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# STUDY ON THE TRICHOMES OF THE PARASITIC WEED BROOMRAPE: MORPHOLOGY AND HISTOCHEMISTRY

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**Summary.** Trichomes are epidermal hairs found on the aerial surfaces of nearly all plants and in different species. They grow in different shapes and act as protectors against insects, microbes and herbivores. Trichomes vary in their morphology, distribution pattern on a given organ and in their histochemical images. Different trichome types were observed in the aerial parts of the two species of broomrape (*Orobanche aegyptica* and *Orobanche crenata*), using both light and fluorescent microscopy techniques. Samples of the parasitic weed broomrape were collected from El-Aiat (Giza) and Helwan (South Cairo) in Egypt. Following standard methods, the samples were washed and prepared for light and epifluorescent microscopy. The examination revealed that the peduncles of both broomrape species were covered with trichomes. Their density varied according to their location on the peduncles. High trichome density was observed on the apex of broomrape peduncle. In the examined samples, all trichomes belonged to the multicellular uniseriate category and were classified as glandular and non-glandular types. Glandular trichomes were found to cover different parts of peduncles of the parasitic plant broomrape in both species, whereas non-glandular trichomes outgrew the corolla and anderoecium of *O. aegyptiaca*. On the other hand, crisped hairs were observed only on the anderoecium of both species. The histochemical tests showed positive reactions to lignin, phenolic, lipid and suberin materials in the outer layer of glandular trichomes, while the phenolic substances were detected in the neck cell and gland secreted cells.

***Keywords:*** broomrape, histochemistry, morphology, phenolics, trichomes.

## INTRODUCTION

Trichomes are epidermal hairs detected on aerial surfaces of nearly all plants (Wagner, 1991). The epidermis of plant organs is commonly covered either by non-glandular or glandular trichomes. It was noted that the importance of plant trichomes is based on their protective function and in taxonomy. When non-glandular trichomes form a dense indumentum, they may serve as a mechanical barrier against various external stress factors, such as the attack with herbivores and pathogens, UV-B radiation, extreme temperatures and excessive water loss (Werker, 2000; Gonzales et al., 2008). However, glandular trichomes, which secret lipophilic substances (terpenes, lipids, waxes and flavonoid aglycones), may provide chemical or physicochemical protection against various types of herbivores and pathogens by entrapping or

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poisoning them (Wagner, 1991; Cipollini and Bergelson, 2002).

The shape, size, structure, distribution/ location of trichomes, and the composition of exudates produced by them, vary greatly among species. Such characters are used in plant taxonomy to distinguish between closely related species or hybrids (Behnke, 1984; Spring, 2000). In this respect, Raman (1991) used trichome variation in the classification of parasitic weeds.

Vaughn (2002) observed the enlargement of epidermal cells of the dodder parasitic weed, which then differentiated into secretor type of trichomes. This, however, implicated the mechanism of its penetration into host tissues. In parallel that the trichome cell walls are malleable, allowing them to elongate towards the host and bend their walls to conform to the shape of the host cell surface.

In histochemical studies conducted by El-Akkad et al. (2002) and Hassan et al. (2004)**,** the occurrence of lignin-like substances, other phenolics, certain lipids and suberin, were observed in the epidermal cell layer of broomrape parasitising faba bean. In addition, corners thickening of the cortical and pith cells in the broomrape (*Orobanche crenata* Forsk.) were recorded. In a corresponding chemical analyses using HPLC, the existence of chorogenic, caffeic, ferulic, and coumaric acids was detected. The analyses revealed the dominance of chlorogenic acid in both aerial and corm-like structure of the broomrape. The root connection zones between different host roots (i.e. peas, tomato, chamomile, dill and Indian cress) and the attached broomrape species were also included in their investigation.

In the present work we aimed to study the trichomes of the parasitic plant *Orobanche aegyptiaca* Frosk and *Orobanche crenata* L. The study included their morphology, anatomy, distribution and density. In addition, a histochemical study was carried out to localize the existence of certain chemicals, i.e. lignin-like substances, phenolics and lipid compounds in the trichomes outgrow the broomrape tissues.

It is suggested that the study of such trichomes, taxonomy, chemistry in addition to other biological aspects of the parasitic weeds may help in the methods to be applied in their control (Salle et al., 1995).

## MATERIALS AND METHODS

Plants of two broomrape species, *Orobanche crenata* Forsk on peas plants and *Orobanche aegyptica* L. parasitizing the roots of faba bean were collected at different growth stages from natural infested fields at El-Aiat, Hellwan Governorate, Egypt. Growth stages included the under ground stage characterized by tumor-like and lobed irregular undifferentiated mass of tissues. The emergence stage was the stage when the growing tip of broomrape appeared through the soil crakes. At the vegetative stage the parasite stalks acquired their characteristic pigmentation. The blooming stage included bud formation, unopened flowers and full blooming (Fig. 1; Hassan, 1996). Both species were identified and distinguished according to the taxonomic description of Parker and Riches (1993).

The trichome distribution and density were traced on the outer surface of both broomrape peduncles parts, stem, scale leaves on the stem and different floral organs. For this purpose, free hand sections, slough or intact specimen were prepared and examined under the light microscope.

A histochemical test was then carried out. In this respect the sections were prepared using paraffin wax embedded

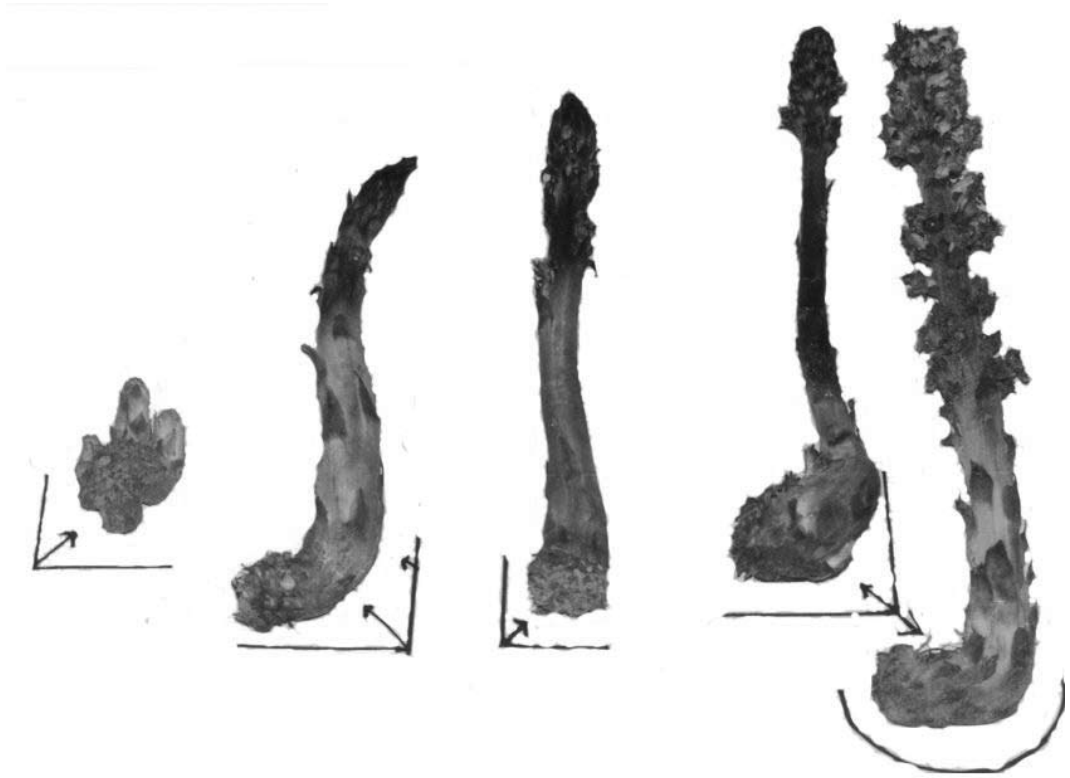

Fig. 1. Different growth stages of broomrape (I-V; vegetative to blooming) according to Hassan (1996). Arrows point to hairless parts (corm like organ and 1-2cm of stem).

methods (Johansen, 1940; Jensen, 1962). This was followed by the examination under epifluorescent microscope. The test included non-stained and/or stained with fluorol yellow 088 (Brundrett, et al. 1991) and mixed sudan III&IV (O'Brien and McCully, 1981). Stained sections with toludine blue O (O'Brien and McCully, 1964) double (safranin / light green) stain (Jensen, 1962) and fat red 7B (Brundrett et al., 1991) were examined under the light microscope. Stains preparation, procedures, stain light-colour reaction and microscope examinations followed the methodology described in detail by El-Awadi (2001).

Nikon microscope Optophoto-2 (Nikon-Japan) was equipped with:

A: A xenophot long life 12V 1000W lamp (Osrama-Germany), neutral colour balance (NCB) filter (for visible light) and CFE plan achro objective lens.

B: Epifluorescent attachment;

1- High-pressure mercury lamp 1000w (USH, 102DH, USHIO INC. Japan).

2- Epi-filter blocks for fluorescent light; ultraviolet (UV-A; 330-380 nm excitation filter), blue (B-2A, 450-490 nm excitation filter) and Green (G-2A, 51-560 nm excitation filter).

## RESULTS

### Trichome distribution and morphology

It was observed that the trichomes covered all parts of the two examined broomrape species peduncles with the exception of the underground organ and 1-2cm of the stem basal part (Fig. 1, Table 1).

Fig. 2. Trichome density outgrowing the surface cell layer of broomrape peduncle (*Orobanche crenata* Forsk and *Orobanche aegyptica* L.) 4X. A, Few trichome; B, Moderate trichome density; C, Dense trichome.

The distribution of tichomes ranged from few trichomes on stem basal part (Fig. 2A), to dense on the other parts (Fig. 2C), i.e. stem apex, scales and different floral parts. Moderate density was recorded on the middle part of stem (Fig. 2B).

All observed trichomes belonged to the multicellular uniseriate category and were classified as glandular and/or non-glandular types. Glandular trichomes outgrew all organs of the two broomrape species except that they were absent on the anthers, ovary and stigma (Table 1).

Morphological description of glandular trichomes is shown in Fig. 3A. Glandular trichomes consisted of a multicellular uniseriate stalk and a gland. The stalk included one-foot cell (epidermal cell)

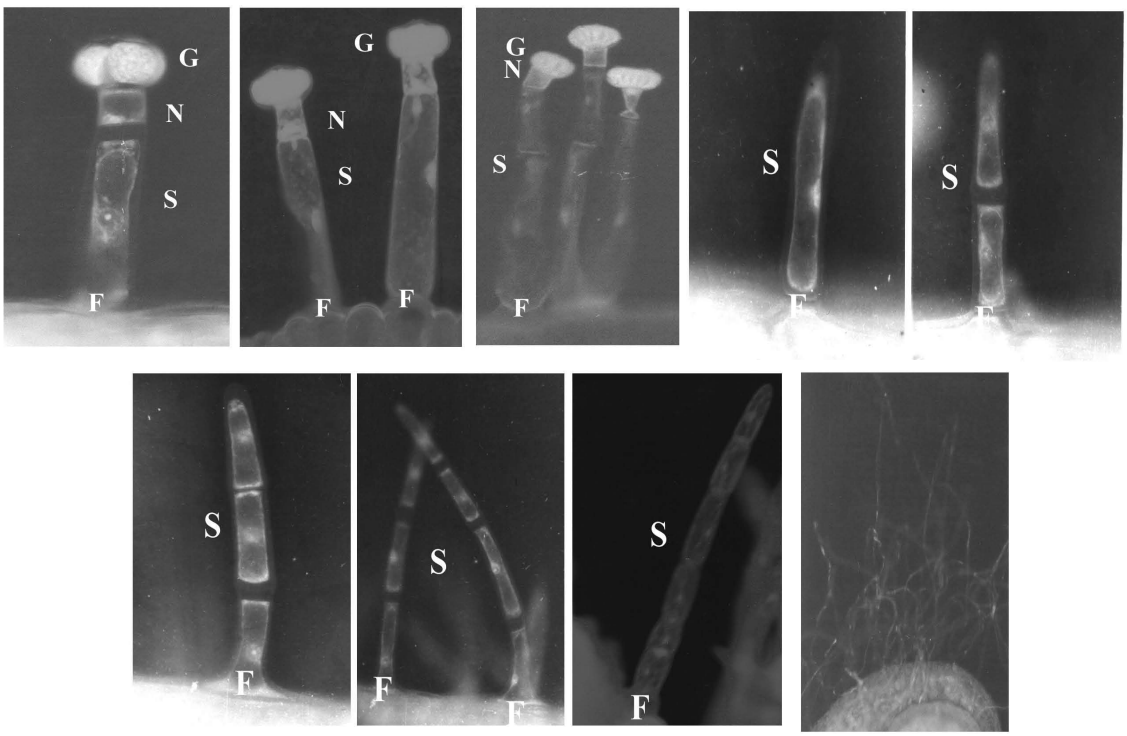


Fig. 3. Types and structure of the trichomes observed on the outer surfaces of the two broomrape species. G, Gland; N, Neck cell; S, Stalk cells; F, Foot Cell; Glandular; Non-glandular; Crisped hairs.

Table 1. Trichome distribution, density and kind on different peduncle parts in *Orobanche aegyptica* and *Orobanche crenata*.

| | | | Trichome existence | | Trichome density | | Trichome type | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Glandular | | Non-glandular | | Crisped hairs | |
| | | | *O. aegyptica* | *O. crenata* | *O. aegyptica* | *O. crenata* | *O. aegyptica* | *O. crenata* | *O. aegyptica* | *O. crenata* | *O. aegyptica* | *O. crenata* |
| Stem | Base | | + | + | * | * | + | + | - | - | - | - |
| | Middle | | + | + | ** | ** | + | + | - | - | - | - |
| | Apex | | + | + | *** | *** | + | + | - | - | - | - |
| Scales | | | + | + | *** | *** | + | + | - | - | - | - |
| Flower | Bract | | + | + | *** | *** | + | + | - | - | - | - |
| | Bracteoles | | + | - | *** | - | + | - | - | - | - | |
| | Sepals | | + | + | *** | *** | + | + | - | - | - | - |
| | Corolla (Petals) | Claw | + | + | *** | *** | + | + | - | - | - | - |
| | | Limb | + | + | *** | *** | + | + | - | - | - | - |
| | Androecium | Filament Base | + | + | *** | *** | + | + | + | - | - | + |
| | | Filament End | + | + | ** | *** | + | + | + | - | - | - |
| | | Anther | + | - | *** | - | - | - | - | - | + | - |
| | Gynoecium | Ovary | - | - | - | - | - | - | - | - | - | - |
| | | Style | + | + | *** | *** | + | + | - | - | - | - |
| | | Stigma | + | - | - | - | - | - | - | - | - | - |

+= Existence -=Absent * =Few trichome **= moderate trichome ***=dense trichome

with one or two body cells and one neck cell-bearing a gland. The gland contained, however, two to twenty secreting cells (Fig. 3A).

On the other hand, non-glandular uniseriate trichomes were characteristic for *O. aegyptica* and were located in androecium filament (base and tip) as shown in Table 1. Microscopic observations showed that the morphology of non-glandular uniseriate trichome included one-foot cell (epidermal) with one or more (1-5) body cells (Fig. 3B).

Crisped hairs were found on the filament base of androecium of *Orobanche crenata* and on anthers of *Orobanche aegyptica* (Table1; Fig. 3C).

**Autofluorescence and histochemical tests of the glandular trichomes in the two species**

The whitish (UV-2A), bright yellow (B-2A) and bright red (G-2A) autofluorescence on the outer layer of trichome stalk and gland (Table 2; Fig. 4A-C) indicated the presence of lignin and/or phenolic substances in the trichomes` tissues` structures.

Secreting cells inside the gland emitted the whitish autofluorescence (UV-2A) of lignin and phenolic substances as shown in Fig. 4A.

The histochemical stains (Table 2; Fig. 5A-D) revealed the presence of lignin (red, double stain), phenolics (blue, toludine blue O), lipid (yellow, fluoroll yellow 088 or red, fat red 7B) and suberin (red, mixed Sudan III&IV). These substances were located in the outer layer of trichomes and in the neck cell. In secreting cells of the examined gland the phenolic substances were detected by the toludine blue O and the double stains (Fig. 5A-B).

## DISCUSSION

The results of the present investigation indicated that the trichomes existed on all parts of broomrape peduncle while they were absent on the under-ground parts,

Table (2): Detection and localization of lignin, phenolics, lipid and suberin compounds in broomrape glandular trichome

| Histochemical tests | | Target compounds | Observed colour | Outer layer of trichome | Neck cell | Gland (secreting cells) |
|---|---|---|---|---|---|---|
| Autofluo-rescence of non-stained sections | UV-2A | Lignin and or phenolics | Bright blue to whitish | + | + | + |
| | B-2A | Lignin and or phenolics | Bright yellow | + | + | + |
| | G-2A | Phenolics | Bright red | + | + | + |
| Double stain | | Lignin | Red | + | + | + |
| Toludine blue o | | Phenolics | Blue | + | + | + |
| Fat red 7b | | Lipid or suberin | Bright red | + | + | - |
| Fluorol yellow 088 | | Lipid | Bright yellow | + | + | - |
| Mixed stain (Sudan III&IV) | | Suberin | Bright red | + | + | - |

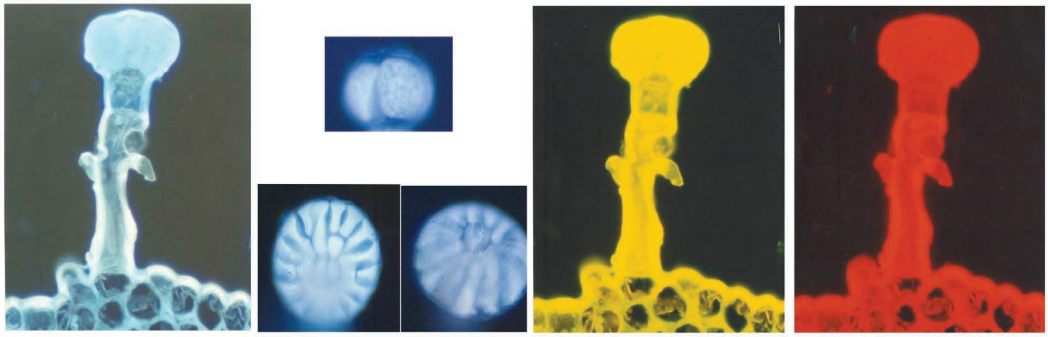

Fig. 4. Autofluorescence of non-stained sections showing the light-colour reaction of broomrape glandular trichome under A-UV-2A; B-B-2A; C-G-2A.

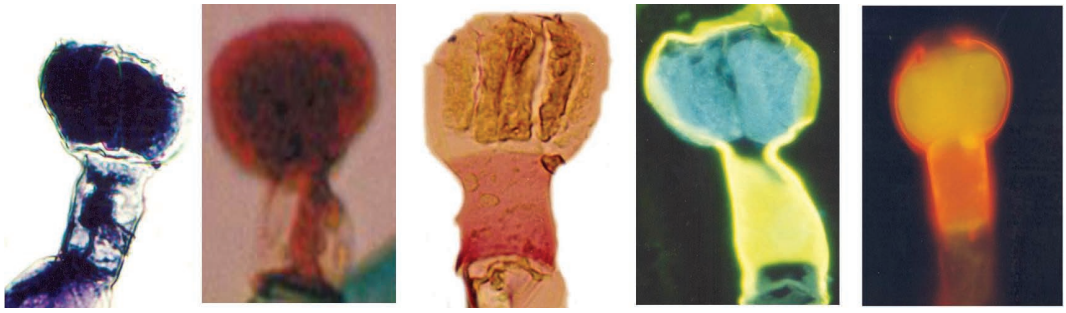

Fig. 5. Histochemical tests showing the chemical composition of broomrape glandular trichomes. A, Stained with double stain under visible light; B, Stained with toludine blue O under visible light; C, Stained with fat red 7B under B-2A light; D, Stained with fluorol yellow 088 under UV-2A light; E, Stained with Sudan III&IV under B-2A light.

as well as on the basal part (1-2 cm) of the stalk. Their density ranged from few trichomes on the lower part of the stalk, followed by moderate appearance on the middle and increased upwards on other stem apex, scales and different floral parts. In this connection, the presence of trichomes was found to exist on the surface of different terrestrial plant parts, leaves, petals, stems, petioles, peduncle and seed coats as previously reported (Wagner, 1991; Fahn, 2000; Amme et al., 2005; Raman, 1991. However, the variation in the trichomes was used in the classification of the parasitic weed in the family Scrophoulariaceae.

The observation herein indicated that the trichomes detected in broomrape species belonged to the multicellular uniseriate category and were classified as glandular and non-glandular types. In addition, the glandular trichomes were found to outgrow the epidermal layer of different broomrape organs with the exception of the anthers, ovary and stigma.

Microscopic test revealed that the glandular trichome was composed of multicellular uniseriate stalk and gland. The stalk included one-foot cell (epidermal cell) with one or two body cells and one neck cell-bearing the gland. The gland contained two to twenty secreting cells. In this respect, the glandular trichomes were found on ca. 30% of the vascular plants (Fahn, 2000; Wagner et al., 2004). Glandular trichomes, which secret lipophilic substances (terpenes, lipids, waxes and flavonoids), may provide chemical or physicochemical protection of a given plant organ against various types of

herbivores and pathogens by entrapping or poisoning them (Wagner, 1991; Cipollini and Bergelson, 2002).

Our results showed that the non-glandular multicellular uniseriate trichomes were characteristic for *O. aegyptica* and were located on androecium filaments (base and tips). As a consequence, the microscopic observations showed that the morphology of a non-glandular trichome included one-foot cell (epidermal) with one or more (1-5) body cells. It can be suggested that the presence of the non-glandular trichomes on the androecium may guide the path of the pollinators (Rodriguez, 1984). This was in agreement with our results since the crisped hairs were shown on the androecium of both broomrape species under test**.**

The autofluorescence and histochemical tests indicated a positive reaction to the existence of lignin, lipid and phenolic substances as characteristic for the glandular trichomes. In secreting cells of the gland the phenolic substances were solely detected. These results were supported by the previous studies of El-Awadi (2001) and El-Akkad et al. (2002). They proved histochemically the occurrence and localization of lignin and other phenolics, as well as lipid compounds and suberin, in the epidermal layer of broomrape parasitised faba bean plant. Similar results were observed in corner thickening of the cortical and pith cells. In accordance, these substances were chemically determined by proving the existence of chorogenic, caffeic, ferulic, and coumaric acids whereas chlorogenic acid was reported as dominant in both aerial and corm- like structures of broomrape.

Our results in support to others (Close and McArthur, 2002; Boudet, 2007) may implicate the role of phenolic substances as classic defense compounds protecting plants from the attack of other organisms. However, including the structural role of phenolic substances as supportive or protective factors in plant tissues showed their involvement in strategies, and signaling properties, particularly in the interaction between plants and their environment.

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