Leaf morpho-anathomical characteristics of five potato clones

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Abstract

Host genetic resistance against pathogens and insects can be directly associated with leaf morpho-anathomical characteristics. The aim was to study leaf morph-anatomical characteristics of five potato (*Solanum* spp.) clones differing in ploidy level and genetic background. The epidermis and mesophyll were characterized and stomata and trichome frequencies determined in both adaxial and abaxial surfaces of plant main leaflets. There were no evident differences in cuticle thickness and epidermis composition among evaluated clones. Clones of S. *microdontum* Bitter and SMII had a palisade parenchyma with uniform, long and compact arranged cells compared to other clones. There were no morphological differences between stomata subsidiary and other epidermal cells. The S. *microdontum* clone showed the highest frequency of stomata, on the abaxial surface, and trichomes, on both leaflet surfaces. Differences in stomata and trichome frequencies among clones need to be accessed to find possible relationship with disease and/or pest resistance.
Key words: *Solanum tuberosum*, foliar anatomy, stomata and trichome frequencies, resistance breeding.

Resumo

A resistência genética de plantas a patógenos e insetos pode estar diretamente associada a características morfo-anatômicas das folhas. O objetivo foi estudar as características morfo-anatômicas das folhas de cin-

co clones de batata (*Solanum* spp.) de diferentes ploidias e bases genéticas. Foram caracterizados a epiderme e o mesofilo e determinadas as freqüências de estômatos e pêlos das faces adaxial e abaxial do folíolo principal das plantas. Não foram observadas diferenças na espessura da cutícula e nem na composição da epiderme entre os clones estudados. Os clones de S. *microdontum* Bitter e SMII apresentaram células do parênquima paliçádico bastante uniformes, longas e arranjadas compactamente quando comparados aos demais clones. As células subsidiárias aos estômatos não diferiram morfologicamente das demais células da epiderme. O clone de S. *microdontum* apresentou a maior freqüência de estômatos na face abaxial e de pêlos em ambas as faces. Novos estudos deverão ser conduzidos para determinar a relação entre as diferentes freqüências de estômatos e pêlos encontradas nesses clones e a resistência a doenças e insetos.
Palavras-chave: *Solanum tuberosum*, anatomia foliar, freqüência de estômatos e pêlos, melhoramento para resistência.

Introduction

Cultivated potato (*Solanum* spp.) has the highest carbohydrate and the second, after soybeans (*Glycine max* L. Merrill), protein yield per area (MURPHY et al., 1999). Potato probable has one of the highest genetic diversity in wild species among all cultivated crops. The great majority of the wild species are diploid (74%) and the others are triploid, tetraploid, pentaploid and hexaploid (HAWKES & JACKSON, 1992). This genetic diversity is source for resistance to diseases and insects and for agronomic traits in breeding programs (ROSS, 1986; GALARRETA et al., 1998; PAVEK & CORSINI, 2001).

Disease and insect resistances are among the most important traits to consider in a potato breeding program, because host genetic resistance is the most appropriate and cost effective means of control (MURPHY et al., 1999; NIKS & RUBIALES, 2002). Broad resistance, effective against several pathogens and pest species, is based upon avoidance and/or elevated concentrations of secondary metabolites (NIKS & RUBIALES, 2002). Physical mechanisms of resistance are leaf morphological characteristics that can suppress or difficult pathogen infection and/or tissue colonization (RUBIALES et al., 1996).

The phase immediately before penetration of the first host cell is the most critical step to resist oomycete colonization and propagation (KRAMER et al., 1997). Leaf morphological features change leaf wettability and humidity in the canopy that interfere in spore germination, germ tube orientation and stomata localization to get into plant tissue. Trichomes (length and frequency) can reduce or hamper water film formation on leaf

surface, making difficult spore germination and bacteria multiplication (NIKS & RUBIALES, 2002). Trichomes can keep spores away avoiding infection (NIKS & RUBIALES, 2002) or entrap plague or vector insects of pathogenic agents through physical action or toxin production (RANGER & HOWER, 2001; MEDEIROS et al., 2004). Stomata (frequency, morphology, leaf surface level) can improve host disease resistance. The presence of a thick cuticle layer can difficult stomata identification, necessary for germ tube differentiation and appressorium formation of oomycetes (RUBIALES et al., 1996; BIRCHER & HOHL, 1997; VAZ PATTO & NIKS, 2001; NIKS & RUBIALES, 2002), reducing pathogen infection (NIKS & RUBIALES, 2002). The oomycete *Phytophthora infestans* (Mont.) de Bary, the causal agent of late blight, is one of the most serious diseases in potato and can infect leaves, stems and tubers of any plant age, devastating a field of a susceptible cultivar in a few days (HENFLING, 1987).

Besides physical mechanisms, some constitutive-chemical substances are present in diverse host tissues. Potato glycoalkaloids are secondary metabolites with antimicrobial and pesticide properties (TINGEY, 1984). High insect resistance was associated with increased trichome density in potato (MEDEIROS et al., 2004), but they should have the appropriate chemical constituents. Alone and synergistically, the exudates of the two types of trichomes avoid and/or reduce digestion and reproductive performance of insects (BONIERBALE et al., 1994; MALAKAR & TINGEY, 2000; PEETERS, 2002). High content of glycoalkaloids in foliage, necessary for resistance, can result in undesirable or even hazardous levels in tubers (TINGEY, 1984), reducing palatability and nutrition qualities (NIKS & RUBIALES, 2002).

The objective was to study leaf morph-anatomical characteristics to identify physical mechanisms of resistance present in five potato clones differing in ploidy level and genetic background.

Material and methods

The experiment was carried out in greenhouse and laboratory of the Department of Fitotecnia, Universidade Federal de Santa Maria, from March, 2002 to August, 2003. Plants were grown from tubers planted in a plastic pot of 15cm diameter, filled with organic substrate. Physical mechanisms of resistance were assessed in one diploid (2n=2x=24) (PI595511 of S. *microdontum* Bitter) and four tetraploid (2n=4x=48) (SMIJ274-3, SMIJ461-1, SMII and Macaca) clones. The tetraploid clones SMIJ274-3 and SMIJ461-1 derived from the hexaploid (2n=6x=62) S. *demissum* Lindley species. The SMII clone derived from the diploid S.

microdontum species (PI595511), which is source for late blight resistance (BISOGNIN et al., 2005). The SMIJ274-3 and SMIJ461-1 are highly resistance to the US8 genotype of *P. infestans* (BISOGNIN & DOUCHES, 2002; BISOGNIN et al., 2002). The cultivar Macaca is susceptible to late blight, has no genetic relationship to the other clones and is the most important cultivar in Rio Grande do Sul State.

One plant per clone was sampled to study leaf cross section. Main leaflets were collected from the middle portion of plants with approximately ten leaves. Leaflets were fixed in a solution of $FAA_{50\%}$ for 72h (JOHANSEN, 1940) and kept in alcohol 70%. Samples of leaflet middle portion were washed in buffer solution, submitted to dehydration in an alcoholic-ethylic series and stored in an infiltration solution for 24h. The leaflet sample was then transferred to a glass test tube with catalyst solution, mixed for 1min and transferred to a cold-curing resin (Resin Historesin, Leica) following the manufacturer's recommendations. Transversal cuts of six μm were made with a microtome (Ancap, model 781). Leaflet cuts were colored with toluidine blue for 3min and permanent slides were prepared with Entelan. Pictures were taken in a light microscope (Olympus, model CH30RF200) for permanent records.

Four plants per clone were used to study the leaf paradermic sections. Four main leaflets were collected from the middle portion of plants with approximately ten leaves. Semi-permanent slides were prepared from both leaflet surfaces following the epidermal fingerprint technique (SEGATTO et al., 2004). Stomata and trichome frequencies were quantified with a light microscope (Carls Zeiss) in an area of 0.0416mm^2 of the abaxial and 0.2463mm^2 of the adaxial surfaces. Frequency was estimated as an average of nine visual fields (three fields each from the basal, central and apex of the leaflet) of each leaflet surface, in a total of 288 visual fields per evaluated clone. The experiment was carried out as a completely randomized design with four replications of one plant. Data from adaxial and abaxial surfaces of each clone were submitted to analysis of variance (F test) and treatment means compared by Duncan´s test ($\alpha < 0.05$).

Results and discussion

The epidermis of the adaxial and abaxial surfaces of all evaluated clones had one stratum of cells with external anticlinal walls coated by a thin, smooth and uniform cuticle (Figure 1). Stomata were present in both surfaces. All evaluated clones had similar stomata morphology of the cuticle crest and position. Stomata were positioned either at the same epidermal cell level (Figure 2). Leaf mesophyll of all evaluated clones had a dorsal-ventral structure with palisade parenchyma constituted by one cell stratum.

Solanum microdontum (Figure 1A) and SMII (Figure 1B) clones presented palisade parenchyma cells very uniform, long and compactly arranged when compared with other clones. These similarities can be explained by the fact that *S. microdontum* is one of SMII progenitors. Lacunose parenchyma of *S. microdontum* clone was formed by three or four cell stratus. Such cells had regular format, resulting in few intercellular spaces and a compact conformation of mesophyll layer (Figure 1A). Other clones had lacunose parenchyma formed by a stratum of four-seven layers of cells with irregular formats. This cell arrangement resulted in intercellular spaces of diverse dimensions, giving relaxed tissue morphology (Figures 1B and 1C). Intercellular spaces of lacunose parenchyma were present in all evaluated tetraploid clones, even SMII that has *S. microdontum* as progenitor. Therefore, there were no major differences in leaflet cross sections among evaluated clones. However, cuticle of potato resistant cultivars (Kufri Jyoti and Kufri Badshah) was thicker on both the adaxial and the abaxial surfaces of the leaf than susceptible cultivars (Kufri Ashoka and Kufri Chandramukhi) to late blight. The cuticle-cum-epidermis thickness also showed a similar trend (MAHAJAN & DHILLON, 2003).

All evaluated clones showed epidermal cells with sinuous walls and similar sizes (Figure 3A). Stomata were well distributed in both surfaces, but the clone SMII had a lower frequency in the adaxial surface than Macaca (Table 1). In the abaxial surface, the *S. microdontum* clone showed the highest and SMIG274-3 the lowest stomata frequency. High frequency of stomata in the adaxial surface, as the case of Macaca, might be an indication for pathogen susceptibility. *Phytophothora infestans* usually penetrates host foliar tissues through opened stomata of adaxial surface, grows below cuticle and infects different depths of cell layers (HOHL & SUTER, 1976). Leaflets of the potato cultivar Pimpernel, highly resistant to late blight, had lower stomata frequency in the adaxial surface than the susceptible cultivar Magestic (WILSON & COFFEY, 1980). Stomata of potato susceptible cultivars were bigger, in the abaxial surface, and in higher frequency, in the adaxial surface, than in resistant cultivars to late blight (MAHAJAN & DHILLON, 2003).

Besides stomata, subsidiary cells also play important role in the interaction between potato and *P. infestans*. Epidermal cells of the cultivar Pimpernel were less infected by *P. infestans* than subsidiary cells (WILSON & COFFEY, 1980) and host penetration occurred through subsidiary cells of adaxial surface (COFFEY & GEES, 1991). Based upon two races of *P. infestans* and eight potato cultivars, GEES & HOOL (1988) verified that subsidiary cells were more susceptible to penetration than other cells, both in resistant and susceptible cultivars. Although subsidiary cells were morphologically similar to other epidermal cells (Figure 3A), subsidiary

cells might have a different physiological role due to stomata proximity (MEIDNER & MANSFIELD, 1968). Contact with an inductive topographical surface is the prerequisite for the induction of appressorium formation. Surface irregularities as edges of stomata guard cells and grooves over anticlinal walls provide topographical signals where *P. palmivora* can penetrate without entering the cytoplasm of host epidermal cells (BIRCHER & HOHL, 1997). *Phytophthora infestans* can sense the groove above anticlinal cell wall on leaf surface and orient its germ tube tip towards subsidiary cells (KRAMER et al., 1997). Histological studies of potato and *P. infestans* interactions in these clones are necessary for a better understanding of stomata and subsidiary cell role in pathogen infection.

All evaluated clones had trichome base surrounded by a group of epidermal cells arranged in a radial plan (Figure 3B). Trichomes were erect, multicellular and no glandular (Figures 1A and 3B). Trichomes were either tetralobulate, morphologically similar to type A, or stalks of long, multicellular with bent apex, morphologically similar to type B. There were trichome frequency differences among potato clones and between leaf surfaces, being more frequent in the abaxial surface (Table 1). The SMIJ461-1 clone had less trichome in the abaxial surface than *S. microdontum* and SMII clones. *Solanum microdontum* had the highest frequency of trichomes in both surfaces. Differences in trichome frequency between cultivars and leaf surfaces were already found in potato (MALAKAR & TINGEY, 2000). High trichome frequency in abaxial surface seems to be common in potato, even in different seasons (MEDEIROS et al., 2004) and cultivars (MALAKAR & TINGEY, 2000). MAHAJAN & DHILLON (2003) found that resistant cultivars had bigger trichomes, on both adaxial and abaxial surfaces, and higher frequency, on adaxial surface, than susceptible cultivars to late blight. Trichome frequency and type has been more associated to insect resistance, either as physical or chemical mechanism (BONIERBALE et al., 1994; RANGER & HOWER, 2001; MEDEIROS et al., 2004). High trichome frequency might also be associated to late blight resistance, since high trichome density can difficult spore germination on leaf surface and disrupt normal germ tube growth (NIKS & RUBIALES, 2002).

It was expected that different physical mechanisms of resistance were present in these clones, since there was genetic diversity from different wild species and ploidy levels. The S. *microdontum* clone presented the palisade parenchyma cells compactly arranged, which was not found in any other clone, even in its progeny (SMII clone). There were no differences in stomata morphology and position at epidermal cell level and between leaflet surfaces. Stomata subsidiary cells were also similar among clones. Subsidiary cells should have different physiological role than epidermal common cells in *P. infestans* penetration (MEIDNER & MANSFIELD, 1968; GEES &

HOOL, 1988; COFFEY & GEES, 1991; KRAMER et al., 1997). Also, a combination of physical mechanisms can act to increase plant protection, since susceptibility to sucker insects was correlated with thin cuticle and high density of small stomata (PEETERS, 2002). Specific complementary studies are necessary for each clone and pathogen/insect interaction to corroborate the association between physical mechanisms and genetic resistance.

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Table 1. Stomata and trichome frequencies per mm2 of the adaxial and abaxial leaflet surfaces of five potato clones

Evaluated	Stomata		Trichomes	
clones	Adaxial	Abaxial	Adaxial	Abaxial
Macaca	17.79a*	96.98b	0.73b	6.02bc
SMIG274-3	15.18ab	65.70c	1.18b	6.26bc
S. *microdontum*	14.53ab	185.80a	16.24a	32.00a
SMIJ461-1	14.53ab	99.40b	1.22b	4.09c
SMII	10.64b	85.44b	1.18b	8.90b
Average	14.53	106.67	4.11	11.45
CV%	16.11	7.92	31.46	40.20

* Means in columns followed by the same letter were not significantly different at $P < 0.05$ using Duncan´s test.

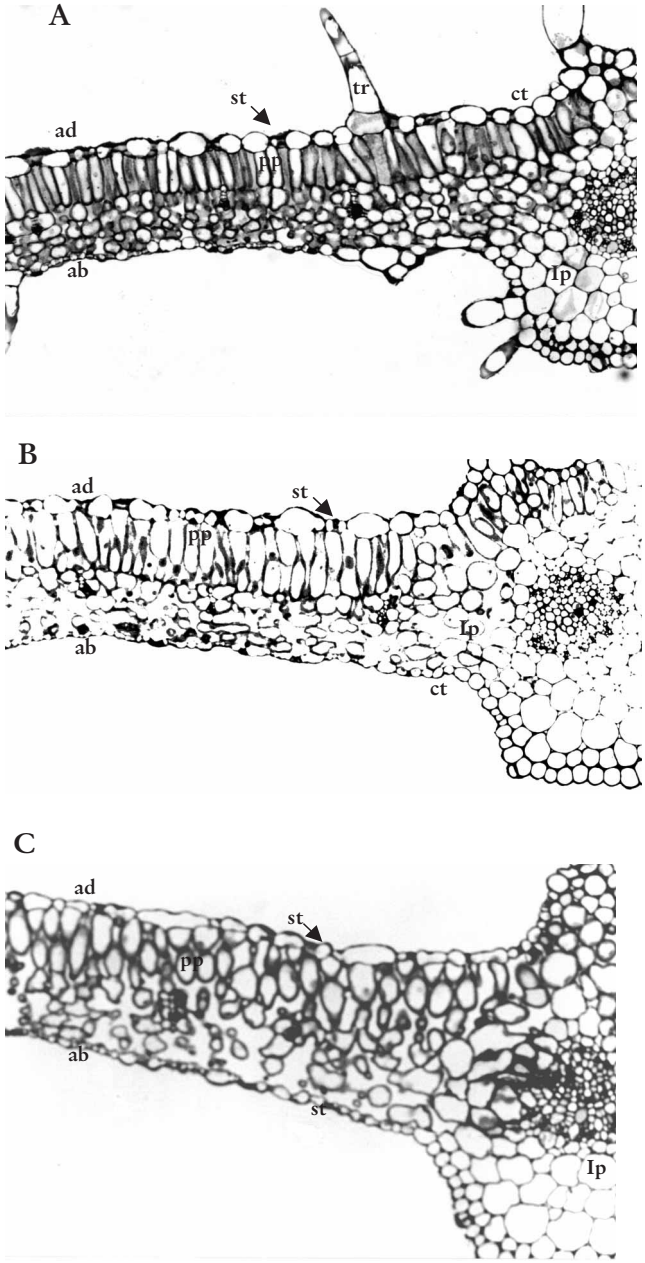

Figure 1. Leaflet cross sections (x 200) of the *Solanum microdontum* Bitter clone PI595511-5 (A) and the clones SMII (B) and SMIJ461-1 (C). ad adaxial surface, ab abaxial surface, lp lacunose parenchyma, pp palisade parenchyma, st stomata, tr trichome, ct cuticle

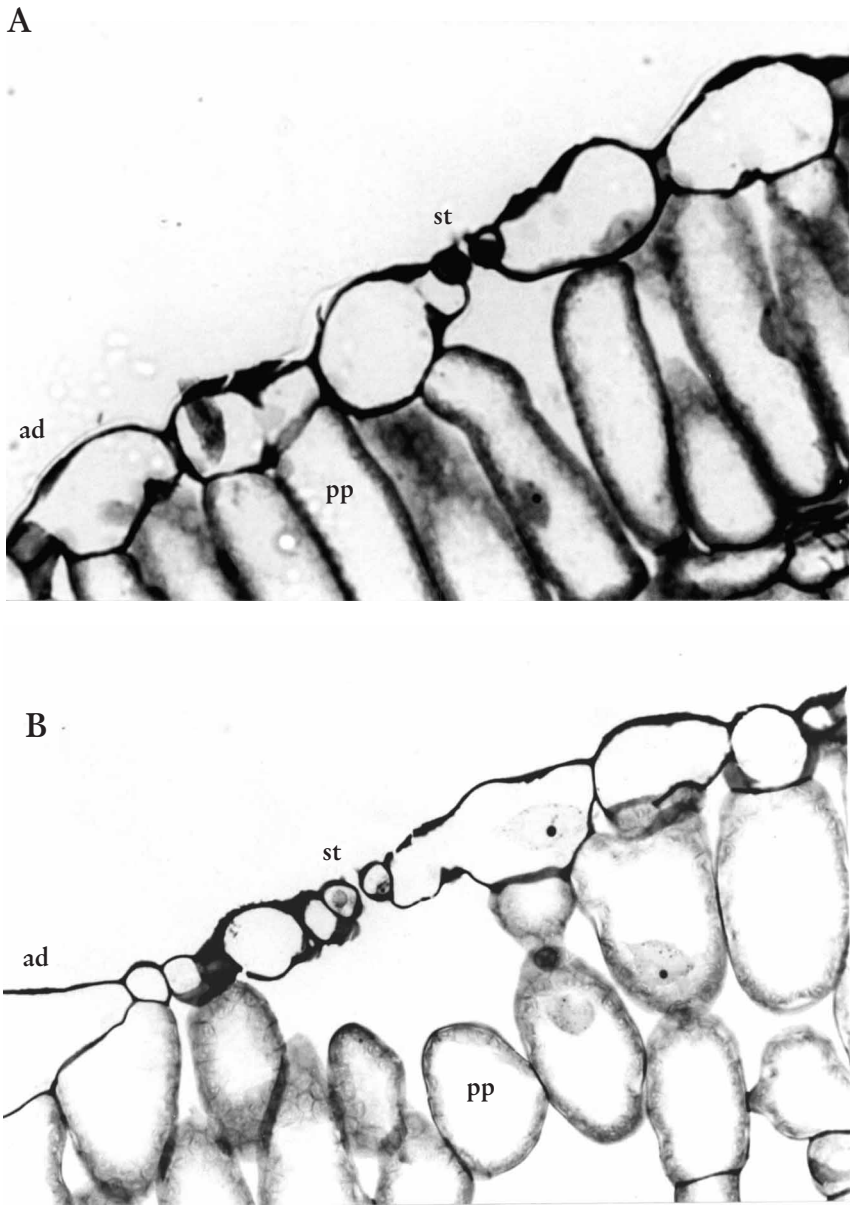

Figure 2. Leaflet cross section (x 800) of the clones SMII (A) and SMIJ461-1 (B). ad adaxial surface, st stomata, pp palisade parenchyma

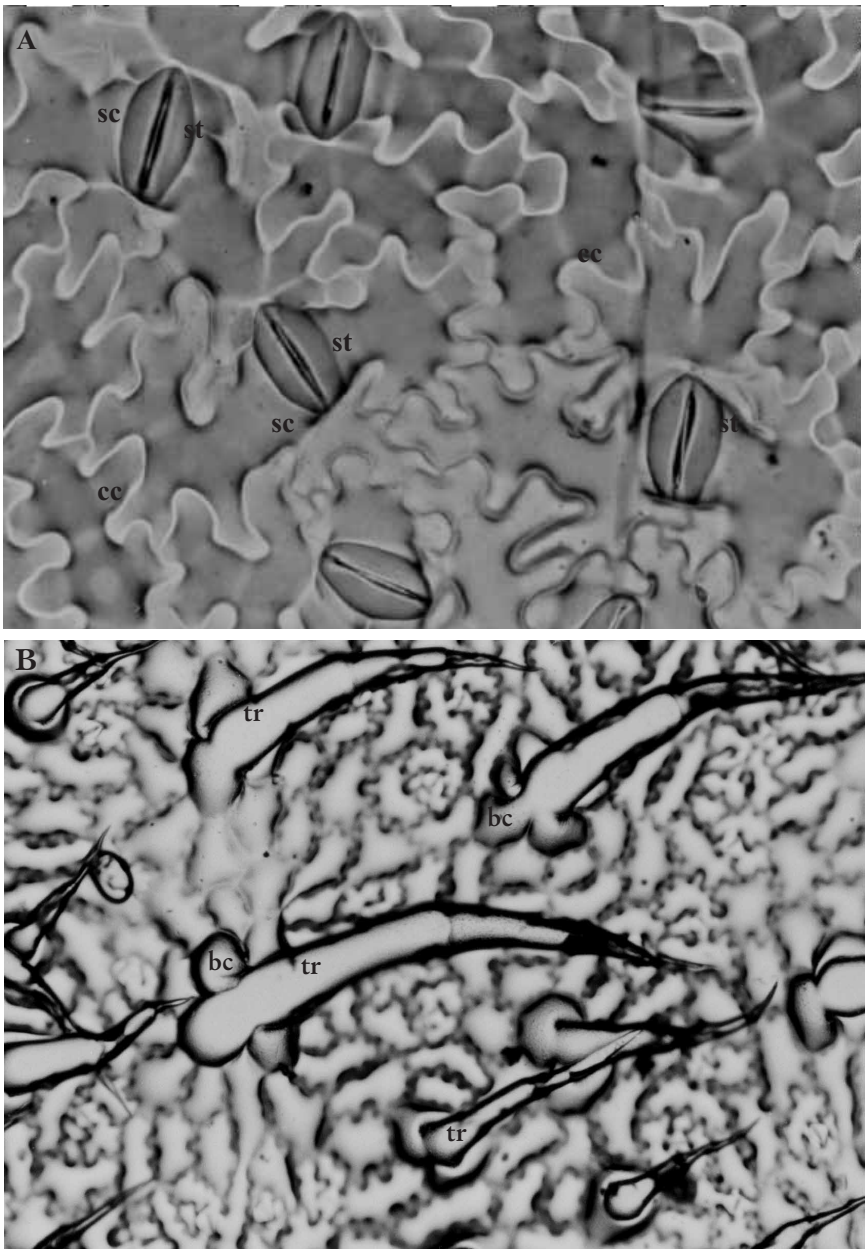

Figure 3. Leaflet paradermic section of the abaxial surface (x 800) of the clone SMII (A) and adaxial surface (x 200) of the *S. microdontum* Bitter clone PI595511-5 (B). cc epidermical common cells, sc subisidiary cells, st stomata, tr trichomes of different sizes and types, bc basal cells of trichomes