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Breeding for postharvest performance in chrysanthemum by selection against storage-induced degreening of disk florets

Geert van Geest[a,b,c,*], Aike Post[b], Paul Arens[c], Richard G.F. Visser[c], Uulke van Meeteren[a]

[a] Horticulture & Product Physiology, Department of Plant Sciences, Wageningen University, P.O. box 16, 6700 AA, Wageningen, The Netherlands
[b] Deliflor Chrysanten B.V., Korte Kruisweg 163, 2676 BS, Maasdijk, The Netherlands
[c] Wageningen UR Plant Breeding, Wageningen University and Research Centre, P.O. Box 386, 6708 PB, Wageningen, The Netherlands

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ABSTRACT

Breeding for postharvest performance in ornamentals is challenging, since many different deteriorative processes determine vase life. In order to improve postharvest performance by breeding, selection should take place on these processes separately. To define processes that are important for chrysanthemum postharvest performance, vase life was assessed after two weeks of cold storage in a set of 44 chrysanthemum cultivars. Since disk floret degreening was the most frequent reason for ending vase life, we further investigated this trait in a large biparental population (n = 381). To quantify disk floret degreening in this large number of genotypes, we developed a high-throughput phenotyping method. The method consists of the quantification of loss of green color as expressed by an increase of intensity of red divided by the intensity of green (R/G) in dark-held detached capitula. R/G increases when disk florets lose green color. The increase in R/G correlated significantly with the number of days until disk floret degreening occurred during vase life. This was the case for the 44-cultivar cultivar panel (Pearson's correlation coefficient (ρ) of −0.70; $p < 0.0001$) as well as in a subset of the biparental pulation (n = 145; ρ = −0.67; $p < 0.0001$). R/G increase segregated in a quantitative manner in the full biparental population, and had a moderately high heritability of 0.73. Carbohydrate content after harvest was measured in a smaller subset of the biparental population (n = 55). The R/G increase correlated with carbohydrate content (ρ=-0.56; $p < 0.0001$). Since carbohydrate content did not explain all variation in degreening sensitivity, we discuss different possible mechanisms to cope with carbohydrate starvation and avoid degreening. In conclusion, disk floret degreening is an important postharvest trait in chrysanthemum, and it is related to carbohydrate starvation. The quantitative segregation suggests involvement of multiple alleles, probably at multiple loci. The moderately high heritability makes it a suitable trait for QTL mapping, which we will commence in the near future.

1. Introduction

In many crop plants, and specifically ornamental crops, breeding for postharvest quality is challenging. A reason for this, is that vase life and shelf life terminating symptoms vary within a crop (Fanourakis et al., 2013; Ferrante et al., 2015; Rico et al., 2007) implying that many different unrelated processes can explain genotypic variation. In addition, a wide range of pre- and postharvest environmental variation can interact with the time to occurrence and severity of these symptoms (Fanourakis et al., 2013). The interaction between genotype and environment is therefore an important factor to take into account while investigating postharvest performance.

Breeding aims to maximize the number of favourable alleles in a certain germplasm or genotype. Timing of occurrence and extent of different postharvest-related deteriorative processes is generally encoded by a broad set of alleles and loci (Carvalho et al., 2015; Moreno et al., 2008; Zhang et al., 2007). This large variation strongly impairs the development of breeding tools that allow estimation of the phenotype from a genotype. The overall postharvest performance of a certain crop should therefore be divided in specific parameters that can be measured reliably and are potentially encoded by a limited set of alleles. Genotypic improvement should take place at the level of these parameters.

* Corresponding author at: Horticulture & Product Physiology, Department of Plant Sciences, Wageningen University, P.O. box 16, 6700 AA, Wageningen, The Netherlands.
E-mail addresses: geert.vangeest@wur.nl, geert.vangeest@gmail.com (G. van Geest).

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There are successful examples of breeding for specific mechanisms to increase postharvest performance in cut flowers. An example is described by Onozaki et al. (2001) in which the authors could improve carnation vase life by specifically selecting against ethylene sensitivity. Another is described by Carvalho et al. (2015) in which the authors successfully located QTLs explaining vase life by assessing the stomatal control in rose using detached leaves.

During the postharvest phase, plants and their harvested products are often kept in low light environments or even in the dark. Production of carbohydrates is therefore strongly impaired while respiration continues, eventually leading to a state where carbohydrates are limiting. This results in senescence-like processes, for example loss of proteins (King et al., 1990) and chlorophyll (Buchanan-Wollaston et al., 2005; Trivellini et al., 2012), DNA fragmentation (Devaux et al., 2003), ammonium accumulation (Devaux et al., 2003; King et al., 1990), and eventually cell death. Reducing carbohydrate starvation or its symptoms has large potential to improve postharvest performance, as these processes lead to unwanted color changes and eventually increased susceptibility to necrotrophic microorganisms.

Variation in postharvest quality of cut chrysanthemum has been related to several different problems: leaf yellowing (Satoh et al., 2008), leaf wilting (van Doorn and Cruz, 2000; van Meeteren, 1992), flower wilting (Adachi et al., 2000), and disk floret degreening (van Geest et al., 2016). In order to define specific measurable parameters that explain vase life after storage in chrysanthemum, we assessed vase life of a genotype panel for a broad range of vase life terminating symptoms. Following two weeks of cold storage, disk floret degreening was the most frequently occurring vase life terminating symptom; therefore we developed a method to rapidly measure sensitivity for disk floret degreening derived from the *Arabidopsis* Infloresence Degreening Assay (AIDA; Jibran et al., 2015; Trivellini et al., 2012). In our previous paper (van Geest et al., 2016), in which we studied three genotypes, we suggest that carbohydrate content might play a role in explaining genotypic differences in disk floret degreening, but that it is not the only factor. In this study, we analysed a much larger set of genotypes as well as a biparental population to investigate the roles of initiation of senescence and carbohydrate metabolism in degreening sensitivity.

2. Materials and methods

2.1. Plant materials

This study describes two groups of genotypes: a cultivar panel and a biparental population. The cultivar/breeding line panel consisted of 44 genotypes with single (daisy-like) white flowers. The biparental population consisted of 381 offspring of a biparental cross (Fig. 1) between Deliflor breeding lines DB36451 and DB39287 (Deliflor chrysanten B.V. Maasdijk, the Netherlands). The parents were part of the cultivar panel. Plants were maintained and propagated vegetatively. Cuttings were rooted during two weeks and after that planted in a greenhouse in Maasdijk, the Netherlands (latitude: 51.959311; longitude: 4.214427). Harvest took place when a field consisting of plants of the same genotype reached commercial maturity. Plants growing at the border of a field were not used.

2.1.1. Cultivar panel

From the 44 members of the panel, 19 genotypes were grown in three replicates in a randomized design and the remaining 25 genotypes were grown in one replicate (Table 1). A replicate consisted of a field ranging from 20 to 100 plants, depending on the availability of cuttings. Of those, up to six stems were used for experiments (see below). Plants were grown from November 2015 to January 2016 in 16 h photoperiods for 14 days and after that in 12 h photoperiods to induce flowering.

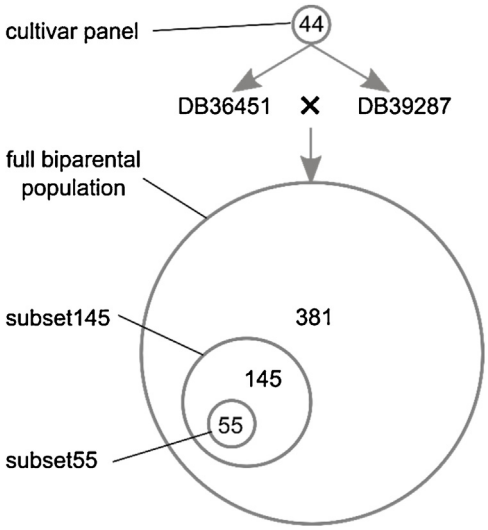

Fig. 1. A schematic overview of used plant material. The x represents a cross between genotypes DB36451 and DB39287. The numbers in the circles describe the number of genotypes in the population and subsets. Circle size is relative to the number of genotypes. The parents (DB36451 and DB39287) are included in all populations.

Table 1
Genotypes used in the cultivar panel.

Name	Breeder	Replicates
Astun	Deliflor Chrysanten B.V.	3
Bacardi	Dümmen Orange	3
Breeding lines[a]	Deliflor Chrysanten B.V.	1–3
Chic	Dümmen Orange	3
Delilah white	Deliflor Chrysanten B.V.	3
Gletsjer	United Selections	1
Goethe	Deliflor Chrysanten B.V.	3
Major White	Dekker Chrysanten B.V.	3
Neva	Deliflor Chrysanten B.V.	3
Pinot Blanc	Dümmen Orange	3

[a] Breeding lines represent 35 genotypes used in the breeding programme of Deliflor chrysanten B.V. They have no name, only a number. Of those, 11 genotypes were grown in three replicates, the rest in one replicate.

2.1.2. Biparental population

The offspring and parents were grown in a randomized block design with three replicates in three different seasons: summer (May to July 2015), late summer (August to October 2015), and autumn (September to November 2015). For the parents, three fields per flower trial were planted. Depending on the viability of the mother plants, fields consisted of 10 to 50 plants. Of those, up to six stems were used for experiments (see below). Plants were grown in 12, 12 and 14 days of 18, 21 and 21 h photoperiods for the summer, late summer and autumn replicates, respectively. After that, they were grown in 11 and 12 h photoperiods (depending on the season) to induce flowering. Since resources for carbohydrate measurements and vase life tests were limiting, we used a subset of 55 genotypes (including parents) for the carbohydrate measurements (here after referred to as 'subset55') and a subset of 145 offspring genotypes (including parents) for the vase life tests (here after referred to as 'subset145'; Fig. 1). Subset55 was completely included in the subset145, and vase life of subset55 was assessed in triplicate. The remaining genotypes in subset145 were measured