

Comparative study of trichomes in three parental *solanum* species and their somatic hybrids, cultivated in greenhouse or phytotron

Antonia-Maria Mărgineanu¹, Imola Erdelyi-Molnár¹
and Elena Rakosy-Tican^{1,✉}

SUMMARY. Trichomes represent one of the most important anatomical structure of plants, involved in protecting them against herbivores or other pathogens. The aim of our study was to compare trichome morphology in *Solanum* parental species and sexual or somatic hybrids cultivated in a greenhouse or phytotron. The analysis were performed on the leaf abaxial epidermis by using light and epifluorescent microscopy. In total, six out of the eight types of *Solanum* specific trichomes, firstly described by Luckwill (1943), were found. There were not significant differences between the plants grown in phytotron or greenhouse. However, we observed variation in the trichomes morphology in different hybrid plants as compared to their parents.

Keywords: Colorado potato beetle, *Solanum chacoense*, somatic hybrids, trichomes.

Introduction

It is necessary to use the most valuable cultivars of potato to obtain an extended crop production. Genetic resources which are useful to improve the quality of commercial species were exploited just to a limited degree along the time mainly because sexual incompatibility. The literature and technology available until this moment, allow us to find new possibilities to operate with the existing resources (Bradshaw *et al.*, 2006).

Trichomes arise from epidermal cells and they are common in all terrestrial plants. They have different shapes, can be easily observed and also, they serve as an excellent model system to analyse molecular mechanisms corresponding to plant cell differentiation, such as cellular death, supervision of the cellular cycle and morphogenesis (Yang and Ye, 2013).

¹ Faculty of Biology and Geology, Plant Genetic Engineering Group, “Babeș-Bolyai” University, Cluj-Napoca, Romania

✉ **Corresponding author: Elena Rakosy-Tican;** Faculty of Biology and Geology, Plant Genetic Engineering Group, “Babeș-Bolyai” University, Cluj-Napoca, Romania, E-mail: arina5744@yahoo.com

In 1943, Luckwill was the first who grouped the *Solanum* trichomes in eight different types, based on their different morphological characteristics. Later, Channarayappa *et al.* (1992) revised the classification made by Luckwill. However, in our study, we used Glas *et al.* (2012) trichome phenotypical traits table, which is a summarised version of the above mentioned researchers results (Table 1). Properly, there can be distinguished: four types of glandular (I, IV, VI, VII) and four non-glandular hairs (II, III, V, VIII) (Glas *et al.*, 2012).

Table 1.

Description of glandular and non-glandular trichomes types,
based on Luckwill (1943) and Glas *et al.* (2012).

TRICHOME TYPE	GLANDULAR/ NON-GLANDULAR	DESCRIPTION
I	GLANDULAR	Tiny trichomes, made by six to eight cells with a small, round cell at the tip and a globular, multicellular base.
II	NON-GLANDULAR	Similar to the first type, but shorter, with a globular and multicellular base.
III	NON-GLANDULAR	Fine hair, made by 4-8 cells and a unicellular, horizontal base.
IV	GLANDULAR	Analogous to type I, but shorter, the head is constituted by a glandular cell.
V	NON-GLANDULAR	Similar to type IV, but has no secretory cells.
VI	GLANDULAR	Short and delicate, with four cells head.
VII	GLANDULAR	Undersized trichomes, the heads are composed by 4-8 cells.
VIII	NON-GLANDULAR	Unicellular base and a bending tip, made by one single cell.

Glandular trichomes possess a large variety of glands. Differences between them consist in chemical composition of the secreted, accumulated or absorbed substances and in the way they are produced. The chemical compounds in glandular trichome cells are secreted through their secretive extremity. A typical glandular hair is composed of uni- or multicellular base, uni- or multicellular stalk and uni- or multicellular head. Sometimes, a binding cell between the head and the stalk part can be identified. However, some uncommon, specific shaped trichomes were also described (Werker, 2000).

Non-glandular hairs have different morphology and anatomy. Generally, their classification is made according to their morphology. They can be uni- or multicellular, branched or unbranched. The connections between trichome cells can be visible or not. There are differences concerning the trichome cells shape, length, size, vertical symmetry or asymmetry and uniformity of the hairs width. The diameter of trichomes could be variable along the hair, therefore they can have sharpened, cut or rounded vertices (Werker, 2000).

It is well known that wild *Solanum* species represent important sources of resistance to Colorado potato beetle (CPB) (*Leptinotarsa decemlineata*) (Jansky *et al.*, 1999) and other diseases and pests.

Somatic hybridization is among the most utilized and efficient methods for a successful transfer of genetic resistance from the wild species into potato cultivars gene pool (Chen *et al.*, 2004; Pelletier *et al.*, 2011). That way the development of CPB resistance to insecticides is avoided by using resistant host plants (Austin *et al.*, 1985; Deimling *et al.*, 1988; Helgeson *et al.*, 1988; Jansky *et al.*, 1999). Colorado potato beetle is one of the most dangerous herbivore of potato. Even a single generation can destroy more than 40% of the annual production, causing large financial losses every year (Noronha *et al.*, 2002; Pelletier and Dutheil, 2006). One of the most effective wild *Solanum* species against CPB attacks is *Solanum chacoense*.

The purpose of this study was to reveal some morphological details about different trichome types found in *S. chacoense*, two *S. tuberosum* cultivars and some of their derived somatic or sexual hybrids. Somatic hybrids (SHs) have been previously obtained by mesophyll protoplast electrofusion. The trichomes were analysed by optical microscopy and compared in the plants grown in a greenhouse or phytotron.

Materials and methods

The plant material consisted in twenty genotypes, grown both in phytotron and a greenhouse, and as such used to compare their trichomes types and morphology.

In the phytotron, day-night alternation was of 16 h light period and 8 h dark. Other environmental factors were represented by 40% humidity, 21°C temperature and 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light intensity. The growing conditions in the greenhouse were: photoperiod of 16 h, temperature maintained at 24°C, sunlight intensity and the proper humidity which was reached with daily soil watering.

In our experiment, two accessions of *Solanum chacoense* were used: PI 458310 (the high leptine producer) grown in phytotron, and 138, from Groß Lüsewitz Genebank, grown in a greenhouse. From *S. tuberosum* ssp. *tuberosus* two commercial cultivars Delikat and Desiree were also used. Besides these genotypes, a series of somatic hybrids were studied too.

Table 2.

The somatic hybrids which were assessed for trichome morphology.

HYBRIDS/BCs	PARENTAL GENOTYPES	OBSERVATION
1552/1	<i>S. tuberosum</i> cv. Delikat <i>S. chacoense</i> 138	
1552/1/1 1552/1/2 1552/1/3 1552/1/4 1552/1/7 1552/1/18	1552/1 <i>S. tuberosum</i> cv. Sonate	The hybrid was backcrossed with the cultivar Sonate and the resulted hybrids were named BC1s
1553/1/7	1553/1 <i>S. tuberosum</i> cv. Sonate	The hybrid was backcrossed with the cultivar
1552/1/7/1 1552/1/7/2	1552/1/7 <i>S. tuberosum</i> cv. Romance	The hybrid was also backcrossed with cultivar and were obtained BC2s
Dk.S10.5 Dk.S10.13 Dk.S10.35 Dk.S10.43 Dk.S10.61	<i>S. tuberosum</i> cv. Delikat <i>S. chacoense</i> PI 458310	<i>S. chacoense</i> PI 458310 is a DNA mismatch repair (MMR) deficient accession, which contains a mutant <i>Atmsh2</i> gene in antisense orientation, from a <i>Arabidopsis thaliana</i> gene
De.C7	<i>S. tuberosum</i> cv. Desiree <i>S. chacoense</i> PI 458310	
De.P5.5 De.P11.5	<i>S. tuberosum</i> cv. Desiree <i>S. chacoense</i> PI 458310	<i>S. chacoense</i> PI 458310 is MMR deficient, with complementary negative <i>Atmsh2</i> gene.

In our study, abaxial epidermis of the third and fourth leaves was removed, placed in distilled water on a microscope slide and investigated by optical microscopy.

We intended to succeed with identification of all type of trichomes which are present on each potato genotypes.

This method allow us to observe a series of morphological features, like contact areas between trichome parts, the segments where the hair is attached to the epidermis, tip form and the number of cells in the glandular cap.

Pictures were taken using Olympus digital camera Camedia C-5060 and then, processed using LabSens software, with the adjustment of the adequate parameters such as light, exposure, image size or contrast.

Results and discussion

More types of trichomes were recognized in the greenhouse grown plants, than in a phytotron (Table 3). The following genotypes: 1552/1, 1552/1/1, 1552/1/2, 1552/1/18, Dk.S10.35, Dk.S10.61, De.C7 and De.P11.5 had the same types of hairs.

Table 3.

Trichome types identified in the plant genotypes cultivated both in greenhouse and in a phytotron. Types I and VIII were not present in any of the genotypes analysed.

GENOTYPE	PLANTS GROWN IN PHYTOTHRON						PLANTS GROWN IN GREENHOUSE					
	TRICHOME TYPE						TRICHOME TYPE					
	II	III	IV	V	VI	VII	II	III	IV	V	VI	VII
1552/1	+	+	+		+	+	+	+	+		+	+
1552/1/1	+	+			+	+	+	+			+	+
1552/1/2	+	+		+	+	+	+	+		+	+	+
1552/1/3	+	+	+	+	+		+	+	+		+	+
1552/1/4	+	+		+	+	+	+	+	+	+	+	+
1552/1/7	+	+	+		+	+	+	+			+	+
1552/1/7/1	+	+	+		+	+	+	+	+	+	+	+
1552/1/7/2	+	+			+	+	+	+	+		+	+
1552/1/18	+	+	+		+	+	+	+	+		+	+
1553/1/7	+	+	+		+	+	+	+		+	+	+
<i>S. chacoense</i> PI 458310	+	+		+	+	+						
<i>S. chacoense</i> 138							+	+	+		+	+
DELIKAT	+	+			+	+	+	+	+	+	+	+
DESIREE	+	+			+	+	+	+	+	+	+	+
Dk.S10.5	+	+	+		+		+	+	+		+	+
Dk.S10.13	+	+			+	+	+	+		+	+	+
Dk.S10.35	+	+	+		+	+	+	+	+		+	+
Dk.S10.43	+	+		+	+		+	+	+		+	+
Dk.S10.61	+	+	+		+	+	+	+	+		+	+
De. C7	+	+		+	+	+	+	+		+	+	+
De.P5.5	+	+	+	+	+		+	+	+		+	+
De.P11.5	+	+	+		+	+	+	+	+		+	+

In some cases, distinguishing between type V and II or III of non-glandular trichomes was difficult if they were incompletely developed *i.e.* in different growth stages. Because of this, we have compared only the trichomes that we have considered completely developed. In all analysed genotypes, trichomes types II, III and VI were present, regardless of the plant developmental stage. Type VII trichomes occurred in all greenhouse grown genotypes, but this type was not observed in some phytotron grown plants (1552/1/3, Dk.S10.5, Dk.S10.43 and De.P5.5) (Table 3). Trichomes type I and

VIII were not identified either in the parents or within their derived somatic hybrids or back-crosses. The two cultivars of potato used in our experiments showed very similar types of trichomes, with additional trichomes IV and V when the plants were grown in a greenhouse. The two accessions of *S. chacoense* could not be compared because each was grown either in phytotron or greenhouse. But, the main trichomes types *i.e.* II, III and VI and VII were present. The hybrids, with or without MMR deficiency, presented also the main trichome types, but there were some variations between them, with some missing or some having additional trichome types (Table 3).

The length of non-glandular trichomes was higher in the greenhouse grown plants than in the phytotron plants. Most of the plants grown in phytotron had 3-5 cells and only those which were developed in greenhouse had six or more cells.

The hair's fluorescence is a feature that could be detected in all the analysed genotypes, in some cases was more intense and less powerful in others. The reflected colours of the trichomes were also extremely interesting characteristics, because in most observation the colour was green, but occasionally the sixth type of hairs revealed red fluorescence (Fig. 1). The green fluorescence of the hairs might be caused by some kind of phenolic compounds, as such chemicals are produced in tomato trichomes or other *Solanum* species. On the other hand, red fluorescence, which was encountered rarely, in some genotypes, can indicate the biosynthesis of anthocyanins, azulenes or related compounds, since it does not look like chlorophyll fluorescence and these compounds were described as fluorescing in red (Roschina, 2008; Roshchina, 2012).

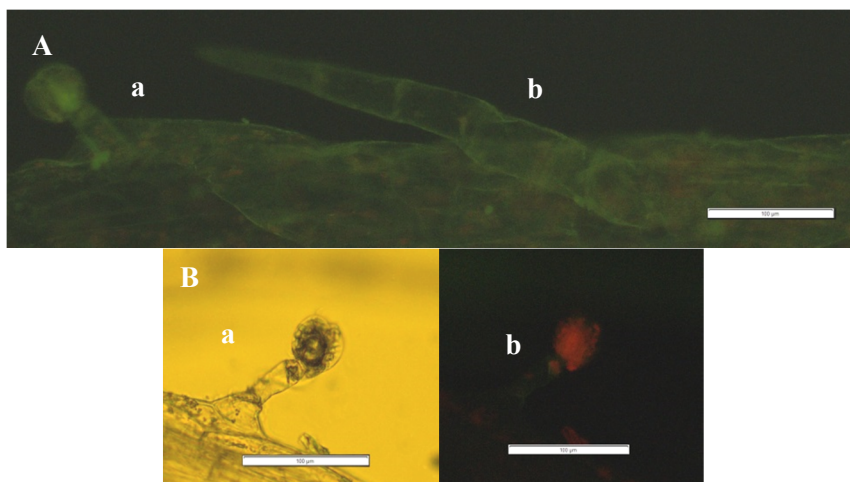


Figure 1. A - Types VI(a) and III(b), analysed in the MMR deficient somatic hybrid DK.S10.20 grown in greenhouse; B – Type VI, observed in direct light (a) and epifluorescence, with red fluorescence (b), in the MMR deficient somatic hybrid DK.S10.40, which was also grown in greenhouse; Bar = 100 μ m.

After performing the microscopically examination, we observed that the cuticula which cover both glandular and non-glandular trichomes, contained some small modifications on cell wall like granules. These can provide a harsh (scabrous) surface to the trichomes. At the non-glandular hairs, these granulations were easier to be observed (Fig. 2).

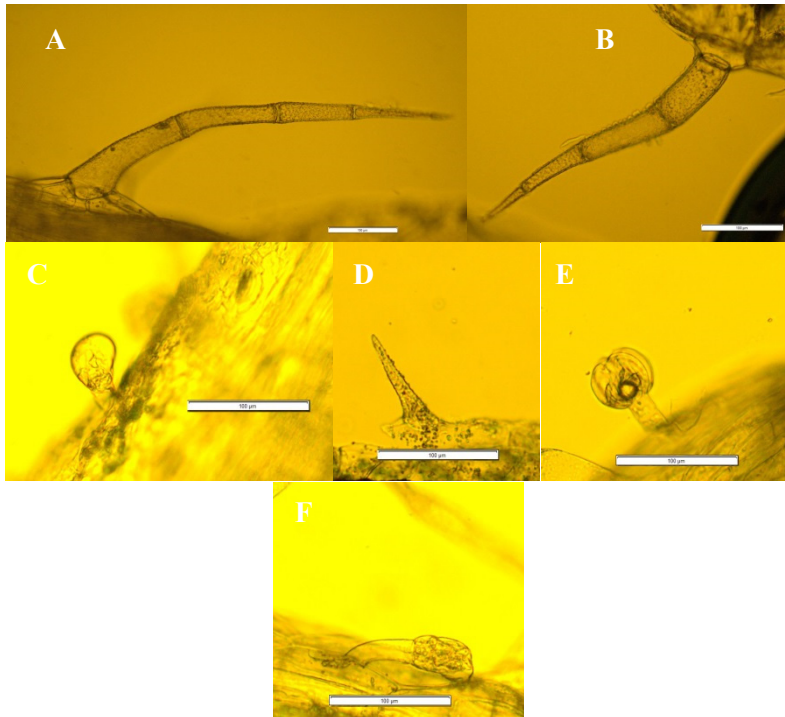


Figure 2. Trichomes types observed in plants cultivated in a greenhouse; A -Type II, in the sexual hybrid, *Solanum tuberosum* cv. Pannonia; B - Type III in *Solanum chacoense* 138, Bar = 10 μ m; C - Type IV in SH 1552/1; D - Type V in BC1 1552/1/4; E -Type VI in DK.S10.5; F – Type VII, in SH 1552/1/2; Bar = 100 μ m.

In hair types VI and VII, the secretory area was darker than in type IV, where this part of the cap was more transparent. The secretory part of type IV trichomes had unicellular construction. The types VI and VII contained multicellular secretory head with four cells. Lengths of secretory cells in type VI trichomes was larger than their width. The multicellular head of the type VII was more voluminous than in the one of the other types of glandular trichomes. In this context, our results are in accordance with those of the authors that we have cited in the introduction.

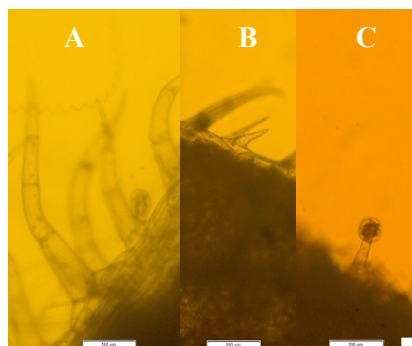


Figure 3. Plants grown in phytotron; A - Type II, *Solanum tuberosum* cv. Desiree; B – Type V, SH 1552-1-4; C - Type VI, somatic hybrid DK.S10.5; Bar = 100 µm.

Within the current study, the glandular hairs had relatively constant dimensions in almost all the genotypes, but this was not the case for non-glandular trichomes.

In the literature, it is certified that glandular and non-glandular hairs are thought to be important assets for plants, because they are supporting their defensive reactions against insects or pathogens. Compared to other biological structures, these epidermal extensions present an impressive diversity of shapes that confer an original appearance.

Nevertheless, *Solanum* trichomes are still classified in eight distinct types and two major classes, depending if their secretory function is available or not. Luckwill (1943) was the first to describe and compile trichomes forms in the genus *Solanum*, which were especially based on analysing the tomato hairs. Later, this paper was modified and adapted to more species in the genus *Solanum* (Glass *et al.*, 2012). In our studies we tried to compare what is known from the tomato trichomes with the types of morphologies we described in potato cultivars Delikat and Desiree, a wild species representing the best source of resistance to Colorado potato beetle and their somatic or sexual hybrids.

According to Dai *et al.* (2010), Reeves (1977) and Lemke and Mutschler (1984), *Solanum habrochaites*, *S. lycopersicum* and *S. pennellii* are three representative species as concerning trichome morphology in *Solanum* genus. Dai *et al.* (2010) distinguished and shortly described the following types: I, III, IV, V, VI and VII. It can be observed that the non-glandular type II, which is present in all genotypes we analysed, was missing in these species of tomato. By the contrary, we have not found type I. Another major difference is that tomato type V conformation is constituted by one to four cells, unlike that of potato, which has a unique, elongated cell. Comparing with tomato, all the potato leaves possessed type VI with four terminal cells. Trichomes with bi-cellular heads, specific for above mentioned tomato species, were not observed in our genotypes. Tian *et al.* (2012) related in their article that the

presence of trichomes on tomato (*Solanum lycopersicum*) mutants leaves surfaces may prevent Colorado potato beetle's activity, even inhibit their appetite or mobility. In potato it is thought that mainly glandular trichomes represent a defence mechanism by repelling the beetles, but from our assays, glandular trichomes density corellates well with hybrid plant resistance to Colorado potato beetle (Mărgineanu *et al.*, 2014).

Due to the trichomes positioning on the leaf, Pelletier *et al.* (2011) also considered them a real way of resistance against herbivore actions. Another opinion speculated in the same paper, is that glandular trichomes could increase potato plants resistance degree (Flanders *et al.*, 1992, Pelletier *et al.*, 2011). They have classified glandular hairs of potato in only two types: A with four cells in the tip and B, which is longer than the previous one (Flanders *et al.*, 1992, Pelletier *et al.*, 2011). *Solanum berthaultii* is another *Solanum* wild species studied in relation to CPB resistance, more than *S. chacoense*. Many of the cultivated potato varieties are hybrids of *S. berthaultii*, because it possesses glandular trichomes and also because its way of resistance is well-known (Pelletier *et al.* 2011). Resistance tests made and described by some authors prove that trichomes, especially the glandular ones, symbolize a natural approach instrument to reduce the injuries caused by insects. Plants defensive reactions achieved through glandular trichomes, are performed by secretion of different chemical compounds. These substances could interfere with the feeding process or could induce important damages in the beetle larvae or adults (Pelletier *et al.* 2011).

Conclusions

At all the analyzed genotypes, we could identify types II, III and VI of the trichomes.

Types II and III of trichomes are very similar, the only difference between them was their base. The secretory head of the type VI consisted of four cells. Thus, we can consider that our results are not different from those indicated in the literature.

We can conclude that the cells are very well delimited from each other and we observed a series of morphological details like cell junctions or binding zones where the hairs attach to the epidermis, and a specific fluorescence due to the accumulation of secondary metabolites, most probably involved in plant defencing against herbivores.

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