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Research Article

# Isolation of Trichomes from Wheat and Other Species of Flowering Plants

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**Abstract:** Plant hairiness or pubescence as a specific phenotypical feature related to dehydration tolerance and resistance to leaf vermin is considered in this paper. A method for the isolation of trichomes, earlier developed for *Arabidopsis* Heynh., was found to be appropriate for the separation of similar polarised cells from pubescent wheat, *Triticum aestivum* L., lines, as well as some other higher plant species. This procedure thus paves the way for the study of the molecular organisation of trichomes in wheat and other mono- and dicotyledoneous plants.

Key Words: Trichomes, leaf, wheat, *Triticum aestivum* L., resistance, vermin, *Oulema melanopiis* L.

## Introduction

Cellular polarisation plays a key part in almost all cell assortments (Klein & Mlodzik, 2004). Trichomes represent specific type of cells derived from an epidermis (Callow, 2000). This essential cell population is intrinsic to the lower surface (abaxial) or both surfaces of the leaf blade. The development of trichomes brings about, as a rule, the formation of unicellular shoots (appendices), and the simultaneous changes in the sub-epidermal layers leading to the assembly of multicellular groups adjacent to the basal parts of trichomes. Cell aggregations forming the basal part are characteristic of typical hair cells, which are usually exposed on the leaf surface. However, the aforementioned hair cells could develop theoretically from any existing or newly emerging plant organs.

According to the online patterns there are smooth uniseriate, dedifferentiated hair cells, containing only one shoot, or differentiated (branched) hair cells. The simplest unbranched trichomes can be found in *Tradescantia* L. More complex, branched trichomes were observed on the leaves of the common ivy, *Hedera helix* L. These trichomes, composed of stellate shoots, were therefore attributed as tentaculiform (multipalmate, or fingered) hair cells. Sensor-like, polytentacular structures of trichomes were identified in the leaf hair cells of an Australian bush, *Ptilotus* R.Br. sp. The abaxial surface of leaves and sepals belonging to another known plant, the *Rhododendron* L. sp. is literally covered with complex trichomes of a specific, umbrella-like composition. An online digital picture posted earlier of the transverse section of a leaf of *Verbascum thapsus* L. depicts that it is circled by myriads of tiny trichomes which 'pollinate' around the epidermis to regulate leaf micro-climate, which is required for a number of the leaf's principal functions that occur on its surface.

There are 21 online canonic types of hairiness observed on the leaf blade. Classification of each type of hairiness depends on the form, colour and surface distribution of trichomes, apart from their particular structural organisation.

The key functions of trichomes can be described as the attainment of moisture under continuous light during sunny days and protecting the plant from leaf-consuming vermin. Nonetheless, cereals are known to possess trichomes distributed, apart from the leaves, on the stigma of the pistil. This peculiarity distinguishes the *Poaceae*

from other plant families. Hence, by 'harbouring' the pollen on a pubescent stigma (Vishnyakova, 1997) trichomes may be involved directly in double fertilisation of the flower. On the other hand, water inflow to the leaves of cereals is supplied in part by a specific organisation of leaf trichomes, since in most of the grasses the basal part of each hair cell consists of so-called bulliform cells, which absorb and store the water, being of the same epidermal origin as the trichomal appendix itself.

Another specific function of some hair cells, e.g., trichomes of the common nettle, *Urtica dioica* L., is the excretion of certain chemical substances fostering non-invasive biochemical cell-to-cell reactions that take place along with ordinary plant protection.

In this study we suggest that trichomes of wheat, *Triticum aestivum* L., as well as hair cells isolated from other mono- and dicotyledonous plant species, could be extracted by the method proposed earlier for the isolation of trichomes from *Arabidopsis* (Zhang and Oppenheimer, 2004).

## Materials and Methods

### Genetic stocks

Leaves of 10-day-old seedlings or 3-month-old plants of wheat, *T. aestivum* L. (cvs. Arai, Lutescens 694 and Otan, Omskaya 9, as well as some other chosen super-pubescent wheat hybrids revealing distinct resistance to the cereal herbivorous beetle, *Oulema melanopiis* L.), were used in addition to leaves, stems, pericarps and sepals detached from some other mono- and dicotyledons (e.g., *Asclepias syriaca* L., *Corylus avellana* L., *Lycopersicon esculentum* L., *Polygonatum sewerzowii* Regel, and *Salvia officinalis* L.) for the isolation of trichomes.

Leaves (or stems, green pericarps and closed inflorescences) were fixed in a mixture of ethanol:acetic acid:water (0.40:0.15:0.45 v/v) for 40-120 min. Then they were rinsed twice in water and once in PBS, pH 7.4, containing 0.02% TX-100. The leaves (and other plant organs as listed above) were further incubated in 50 mM EGTA in the same PBS pre-heated at 50 °C, as previously described (Zhang and Oppenheimer, 2004). After incubation and periodic shaking at room temperature for 1 h, all the extracts were subjected to an additional storage at 4 °C for 12-18 h. Then the leaves (and other plant organs) were treated with clean paintbrushes and discarded. The extracts were further spun at 3500 rpm for 4 min (Eppendorf 5417R). The resulting pellets were washed 3 times with PBS, pH 7.4, lacking EGTA, centrifuged again and re-suspended in small portions (150-200 µl) of the same buffer containing 0.005%-0.02% TX-100. Aliquots (20-25 µl) of the final suspensions were dropped on clean pre-heated glasses and dried at a sustainable temperature regime. Trichome quality was checked by microscopic visualisation using Axioaskop-40 (Zeiss). To achieve image contrasting, 40-min staining with 0.02% methylene blue was undertaken either by direct addition of dye microamounts (2-10 µl) to a 100-excess of the preparations or by the addition of greater dye volumes with 3 subsequent washing-spinning cycles in PBS, as described above. Supernatants ($S_{3.5}$) were additionally centrifuged at 10,000 rpm for 4 min to verify the presence of trichomes in the final pellets by microscopic observations, as indicated above.

The following abbreviations are used in the text: - EGTA: ethylene glycol-bis-(beta-aminoethyl ether)-N,N,N',N'-tetra-acetic acid; PBS: phosphate-buffered saline; TX-100: Triton X-100.

## Results and Discussion

As shown in Figure 1(A), control glabrous leaves of cv. Kazakhstanskaya 10 contained a very small range of trichomes. The scope chosen for photography appeared to be exceptional due to the registration of a single occasional trichome. The following pictures 1(B) and 1(C) show that hairy leaves characteristic of cvs. Arai and Lutescens 694 would contain trichomes, which can be easily separated from leaf surface with the manipulation of higher concentrations of EGTA (50 mM of hot buffer-adjusted reagent). The same chelating agent was earlier used for obtaining trichomes from leaves of *Arabidopsis thaliana* (L.) Heynh. (Zhang and Oppenheimer, 2004). However, the authors erroneously referred to their method as unsuitable for the extraction of hair cells from monocotyledons. Our experimental data refuted such a conclusion, as the method turned out to be appropriate for obtaining hair cells from a number of plant species, including pubescent wheat cultivars (Figure 2), and super-pubescent wheat hybrids (Figure 3). As trichomes were not detected in the pellet after spinning $S_{3.5}$ at 10,000 rpm for 4 min, centrifugation of hair cells at low speed (3500 rpm) appeared to be sufficient for complete precipitation of wheat trichomes.

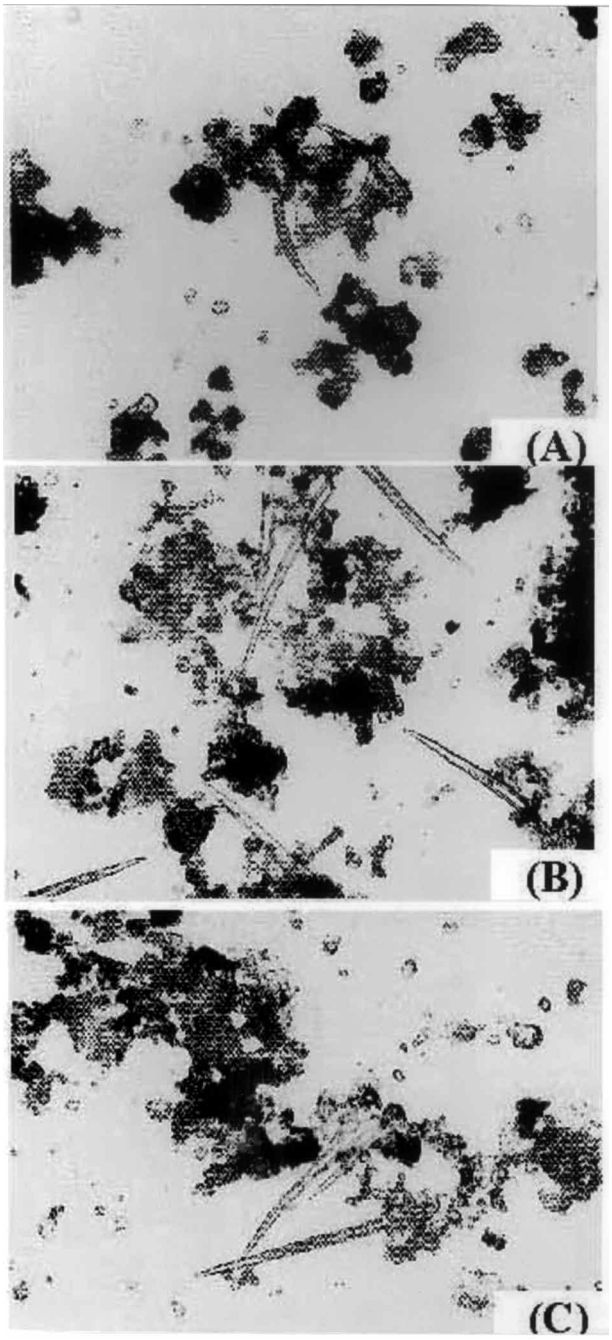


Figure 1. Wheat trichomes: (A) glabrous control (cv. Kazakhstankaya 10); (B) cv. Arai; (C) cv. Lutescens 694. Light microscopy under X36 magnification.

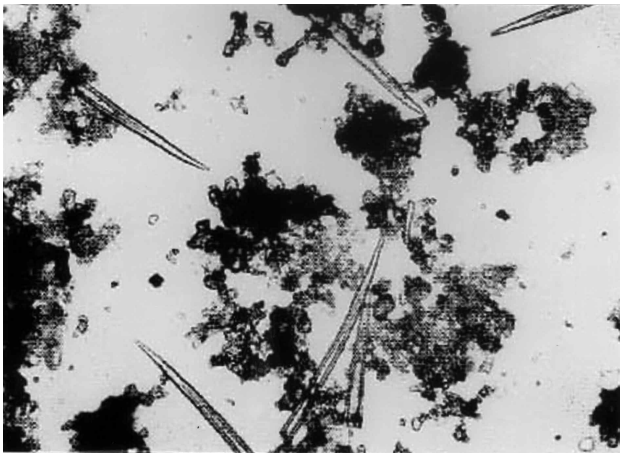

Figure 2. Trichomes of wheat leaves from cv. Arai. Light microscopy under X62 magnification.

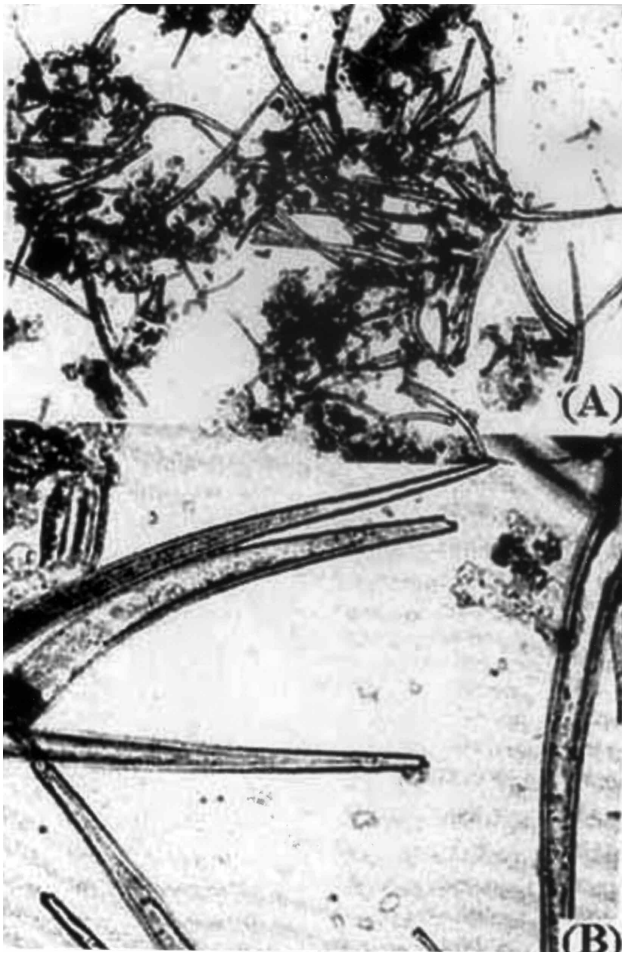


Figure 3. Trichomes from super-pubescent leaves of wheat hybrid line No. 89. (A) Magnification X20. (B) Magnification X40.

Furthermore, methylene blue staining of hair cells after the procedure resulted in virtually no improvement in the quality of cell images. Unlike hair cells of *Arabidopsis* (Zhang and Oppenheimer, 2004), trichomes from wheat and other species were noticeable even in the colourless preparations.

Glass drying at high temperature always led to the degradation of trichomes. As indicated in Figure 3, extraction of hair cells could be especially easy if leaves of super-pubescent wheat hybrids were used as a pattern for isolation.

Judging by trichome images obtained for other plant species tested in the course of the current study, extensive brushing of rigid stems of *Salvia officinalis* (see Figure 4) as firm pericarps of unripened nuts of *Corylus avellana* (Figure 5) is supposedly more effective, as compared with the same step of brushing, applied in this study to leaves of *Salvia officinalis* (Figure 6) or gentle leaves of *Lycopersicon esculentum* (Figure 7). However, it becomes clear that the procedure as a whole is applicable not only to wheat (Figures 1-3), but also to other mono- and dicotyledonous species (Figures 4-10). It is noteworthy that pericarpic trichomes covering immature nuts of *Corylus avellana,* which grows along the riverbed of the Ural in Western Kazakhstan, reveal a specific thickening at the basal part of hair cells (Figure 5). Trichomes from leaves of the common weed, *Polygonatum sewerzowii* (see Figure 9), appear to be heterogeneous in their lengths, which may suggest that this weed would demonstrate the specific features of both retaining moisture under arid conditions, and successful withstanding the attacks of herbivorous vermin.

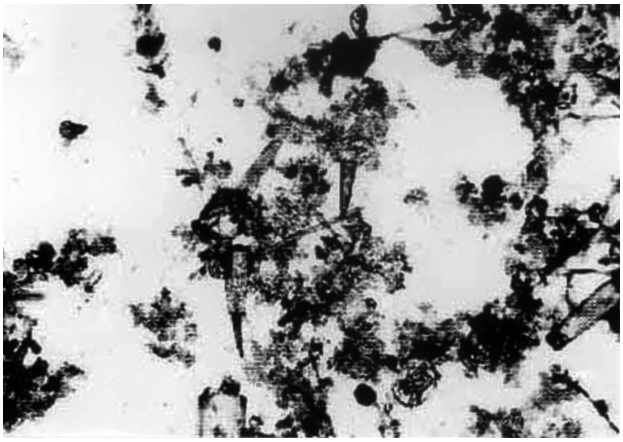

Figure 4. Trichomes from stems of *Salvia officinalis* L. Magnification X20.

Figure 5. Trichomes from green pericarps of *Corylus avellana* L. Magnification X20.

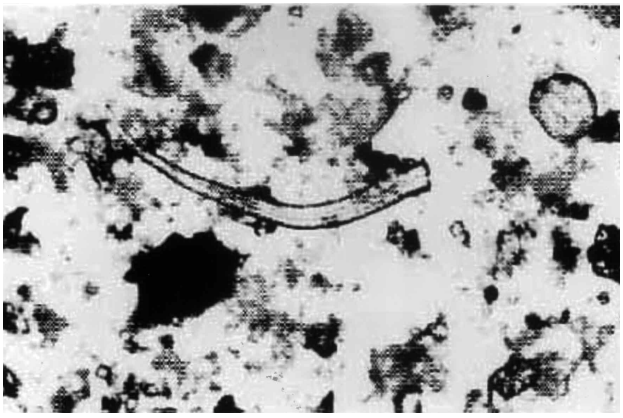

Figure 6. Trichomes from leaves of *Salvia officinalis* L. Magnification X40.

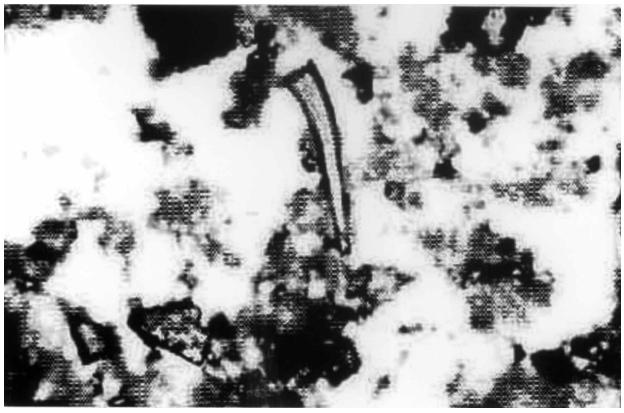

Figure 7. Trichomes from leaves of tomato, *Lycopersicon esculentum* L. Magnification X20.

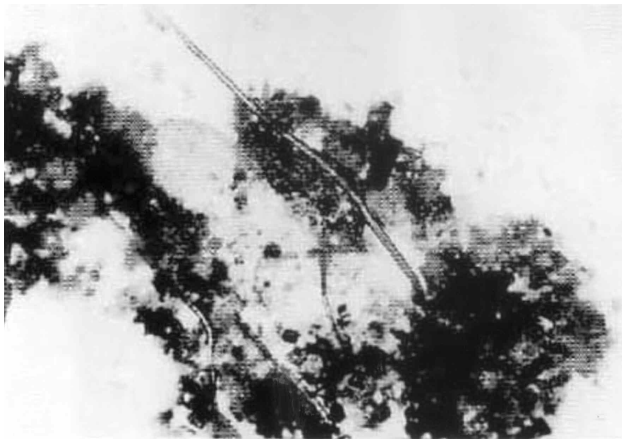

Figure 8. Trichomes from leaves of *Corylus avellana* L. Magnification X20.

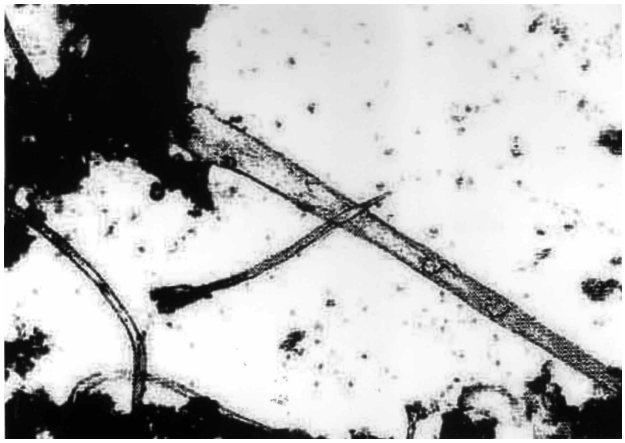

Figure 9. Trichomes from leaves of *Polygonatum sewerzowii* Regel. Magnification X40.

Figure 10. Trichomes from sepals of *Asclepias syriaca* L. Magnification X40.

Techniques developed for trichome investigation are mainly based on detailed proteome analysis and specific expression of foreign genes in definite types of hair cells. Over the last decade, trichome formation in *Arabidopsis* has been ascertained to occur in both a genetic and molecular model system to study key developmental and cellular mechanisms (Larkin et al., 1997; Huelskamp et al., 1999). The development of root and leaf hair cells provides a useful model for the investigation on general cell fate determination in plants (Kirik et al., 2004). Substantial efforts have been focused on comparing and contrasting these patterning pathways based on the orientation of cell expansion and mechanisms of lateral inhibition (Larkin et al., 2003). The latest models of trichome initiation (Szymanski et al., 2000) and lateral inhibition (Schellmann et al., 2002) at the molecular level in *Arabidopsis* have contributed to a new insight into the genetic control of cell fate and morphogenesis. The positional modes regulating trichomal spacing appear to be unique (Marks and Esch, 2003).

Research on wheat with regards to other plants' leaf morphogenesis, evolutionary changes in leaves with reference of trichome formation and functional activities opens new perspectives of detailed investigation on various interactions between superficial and inner cells, polarised and unpolarised cell populations, as well as assisting in solving problems of transpiration, complex resistance and double fertilisation (especially among cereals).

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