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Morphological and Histochemical Investigation on Glandular Trichomes of *Orobanche ramosa* subsp. *nana* (*Orobanchaceae*)

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With 9 figures

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S u m m a r y

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Glandular trichomes present in the stems and flower parts of *Orobanche ramosa* L. subsp. *nana* (REUTER) COUTINHO were examined under conventional microscopy, UV microscopy and scanning electron microscopy. The ontogenesis and morphology of the glandular trichomes appeared to be identical to those reported for other species belonging to *Geraniaceae*, *Solanaceae*, *Cannabaceae* and *Scrophulariaceae*. Histochemical investigations were performed to qualitatively detect the main classes of compounds occurring in the glandular hairs and in the secretion. The results achieved suggested the presence of terpenes and flavonoids as the main chemicals. The

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absence of positive reactions to sesquiterpenes contrasts with similar previously written papers which report sesquiterpenes, together with phenylpropanoids and iridoid glycosides as characteristic chemicals of the genus.

Zusammenfassung

SACCHETTI G., BALLERO M., SERAFINI M., MUZZOLI M., TOSI B. & POLI F. 2003. Morphologische und histochemische Untersuchungen an Drüsenhaaren von *Orobanche ramosa* subsp. *nana* (*Orobanchaceae*). – Phyton (Horn, Austria) 43 (1): 207 – 214, 9 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Drüsenhaare aus dem Stamm und Blütenbereich von *Orobanche ramosa* L. subsp. *nana* (REUTER) COUTINHO wurden mit konventioneller, UV- und Rasterelektronenmikroskopie untersucht. Die Entwicklung und Morphologie der Drüsenhaare erwiesen sich ident mit denen von *Geraniaceae*, *Solanaceae*, *Cannabaceae* und *Scrophulariaceae*. Um die Hauptbestandteile in den Drüsenhaaren und den Sekreten festzustellen, wurden qualitative histochemische Untersuchungen durchgeführt. Terpene und Flavonoide erwiesen sich als die Hauptkomponenten. Das Fehlen von positiven Reaktionen auf Sesquiterpene steht im Gegensatz zu ähnlichen früheren Arbeiten, die Sesquiterpene gemeinsam mit Phenylpropanen und Iridoidglykosiden als charakteristische Verbindungen in diesem Genus bezeichnen.

Introduction

Many parasitic herbaceous plant species belonging to the genus *Orobanche* are widespread in the temperate areas of southern Europe (PIGNATTI 1982, TUTIN & al. 1972). These species are phytochemically characterized by the presence of iridoid glycosides, sesquiterpenes (HEGNAUER 1990) and phenylpropanoid glycosides (SERAFINI & al. 1995). In particular, phenylpropanoid glycosides are considered chemosistematic markers of the genus *Orobanche* (NICOLETTI & al. 1987) and they are responsible for the anti-inflammatory, antibacterial, antihypertensive, antitremor and analgesic activities ascribed to some *Orobanche* species (LAHLOUB & al. 1991).

This paper reports a morphological investigation of glandular trichomes of *Orobanche ramosa* L. subsp. *nana* (REUTER) COUTINHO, performed by employing conventional light, fluorescence and scanning electron microscopy (SEM). Histochemical analyses were performed to evaluate the main classes of chemical compounds occurring in the cells of the trichomes and in the secretion.

Material and Methods

Plant material

Orobanche ramosa subsp. *nana* plants, spontaneously grown in the Botanical Garden of the University of Cagliari, were collected during full flowering and identified following the taxonomic keys reported by PIGNATTI 1982. Samples of the collected species are deposited in the Herbarium of the Department of Botanical Sciences, University of Cagliari (CAG: no. 1064/C).

Conventional and fluorescence microscopy

Fresh samples of stems and flowers (petals and sepals) were prepared for the examination of the glandular trichomes at conventional and fluorescence microscopy and for being subjected to

the following histochemical reactions: a) NADI reagent, $SbCl_3$ and α-naphtol for terpenes (DAVID & CARDE 1964); b) ferrous thiocianate and H_2SO_4 for sesquiterpenes; c) $AlCl_3$ and neutral lead acetate for flavonoids (JENSEN 1962); d) Sudan Black B for lipids and essential oils; e) $FeCl_3$ and potassium bichromate for tannins (GAHAN 1984).

Fresh samples of stems and flowers were fixed and embedded for optical microscopy as reported in POLI & al. 1995. Sample semi-thin sections were then re-hydrated by means of a decreasing ethanol series and stained with PAS-F solution, specific for polysaccharides (GAHAN 1984).

All the observations were made employing a Zeiss stereomicroscope and a Zeiss Axiophot photomicroscope equipped with an epi-illuminator set with UV01 (BP 365/12 FT 395 LP 397) and UV06 (BP436/10 FT 460 LP 470) Zeiss filters.

Scanning electron microscopy

Part of the same samples employed for conventional and fluorescence microscopy were subjected to fixation, dehydration by means of increasing acetone series and critical point dried, following the suggestions given in BRUNI & al. 1987. The samples were observed employing a Scanning Electron Microscope Stereoscan Cambridge (Electronic Microscopy Center - University of Ferrara).

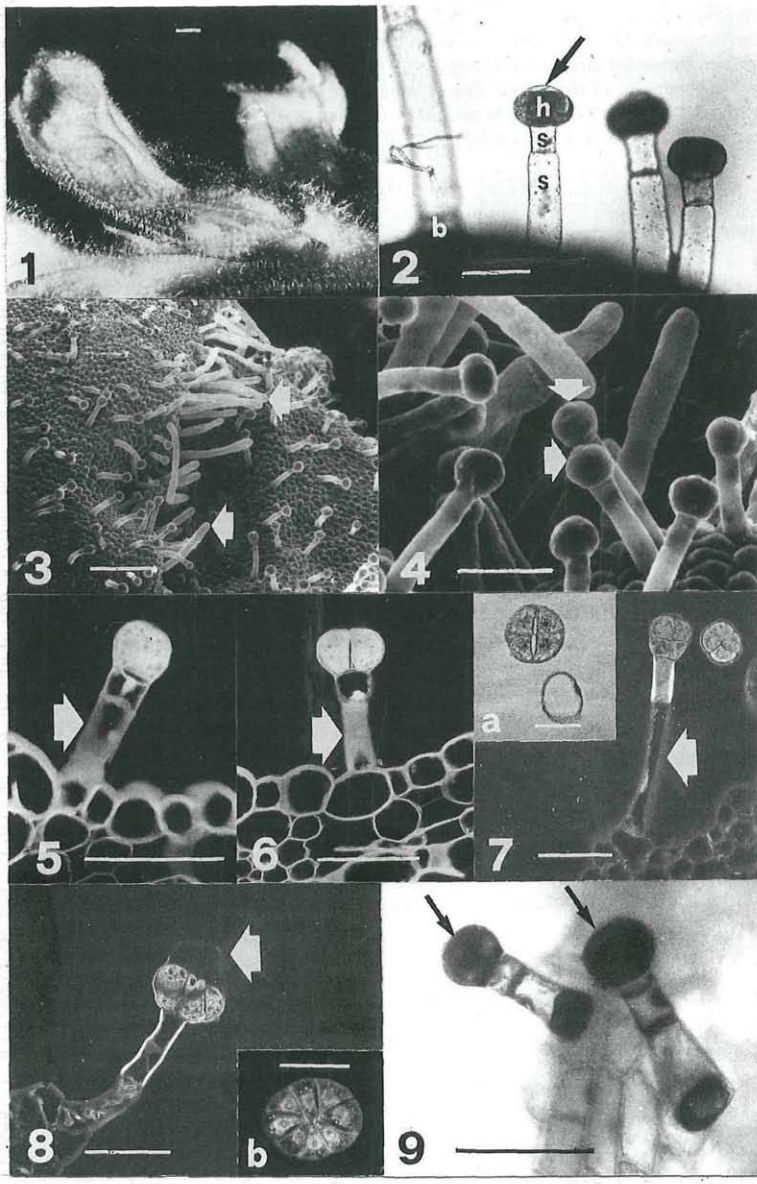
Results

Fresh samples of stems and flowers (petals and sepals) of *Orobanche ramosa* subsp. *nana* presented glandular and non-glandular trichomes on all their surfaces (Fig. 1). Under the conventional microscope, the glandular trichomes appeared to be characterized by a basal cell, two stalk cells and a glandular multicellular head, yellow-brown in color. The translucent secretion was stored in the sub cuticular space of the upper part of the glandular head (Fig. 2).

Table 1. Autofluorescence and histochemical reactions with the glandular trichomes of *Orobanche ramosa* subsp. *nana*.

Target compounds		Response		
Autofluorescence		Cells of the stalk	Cells of the head	Secretion
λ= 436 nm - bright yellow		-	++	++
λ=365 nm - bright blue		-	++	++
Staining				
Sudan Black B	*Lipids and essential oils*	-	-	-
NADI	*Terpenes*	-	++	++
$SbCl_3$	*Terpenes*	-	+-	+-
α-naftol	*Terpenes*	-	+-	+-
$Fe(SCN)_2$	*Sesquiterpenes*	-	-	-
$AlCl_3$	*Flavonoids*	-	+-	+-
Neutral lead acetate	*Flavonoids*	-	+-	+-
H_2SO_4	*Sesquiterpene lactones*	-	-	-
$FeCl_3$	*Tannins*	-	-	-
Potassium bichromate	*Tannins*	-	-	-

++ positive; +- weakly positive; - negative



Samples of petals examined with SEM showed the uniform distribution of the glandular hairs on all the surfaces and higher density of non-glandular hairs at the level of the fauces of the corollas (Fig. 3). At higher magnification, it was possible to determine that the secretory head of the glandular hairs was always multi-cellular. At the top of the secretory head there were cuticle blisters which could represent sub-cuticular areas of accumulation of the secreting material (Fig. 4).

Transverse and longitudinal sections of resin embedded samples, treated with PAS-F and examined under fluorescence microscopy, showed that the ontogenesis of the glandular head started with a periclinal division of the single head cell. If there was a one-celled head, the walls of the lower stalk cells positively reacted to the PAS-F solution, specific for polysaccharides (Fig. 5). After the periclinal division, the walls of the stalk cells were already negative to the PAS-F reaction (Fig. 6). Each cell of the head underwent two consecutive periclinal divisions thus forming the completely developed 8-celled head of the mature glandular trichome (Figs. 7-8), where the walls of the lower stalk cells were also PAS-F negative.

Water mounted samples observed under fluorescence microscopy at 365 nm showed a bright blue auto-fluorescence of the glandular head and of the secretion whereas, at 436 nm the auto-fluorescence was bright yellow (Table 1).

Fig. 1. Fresh sample of flower at the stereomicroscope. Trichomes are present on all the surfaces of the sample. Scale bar = 1 mm.

Fig. 2. Fresh sample of stem under conventional microscope. The trichomes appeared to be characterized by a basal cell (b), two stalk cells (s) and a glandular head (h), yellow-brown in colour. Note the translucent secretion in the sub cuticular space (arrow). Scale bar = 25 μm.

Fig. 3. SEM micrograph of flower showing glandular and non-glandular trichomes. Note the high density of non-glandular trichomes (arrows) at the fauces of the corolla. Scale bar = 100 μm.

Fig. 4. SEM micrograph of flower with glandular trichomes presenting cuticle blisters at the top of the secretory head (arrows). Scale bar = 50 μm.

Figs. 5, 6. Semi-thin sections of embedded samples of stem (Fig. 5) and flower (Fig. 6) treated with PAS-F reagent to detect polysaccharides. Note the positive reaction to the PAS-F solution of the walls of the lower stalk cells (arrow), at the stages of development of 1-celled (Fig. 5, Scale bar = 50 μm) and 2-celled (Fig. 6, Scale bar = 50 μm) glandular head.

Fig. 7. Semi-thin sections of embedded samples treated with PAS-F reagent to detect polysaccharides. Note that the trichome at the following stages of development shows a multi-cellular glandular head; the walls of the lower stalk cell negatively react to PAS-F at this stage of trichome development (arrow). Scale bar = 50 μm; a: particular of the glandular head; Scale bar = 25 μm.

Fig. 8. Semi-thin sections of embedded samples of flower treated with PAS-F reagent to detect polysaccharides. The completely developed mature glandular trichome with the cuticle of the glandular head raised (arrow), forming a sub-cuticular space where the secretion was stored. Note the negative reaction of the walls of the lower stalk cell to the PAS-F reagent; Scale bar = 50 μm. b: particular of the glandular 8-celled head. Scale bar = 25 μm.

Fig. 9. Fresh flower sample treated with NADI reagent specific for terpenes and observed under conventional microscope. The reagent solution strongly stained the glandular head (arrows). Scale bar = 50 μm.

Histochemical analyses were performed with the aim to qualitatively characterize the main classes of chemicals in the glandular hairs and in the secretion (Table 1). NADI reagent, specific for terpenes, strongly stained blue-violet the glandular head (Fig. 9; Table 1). Reactions specific for terpenes ($SbCl_3$ and α-naphtol) gave weakly positive results both with glandular head and with secretion. In the same way, the specific reactions for flavonoids ($AlCl_3$ and neutral lead acetate) were weakly positive. The histochemical reactions performed to detect lipids and essential oils (Sudan black B), sesquiterpenes ($Fe(SCN)_2$ and H_2SO_4) and tannins ($FeCl_3$ and potassium bichromate) were all negative (Table 1).

Discussion

Glandular hairs of *Orobanche ramosa* subsp. *nana* stems and flowers (sepals and petals) presented some morphological characteristics similar to those described in related papers for plant species belonging to *Geraniaceae, Solanaceae, Cannabaceae* (FAHN 1988) and Scrophulariaceae (SACCHETTI & al. 1997, 1999). In particular, the ontogenesis and the morphology of the completely developed glandular hairs are the same as those reported for *Calceolaria volckmanni (Scrophulariaceae)*, a south American native species, known to produce and accumulate phenylpropanoid glycosides, flavonoids and terpenic compounds (NICOLETTI & al. 1988, CHAMY & al. 1989, WOLLENWEBER & al. 1989, GARBARINO & al. 1992, DI FABIO & al. 1995).

Glandular hairs of *Orobanche ramosa* subsp. *nana* presented an evident cutinization of the walls of the lower cell of the stalk - as demonstrated by the negative reaction to the PAS-F solution – typical of oil secreting trichomes (FAHN 1988). This particular specialization of the walls could be linked to prevent leakage of the secreted substance back through the apoplast (FAHN 1988, SERRATO-VALENTI & al. 1997, ASCENSAO & PAIS 1998, SACCHETTI & al. 1999).

The fluorescence emitted at the wavelength of 436 nm could be related to the presence of flavonoids both in the secretion and in the glandular head cells (SACCHETTI & al. 1997, 1999). This suggestion is confirmed by the positive reactivity to specific histochemicals, however with a weak staining intensity. The bright blue fluorescence, emitted by the secretion at 365 nm in particular, could however suggest the presence of coumarins or also phenolic compounds (POLI & al. 1995, SACCHETTI & al. 1997). Moreover, the histochemical analyses showed also the presence of terpenic compounds but excluded that of sesquiterpenes and sesquiterpene lactones. This data contrasts with those in related papers which report sesquiterpenes, together with phenylpropanoids and iridoid glycosides as characteristic chemicals of the genus (HEGNAUER 1990).

Finally, the release of the secretion to the environment may occur through the partial rupture of the cuticle caused by natural conditions (temperature, humidity, etc.), by contact with predators (WERKER 1993) and by cuticular exudation as described for many other plant species (WERKER & FAHN 1981, WERKER & al. 1985a, b, SERRATO-VALENTI & al. 1997).

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