TRICHOMES TYPES ANALYSIS AND THEIR DENSITY IN PARENTAL SPECIES SOLANUM TUBEROSUM AND S. CHACOENSE AND THEIR DERIVED SOMATIC HYBRIDS

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Abstract. Trichomes or hairs are appendices of epidermis which represent the first level of interaction between plants and herbivores or pathogens. The aim of this study was to identify differences regarding morphology or density in foliar trichomes corresponding to parental species and somatic hybrids. The third leaf was harvested from the plants of: S. chacoense, two cultivars of S. tuberosum “Delikat” and “Desiree” and 23 of their derived somatic hybrids obtained by mesophyll protoplast electrofusion. The source plants of all genotypes were first grew in vitro, and also transferred ex vitro, in a phytothron. The last type of plant material was used for density determinations. Somatic hybrids tend to maintain the typical hairs from S. chacoense. Differences concerning hairs structure are notable at both in vitro and ex vitro plants, depending on the genotype. The majority of trichomes types identified are corresponding to the Luckwill (1943) table. Mostly glandular trichomes density and the ratio between the two groups (glandular and non-glandular) are correlated with plant resistance to Colorado potato beetle.

Keywords: fluorescence, glandular and non-glandular trichomes, Leptinotarsa decemlineata, Solanum chacoense HL, somatic hybrids.

Introduction

Solanum chacoense Bitter is a wild species of potato sexually compatible to the cultivated species (Solanum tuberosum L. ssp. tuberosum), which is currently situated on the third position in the top of the most cultivated crops at global scale. Solanum chacoense is well known for its resistance to Colorado potato beetle (Leptinotarsa decemlineata L.). Two mechanisms of resistance were identified in this wild species: 1) leptines, a special acetylated form of glycoalkaloids only synthetized in the leaves of some S. chacoense accessions and 2) glandular trichomes.

Wild species of Solanum are often used in hybridization programs, because of their great phenotype diversification and many genes conferring resistance towards both biotic and abiotic stress. They are valuable resources in breeding and obtaining high quality genotypes (McCann et al., 2010, Hirsch et al., 2013).

Somatic hybridization by protoplast fusion, as a biotechnological tool, can assist in faster transfer of multiple resistance traits from wild species into their cultivated relatives (Thieme et al., 2010).

Solanum tuberosum L. possesses an extending capacity of adaptation, production potential and nutritional advantages (Hirsch et al., 2013). Potato presents also considerably quantity of starch in tubers. The potato is being grown at a large scale in the whole world and it has been adaptable to new conditions and forms of tubers. Nearly all the potato varieties are heterozygous autotetraploids (2n=4x=48). Wild species, including Solanum chacoense, are diploids (2n=2x=24) (Hirsch et al., 2013).

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Colorado potato beetle (*Leptinotarsa decemlineata*) belongs to the order Coleoptera, family Chrysomelidae. It is a common pest of Solanaceae family of plants and is capable to destroy the cultures totally (Miller & Parker, 2006; Metzger et al., 2008). In laboratory conditions it reveals resistance to at least 52 different chemical compounds, represented by major classes of pesticides, comprising pyrethroids, organophosphates and neonicotinoids (Alyokhin et al., 2008; Kumar et al., 2014). Insecticides remain the most efficient tool for exterminating beetle populations (Alyokhin et al., 2008; Alyokhin, 2009; Kumar et al., 2014), but their use not only select resistant beetles but are also a threat to the environment and human health.

Trichomes are epidermal protuberances localized on the leaves, petals, stalks, peduncles, stems and seeds coat surfaces. They present some physical properties like size or density, that allow them to protect plant buds against insect attack. They are used for reducing leaf temperature, increase light reflectance degree, prevent desiccation and reduce frictional force on the leaves (Wagner, 1991; Wagner et al., 2004; Dai et al., 2009).

Trichomes, glandular or non-glandular, have different forms and are already accessible, serving like an excellent model system for analyzing molecular mechanisms corresponding to plant cells differentiation. Hairs play a critical role in plant resistance to some pests and they have also the capacity to synthesize, store and secrete many secondary metabolites. Thus, they represent important sources of chemical compounds used in pharmaceutical industry, food additives, perfumes, or cosmetics (Callow 2000; Wagner et al., 2004).

In *Solanum* species trichomes morphology was described for the first time by Luckwill (1943) and later revised by Channarayappa et al., (1992). There can be distinguished eight types of hairs - four of them are glandular, respectively types I, IV, VI and VII, while the others types, II, III, V and VIII are non-glandular. Glandular types can be classified in capitate hairs, like types I and IV, or globular hairs, like VI and VII (Glas et al., 2012).

The aim of our study was to identify differences regarding morphology and density in foliar trichomes, corresponding to parental species and their derived somatic hybrids, in order to better understand their role in inducing resistance to Colorado potato beetle. It was found that mainly the density of glandular trichomes but also the ration between glandular and non-glandular groups correlates to the resistance of some of the somatic hybrids to Colorado potato beetle. The complexity of these small protuberances and their role in the interface between plant and their enemies or abiotic stress will be also discussed.

**Materials and methods**

The plant material was represented by parental species *Solanum chacoense* HL (an accession with the highest content of leptines, PI 458310, provided by NPGS Sturgeon Bay, USA), *Solanum tuberosum* cv. ‘Delikat’ (Dk), and ‘Désirée’ (De) and 23 of their derived somatic hybrids obtained by protoplast electrofusion. Some of the somatic hybrids involved mismatch repair (MMR) deficient *S. chacoense* with transgenic gene *Atmsh2* in antisense (S) orientation or a negative complementary form (noted with P), produced previously by genetic manipulation (Rakosy-Tican et al. 2004). The somatic hybrid clones are coded with numbers and C denotes wild type *S. chacoense*. For instance DkS10-5 is a somatic hybrid (SH) resulting from the fusion of mesophyll protoplasts of potato cultivar ‘Delikat’ and
mesophyll protoplasts of *S. chacoense* HL transformed with the gene *Atmsh2* in antisense orientation, clone 10, and having the number as SH =5.

As a first step, leaves or stalk’s epidermis was prelevated and put into a drop of distilled water, on a slide and covered with a coverslip. The preparation was visualized at the microscope with the aim of identifying the principal types of trichomes in the plants grown *in vitro* (according to the Luckwill’s table - 1943).

The next phase consisted in a density determination. The samples consisted in twenty-six genotypes of potato, including parental species, grown *ex vitro*, in a phytothron. For each genotype four leaves were harvested (situated at the third and the fourth node) and three photos were taken under stereomicroscope. The plant material was preserved in alcohol 70%. Glandular and non-glandular trichomes were counted by means of Adobe Photoshop CS6, Count Tool extension. Then Microsoft Excel T Test was used to calculate density of hairs/ cm² and determine standard deviations (SD). The differences of density and total number of trichomes per each genotype were established.

The results were correlated with each genotype resistance to Colorado potato beetle as determined in laboratory bioassay (Imola Molnár - unpublished data). Leaves maintained in alcohol 70% were also used for hairs morphological analysis, repeating the same procedure described before. This step needs to be repeated and refined, in order to describe in more detail the trichomes types of the plants grown *ex vitro* in a phytothron or in a greenhouse. Trichomes analysis was technically supported by light and fluorescent microscopy (Olympus BX60 with video camera XC50 and LabSens software). A stereomicroscope was used to visualize the leaf abaxial hairs and digital pictures were recorded (Olympus digital camera Camedia C-5060) by using the same magnification of stereomicroscope and computer assisted image assessment. The data were statistically analyzed.

**Results and discussions**

**Trichomes morphology**

It seems that the somatic hybrids tend to maintain basic forms of hairs inherited from *Solanum chacoense*. Regarding *Solanum tuberosum* „Delikat” and „Désirée”, the trichomes present on the *in vitro* leaves and stalks were different. Double light microscopy observations reveal a large series of morphological details which can’t be seen in direct light optical microscopy (Figs. 1-3).

The wild species and hybrids presented fluorescence, while the cultivated ones hadn’t this feature entirely. This fluorescence of both non-glandular and glandular trichomes might be related to specific metabolites, probably secondary ones, wich are biosynthetized and stored in other trichomes, as it was demonstrated by other authors (see Glas et al., 2012 and reference within). Moreover, the morphology of the trichomes in the parental species used in our study was not investigated before and fluorescence studies and its relation with hairs content was also not presented by other authors. It is then evident that the 23 somatic hybrids, which represent a unique plant material obtained by cell fusion, represent a very interesting object of investigation for understanding the roles of trichomes in inducing resistance to herbivory, in that case to consumption by Colorado potato beetle.
Figure 1. A sample of light microscopy images showing some of the differences between parents and somatic hybrids: A - *Solanum tuberosum* ‘Delikat’; B - *Solanum chacoense* HL; C - somatic hybrid DkS10.61 (minimum number of trichomes); D - somatic hybrid DkS10.61 (maximum density of trichomes). Bar = 100 μm

Figure 2. A practical example of trichomes distribution on leaves of *ex vitro* grown plants. Pointed with white arrow are non-glandular hairs and pointed with black arrow, the glandular ones; A – *Solanum tuberosum* ‘Désirée’ has quite a large number of non-glandular trichomes but fewer glandular ones; B - *Solanum chacoense* HL, a species known as being resistant to Colorado beetle which shows a low density of non-glandular trichomes but the glandular small ones are very dense; Bar = 100 μm;
Figure 3. Principal types of trichomes found in the studied genotypes (in vitro plants), were II, III, V, VI and VII. A – *Solanum tuberosum* ‘Désirée’, glandular trichome, type VII; B – *Solanum tuberosum* ‘Delikat’, glandular trichome, type VII; C – *Solanum chacoense* HL, stalk’s glandular trichomes, type VII; D – *Solanum chacoense* HL, observations under epifluorescence, glandular trichome, type VI; E – DK.P11.24 (somatic hybrid); a – non-glandular trichome; b – glandular trichome, type VII; F – DK.P11.24, observations in direct light microscopy; a – glandular trichome, type VI; b – non-glandular trichome, type III, c – non-glandular trichome, type V;

All the different types of trichomes were compared with the results obtained by Imola Molnár in a laboratory bioassay of Colorado potato beetle (CPB) viability when reared on leaves belonging to different somatic hybrids or parental genotypes (unpublished data). There are three groups of genotypes, classified depending on their resistance potential to CPB - the first one with the lowest resistance, followed by one with a moderate resistance and the last one with the highest resistance, comparable or even higher than *S. chacoense* HL. The group with the highest resistance contains also the clones having higher densities of glandular trichomes (Fig. 4). These data correlates with previous other studies in *Lycopersicon hirsutum*, found to produce in the glandular trichomes zingiberene, a sesquiterpene, which was toxic to CPB (Carter et al. 1989; Glas et al., 2012). There are no such data for cultivated potato or its wild *Solanum* relatives known to be toxic or deter this voracious pest (i.e. CPB).

*Solanum chacoense* HL is very resistant to Colorado potato beetle and trichome analysis reveals a great number of glandular ones. Also, genotype 1552/1/7, a back-cross BC1 between potato and another accession of *S. chacoense* proven to be resistant to CPB, has more glandular trichomes then the others clones (Fig. 4). The most balanced ratio between glandular and non-glandular hairs appears to be in the parental cultivars of *S. tuberosum* and the genotypes of DK.S10.13 and BC1-1552/1/18. The first, DK.S10.13 is a somatic hybrid between cv. ‘Delikat’ and MMR deficient *S. chacoense* HL transformed
with antisense \textit{Atmsh2} gene. The 1552/1/18 is again a BC1 clone of the same somatic hybrid as above.

\textbf{Figure 4.} The representation of the total number of glandular and non-glandular hairs – genotypes on the horizontal axis and value thresholds on the vertical one.

\textit{Solanum tuberosum} ‘Delikat’ and ‘Désirée’ are not resistant to CPB and have more non-glandular hairs than glandular ones but the ratio between the two groups is almost equal (Fig. 4). Another characteristic of them is that they have fewer glandular trichomes than \textit{S. chacoense}, but more than other hybrids that seem to be resistant. A correlation between hairs density and insect pest resistance was previously indicated by Levin (1973). Glandular hairs in particular can deter or poison pests or pathogens by their secretions, but the biochemistry of the very diverse types of trichomes is poorly understood (Werker, 2000). Although the principal biochemical groups that are biosynthesized in different trichomes were identified in some plant families, including Solanaceae, they were not yet studied in the cultivated potato and its wild relatives (Glas et al., 2012). Previously, it was thought that only glandular trichomes are biosynthesizing diverse alkaloids, nevertheless the detailed studies in \textit{Artemisia annua} reveal that specific terpene metabolic pathways are present in filamentous, non-glandular, trichomes (Soetaert et al., 2013). Moreover, in \textit{Arabidopsis thaliana}, QTL analysis demonstrated that the density of the trichomes might change in response to wounding and hence herbivores attack (Bloomer et al., 2014). This adaptive strategy has to be demonstrated in other plant species, like \textit{Solanaceae}, as well. In \textit{Solanum} the model species for trihomes investigations is tomato. One of the greatest trichome projects is the \textit{Solanum} Trichome Project, centered on tomato and related wild
species, very representative for the *Solanum* genus and Solanacee’s family (http://www.trichome.msu.edu/about/overview_continued.html). However, with all the data on trichomes biochemistry, metabolism, genome databases on expressed sequence tags or metabolite profiling, *Solanum tuberosum* and its wild relatives excepting *S. habrochaites*, are very rarely used to study trichomes morphology or the metabolites they produce, store or secrete (Glas et al., 2012). Although, the knowledge on tomato will surely prove very useful for comparative studies, the great diversity and specificity of biochemical compounds present in the trichomes, like: terpenes, phenylpropenes, flavonoids, methyl ketones, acyl sugars or defensive proteins (Glas et al., 2012 and references herein), makes it difficult to identify very specific compounds involved in the interactions with each pathogen and pest. Flavonoids, are known to be involved in the protection of the plants from the aggressive UV light (Glas et al., 2012), but they are also autofluorescent compounds. Their presence or polyphenols might be responsible for the green fluorescence we observed in both non-glandular and glandular hairs of the somatic hybrids and *S. chacoense*. Moreover it is likely that besides common biochemical structures very specific ones occur in such a complex interaction. This is why we undertake this present analysis of trichomes in potato cultivars and the species *S. chacoense* and their derived somatic hybrids. *Solanum lycopersycum* cv. Moneymaker, the cultivated tomato, presents trichomes: I, III, V, VI, VII, and VIII, according to Luckwill table (Glas et al., 2012). In comparison to *Solanum tuberosum*, glandular trichomes density is low in both species (Glas et al., 2012). In the same article, image of type VI, and also in the text, reveal that this trichome has the head made by four cells, all of them placed on a bicellular stalk. (McDowell et al., 2011; Glas et al., 2012) The same type could be identified in our genotypes.

**Density – statistical analysis**

The data was statistically analyzed using T Test (Microsoft Excel) and all the genotypes were compared with parental species and the resulted table was uploaded below (Table 1).

Table 1. Statistical significant differences between trichome density, are represented by “+”, when the value is significantly bigger than the density of the parental species with which it is compared and “-“, when the density value is significantly lower. Where the sign is missing the values are approximately equal.

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<tr>
<th>Genotypes</th>
<th>Glandular trichomes density</th>
<th>Non-glandular trichomes density</th>
<th>Glandular trichomes density</th>
<th>Non-glandular trichomes density</th>
<th>Glandular trichomes density</th>
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<td>Comparison with Delikat</td>
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From Table 1, it is to be pointed out that many of the hybrids have significantly higher non-glandular trichomes densities if compared with *S. chacoense*, i.e. 11 genotypes out of 23. Some of them are also having a density significantly higher as the cultivars from which they derive, as DeP11.29 differing from ‘Desiree’ in glandular trichomes density, or DeP11.5 that differ in non-glandular trichomes from cultivated parent. These studies have to be further developed to better understand the role of trichomes in the interrelationship between plants and CPB herbivore, as well as the metabolic pathways and their genetics, as it is partly known for the model plant *Arabidopsis*.

**Conclusions**

Trichomes densities and types varies in the somatic hybrids analyzed, some of them having very high and other very low number of hairs. There is a variation also in the same hybrid. All the data was correlated with the resistance to CPB. There is significant correlation between glandular trichome density and resistance to CPB, demonstrating that glandular hairs play an important role in plant defense to herbivory.

The wild species and the majority of the hybrids present a green fluorescence that might be caused by flavonoids. Biochemical characterization of the trichomes will reveal more data on their role in plant resistance to CPB.
Solanum chacoense HL and the BC1 1552/1/7 score the greatest glandular trichome density than the other genotypes, suggesting their resistance to CPB. Somatic hybrid De.P11.5 reveals the largest number of non-glandular hairs.

Acknowledgements

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REFERENCES


http://www.trichome.msu.edu/about/overview_continued.html