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Genotypic differences in metabolomic changes during storage induced-degreening of chrysanthemum disk florets

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ABSTRACT

Selecting chrysanthemum cultivars with long storability and vase life is a major challenge for breeders. The rate of degreening of disk florets during the postharvest phase is an important determinant of vase life. There is large genotypic variation in susceptibility to disk floret degreening. Our aim was to understand these genotypic differences at the physiological level. Carbohydrate starvation seemed to play a role, since application of sugars prevented degreening and degreening only occurred if florets had a long-term low carbohydrate content. In order to find out which metabolic processes could explain genotypic differences, we used ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy profiling, High Performance Anion Exchange Chromatography (HPAEC) and respiration measurements to compare metabolic responses of three genotypes to carbohydrate starvation. HPAEC and NMR measurements showed that carbohydrate content could not fully explain genotypic differences. A genotype with intermediate sensitivity to degreening showed similar carbohydrate content compared to an insensitive one. However, respiration rate declined faster under carbohydrate starvation in a sensitive and intermediate sensitive genotype compared to an insensitive genotype, suggesting a more abrupt constraint on the mitochondrial electron transport chain and with that oxidative stress. Changes in the metabolic profile under carbohydrate starvation were diverse and revealed candidate processes associated with disk floret degreening. Camphor content increased and correlated positively with degreening insensitivity. Phenylpropanoids and flavonoids also increased upon carbohydrate starvation and the response was genotype specific. We propose the upregulation of the phenylpropanoid metabolism as important source of nitrogen in the form of harmful ammonia during carbohydrate starvation. Our results provide a framework to identify processes associated with genotypic differences in the response to carbohydrate starvation and susceptibility to floret degreening.

1. Introduction

The environment to which plants and their products are exposed during post-harvest life is often quite different from their original growth environment in terms of light, temperature, and availability of nutrients. During production in greenhouses, light intensity and temperature are optimized for fast growth and high yields, whereas the environment during post-harvest storage and transport is aimed at keeping the quality and appearance of the flower product as it was at harvest. Light deprivation often means absence of photosynthesis and therefore no *de novo* production of carbohydrates. However, respiration continues in the dark, especially in products that are harvested in a developmental stage characterized by fast growth and high respiration rate, like flowers and sprouts. Eventually, this leads to a state of carbohydrate starvation, which is often detrimental for plant quality, since carbohydrates are the main source for respiration in plants (Plaxton and Podestá, 2007). Carbohydrate starvation leads to oxidative stress (Couée et al., 2006; Morkunas et al., 2003), which eventually results in programmed cell death (Tiwari et al., 2002). The exact process leading from carbohydrate starvation to cell death remains largely unknown.

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Chrysanthemum (*Chrysanthemum* × *morifolium*) is an economically important cut flower, known for a relatively high postharvest performance compared to other cut flower species. However, after dry, dark, and cold storage during two weeks or more, some genotypes show quick degreening of disk florets, and eventually turn from green to yellow to brown, thereby losing market value. Yellowing is most likely due to loss of chlorophyll, while browning is likely the result of enzymatic oxidation of phenolic compounds into brown polymers, as is the case in many browning processes in plants (Ke and Saltveit, 1988; Pourcel et al., 2007). In unstressed cells, this oxidation is prevented by differential subcellular compartmentalization, but enzymes and substrates come together if membranes are leaky (Pourcel et al., 2007). Membrane damage can occur upon physical damage as in potato bruising (Bachem et al., 1994), during senescence (Thompson et al., 1998), or induced by stress, like carbohydrate starvation (Aubert et al., 1996).

Feeding flower stems with carbohydrates often increases vase life (Ichimura et al., 2005; Yakimova et al., 1996). In addition, feeding florets with sugars prevents degreening in broccoli (*Brassica oleracea* var *italica*) and *Arabidopsis thaliana* (Irving and Joyce, 1995; Trivellini et al., 2012). Finally, feeding chrysanthemum flower stems with sugars also reduces degreening (Van Geest et al., unpublished results). These results indicate that carbohydrate metabolism is very relevant for postharvest performance, specifically for problems related to degreening. There are large genotypic differences in sensitivity to degreening in chrysanthemum. Under the assumption that carbohydrate content plays a role in degreening sensitivity, these genotypes respond differently to a low availability of carbohydrates. Knowledge about genotypic variation in the response to carbohydrate starvation is generally absent. However, it has large potential to aid breeding for postharvest performance.

^{1}H nuclear magnetic resonance (NMR) allows simultaneous quantitation of both primary and secondary plant metabolites (Kim et al., 2010a), and therefore can be used to investigate a wide range of phenomena at the metabolomic level. Characterization of the carbon starved metabolome using ^{1}H NMR of *A. thaliana* cell cultures (Kim et al., 2007) and ^{31}P NMR of *Acer pseudoplatanus* cell cultures (Aubert et al., 1996), demonstrated marked increases of free amino acids, malic acid and phosphorylcholine. These observed metabolic changes are typical for carbohydrate starvation, but their amplitude or occurrence might differ strongly between genotypes that differ for sensitivity to carbohydrate starvation.

In order to investigate the genotypic differences in the metabolic response to carbohydrate starvation, we used a metabolome-wide approach to detect compounds that quantitatively vary during carbohydrate starvation. By constructing metabolomic profiles of disk florets using ^{1}H NMR and high performance anion exchange chromatography (HPAEC) at different time points in three chrysanthemum genotypes during carbohydrate starvation and feeding, we identified genotype specific and general metabolites associated with starvation. This paper discusses the differential metabolic response to carbohydrate starvation between genotypes, and proposes hypotheses that explain how a genotype can affect the tolerance to starvation of carbohydrates by altering its metabolome.

2. Materials and methods

2.1. Plant material and pre- and postharvest conditions

Three chrysanthemum genotypes were obtained from Deliflor chrysanten B.V. (Maasdijk, the Netherlands): 'DB39287', which is a single white type and sensitive to degreening of the disk florets after long storage (S), 'DB32030', a single purple type which is medium sensitive to degreening (MS), and 'DB36451', a single white type which is insensitive to degreening (I). Sensitivity was previously assessed by Deliflor Chrysanten B.V. (Maasdijk, the Netherlands) in commercial vase life tests. Plants were grown in a greenhouse in Maasdijk, the Netherlands using commercial growing practices. For initial respiration measurements, plants were grown from April to June 2014, harvested from large fields in the same greenhouse. For respiration measurements over time, plants were grown from August to November 2014 in a randomized block design. For both experiments, flowers were transported to Wageningen (the Netherlands) on the same day. For NMR, HPAEC and protein measurements, plants grown from July to September 2013 were used. Plots were planted in a randomized block design with five blocks representing five replicates. For each time point, one stem was harvested per plot. For all experiments, harvest took place in the morning, and stems were transported dry in cardboard boxes to Wageningen, the Netherlands. Except for respiration measurements, the combination of the disk florets of the upper three capitula of a flower stem was considered as one biological replicate. For all treatments, per stem the upper three capitula were cut in an angle of approximately 45°, and ray florets were removed. Disk florets sampled at day 0 were frozen in liquid nitrogen upon arrival at the lab. The cut ends of the three capitula were placed in a 35 mL pot containing 25 mg L^{-1} sodium dichloroisocyanurate (DICA) in demineralized water as bactericidal compound for the carbohydrate starvation treatment, or the same amount of DICA with 50 mM sucrose for the sugar feeding treatment. Solutions were replaced on every day on which sampling was performed. Pots with capitula were placed in a dark temperature controlled cabinet at 20 ± 1 °C. Air humidity was buffered using a 2 L saturated sodium chloride solution in the cabinet resulting in a relative humidity of approximately 75%. Samples of disk florets were taken at 0, 5, 10 and 14 days after harvest. When sampled, florets were flash frozen in liquid nitrogen and stored at −80 °C until freeze–drying.

2.2. Color measurements

To determine the change in degreening of disk florets over time, pictures of capitula were taken in a standardized light environment created by fluorescent light scattered by a Perspex plate bent in a half cylinder. A Hitachi HV-C20 video camera with a Tamron SP 35–80 mm objective was used for imaging. The average intensity of red and green of the disk florets was quantified using ImageJ (http://imagej.nih.gov/ij/), using a custom-made macro.

2.3. Respiration measurements

Nine capitula were placed in three 35 mL pots (three per pot) containing 25 mg L^{-1} DICA per biological replicate. For initial respiration measurements, flowers were stored dry at 4 °C overnight before excising the capitula and respiration measurements. For measurements over time, capitula were excised upon arrival in Wageningen, and placed in the 35 mL pots. Respiration measurements took place the next day. For respiration measurements, three 35 mL pots were placed in an airtight 1 L jar at 20 ± 2 °C. A 1 L jar was considered as biological replicate. Jars carried a septum and contained 40 mL saturated sodium chloride solution to buffer air humidity during the respiration measurement. Three jars per genotype were used. Jars were closed after one hour of temperature acclimation. After closure, 3 mL of air were withdrawn every 30 min for three hours. Oxygen concentration of the air sample was measured using a Dansensor CheckMate 3 headspace gas analyser (Dansensor A/S, Ringsted, Denmark). Dry weight was measured after respiration rate measurements by