



## An eco-metabolomic study of host plant resistance to Western flower thrips in cultivated, biofortified and wild carrots

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### ABSTRACT

Domestication of plants and selection for agronomic traits may reduce plant secondary defence metabolites relative to their ancestors. Carrot (*Daucus carota* L.) is an economically important vegetable. Recently, carrot was developed as a functional food with additional health-promoting functions. Biofortified carrots contain increased concentrations of chlorogenic acid as an antioxidant. Chlorogenic acid is involved in host plant resistance to Western Flower Thrips (*Frankliniella occidentalis*), one of the key agricultural pests worldwide. The objective of this study was to investigate quantitative host plant resistance to thrips in carrot and to identify candidate compounds for constitutive resistance. As such we explored whether cultivated carrot is more vulnerable to herbivore attack compared to wild carrot. We subjected a set of 14 biofortified, cultivated and wild carrot genotypes to thrips infestation. We compared morphological traits and leaf metabolic profiles of the three most resistant and susceptible carrots using nuclear magnetic resonance spectroscopy (NMR). In contrast to our expectation, wild carrots were not more resistant to thrips than cultivated ones. The most thrips resistant carrot was the cultivar Ingot which is known to be tolerant against carrot root fly (*Psila rosae*). Biofortified carrots were not resistant to thrips. Plant size, leaf area and number of leaf hairs did not differ between resistant and susceptible carrots. The metabolic profiles of the leaves of resistant carrots were significantly different from those of susceptible carrots. The leaves of resistant carrots contained higher amounts of the flavanoid luteolin, the phenylpropanoid sinapic acid and the amino acid β-alanine. The negative effect of these compounds on thrips was confirmed using *in-vitro* bioassays. Our results have potential implications for carrot breeders. The natural variation of metabolites present in cultivated carrots can be used for improvement of thrips resistance. This is especially promising in view of the candidate compounds we identified since they do not only confer a negative effect on thrips but as antioxidants also play an important role in the improvement of human health.

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### 1. Introduction

Domestication of plants and selection for agronomic traits, such as yield, palatability or nutrition often resulted in a reduction of plant secondary defence metabolites relative to those of the ancestral lines (Wink, 1988; Rosenthal and Dirzo, 1997). Optimal defence models assume that plant defence is based on carbon and nitrogen resources that could otherwise be used for growth and reproduction, i.e. there are metabolic costs to defence (Herms and Mattson, 1992). In addition, greatly reduced phenotypic and

genetic diversity may further reduce resistance or can enhance adaptation to defence compounds by pathogens and herbivores (Kennedy and Barbour, 1992).

Carrot (*Daucus carota* L.) presents an example of a species with a long and well-known domestication history. Carrot has become an economically important vegetable crop worldwide due to its high yield potential and its use as fresh or processed vegetable. Carrot breeding focused on high yield, short growing period and excellent root colour (Stein and Nothnagel, 1995). In contrast, only few varieties resistant to pests or diseases were developed. Today's orange carrot was first observed in The Netherlands in the 17th century (Brandenburg, 1981). The orange colour is thought to be based on a unique mutation. Therefore, a limited gene pool has been utilized for the development of orange cultivars. Baranksi et al. (2012) studying the genetic diversity of carrot showed only a moderate divergence of gene pools of Asian and Western cultivated carrots.

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Genetic diversity of the Asian gene pool was higher than that of the Western gene pool. Also St. Pierre and Bayer (1991) observed a high degree of genetic similarity between commonly grown Western bunching and processing cultivars (St. Pierre and Bayer, 1991). Metabolic fingerprinting indicated quantitative differences in secondary metabolites between wild and cultivated carrot leaves (Grebenstein et al., 2011). Wild carrot leaves contained more terpenoids and phenylpropanoids.

Recently, carrot has gained popularity due to an increased consumer demand of nutritious and functional foods with additional health-promoting functions (Arscott and Tanumihardjo, 2010). Biofortified carrots contain especially high amounts of the antioxidant chlorogenic acid in the roots (Sun et al., 2009). Besides being implicated as an important plant dietary antioxidant chlorogenic acid is involved in host plant resistance to various pests. It affects various caterpillars (Bernays et al., 2000; Beninger et al., 2004), leaf beetles (Fulcher et al., 1998; Ikonen et al., 2002; Jassbi, 2003), leafhoppers (Dowd and Vega, 1996), aphids (Miles and Oertli, 1993) and thrips (Leiss et al., 2009a).

One of the herbivores affecting carrot leaves is Western flower thrips, a key agri- and horticultural pest worldwide causing substantial economic losses (Kirk and Terry, 2003). It is highly polyphagous with about 200 recorded wild and cultivated hosts (Lewis, 1997). Western flower thrips has piercing-sucking mouthparts causing two types of damage: growth damage leading to reduction in growth and yield and silver damage affecting product appearance and market quality (de Jager et al., 1995). In addition it is the main vector of tospoviruses (Maris et al., 2002). Carrot is a host of the tomato spotted wilt virus (TSWV) (Mc Pherson et al., 2003), the economically most important tospovirus transmitted by Western flower thrips. Thrips resistance is based on secondary metabolites as has been shown by Leiss et al. (2011) applying an eco-metabolomic approach. The metabolic profiles of resistant and susceptible plants were compared in order to identify candidate compounds for constitutive host plant resistance. Different compounds were involved in different plant species: pyrrolizidine alkaloids and a flavanoid in the wild plant *Senecio* (Leiss et al., 2009b), phenylpropanoids in the ornamental chrysanthemum (Leiss et al., 2009a) and acylsugars in tomato (Mirnezhad et al., 2010). Young leaves were more resistant to thrips damage compared to older leaves.

The objective of this study was to investigate quantitative host plant resistance to thrips in carrot and to identify candidate compounds for constitutive resistance. As such we explored whether cultivated carrot is more vulnerable to herbivore attack compared to wild carrot. We subjected a set of different carrot genotypes including biofortified, cultivated and wild carrots to infestations with Western flower thrips and compared morphological traits as well as metabolomic profiles of leaves. In particular we wanted to answer the following questions:

1. Are wild carrots more resistant to Western flower thrips compared to cultivated carrots?
2. Is thrips resistance in carrot related to morphological characteristics?
3. Which metabolites are involved in thrips resistance in carrot?
4. Is the amount of chlorogenic acid in carrot roots correlated with the amount of chlorogenic acid in the leaves?
5. Are biofortified carrots, rich in chlorogenic acid, resistant to Western flower thrips?

## 2. Results

### 2.1. Plant resistance to thrips and morphological measurements

A non-choice whole plant bioassay was conducted with the biofortified, cultivated and wild carrots to test for plant resistance to

thrips. Among the total set of carrot genotypes we observed significant differences in thrips damage to leaves, generally described as silver damage ( $F = 2.366$ ,  $df = 13$ ,  $p = 0.014$ , Fig. 1) as tested with one-way ANOVA. Three out of the four biofortified carrots were most susceptible to thrips. The maximum damage of  $199.40 \pm 75.74 \text{ mm}^2$  in the biofortified carrot Orange was ten times as much as the minimum damage of  $19.80 \pm 7.13 \text{ mm}^2$  in the cultivated carrot Ingot. Dry mass had no effect on silver damage ( $F = 0.665$ ,  $df = 1$ ,  $p = 0.418$ ). Excluding the biofortified carrots, silver damage did not differ significantly between wild and cultivated carrots ( $F = 0.024$ ,  $df = 1$ ,  $p = 0.823$ ). Within wild and cultivated carrots we didn't detect any significant differences either ( $F = 2.138$ ,  $df = 8$ ,  $p = 0.055$ ).

To study the relationship between thrips resistance and morphological traits leaf area and number of leaf hairs were measured, which were analysed performing one-way ANOVA. Number of leaf hairs did not differ between carrot genotypes ( $F = 1.565$ ,  $df = 13$ ,  $p = 0.129$ ), while leaf area, based on one single leaf, was significantly different among carrots genotypes ( $F = 2.641$ ,  $df = 13$ ,  $p = 0.007$ ) with the cultivated carrot Chantenay obtaining a maximum of  $5059 \pm 1065 \text{ mm}^2$  and the biofortified carrot purple-orange a minimum of  $1757 \pm 229 \text{ mm}^2$  leaf area. However, leaf area was not significantly correlated with silver damage ( $r = 0.045$ ,  $N = 14$ ,  $p = 0.722$ ).

### 2.2. Metabolic profiling

The amount of chlorogenic acid in roots and leaves of the different carrot types was measured by  $^1\text{H}$  NMR. Contrary to expectations the roots of most carrot genotypes contained only trace amounts of chlorogenic acid (Fig. 2), which could not be further analysed statistically. The contents of chlorogenic acid in the leaves varied from low to medium amounts with the highest amount in the wild carrot D1 (Fig. 2) and was not statistically different between susceptible and resistant carrots ( $F = 1.818$ ,  $df = 1$ ,  $p = 0.235$ ) as tested with nested ANOVA.

The  $^1\text{H}$  NMR leaf metabolomic profiles of the most resistant and the most susceptible carrot genotypes were then analysed by principal component analysis (PCA), an unsupervised method. Although not separating the resistant and susceptible carrot genotypes PCA showed a clustering of the resistant genotype D1 (Fig. S1A). We therefore, used partial least square-discrimination analysis (PLS-DA) to identify the metabolites involved in thrips resistance. This supervised technique not only uses the information of the metabolic matrix, but also takes the resistance matrix into account. PLS-DA showed differentiation between the resistant and susceptible groups (Fig. S1B). However, the variance of  $R^2 = 0.601$  and predictive ability  $Q^2 = 0.280$  showed that this model could not be validated. Usually,  $Q^2$  values greater than 0.5 are generally accepted as good. In order to remove the effect of inter sample variability between the leaves and to describe maximum separation orthogonal signal correction (OCS-PLS-DA) was applied. OCS removes variation from the X-data, the matrix of predictors, which is unrelated to Y, the matrix of responses. OCS resulted in a separation between the metabolites of resistant and susceptible carrot leaves (Fig. 3A) and a validated model with a variance  $R^2 = 0.970$  and a predictive ability  $Q^2 = 0.685$ . The resistant genotype D1 was clearly clustered at the positive side of PC1, while the remaining resistant genotypes were positioned more centrally with some overlap with the susceptible genotypes. A relatively high within genotype variability was observed.

A t-test was applied to the data set to determine the statistically significant signals between the resistant and susceptible carrot groups. From 239  $^1\text{H}$  NMR signals 17 were significant ( $P < 0.05$ ) which are described in Fig. 3B.

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