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# WHITE BLISTER RUSTS AND DOWNY MILDEWS FROM BAJAUR AGENCY FATA, WITH SOME NEW RECORDS FROM PAKISTAN

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**Abstract**

In a species diversity study of Oomycyctes of Bajaur Agency FATA, Pakistan, infection of white blister rusts and downy mildews recorded on three cultivated and four wild plants. *Capsella bursa-pastoris* showed mixed infection of *Albugo candida* and *Hyaloperonospora parasitica* (syn: *Peronospora parasitica*). Similarly, *A. candida* and *H. brassicae* (syn: *P. brassicae*) parasitized *Brassica campestris*. *Wilsoniana portulacae* (syn: *Albugo portulacae*) and *W. occidentalis* com. nov. (syn: *Albugo occidentalis*) recovered from *Portulaca oleracea* and *Spinacia oleracea*, respectively. *Bremia taraxaci, B. sonchicola* and *B. saussureae* recorded on *Taraxicum officinale*, *Sonchus* sp., and *Saussurea* sp., respectively. All these obligate parasites are new records from Bajaur Agency, while *H. parasitica*, *W. occidentalis*, *B. taraxaci*, and *B. saussureae* on the mentioned hosts are new records from Pakistan.

**Key words:** White rust, Downy mildew, New records, Bajaur Agency, Pakistan.

## Introduction

White blister rusts and downy mildews belong to Phylum Oomycota, class Oomycetes and Order Peronosporales of the kingdom Straminopila (Dick, 2001). These biotrophic plant parasites cause diseases in economically important crops and common weeds (Farr *et al*., 1989; Voglmayr, 2008). *Peronospora* is the largest genus of downy mildews and Constantinescu (1991) listed about 600 binomials of it. Constantinescu & Fatehi (2002) segregated the genus *Peronospora* into three genera, *Peronospora s.str*., *Hyaloperonospora*, and *Perofascia.* The white rust producing genus *Albugo* belongs to family Albuginaceae and includes about 50 species (Biga, 1955; Choi & Priest, 1995). Of these, *A. candida* (Pers.) Roussel infects more than 300 host plants (Farr *et al*., 2004).

Although comprehensive lists of saprophytic and parasitic fungi from Pakistan are available, Oomycetes are among the less studied group where 18 genera comprising 115 species have been recorded (Mirza & Qureshi 1978; Shahzad & Ghaffar, 1993; Ahmad *et al*., 1997; Abdul Haq & Shahzad, 1998; Lodhi, 2007; Bala *et al*., 2010; Lodhi *et al.,* 2013). Of these, 42 species produce white blister rust and downy mildew including 5 species of *Albugo,* 2 species of *Bremia,* 31 species of *Peronospora,* 2 species *Plasmopara,* and one each of *Pseudoperonospora* and *Sclerospora.*

Bajaur Agency is a mountainous area of FATA in the northern part of Pakistan. Although the area provides an ideal environment for the growth of Oomycetes, it was somehow neglected by the mycologists working in Pakistan. In a preliminary study, we isolated nine Oomycetes including *Brevilegnia* sp., and 8 species of *Pythium* from the soil of Bajaur Agency (Abdul Haq & Shahzad, 1998) that indicated that the area has great potential for exploration of Oomycetes. The present paper reports white blister rusts and downy mildews form Bajaur Agency with some new records from Pakistan. A new combination in the genus *Wilsoniana* has also been proposed

## Materials and Methods

Infected plants collected from different parts of Bajaur Agency screened for the presence of sporangia and sporangiophores. In case of downy mildews, sporangia and sporangiophores were induced by incubating the plants in humid chamber at 5-10$^{o}$C for two to three days. Slides were prepared in lactophenol and cotton blue. The pathogens identified after reference to Wilson (1907), Tanaka (1919), Skidmore & Ingram (1985), Göker *et al*. (2003) and Thines & Spring (2005).

## Results and Discussion

During the study, 3 species of white blister rust producing genera viz., *Albugo* and *Wilsoniana,* and 5 species of downy mildew fungi including two species of *Hyaloperonospora* and three species of *Bremia* were recorded. Before 2005, all the white blister rusts were placed in a single genus *Albugo*. However, Thines & Spring (2005) based on morphological and molecular phylogenetic investigations segregated white blister rusts into three genera *viz., Pustula, Wilsoniana*, and *Albugo*. *Albugo s.str.* mostly parasitizes Brassicaceae, while *Wilsoniana* and *Pustula* infect Caryophyllales and Asteridae (except for Convolvulaceae), respectively. During the present studies, *A. candida* was recorded on *Brassica campestris* and *Capsella bursa-pastoris. A. candida* is worldwide in distribution. It has also been reported on many brassicacious hosts from Tandojam, Karachi, Rawalpindi, Faisalabad, Dargai and Mingora but *C. bursa-pastoris* appeared to be a new host record from Pakistan. It is also the first record of *A. candida* from Bajaur Agency (Mirza & Qureshi, 1978; Ahmed *et al.,* 1997).

Based on morphological, ultrastructural, and molecular data, Thines & Spring (2005) segregated the species of *Albugo* in to three genera *viz.*, *Albugo, Wilsoniaia,* and *Pustula.* Of the two species of *Wilsoniana* recorded during present studies, W. *portulacae* has been reported earlier from Swat, Karachi, Tandojam, Mianwali, Mirpur, Quetta and Bathora areas of Pakistan (Mirza & Qureshi, 1978; Ahmed *et al.,* 1997). However, this appears to be the first report of *W. portulacae* from Bajaur Agency.

Wilson (1907) described *A. occidentalis* from *Chenopodium capitatum*. It also causes considerable damage to spinach crop (Leskovar & Kolenda, 2002). During the present studies, white blister disease was also recorded on spinach crop. Initailly the fungus was identified as *A. occidentalis.* However, after perusing the work of Thines & Spring (2005), it appeared that morphologically, *A. occidentalis* is close to *Wilsoniana*. In analysis of ITS rDNA sequences of *A. candida, A. occidentalis* and *W. partulaceae* available in GenBank, *A. occidentalis* branched with *Wilsoniana portulacae,* and showed a distant relationship with *A. candida* (Fig. 1). Thines & Spring (2005) also suggested that *A. occidentalis* is basal to *Wilsoniana* but they did not transfer it to genus *Wilsoniana* because no specimen was microscopically examined by them. However, on the basis of the microscopic structures observed during the present studies, transfer of this species from *Albugo* to *Wilsoniana* is proposed. No previous report of the fungus from Pakistan is available (Mirza & Qureshi, 1978; Ahmed *et al.,* 1997).

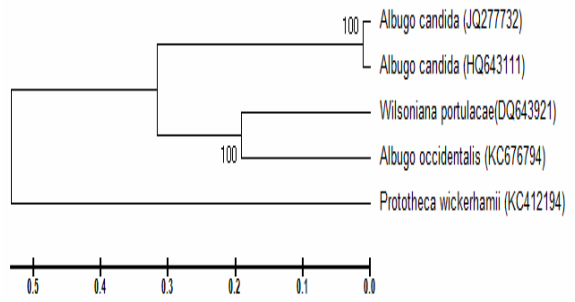


Fig. 1. Phylogenetic analysis of *Albugo* and *Wilsoniana* using ITS rDNA sequences from GenBank.

Following the broad species concept, Yerkes & Shaw (1959) included all *Peronospora* accession parasitic to Brassicaceae into *Peronospora parasitica*. On the basis of molecular and morphological evidence, Constantinescu & Fatehi (2002) segregated genus *Peronospora* into *Peronospora s.str*., *Hyaloperonospora* and *Perofascia.* They accepted six species in the genus *Hyaloperonospora* to include *Peronospora parasitica* and other *Peronospor*a species parasitic on Brassicaceae, Capparaceae, Cistaceae, Limnanthaceae, Resedaceae and Zygophyllaceae. Among the six species accepted in the genus *Hyaloperonospora* by Constantinescu & Fatehi (2002), *H. parasitica* has a wide host range. *H. brassicae* (syn: *P. brassicae)* that was previously merged in *H. parasitica* (Constantinescu & Fatehi, 2002), now has been introduced as a new combination in the genus *Hyaloperonospora* based on molecular phylogenetic investigation (Göker *et al*., 2003). During the present studies, *H. brassicae* on *B. campestris,* and *H. parasitica* on C. *bursa-pastoris* recorded for the first time from Bajaur Agency, FATA. Of these, *H. parasitica* was previously reported on the leaves of *Eruca sativa* from Lahore and Sheikhpura, and on *B. campestris* var. *rapa* and *Raphanus sativus* from Tandojam and Ranipur, but it is reported for the first time on *C. bursa-pastoris* from Pakistan (Mirza & Qureshi, 1978; Ahmed *et al.,* 1997).

A lot of uncertainty prevails regarding species delimitation in *Bremia*, another genus of downy mildews that contains pathogens parasitic to a wide range of plants belonging to family Asteraceae. Before the introduction of molecular phylogenetic studies, two species, *B. lactucae* and *B. graminicola*, respectively parasitic to Asteracea and Poacea, were generally recognized. In an attempt to render *Bremia* monotypic, Thines *et al*. (2006) introduced a new genus *Graminivora* to accommodate *B. graminicola*. Due to lack of distinct morphological characteristics, many species of *Bremia,* like *B. sonchicola, B. saussureae,* and *B. taraxaci* recognized by Sawada (1914), Sydow (1923) and Ito & Tokunaga (1935) were lumped to a single species, *B. lactucae*. On the account of high host specificity, Skidmore & Ingram (1985) treated some of these *Bremia* lineages as *forma speciales* rather than as distinct species. Tao (1998) observed morphological distinctiveness in 11 species of *Bremia*.

Recent molecular phylogenetic studies of Voglmayr *et al.* (2004), Choi *et al.* (2007, 2011), Voglmayr & Constantinescu (2008) and Thines *et al.* (2010) revealed the presence of considerable genetic diversity within the genus *Bremia*. Thines *et al.* (2010) reported that *B. lapsanae*, *B. sonchicola* and *B. taraxaci* were genetically highly distinct from *B. lactucae* parasitic to *Lactuca sativa*. Choi *et al.* (2011) found that *B. microspora*, *B. ovata*, *B. saussureae*, and *B. sonchicola*, which parasitize plants outside the genus *Lactuca* could be established as distinct, host-specific entities. During the present studies, infection by *Bremia* recorded on *Sonchus oleraceous, Saussurea* sp., and *Taraxicum officinale*. Microscopic study of the fruiting structures of *Bremia* on these three hosts showed great variation (Table 1). It indicated that these three different species as also suggested by Thines *et al.* (2010) and Choi *et al.* (2011). These species are therefore treated as three distinct species. All the three species are recorded for the first time from Bajaur Agency, FATA. Of these, *B. sonchicola* was previously reported as *B. sonchi* on *Sonchus oleraceous* from Quetta, Shekhupura, Lahore and Kaghan areas, whereas no previous report of *Bremia* species on *Saussurea* sp. and *Taraxicum officinale* is available (Mirza & Qureshi, 1978; Ahmed *et al.,* 1997).

**Table 1. Comparison of morphological features of *Bremia* species parasitic to three genera of Asteraceae *viz.*, *Sonchus, Saussurea* and *Taraxicum*.**

| Species | Length of sporangiophore (µm) | Length of sporangiophore to 1st branch (µm) | Diam. of vesicle (µm) | Length of sterigmata (µm) | Shape of sporangium | Sporangial dimensions (µm) |
|---|---|---|---|---|---|---|
| *B. sonchicola* | 180-297 (av. 232) | 72-144 (av. 103) | 6.5-9.5 | 4.5-9.0 | Spherical | 18.0-22.5×17.3-20.5 (av. 21.3×20.5) |
| *B. saussureae* | 720-1025 (av. 884) | 670-830 (av. 740) | 7.5-10.0 | 8.1-9.0 | Ellipsoidal | 20-29×16-23 (av. 24.5×19.2) |
| *B. taraxaci* | 414-675 (av. 510) | 216-450 (av. 321) | 7.2-9.9 | 3.6-4.5 | Spherical to ovoid | 15-24×14-20 (av. 21.2×18.0) |

**Description of species**

***Albugo candida*** (Pers.) Roussel, *Fl. Calvados*, Edn 2: 47 (1806)

***Synonymy:*** *Aecidium candidum* Pers., in Gmelin, *Systema Naturae*, Edn 13, 2: 1473 (1792)
*Uredo candida* (Pers.) Pers., *Syn. meth. fung.* (Göttingen), 1: 223 (1801)
*Caeoma candidum* (Pers.) Nees, *Syst. Pilze* (Würzburg): 15, tab. 1: 8 (1816)
*Cystopus candidus* (Pers.) Lév., *Annls Sci. Nat., Bot.*, sér. 3, 8: 371 (1848)

Prominent white sori produced on all parts of the plants except roots, variable in size and shape, sometime confluent, often producing marked distortion of host due to hypertrophy. Sori burst open and release white powdery mass of sporangia. Intercellular mycelium produces sporangiophores sub-epidermally in clusters. Sporangiophores hyaline, clavate, about 24-27 × 12-16 (av. 25.6×14.4) µm in case of *C. bursa-pastoris,* and 23-27×11-16 (av. 25.0×14.2) µm in case of *B. campestris*. Sporangia mostly globular, hyaline, primary sporangia slightly thick-walled than secondary sporangia, smaller in size, 17-21×13-20 (av. 17.3×13.0) µm in *C. bursa-pastoris* and 15-19×14-18 (av. 16.4×14.7) µm on *B. campestris*. Encysted zoospores 7-9 µm in diameter. Oogonia usually confined to stem, abundant in the deformed swollen region, 49.5-64.8 (av. 56.7) µm; antheridia attached laterally, 21.1-32.2×9-16.2 (av. 26.6×13.8) µm; oospores chocolate brown, 29.7-47.8 (av. 39.3) µm, epispore thick with low blunt ridges which are usually confluent and irregularly branched (Fig. 2A, B, C & D).

***Wilsoniana occidentalis*** (G. W. Wilson) Abdul Haq & Shahzad *comb. nov.*

***Synonymy:*** *Albugo occidentalis* G. W. Wilson, *Bull. Torrey bot. Club,* 34: 80 (1907)
*Cystopus occidentalis* (G.W. Wilson) Sacc. & Trotter, *Syll. fung.* (Abellini), 21: 859 (1912)

Symptoms appear as raised white pustules on the lower surface of *Spinacia oleracea* leaf, which coalesce as the disease progresses. Chlorotic spots appear on the upper surface in the corresponding region of the leaf. Sori superficial, round to irregular in outline, sometimes confluent, 0.5-3mm. Sporangiophores cylindrical, slightly curved, 24.8-29.4×12.9-16.6 (av. 26.2×13.9) µm; sporangia discoid with an equatorial thickening, 14.7-20.2×13.8-17.5 (av. 18.0×16.3) µm; oogonia formed in leaf tissues, globular, 56-64 (av. 60.5) µm, oospores yellowish brown, shallowly reticulate, 54-58 (av. 55.2) µm (Fig. 2E, F ).

***Wilsoniana portulacae*** (DC.) Thines, *Mycotaxon,* 92: 456 (2005)

***Synonymy:*** *Uredo portulacae* DC., in de Candolle & Lamarck, *Fl. franç.*, Edn 3 (Paris), 5/6: 88 (1815)
*Cystopus portulacae* (DC. ex Duby) Lév., *Annls Sci. Nat.*, Bot., sér. 3, 8: 371 (1848)
*Albugo portulacae* (DC. ex Duby) Kuntze, *Revis. gen. pl.* (Leipzig), 2: 658 (1891)

Numerous white or yellowish pustules produced on all parts except the roots of *Portulaca oleracea* plants. On leaf, pustules appear on the upper surface. Sori rounded to irregular in outline, up to 4 mm in diameter. Sporangiophores produced in clusters, clavate, 22.5-29.4×12.6-13.5 (av. 25.0×12.7) µm in size. Sporangia dissimilar in size and shape; the terminal ones larger and cylindrical, 18.5-23.9×10.9-19.3 (av. 21.4×13.8) µm, whereas, the basal ones smaller and sub-globular, 12.0×9.0 µm in size. Oogonia and oospores produced in stems and leaf tissues. Oogonia 64.4-81.9 (av. 72.4) µm in diam. Oospores globular, yellowish brown, 50.6-55.2 (av. 53.2) µm; epispore with short ridges (Fig. 2G, H).

***Hyaloperonospora brassicae*** (Gäum.) Göker, Voglmayr, Riethm., Weiss & Oberw., *Can. J. Bot.,* 81(7): 681 (2003)

**Synonymy:** *Peronospora brassicae* Gäum., *Beih. bot. Zbl.*, Abt. 1 35: 131 (1918)
*Peronospora brassicae f. brassicae-nigrae* Săvul. & Rayss, (1934)
*Peronospora brassicae f. major* Savul. & Rayss, *Palest. J. Bot.*, Jerusalem Series 1: 154 (1938)
*Peronospora parasitica* subsp. *brassicae* (Gäum.) Maire, *Mém. Soc. Sci. Nat. Maroc.* 45: 14 (1937)

Symptoms appear in the form of discrete and irregular chlorotic to yellowish brown spots on the dorsal surface of *B. campestris* leaves, especially the radical leaves, and grayish white areas on the ventral surface. The spots increase in size and number, and ultimately cover a large area of the foliage that later on becomes necrotic. The pathogen produces hyaline, coenocytic mycelium that grows intercellularly sending lobed haustoria into the cells. Sporangiophores emerge from stomata on the under surface of the leaf, dichotomously branched, ending in slender curved tips, 279-558 (av. 398.5) µm. Branching starts at a distance of 216-459 µm from the base; ultimate branches length 10.8-39.6 µm. Each branch bears a single apical sporangium. Sporangia ovoid, 17.1-27.9×14.4-23.4 (av. 21.9×18.7) µm. Oogonia and oospores produced in leaf tissues; Oogonia monosporous, yellow to orange in color, globose to sub-globose, 41.8-59.4 (av. 49.7) µm in diam.; Oospores globose, 26.4-48.4 (av. 31.8) µm in diam., (Fig. 3A, B).

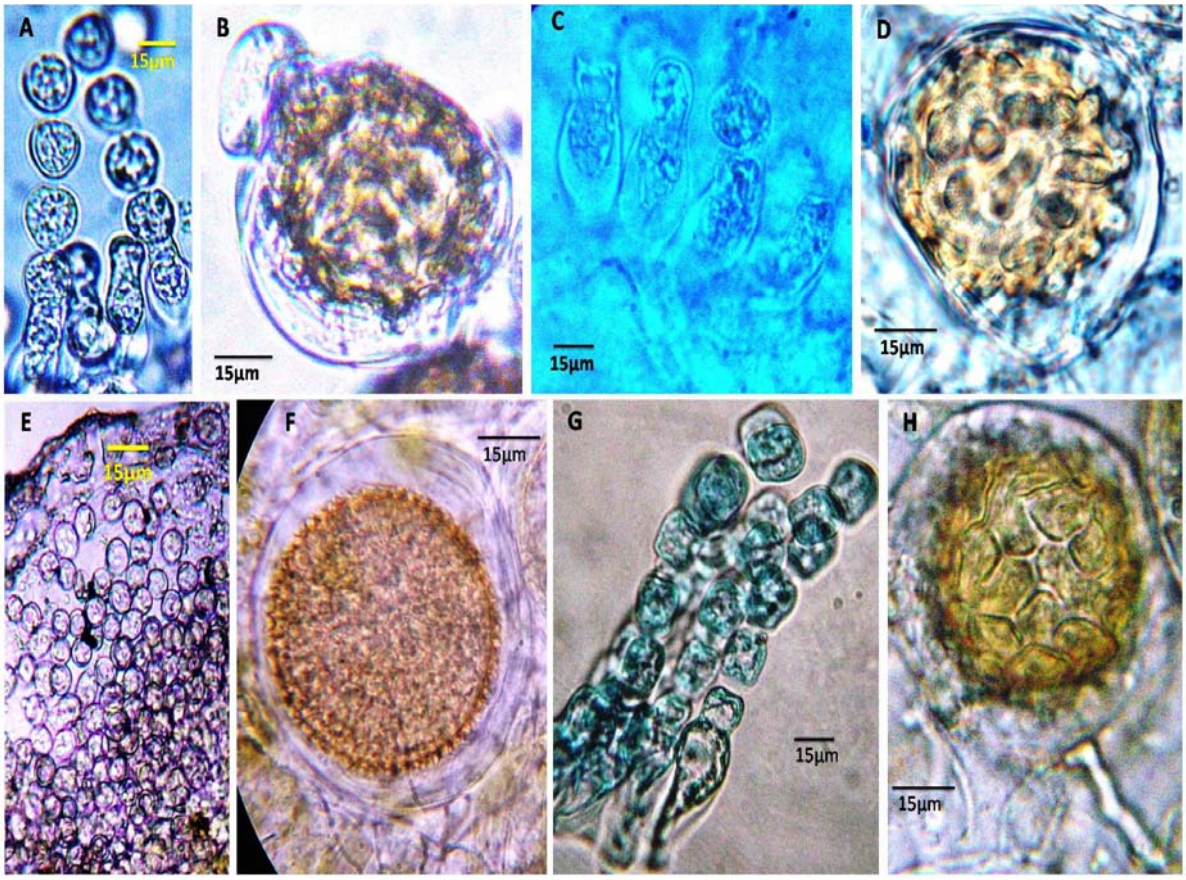


Fig. 2. *Albugo candida* on *C. bursa-pastoris*: A= Sporangiophores and sporangia, B= Oogonium with oospore and antheridium; *A. candida* on *Brassica campestris*: C= Sporangiophores and sporangia, D= Oogonium with oospore and antheridium; *Wilsoniana occidentalis* on *Spinacia oleracea*: E= Sporangiophores and sporangia, F= Oogonium with oospore and antheridium; *W. portulacae* on *Portulaca*: G= Sporangia and sporangiophores, H= Oogonium and oospore.

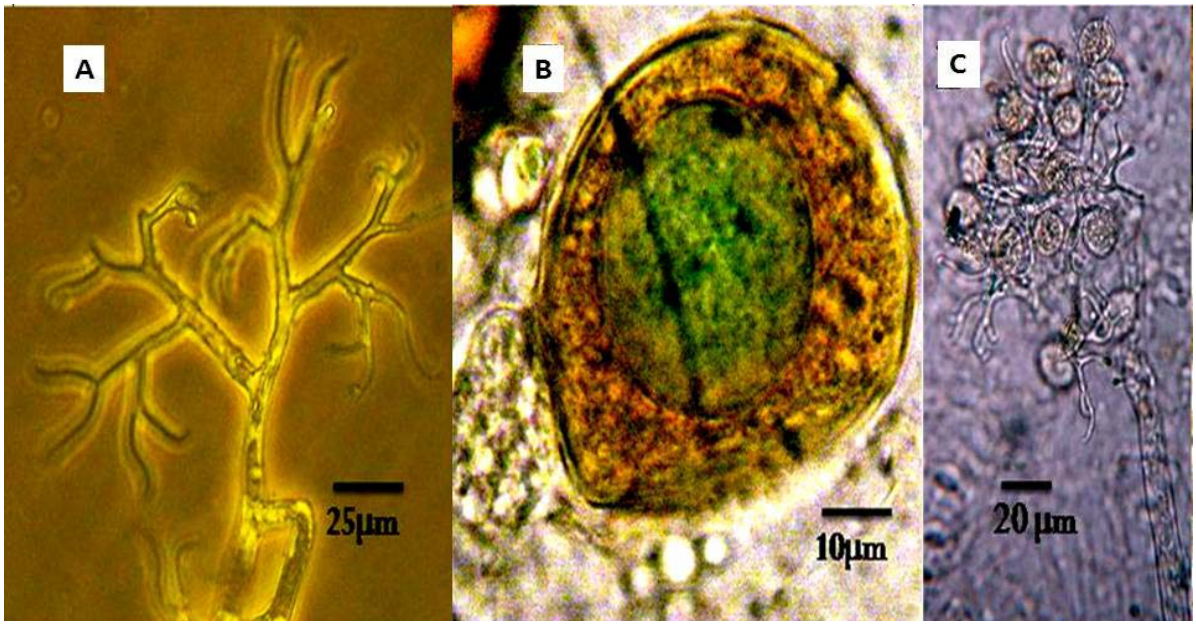


Fig. 3. *Hyaloperonospora brassicae* on *B. campestris*: A= Sporangiophore, B= Oogonium and oospore with antheridium. *H. parasitica* on *C. bursa-pastoris*: C= Sporangiophore and sporangia.

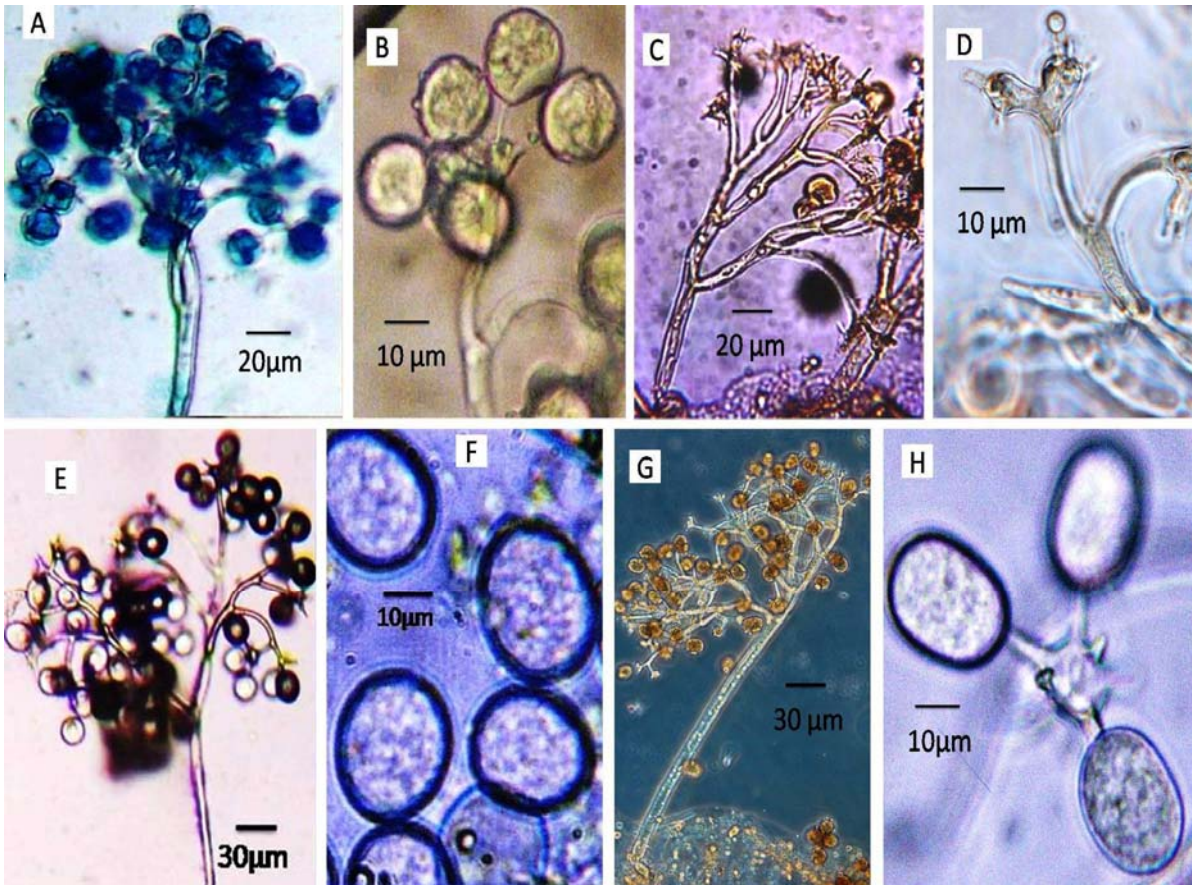


Fig. 4. Sporangia and sporangiophores of *Bremia* species: A, B, C & D= *B. sonchicola,* E & F= *B. saussureae,* G & H= *B. taraxaci.*

***Hyaloperonospora parasitica*** (Pers.) Constant. in Constantinescu & Fatehi, *Nova Hedwigia,* 74(3-4): 310 (2002)

**Synonymy*:*** *Botrytis parasitica* Pers., *Observ. mycol.,* (Lipsiae) 1: 96 (1796)
*Peronospora parasitica* (Pers.) Fr., *Summa veg. Scand.*, Section Post. (Stockholm): 493 (1849)
*Peronospora parasitica var. capsellae* Y.C. Wang [as '*parasitica Capsellae*'], 1(4): 254 and 257 (1944)

The sporangiophores found only on the stems of *Capsella bursa-pastoris*. Mycelium coenocytic and hyaline, growing intercellularly and sending lobed haustoria into the cells. Sporangiophores emerge from stomata, dichotomously branched, ending in slender curved tips, 270-580 (av. 390.1) µm in length; branching started at 210-450 µm from the base; ultimate branches length 10.0-35.5 µm; each branch bearing a single sporangium. Sporangia ovoid, 16.5-28.4×15.5-23.5 (av. 21.0×18.5) µm; oogonia and oospores not recorded (Fig. 3C).

***Bremia sonchicola*** (Schltdl.) Sawada, *Descriptive Catalogue of Formosa Fungi*, 3: 47 (1927)

**Synonymy:** *Bremia lactucae* Regel, *Bot. Ztg.,* 1: 666 (1843)
*Bremia sonchi* Sawada, *Bot. Ztg.,* 10: 620 (1852)

Polygonal chlorotic spots, bounded by veins, appear on the upper surface of the leaves, which sometime cover the entire surface in lower leaves. Older leaves close to the ground are affected first. Under side of the leaf opposite to the yellow patches shows white mass of sporangiophores that grow larger and take on a white downy appearance. As the disease progresses, the spots become necrotic and dark brown. Mycelium intercellular, coenocytic and hyaline; producing spherical to ovoid haustoria. Sporangiophores arise singly or in group of 2-4 from stomata, 180-297 (av. 232.3) µm in length, upper half 3-6 times dichotomously branched, ending in a vesicle (6.5-9.5 µm), bearing 3-5 sterigmata, 4.5-9.2x2.5-3.7µm. Branching started at a distance of 72-144 (av. 103.0) µm from the base, basal portion swollen, 11-17 µm; sporangia spherical, 18.0-22.5×17.3-20.5 (av. 21.3×20.5) µm (Fig. 4A-D).

***Bremia saussureae*** Sawada, *Bot. Mag.*, Tokyo 28: 138 (1914)

**Synonymy*:*** *Bremia lactucae* Regel, *Bot. Ztg.,* 1: 666 (1843)

Mycelium intercellular, sending ovoid to obovoid haustoria to the cells; sporangiophores long, single or caestipose, arising from stomata, 720-1025 (av. 884.1) µm long, branching dichotomous, rarely monopodial, ending

in a vesicle 7.5-10.0µm, bearing 3-6 sterigmata, 8.1-9.0µm long, branching started at 670-830 (av. 740.5) µm from the base; sporangia ellipsoid, 20-29×16-23 (av. 24.5×19.2) µm (Fig. 4E, F).

***Bremia taraxaci*** S. Ito & Tokun, Notae mycologicae, Asiae orientalis. I. *Trans. Sapporo Nat. Hist. Soc.,* 14: 11-33 (1935)

**Synonymy:** *Bremia lactucae* Regel, *Bot. Ztg.,* 1: 666 (1843)

Symptoms were not well marked, however, white patches of downy appearance frequently observed on the ventral side of leaves near the ground. Mycelium intercellular, sending spherical to ovoid haustoria to the cells; sporangiophores single or caestipose, arising from stomata on the lower surface, 414-675 (av. 510) µm long, dichotomously branched, ending in a vesicle 7.2-9.9µm in diam, bearing 3-6 sterigmata 3.6-4.5µm, first branching 216-450 (av. 321) µm; Sporangia spherical to ovoid,15-24×14-20 (av. 21.2×18.0) µm; no oogonia and oospores observed (Fig. 4G, H).

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