

Article Addendum

Two independent resistance genes in the *Medicago truncatula* cultivar jester confer resistance to two different aphid species of the genus *Acyrtosiphon*

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In recent years the biology of resistance to aphids and other sap-sucking insects has been studied in detail, whereas the genetic basis underlying this resistance is still poorly understood. Genetic resistance to *Acyrtosiphon kondoi* Shinji (bluegreen aphid; BGA) has been identified in *Medicago truncatula* Gaertn and backcrossed into susceptible cultivars. One of these *M. truncatula* cultivars named Jester also has good resistance to an Australian biotype of pea aphid (PA; *A. pisum* Harris). Until now it has been unclear whether resistance to each aphid species of the genus *Acyrtosiphon* is conferred by distinct genes or the same gene termed *AKR* for *A. kondoi* resistance. Infestation of the progenitors of the cultivar Jester with both aphid species revealed that resistance to BGA came from a different donor than resistance to PA, demonstrating that resistance to these aphid species is mediated by different resistance genes. However, an interaction between these genes for resistance to *Acyrtosiphon* species remains a possibility, given that PA resistance was not one of the parameters selected for in the creation of Jester. The identification of resistance to the model aphid, PA, and a closely related aphid BGA in the same genetic background of the model legume *M. truncatula* makes this system an attractive model for the study of plant-aphid interactions, as well as *R* gene specificity and evolution.

Introduction

Aphids are phloem-feeding insects of the Hemiptera suborder Sternorrhyncha and cause significant damage in agriculture

worldwide through direct phloem sap ingestion and transmission of pathogenic viruses.¹ The understanding of the genetic and molecular mechanisms underlying plant defense against phloem feeding insects such as aphids is thus of great importance. *Medicago truncatula*, a model plant species for plant biology studies,^{2,3} has also been shown to be an excellent model plant for studying plant-aphid interactions.⁴⁻⁸ The pea aphid (PA, *Acyrtosiphon pisum*) was selected by an international consortium as the model aphid species for genetics and genomics studies and the PA genome sequencing project has just been completed (www.hgsc.bcm.tmc.edu/projects/aphid/). With the wealth of genetic and molecular resources becoming available, *M. truncatula* and PA provide a powerful system to identify key factors involved in aphid resistance as well as aphid host manipulation factors.

To date, our research focus has been on the biological, genetic and molecular characterization of aphid resistance in *M. truncatula* cultivars, which are host to a range of legume pests, including bluegreen aphid (BGA; *A. kondoi*), pea aphid and spotted alfalfa aphid (SAA; *Therioaphis trifolii*). The *M. truncatula* cv Jester is resistant to BGA, PA and SAA and resistance to all three aphid species in Jester has been characterised in detail and involves antibiosis and antixenosis, and in all three cases is phloem based.⁶⁻⁸ Resistance to BGA in Jester is conferred by a dominant gene called *AKR* (*Acyrtosiphon kondoi* resistance) located on the short arm of chromosome three in a region rich in CC-NBS-LRR genes.⁷ Similarly, SAA resistance in Jester is conferred by a dominant gene termed *TTR* (*Therioaphis trifolii* resistance) and is also located on chromosome three approximately 22.8 cM distal of *AKR*.⁸ Ten F₃ families descended from a cross A17 x Jester, with recombination events tightly linked to the *AKR* locus controlling BGA resistance were assayed for their PA resistance and the segregation ratios for PA resistance were correlating with the F₂ genotypes for the molecular markers linked to *AKR*.⁶ This data led to the hypothesis that the same gene might be involved in the resistance to both BGA and PA. However, the transcriptional analysis of genes representing various defense signalling pathways showed that genes involved in the octadecanoid pathway were highly and exclusively induced

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following BGA infestation in the resistant Jester plants but not in the susceptible A17 plants which are near-isogenic to Jester.⁴ In contrast, these genes were not induced in either Jester or A17 following PA infestation.⁶ Here, we provide evidence that resistance to PA is controlled by an independent gene from *AKR*.

M. truncatula cv Jester's Origin, Breeding and Aphid Resistance Characteristics

To determine whether BGA and PA resistance came from the same *M. truncatula* donor, we first investigated the origin of Jester. Jester has a total of four progenitors, being A17 (Jemalong), Cyprus, SA1499 and SA10733 and was generated to introduce BGA and SAA resistance to the elite *M. truncatula* cultivar, Jemalong (Fig. 1).⁹ Jester constitutes approximately 1.6% Cyprus, 3.1% SA1499 and 6.3% SA10733 and is thus 89.0% near isogenic to A17. The breeders reported that Cyprus and SA10733 were the sources of SAA resistance, whereas SA1499 was the source of BGA resistance.⁹ Our research has shown that resistance to PA was also introgressed into Jester, apparently in the absence of selection against this species, but the donor of this resistance was unclear.

PA Resistance comes from a Different Progenitor than BGA Resistance

To identify the donor of PA resistance and to confirm the source of resistance for BGA in Jester, the plant tolerance of Jester and its progenitors against BGA and PA was monitored for 28 days following infestation with two apterous adults of BGA or PA on two-week-old plants. The *M. truncatula* cv Borung has previously been shown to be one of the most susceptible accessions to both BGA and PA, and was therefore included as a susceptible control.⁵ Twenty-eight days after infestation with BGA, Jester and SA1499 plants were relatively healthy, whereas Cyprus, SA10733 and the susceptible control Borung died (Fig. 2A). The accession A17 showed an intermediate BGA resistance phenotype, with a significantly different level of plant damage compared to its near isogenic line Jester.

In the case of PA, Jester and SA10733 survived 28 days following PA infestation, in stark contrast to the susceptible control Borung, A17, Cyprus and SA1499 (Fig. 2B). The accession A17 was somewhat more resistant than Borung, Cyprus and SA1499 in the early stages of infestation, but by 28 days the majority of the plants died (Fig. 2B). These results clearly demonstrate that *AKR* in Jester was derived from the accession SA1499, whereas PA resistance was derived from SA10733. It is obvious that *AKR* is not the main locus conferring resistance to PA, and that PA resistance is controlled by a different gene in Jester, the *Acyrtosiphon pisum* resistance gene (*APR*).

Discussion

The value of *M. truncatula* as a model system to study plant aphid interactions has been demonstrated and three single-gene resistances against three major legume aphids in *M. truncatula* have been characterised in detail. For BGA and SAA these resistance loci have been fine mapped.^{7,8} It will therefore be of importance

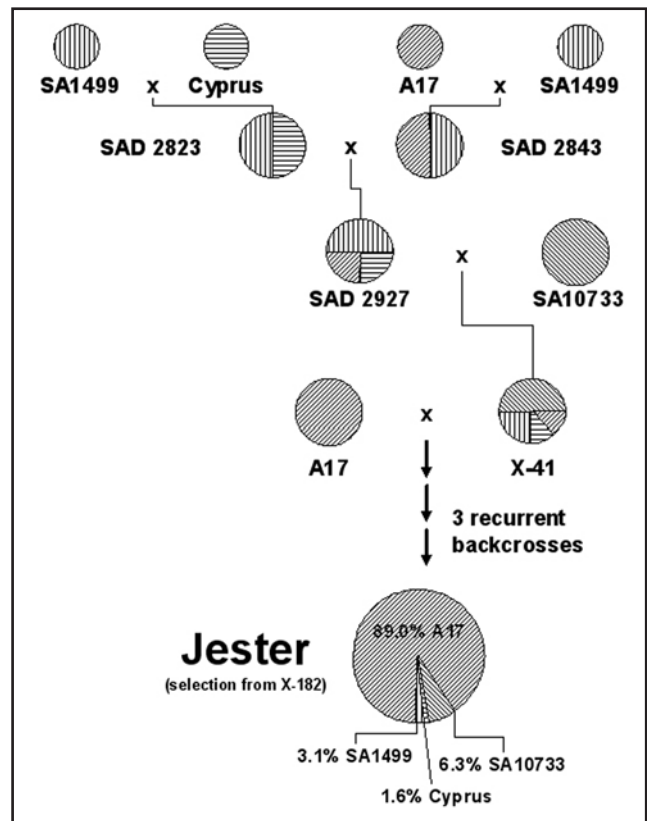


Figure 1. Origin and breeding of the *M. truncatula* cultivar Jester.

to pursue the fine-mapping of the PA resistance gene, *APR*. *APR* could be tightly linked to *AKR* or *APR* could have an additive effect on *AKR* and/or *TTR*. Either one of these hypotheses could explain why PA resistance was retained in Jester, given that PA resistance was not one of the parameters selected for by the breeders. The presence of an independent resistance gene *APR* is supported by the fact that different defense related pathways were induced by BGA infestation compared to PA infestation.⁴ This is also consistent with, the reciprocal pre-infestation experiments with BGA and PA on Jester which suggested that different downstream defense mechanisms act against these two closely related aphid species.⁶

With the PA genome sequence completed and various PA genomics and proteomics resources available (www.aphidbase.com/aphidbase/), the *M. truncatula*/PA system has become an even more attractive model to study plant-aphid interactions. It will allow us to study the evolution of *R* genes that confer resistance to closely related species (and biotypes) as well as the differences in the salivary gland secretome,^{10,11} the suite of effector proteins which collectively manipulate host plant defence signaling and responses to allow successful feeding. Overall these studies should help shed light on the intimate relationship between plants and phloem-feeding insects.

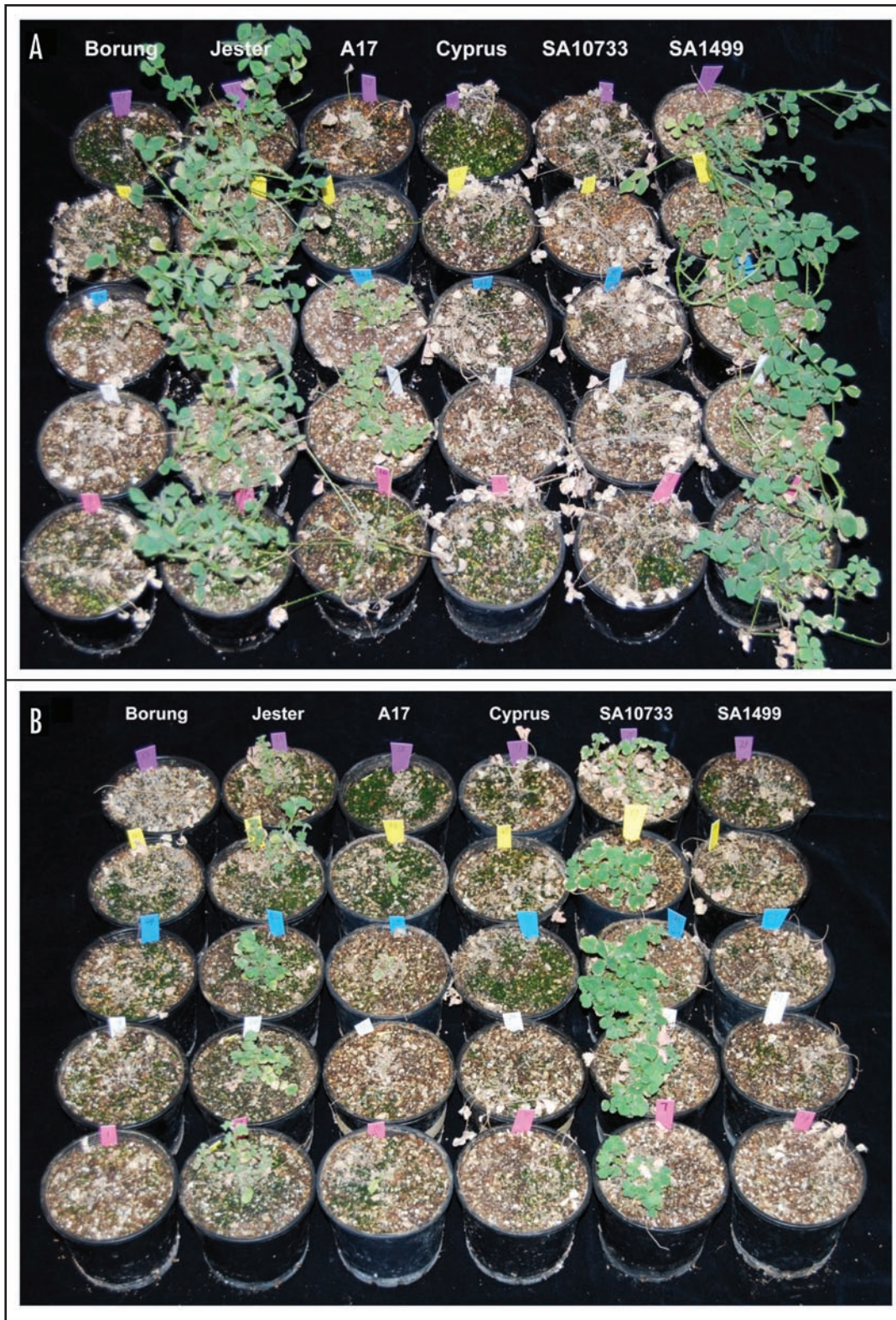


Figure 2. Phenotypes of *M. truncatula* accessions 28 days post infestation with either (A) bluegreen aphid or (B) pea aphid. Five replicates of two-week-old plants of each accession were infested with two apterous aphids of BGA or PA and monitored up to 28 days post infestation.

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