes that are involved in the degradation of lipids, protein, and complex carbohydrates such as cell walls. Several genes involved in signal transduction and in transcription were observed to change expression levels in both species, but none were the same in both species, as judged from the limited sequence information. A novel EIN3 (EIL) transcription factor was discovered in carnation. The expression pattern of some putative transcription factors in carnation were expressed independently of ethylene treatment, and may be candidates for early regulators of traits such as ethylene senstivity.

The detailed results on Iris have been published in the December 2003 issue of Plant Molecular Biology (53: 845-865); the results on carnation have been submitted.

INTRODUCTION

Gene expression studies of flower senescence have been reported, for example, in carnation and in day-lily, in which senescence is and is not regulated by ethylene, respectively. These studies cover one or a just few genes at a time. Applying micro-array technology it is now feasible to evaluate the expression pattern of numerous genes at the same time. We used dedicated cDNA microarrays to study gene expression in the distal edge of Iris flag tepals, in which senescence is not regulated by ethylene, and in whole carnation petals. The details of this work are published elsewhere. Those on Iris can be found in Plant Molecular Biology 53 (6): 845-863 (2003), while the results on carnation have at the time of writing been submitted.

MATERIALS AND METHODS

For the methods we refer to the detailed publications.

RESULTS AND DISCUSSION

A main surprise was the discovery, in Iris, of several defence-related genes appearing to play a role in flower development. To gain some insight into the nature of the genes involved, 220 of the most differentially expressed genes were sequenced. Several clones were found of most defence-related genes, and in no other class of genes was the redundancy found to be so high. The genes were similar to: MtN3 (a senescence-associated defense gene), ribosome-inactivating protein (a toxin, involved in inhibition of protein synthesis), sesquiterpene synthase (related to terpenoid synthesis), thionin (a toxin, involved in membrane disruption), and tropinone reductase (involved in alkaloid synthesis). In carnation the number of defence-related genes detected was much smaller.

The absence of ethylene-related genes amongst the differentialy expressed genes sequenced in Iris was not surprising. However, the rather low number of ethylene-related genes in carnation was unexpected. It is possible that most of these genes had been eliminated in the subtraction process. If so ACO1, which we could detect, seems specific for the climacteric ethylene peak.

Genes encoding enzymes involved in the degradation of macromolecules were found in both species. The gene products were apparently involved in breakdown of lipids, proteins and carbohydrates. Some examples are:

- Lipid degradation. Acyl CoA oxidase, acyl CoA synthase, and GDSL-motive lipase. The acyl CoA enzymes are involved in beta-oxidation, that is, the conversion of lipids into sugars; and the lipase is involved in lipolytic activity.
- Protein degradation. Clp protease, and a cysteine protease.
- Carbohydrate metabolism. Invertase, sucrose synthase, triose phosphate isomerase.
- Cell wall degradation. Beta-galactosidase, pectinesterase, and xyloglucan endotrans-glycolase (XET).

Carnation

- Protein degradation: several cysteine proteases. Several proteins with homology to four different proteins that are involved in 26S proteasome-mediated protein degradation (This result supports a role for the ubiquitin system in petal senescence).
- Cell wall degradation: a beta-D-xylosidase-like gene was strongly upregulated, and the beta-D-galactosidase gene SR12 was also upregulated. Similar upreglation of expansin 2, an extracellular protein probably involved in cell wall breakdown.

We detected several genes putatively involved in the process of signal transduction and transcription. Some examples are here described.

Iris

1. AGAMOUS-like MADS-box factor. Most of these are important in early flower development, but some are upregulated in petals/tepals at a late developmental stage. In Iris, expression, on day 2, coincided with irreversibiltiy of the senescence program: senescence can then no longer be postponed by chemicals. The factor is expressed too late to control the onset of senescence.
2. Cyclic nucleotide channel protein. It is related to influx of Ca^{2+}; has reactive sites to cyclic nucleotides (e.g. cAMP), and to phosphorylation. Most of these proteins bind to calmodulin, and are known to be upregulated early during the leaf and petal senescence in *Arabidopsis*. In Iris a large increase occurred from day 0 to 2. It may therefore be a regulator of senescence in the epidermis cells but the increase seems too late for the control of the onset of senescence in the mesophyll cells. In the latter cells the first signs of senescence are visible, using electron microscopy, on day 0 (day of harvest).
3. Caseine kinase. This is a protein kinase of the CK type, some of which are key regulators of the cell survival pathway. In Iris it was highly expressed on day - 2 and -

1, and then decreased. It may be an early regulator of Iris tepal senescence.

Carnation

1. A novel EIL (environment condition induced lesion) transcription factor, part of the ethyene signal transduction pathway. EIL was upregulated by ethylene. The normal upregulation was prevented both by STS and by sucrose treatment.
2. CIPK6, a serine - threonine protein kinase. This protein interacts with calcium binding proteins. It is a regulator of phosphorylation cascades that transmit calcium signals.
3. An Aux/IAA like gene. This gene is, in other tissues, specifically induced by auxin. It is a nuclear protein, involved in auxin signalling.
4. Some proteins that were not affected by ethylene treatment, and therefore possibly early regulatory factors or factors that act independently of ethylene. These include several proteins of unknown function, a MYC-like DNA binding protein, and an F-box protein. Some of the latter types of proteins are known to initiate leaf senescence. The isolated F-box protein increased about two-fold just before the rise in autocatalytic ethylene biosynthesis.

It is concluded that cDNA microarray analysis of petal senescence is a very promising technique. It resulted in isolation of several proteins whose putative function had hitherto not been associated with senescence. The work also led to some speculation about the possible role of a calcium signal in the senescence of both Iris and carnation petals, and about a possible role of an auxin signal in carnation senescence.

The exact role of the most interesting genes, mainly those that are putatively involved in signal transduction and in transcription, need to be further evaluated by constructing plants in which these genes are silenced and/or overexpressed.

Literature Cited

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