



# *Brassica villosa* a Potential Tool to Improve the Insect or Disease Resistance of Brassica Crop Species

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## Abstract

Outward growths on plant epidermal cells are known as trichomes or hair cells and they act as a barrier for plant predators. *Brassica napus* (canola) is the major oil seed crop in the world but lacks trichomes on them but a wild relative of canola, *Brassica villosa* has dense trichomes on them. Transcriptomic study of *B. villosa* indicated differential expression of trichome, photosynthesis light reactions, major carbohydrates, cellulose, lipid and amino acid metabolism, sulfur assimilation, metal handling/binding, hormones, biotic stress, redox, RNA regulation/transcription, post-translational modification, signalling, cell vesicle transport, development, secondary metabolism and miscellaneous genes. And bio-chemical results from *B. villosa* trichomes confirmed accumulation of metals and a unique alkaloid-like compound in them. These results from *B. villosa* opened the doors for using this species as a potential tool for the improvement of insect or disease resistance in Brassica crop species.

**Keywords:** Trichomes; Hormones; Metabolites; Microarray

## Short Communication

Outward growths on plant epidermal cells Levin [1], Mathur [2] are known as trichomes or hair cells. Trichomes can act as a barrier for plant predators, such as insects. As well, they provide a “blanket” that assists plants against dehydration Levin [1]. In nature, there are two types of trichomes, one with glands which excrete defensive or attracting metabolites and accumulate metals, and a second non-glandular type that mainly provides a physical barrier.

*Brassica napus* is one of the world’s major oil seed crops and has very sparse (i.e., semi-glabrous) trichome coverage and susceptibility to insect pests and disease. Its wild relative *B. villosa* is a perennial weed with an exceptionally dense coverage of trichomes (>2,172 per cm<sup>2</sup>) Gomez Campo and Tortosa [3] and resistance to many pests. Attempts at reciprocal crossing between *B. villosa* and *B. napus* have resulted in only a few flowers and empty pods (Nayidu, unpublished), but segments of the *B. villosa* genome have been introduced into *B. oleracea* (broccoli) using QTL tracking Mithen et al. [4].

Therefore, global gene expression data from the first emerging leaf of these two species was compared using a *B. napus* microarray to determine whether *B. villosa* could serve as a candidate species for characterizing trichomes in Brassica species. Preliminary data indicated that 78.5% of *B. villosa* paired-end RNA reads easily mapped to a *B. oleracea* reference genome, a value similar to mapped *B. oleracea* (81.9%) and *B. napus* (73%) young leaf transcripts Nayidu et al. [5], in preparation.

Although not all *B. napus* genes were represented on the microarray (since *B. napus* was not sequenced at the time), a total of 94 trichome genes showed differential expression, with 35 more strongly expressed and 59 more weakly expressed in *B. villosa* emerging leaves compared with *B. napus* leaves Nayidu et al.[6]. Trichome positive regulatory genes (GL1, GL2, and EGL3) and two negative regulators (TRY and ETC1) also had more abundant transcripts in *B. villosa* than in *B. napus* leaves, suggesting a different mode of regulation than in the Arabidopsis trichome model. Other non-trichome differentially expressed transcripts in trichome-bearing *B. villosa* leaves included those coding for photosynthesis light reactions, major carbohydrates, cellulose, lipid and amino acid metabolism, sulfur assimilation, metal handling/binding, hormones, biotic stress, redox, RNA regulation/transcription, post-translational modification, signalling, cell vesicle transport,

development, other transporters, secondary metabolism (particularly phenolics, glucosinolates, and alkaloids), and miscellaneous genes. Although protocols for plant transformation are not available for *B. villosa*, regulatory genes for specific traits in this species can be studied indirectly by selection for strong differential expression relative to *B. napus* in leaf microarray data or leaf and cotyledon RNA sequencing data (“The latter has recently been deposited in NCBI databases”). Selected genes could then be used for identifying useful phenotypes in *B. napus* expression lines featuring *B. villosa* transgenes or knockdown cassettes of homologous *B. napus* genes. Five of the major trichome regulatory genes from *B. villosa* Nayidu et al. [6], have been already introduced into *B. napus*. Alternatively, *B. villosa* mutant populations could be developed to study genes directly if a shorter method for generating large amounts of seed was in place. At present, *B. villosa* seeds are either collected from natural sources or produced in small quantities after repeated rounds of vernalization to induce *B. villosa* flowering.

Trichomes are considered to be organs that store metals and secondary metabolites or detoxify undesirable metal concentrations in plants. Isolated *B. villosa* trichomes were confirmed to accumulate metals, using ICP/MS analysis, and HPLC-MS and NMR confirmed a unique UV-absorbing 4-N alkaloid-like compound (C<sub>25</sub>H<sub>26</sub>N<sub>4</sub>O<sub>2</sub>; absolute mass 414.2046 Da) Nayidu et al.[5]. Metal accumulation in trichomes may assist *B. villosa* to manage the calcium-laden rocky slopes of its native habitat Snogerup et al. [7]. Heavy metals and alkaloids are known to impact negatively on insects van Ooik et al.[8]; Wink [9,10].

The firmly attached, flexible trichomes, their contents, and the presence of transcripts specifying trichome development and a host of other relevant

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genes indicate that *B. villosa* leaves have a variety of protection strategies. The data confirms this species as a biochemical system for studying genes, proteins and metabolites of importance to plants.

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