A phenomics and genomics approach to the use of landraces and crop wild relatives for crop improvement

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At present it is not possible to feed the world population without the application of insecticides. Worldwide yield losses caused by insects would be at least 30–50 % when no insecticides are used. However, the use of pesticides is hazardous to the environment and usually not very durable as insects may develop resistance against pesticides very rapidly. Additionally, on the 13th January 2009 the European Parliament proposed legislation placing controls on crop spraying and banning 22 pesticide chemicals. Therefore, crop production is in need of alternative control measures of which the use of resistant varieties is the most promising. To be able to develop such varieties one first needs to identify resistant sources. Often such resistances are found in crop wild relatives (CWR) and landraces (LR). Once the accessions containing the resistance traits are known, the chromosomal region or preferably the genes involved need to be identified to facilitate transfer to the crop species using molecular markers. Novel phenomics, genomics and transcriptomics technologies can speed up the identification of such markers (Broekgaarden *et al*., 2011).

Figure 1 Kale plant heavily infested with cabbage whitefly (Source: Greet Steenhuis of Plant Research International)

In the PGR Secure project we focus on the identification of resistance factors against the cabbage aphid (*Brevicoryne brassicae*) and the cabbage whitefly (*Aleyrodes proletella*), which are both specialist insects that feed only on members of the Cruciferae family, to which *Brassica oleracea* varieties and their wild relatives belong. These two insect species are phloem feeding and can cause serious problems in cultivation of *B. oleracea* crops in Europe. In particular, Brussels sprouts, kale and Savoy cabbage can be heavily infested by these herbivorous insects (Fig. 1). Aphid feeding causes chlorosis and leaf curling, whereas whitefly females lay eggs in circular patterns that are visible as white patches. Besides this cosmetic damage, both insects excrete a sugary substance (honeydew) that allows the growth of sooty mould. Both types of damage seriously reduce the marketability of the crop.

Plants can defend themselves against herbivores through physical and chemical barriers that can be constitutively present (i.e. present regardless of attack and forming a first line of defence) or induced upon herbivore attack (Schoonhoven *et al*., 2005; Alvarez *et al*., 2006). Plant morphological features, such as a wax layer or leaf toughness, form a first line of defence by preventing herbivores from settling or feeding on a plant. In addition, plants can deter herbivores through the production of repellent volatile secondary metabolites and defensive compounds or the production of proteins that directly affect herbivore performance. Glucosinolates, a group of secondary metabolites that are almost exclusively found in *Brassica* species, are well studied defensive compounds (Hopkins *et al*., 2009). When plant cells are disrupted, glucosinolates are hydrolyzed by the enzyme myrosinase resulting in the formation of a variety of toxic compounds such as isothiocyanates (Halkier and Gershenzon, 2006). However, most specialist insects have evolved enzymes to detoxify glucosinolates and/or their breakdown products (Ratzka *et al*., 2002). Therefore, plant resistance towards specialist herbivores is probably based on defensive compounds or proteins other than glucosinolates. Several proteins/compounds and the genes encoding them have been shown to play an important role in plant resistance towards herbivores. To elucidate the resistance mechanisms present, the electrical penetration graph (EPG) technology can be used, which allows a close analysis of the detailed mechanisms of resistance to sap-feeding pests (Alvarez *et al*., 2006). Such information complements the analysis of secondary metabolites and in combination with the gene expression data (Couldridge *et al*., 2007) allows informed hypotheses of gene function to be generated.

General approach in PGR Secure

Phenotyping is time and space consuming, which is a big problem when large collections have to be evaluated for a particular trait. This is especially true for the evaluation of plant material for insect resistance. Starting from a collection of about 3700 *Brassica* accessions in BrasEDB, a selection of around 400 has been made for the phenotyping (Pelgrom *et al*., this issue). From these, some 125 accessions will be selected for further analysis using metabolomics and a further subset of these will be assessed in terms of resistance/susceptibility using the EPG. This will determine the underlying mechanisms of resistance by measuring insect feeding behaviour, as we have already done for rice (Bahagia *et al*., 2009a). Based on the resistant and susceptible subsets that are identified, next generation sequencing technologies will be used to access the total gene transcriptome content of around 15 accessions of *Brassica* CWR and LR which will allow the identification of novel genes (and allelic variation) in this plant material—again as already shown in rice (Bahagia *et al*., 2009b). Trans-criptomics will be carried out using Affymetrix *Arabidopsis* genechips to assess gene expression variation across different populations of the accessions to determine within species variation and response to insect attack. In a second line we will develop segregating populations derived from crosses between plant material that is resistant and susceptible towards cabbage aphid and the cabbage whitefly. These populations will be used for quantitative trait loci (QTL) analy-

sis. A much smaller study on *Medicago sativa* populations/ accessions as proof of applicability of the technologies to CWR other than *Brassica* species will be carried out.

Partners in the project and contribution:

- DLO, Wageningen, the Netherlands: resistance screens, metabolomics, QTL analysis
- University of Birmingham, UK: resistance screens, EPG, gene expression
- University of Nottingham, UK: gene expression
- ServiceXS, Leiden, the Netherlands: sequencing, genotyping

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Looking for resistance to phloem feeders in *Brassica oleracea*

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There are several ways to protect crops against insects. One of them is to look for natural resistance in crop wild relatives (CWR) and landraces (LR). Because it is not possible to test all CWR and LR that are known, we made use of the core collection that was established by Boukema *et al*. (1997). This collection contains representatives of each *Brassica* crop type and from different ecogeographical origins. In total, 434 accessions (105 CWR and 329 LR) were screened for resistance against the cabbage aphid (*Brevicoryne brassicae*) and the cabbage whitefly (*Aleyrodes proletella*); both phloem feeding insects (Table 1). The field experiment of 2011 was conducted at two different locations: one in Wageningen, the Netherlands (Fig. 1) and the other in Stratton Audley near Bicester (Fig. 2), run by Oxford Agricultural Trials, in the United Kingdom. At both locations five week old plants were transplanted in the field. Plant growth and natural infestation (a choice test) of cabbage aphids and whiteflies were monitored.

"105 CWR and 329 LR were screened for resistance against the cabbage aphid (*Brevicoryne brassicae*) and the cabbage whitefly (*Aleyrodes proletella*)"

Figure 1 Cabbage field near Wageningen. Plants were planted in a complete randomized block order with ten replications.

Wageningen field experiment, the Netherlands

In Wageningen, the complete set of 434 accessions was planted. In this field experiment the focus was on cabbage whitefly, of which the natural infection was very low, probably due to the wet and cold summer of 2011 in the Netherlands. It was not possible to distinguish susceptible from resistant accessions; therefore, no-choice tests with clip cages containing five female whiteflies were performed on the field grown cab-