

Mining mustard (*Brassica juncea*) diversity for virus resistance

1. Introduction

Brassica juncea

Brassica juncea (mustard, family *Brassicaceae*), is an amphidiploid plant species with the genome AABB, comprising the genomes of the two diploid species, *Brassica rapa* (AA) and *Brassica nigra* (BB). It is widely grown and has various uses including as a leaf, stem, or root vegetable, oilseed crop, forage crop and condiment (Fig. 1a-c).

Turnip mosaic virus (TuMV)

Turnip mosaic virus (Fig. 1d) (TuMV, family *Potyviridae*, genus *Potyvirus*) has the widest host range amongst potyviruses. Globally, it is the second most important virus infecting vegetables and is particularly damaging to brassicas in Asia, North America and Europe.

Most *B. juncea* cultivars are very susceptible to TuMV (Fig. 1e), resulting in severe losses. Research on TuMV resistance and the identification and mapping of natural plant resistance genes would be very useful in speeding up breeding virus-resistant crops, through marker-assisted selection.

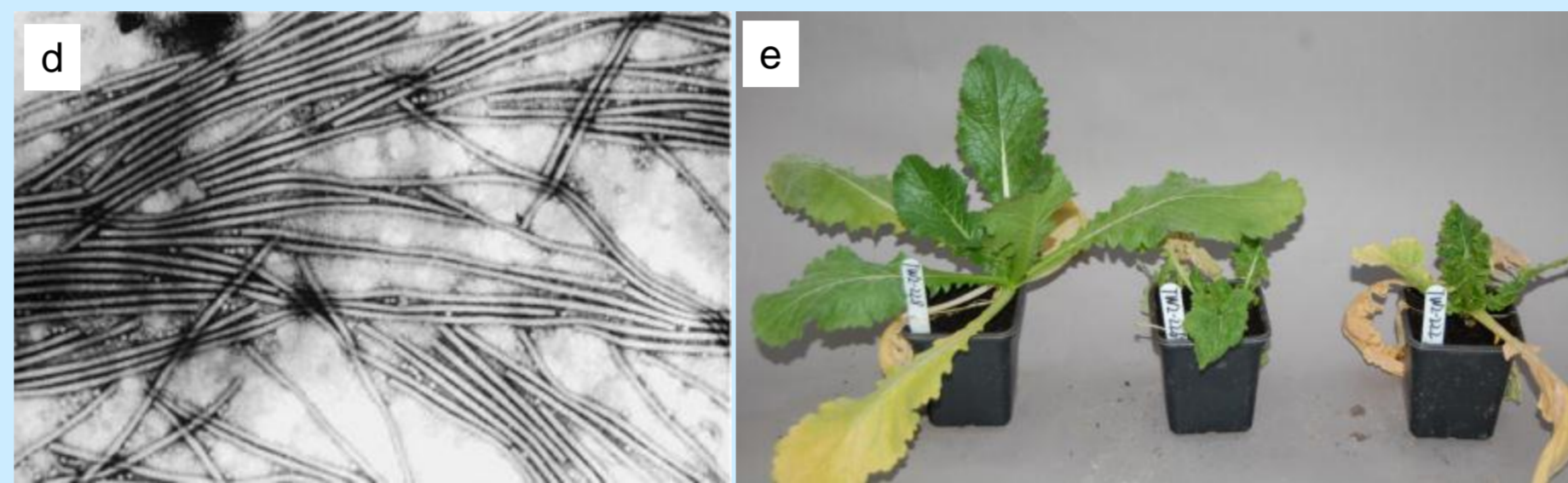
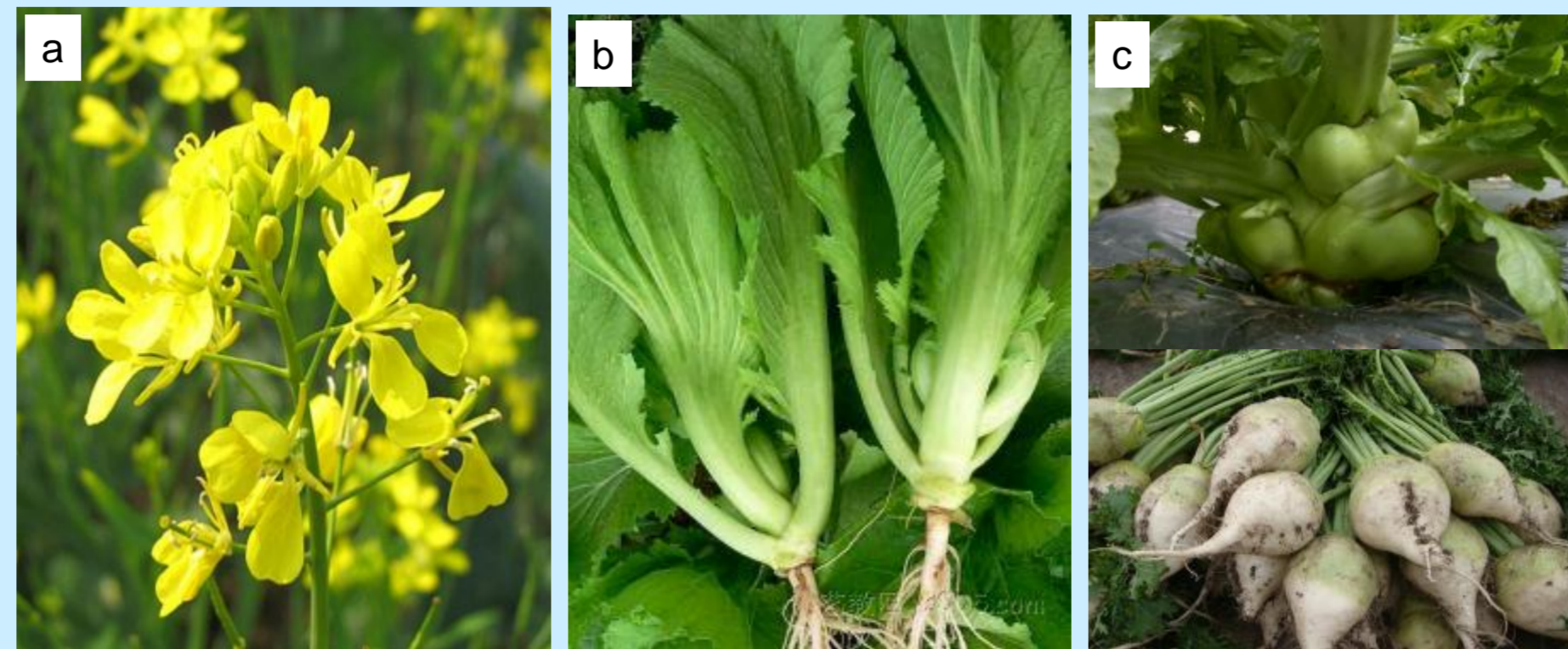


Fig. 1a, b and c, different types of *B. juncea*; Fig. 1d, Electron micrograph of TuMV virions stained with methylamine tungstate (by Colin Clay); Fig. 1e, TuMV infection of *B. juncea* (two infected plants on the right and an uninoculated plant on the left).

2. Aims

- Identify sources of resistance to TuMV in *B. juncea* and *B. nigra*.
- Characterise and map the genes involved in the TuMV resistances.
- Introgress TuMV resistance from *B. rapa*, or *B. napus* and any identified in *B. nigra* in to *B. juncea*.

3. Methodology

- Mechanical inoculation of viruses to plants.
- Symptom assessments of inoculated plants.
- ELISA (Enzyme-linked immunosorbent assay) of plants to detect virus infection / resistance.
- Crossing resistant and susceptible plant lines to develop segregating backcross (BC_1) populations for gene mapping.
- Genetic marker analysis of segregating populations, statistical analysis of genetic linkage data and mapping of resistance gene(s).

4. Results

A. Testing *B. juncea* and *B. nigra* for resistance to TuMV.

In total, 35 lines of *B. juncea* and 27 lines of *B. nigra* have been tested for resistance to the pathotype 1 isolate of TuMV, UK 1. All 27 lines of *B. nigra* were very susceptible (Fig 2). Resistance to TuMV was identified in 5 lines of *B. juncea* (Figs. 3-5).



Fig. 2. Necrotic infection of *Brassica nigra* plants (phenotype $+_N$) following sap inoculation with TuMV, isolate UK 1. The plant on the left is the uninoculated control.



Fig. 3. Reaction of a TuMV-resistant *Brassica juncea* line that was segregating for resistance to TuMV isolate UK 1. The plant on the left is susceptible (phenotype $+$) and the one on the right is resistant (phenotype 0).



Fig. 4. Reaction of another TuMV-resistant *Brassica juncea* line that was segregating for resistance to TuMV, isolate UK 1. The plant on the left is resistant (phenotype 0) and the one on the right is susceptible showing necrosis (phenotype $+_N$).



Fig. 5. Reaction of a TuMV-resistant *Brassica juncea* line that was uniformly resistant to TuMV isolate UK 1 (phenotype 0). The plant on the left is the uninoculated control.

B. Developing mapping populations.

Crosses have been made between TuMV-resistant plants from four of the *B. juncea* lines showing resistance and a well-characterised, TuMV-susceptible DH line (Fig. 6). F_1 seeds were obtained and tested for TuMV resistance. BC_1 populations will be developed.



Fig. 6. Crosses and selfs of *Brassica juncea* in the glasshouse.

C. Identification of *TuRB01* in the A genome of *B. napus* for introgression in to mustard.

TuRB01 is a single dominant gene originally identified and mapped in *B. napus* (Walsh et al. 1999). It confers extreme resistance to pathotype 1 isolates of TuMV. Subsequently *TuRB01b* which appears to be the same gene as *TuRB01* was identified in *B. rapa*. *TuRB01* has been mapped to an interval containing a number of conventional resistance (R) genes. Two candidates for *TuRB01* have been amplified by PCR and sequenced from a number of plant lines previously. A third candidate has now been amplified from a number of *B. napus* lines (Fig. 7) and sequenced. Sequences of alleles of this *TuRB01* candidate from resistant and susceptible plants were identical, suggesting it is not *TuRB01*.

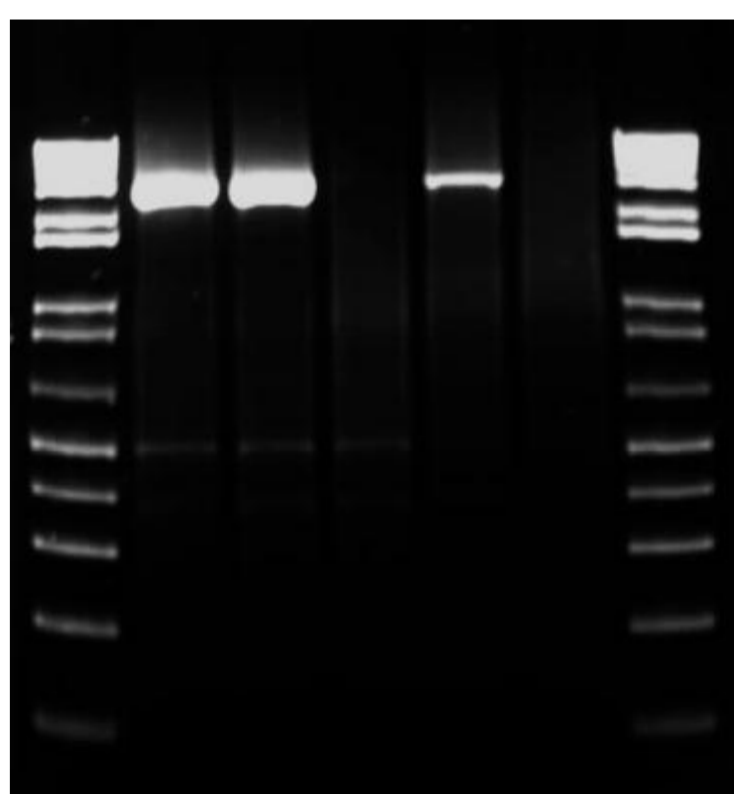


Fig. 7. PCR amplification of a *TuRB01* candidate from different *Brassica napus* lines.

D. Introgression of *TuRB01* and *TuRB01b* in to *B. juncea* through inter-specific crosses.

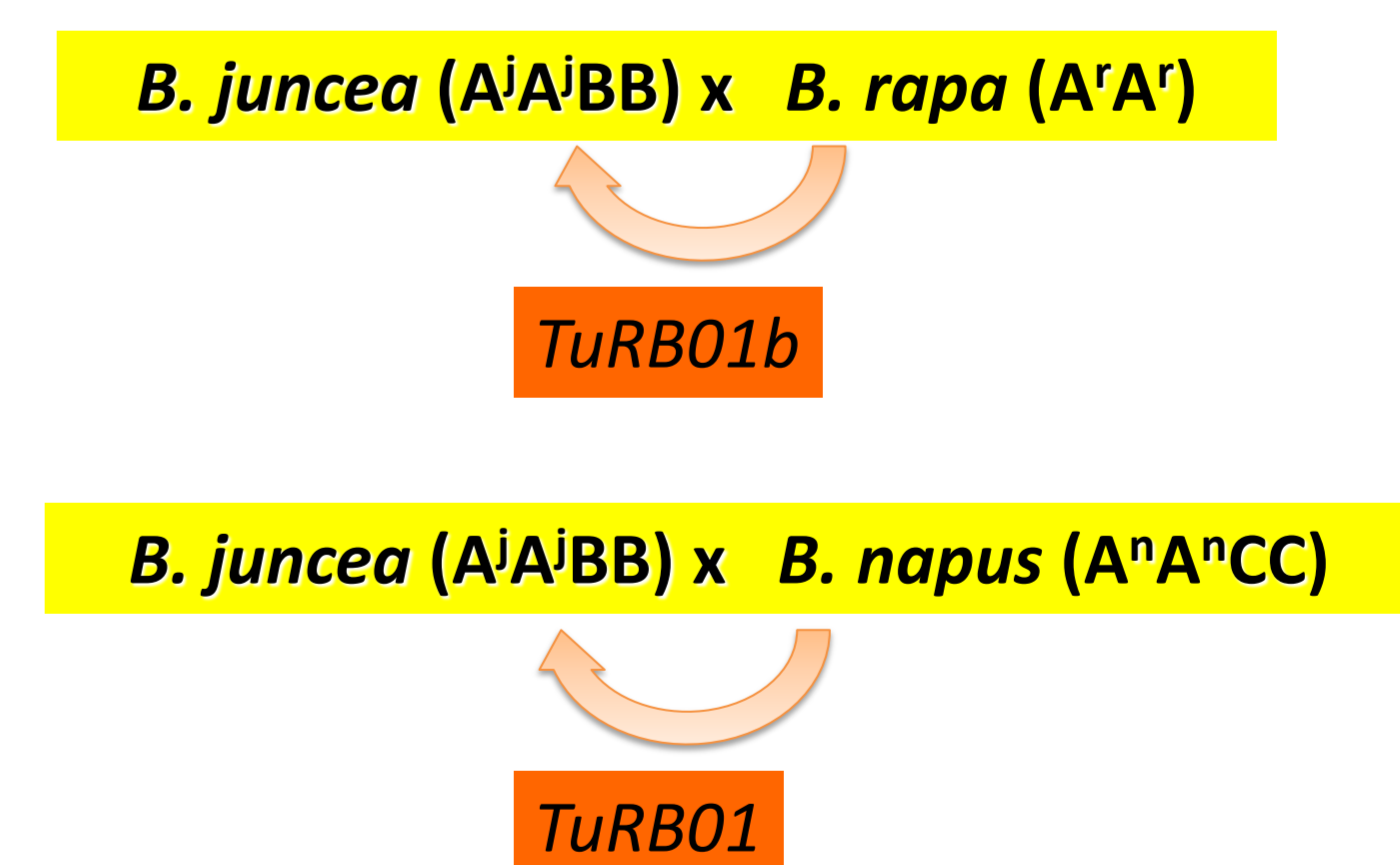


Fig. 8. Scheme of inter-specific crossing and introgression of *TuRB01* and *TuRB01b* in to *Brassica juncea*.

5. Future work

- The *B. juncea* resistant lines will be tested against a range of TuMV isolates to determine their spectra of resistance and hence the potential durability of the resistances in the field.
- The genetic inheritance of the TuMV resistances in *B. juncea* will be characterised from the phenotypes of the F_1 , BC_1 and F_2 generations from the different crosses.
- The gene(s) conferring resistance to TuMV in *B. juncea* will be mapped, based on the phenotypes and genotypes of the BC_1 plants.
- The TuMV resistance gene *TuRB01* will be identified, allowing the development of within gene molecular markers.
- Attempts will be made to introgress *TuRB01* / *TuRB01b* from *B. napus* and *B. rapa* in to *B. juncea* to establish the viability of this approach for introgression of other TuMV resistance genes in to *B. juncea*.

Acknowledgement

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References: Walsh et al. (1999). Theoretical and Applied Genetics 99, 1149-1154.