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Possible role of trichomes in resistance of strawberry cultivars against spider mite

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Abstract

The trichome types on leaves of garden strawberry (*Fragaria* × *ananassa*) were analyzed. Two types of trichomes was described. The first were unicellular long and thin simple trichomes, located mainly on leaf veins and leaf margins, mostly on the underside of the leaf. The second were smaller multicellular uniseriate glandular trichomes. These trichomes consisted of one basal epidermis cell, several stalk cells, and a single rounded head cell. Strawberry cultivars with different resistance against two-spotted spider mite (*Tetranychus urticae* Koch) were used to test a hypothesis that higher pubescence in strawberry cultivars is correlated with a higher degree of resistance against the herbivore. Nonglandular trichomes were not the resistance factor for strawberry cultivars against spider mite because trichome density was affected by growth conditions and developmental state. It was concluded that gladular trichome-localized inducible responses are among the potential resistance mechanisms against spider mite in garden strawberry.

Key words: Glandular hairs, polyphenol oxidase, garden strawberry, spider mite, trichomes.

Introduction

Morphological features of plant leaves are known to affect herbivores (Peters, Berry 1980). Among them, foliar trichomes are unicellular or multicellular structures arising from the epidermal tissues (Larkin et al. 1996). From a point of view of constitutive plant defences against herbivores, trichomes can be treated as a structural defence detering herbivores. Due to the large diversity of trichome types on different plant species, it is difficult to generalize any specific role of these structures in herbivore defence. However, it is generally believed that pubescent leaves will be more resistant against herbivores than less pubescent ones of the same species due to mechanical restrictions to herbivore activities caused by a high density of trichomes (Levin 1973). Thus, nonglandular trichomes are expected to act as a physical barrier to herbivores. However, experimental data does not always support this relationship.

Spider mite, *Tetranychus urticae*, is one of the most widespread pests on strawberry foliage. The data on the correlation between pubescence (the number of nonglandular trichomes) and resistance against spider mites are mostly contradictory. Resistance to *T. urticae* is positively correlated with increased pubescence in *Buddleia* L. taxa (Gillman et al. 1999). In addition, removal of trichomes increased oviposition of mites. It has been shown also that an increase in density of nonglandular trichomes for *Fragaria chiloensis*

Duch. decreased the number of eggs laid by *T. urticae* (Luczynski et al. 1990). Further, for cotton plants, cultivars with a higher number of highly branched trichomes had higher resistance against *T. urticae* (Kamal, Elkassaby 1965). However, experiments with other cultivated plants revealed a negative correlation between the density of nonglandular trichomes and the resistance against *T. urticae* (Kishaba et al. 1972; Yiem et al. 1993).

Chemical defence against herbivores may be associated with a different type of trichome e.a., glandular trichomes, usually containing putative defense metabolites (Levin 1973). However, the information in the literature on particular roles of glandular trichomes in plant resistance against herbivores is extremely limited.

Several studies have indicated significant differences in the tolerance levels of garden strawberry cultivars to *T. urticae* (Ferrer et al. 1993; Shanks et al. 1995; Shanks, Moore 1995; Petrova et al. 2000). Recently we have shown that cv. 'Zephyr', partially resistant to *T. urticae*, is characterized by a higher activity of wound-induced polyphenol oxidase and peroxidase in comparison to the more susceptible cv. 'Korona' (Steinite, Ievinsh 2002). Since the main proteinaceous component of glandular trichomes is polyphenol oxidase (Kowalski et al. 1992), it can be to suggested that the higher resistance of certain strawberry cultivars against *T. urticae* is associated with a trichome-localized inducible increase of polyphenol oxidase and peroxidase activities.

In spite of the idea that foliar pubescence might be related to spider mite resistance in strawberry (Kishaba et al., 1972), there is no detailed data available in the literature on trichome morphology in garden strawberry. Therefore, the aim of the present investigation was to analyse trichome types on leaves of strawberry and to test the hypothesis that higher pubescence in strawberry cultivars is correlated with a higher degree of resistance against the spider mite, *T. urticae*.

Materials and methods

Eight commercially grown garden strawberry (*Fragaria* × *ananassa*) with different resistance against two-spotted spider mite (*Tetranychus urticae* Koch) were used for the experiments: 'Zephyr', 'Venta', 'Tenira', 'Induka', 'Bogota', 'Senga Sengana', 'Kokinskaja Pozdnaja', and 'Korona' (Petrova et al. 2000). Plants were propagated in plant tissue culture on Murashige-Skoog micro- and macro-salt medium without hormones supplemented with glycine and vitamins (Steinite, Ievinsh 2002). Plants were grown in a greenhouse under natural light and temperature conditions for 3 months in plastic boxes containing commercial peat mixture with mineral nutrients. The experiment was conducted in June and randomly selected leaves were collected from plants to use in a disk bioassay. A sample for bioassay consisted of three leaf disks (14 mm in diameter) cut from adult leaves at the base of each leaf using a cork borer. All leaf disks were examined under a binocular microscope MBC-10 (magnification 14 x) from both surfaces. An additional leaf disk bioassay was performed with 'Zephyr' and 'Korona' plants in October.

To investigate the morphology of trichomes, fresh plant material from tissue culture as well as from greenhouse-grown strawberry plants was subjected to light microscopy. Epidermal peels from the underside of leaves were examined (magnification 250 x for Fig. 1 and 1000 x for Fig. 2).

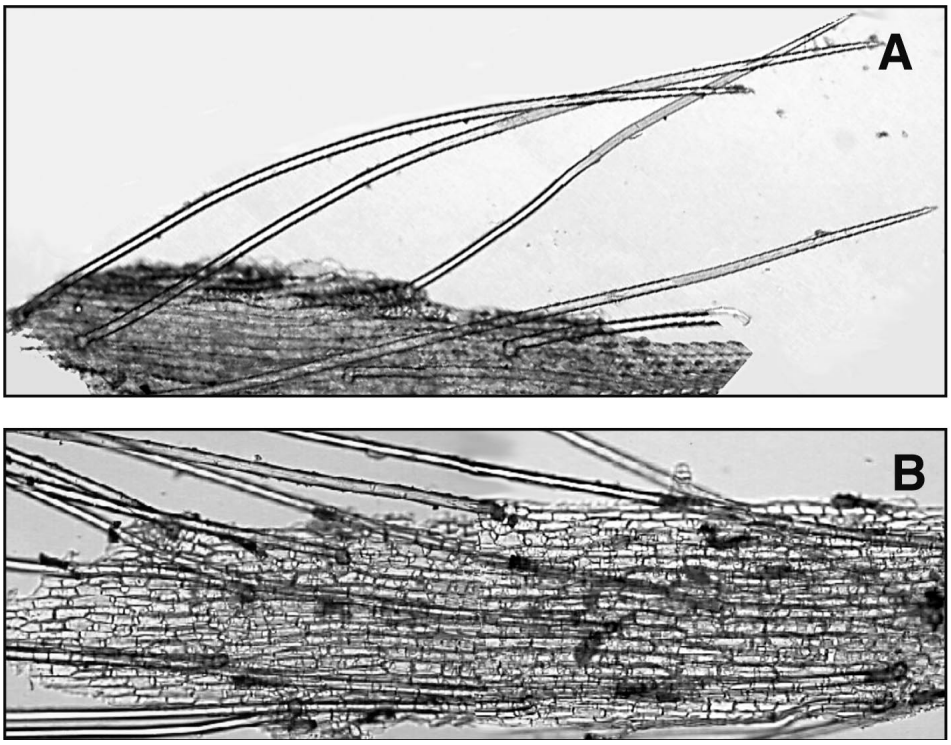

Fig. 1. Unicellular trichomes on leaves of strawberry cultivars 'Korona' (A) and 'Zephyr' (B).

Results

Strawberry leaves had two types of trichomes. The first were unicellular simple trichomes, long and thin, 0.8÷1.0 mm in length (Fig. 1). These trichomes were located mainly on leaf veins and leaf margins, mostly on the underside of the leaf. The second were smaller multicellular uniseriate glandular trichomes, 0.04÷0.05 mm in length, belonging to a type of a capitate glandular trichome (Fig. 2). These trichomes consisted of one basal epidermis cell, several stalk cells (3÷4), and a single rounded head cell.

Glandular trichomes differed in number and colour. Strawberry plants grown in vitro had a low number of glandular trichomes with colourless head cells on their leaves (Fig. 2A and B). For greenhouse-grown plants of the susceptible cv. 'Korona', the glandular trichomes were mostly colourless (Fig. 2C). In contrast, plants of the resistant cv. 'Zephyr' had a high number of glandular trichomes with dense red-brown colouring in their head cells (Fig. 2D).

To test for a relationship between the number of unicellular trichomes and resistance against spider mite, trichome density was measured for different strawberry cultivars grown in the greenhouse. For greenhouse grown plants in June, more susceptible cultivars in general had a higher number of trichomes on the underside of leaves than resistant

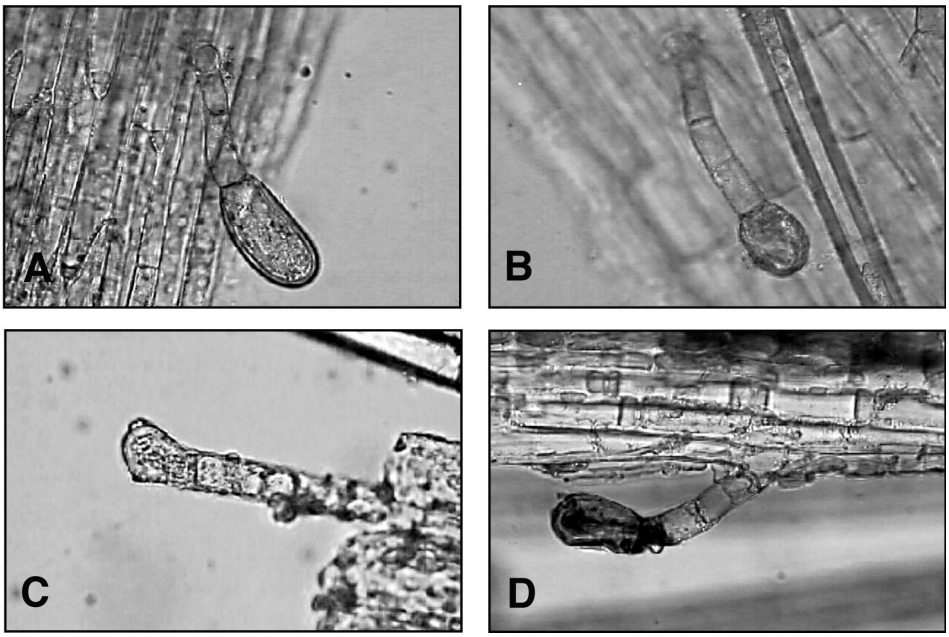

Fig. 2. Glandular trichomes on leaves of strawberry cultivars 'Korona' (A) and 'Zephyr' (B) grown in tissue culture and cultivars 'Korona' (C) and 'Zephyr' (D) grown in the greenhouse.

cultivars (Table 1). However, the relationship was not supported for several cultivars with partial resistance vs. partial susceptibility. When strawberry leaves were collected from greenhouse grown plants in October, the resistant cv. 'Zephyr' had a higher number of unicellular trichomes than the susceptible cv. 'Korona' on both sides of different types of leaves (Table 2).

Table 1. Number of unicellular trichomes on different sides of leaves of strawberry cultivars with different susceptibility vs. resistance against *T. urticae* grown in greenhouse conditions in June. Data are the means from 20 samples, 3 measurements each, for every data point ± S.E.

Cultivar	Susceptibility vs. resistance	Number of trichomes cm^{-2}	
		underside	overside
'Senga Sengana'	susceptible	309±38	0
'Kokinskaja Pozdnaja'	susceptible	289±21	0
'Korona'	susceptible	217±17	<1
'Venta'	resistant	200±26	15±4
'Zephyr'	resistant	188±21	22±4
'Tenira'	resistant	174±18	2±1
'Induka'	partially resistant	85±13	0
'Bogota'	partially susceptible	71±8	2±1

Table 2. Number of unicellular trichomes on different sides of leaves of strawberry cultivars grown in greenhouse conditions and in October. Data are the means from 20 samples, 3 measurements each, for every data point ± S.E.

	'Zephyr', resistant		'Korona', susceptible	
	underside	overside	underside	overside
On the leaflet of the lower leaf	2385±180	51±6	1298±128	9±3
On 1 disk of the lower leaf	244±20	49±5	62±9	3±2
On 1 disk of a growing leaf	132±15	37±4	132±20	<1

Discussion

Spider mites commonly feed in an inverted position on the underside of leaves. Therefore, to fulfill the hypothesis that higher pubescence is correlated with a higher degree of resistance against the spider mite, only trichomes located on the underside of the leaves should to be taken into the account. For strawberry plants grown in the greenhouse and collected in October, the resistant cv. 'Zephyr' had a higher number of unicellular trichomes on both sides of leaves than the susceptible cv. 'Korona' (Table 2). This relationship was not found for strawberry plants grown in greenhouse conditions in June, where more susceptible cultivars in general had a higher number of unicellular trichomes on the underside of their leaves than the more resistant ones (Table 1). However, for other arthropod herbivores, there is evidence of a defensive role of nonglandular trichomes. For example, a study on the role of soybean pod trichomes demonstrated that densely pubescent soybean has the potential to resist bean leaf beetle feeding on pods (Lam, Pediogo 2001).

Based on the present experiments, it appears that growth conditions and developmental state are among the significant factors regulating unicellular trichome density in strawberries. In Arabidopsis, trichome development is under a tight genetical control (Larkin et al. 1996). On the other hand, an increase of trichome density after herbivore attack is one of the induced defence responses (Mauricio et al. 1997; Agrawal 1998).

In light of the present experiments, it appears that nonglandular trichomes are not the resistance factor for strawberry cultivars but rather, the glandular trichomes containing oxidative enzymes. Although the number of glandular trichomes was not measured for strawberry cultivars with different susceptibility against *T. urticae*, plant leaves of the resistant cv. 'Korona' had dense red-brown colouring in head cells of glandular trichomes in contrast to colorless head cells for trcihomes on the susceptible cv. 'Zephyr' (Fig. 2). So far, only a few investigations have focused on the relationship between glandular trichomes and mite life histories. It has been shown that glandular hairs are associated with resistance in tobacco (Patterson et al. 1974) and tomatoes (Rodriguez et al. 1972). In addition, the glandular trichomes on alfalfa appeared to provide the major host resistance factor, with the resistance to adult leafhoffers being chemically based and resistance to nymphs being chemically and mechanically based (Ranger, Hower 2001).

It was shown that the main proteinaceous component of glandular trichomes of cultivated solanaceous plants is polyphenol oxidase, accounting for as much as 50-70 % of the total protein (Kowalski et al. 1992; Yu et al. 1992). Glandular trichomes contain

also peroxidase activity which further contributes to oxidative reactions (Levin 1973). Most likely, oxidative enzymes in trichomes are involved in oxidation and polymerization of phenolic constituents released from herbivore-damaged trichomes, which in turn act as a physical barrier preventing them from feeding. Previously it was thought that trichomes function primarily as a constitutive defense (Levin 1973). However, recently it was demonstrated that rapid trichome-localized chemical changes are part of induced defense against herbivores (Laue et al. 2000; Traw, Dawson 2002). The present data, together with previous results that strawberry cultivars more resistant to *T. urticae* are characterized by a higher inducible activity of oxidative enzymes (Steinite, Ievinsh 2002), indicate that gladular trichome-localized inducible responses are among the potential resistance mechanisms against spider mite in garden strawberry. More experimental evidence is needed to verify the hypothesis that induction of oxidative enzymes in glandular trichomes on strawberry leaves by *T. urticae* feeding is a main resistance factor against the herbivore.

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Lapu matiņu iespējamā piedalīšanās zemeņu šķirņu izturībā pret tīklērci

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Kopsavilkums

Analizēja lapu matiņu veidus uz dārza zemeņu (*Fragaria* × *ananassa*) lapām. Aprakstīti divu veidu lapu matiņi. Pirmkārt, gari un slaidi vienšūnas matiņi, kas izvietoti uz lapu dzīslām un malām pārsvarā lapu apakšpusē. Otrkārt, mazāki daudzšūnu dziedzermatiņi. Šie matiņi sastāvēja no vienas epidermālās pamatšūnas, vairākām kāta šūnām un vienas apaļas galviņas šūnas. Zemeņu šķirnes ar dažādu izturību pret tīklērci (*Tetranychus urticae* Koch) tika izmantotas, lai pārbaudītu hipotēzi par to, ka paaugstināts matiņu daudzums korelē ar attiecīgās šķirnes palielinātu izturību pret šo augēdāju. Vienšūnas matiņi nebija zemeņu šķirņu izturības faktors pret tīklērci, jo to biežums bija atkarīgs no augšanas apstākļiem un attīstības stadijas. Secināja, ka dziedzermatiņos lokalizētas inducējamās atbildes reakcijas ir viens no tiem mehānismiem, kas nodrošina dārza zemeņu izturību pret tīklērci.