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NLRs are highly relevant resistance genes for aphid pests

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Abstract

Since the beginning of the 20th century, when plant resistance to aphids has been available, it has been widely used by farmers. This has boosted breeding programs for resistant cultivars. More recently, the inheritance of plant resistance has been understood for several crops. However, it is only when the plant-aphid relationship was compared to that of microbial pathogens, that aphid resistance has begun to be understood and integrated into the plant immune network. Until now, genes have only been identified for plant resistance in four crops and, for three of them (tomato, melon and wheat), these genes encode nucleotide-binding site LRR receptor (NLR) proteins responsible for aphid-effector triggered immunity. Aphids are vectors for many plant viruses, and aphid-effector triggering immunity when they pierce plant cells are expected to trigger resistance to the viruses that are transmit to the plant with effectors, as has been shown for aphid resistance in melon. This dual phenotype increases the interest of NLRs in the control of aphids. New NLRs and genes belonging to other families are also obviously involved in aphid resistance. Genome Wide Association Studies (GWAS) are promising strategies to identify these genes when high throughput phenotyping is available.

Highlights

- After breeding success in resistance to aphids, resistance in tomato, melon and wheat was shown conferred by NLR genes
- NLR resistance triggered by aphids puncturing is expected efficient to control viruses inoculated during puncturing
- GWAS is a promising strategy to discover new resistance gene in crops' diversity

Farmers with access to aphid resistant varieties use them to a substantial extent because it is the simplest way to control aphid infestations and this has fostered breeding programs for plant resistance to aphids. For example, to control *Diuraphis noxia* aphids feeding on several row crops, a wheat breeding program was launched in South Africa in 1978 and the resulting aphid resistant cultivars, carrying the *Dn* gene, were later used in USA when *D. noxia* became invasive (Porter et al. 2009). Many other *Dn* genes/alleles are currently employed to control this pest (Radchenko, Abdullaev, and Anisimova 2022), and in 2022 USDA released 'USDA Fortress', a multiple aphid-resistant, high yielding winter feed barley that is highly resistant to *D. noxia* and *Shizaphis graminum*, while also being resistant at the seedling stage to *Sipha maydis* (Mornhinweg, Carver, and Springer 2022), a new invasive aphid. Regarding horticultural crops, the cultivation of melons resistant to *Aphis gossypii* in France is emblematic of the success of aphid resistance breeding. Margot, the first aphid resistant melon cultivar, was registered in the 1987 in France {Boissot, 2016 #3001}, and since then farmers' demand has increased to the extent that all new melon cultivars recently proposed for registration are declared resistant to aphids (GEVES data).

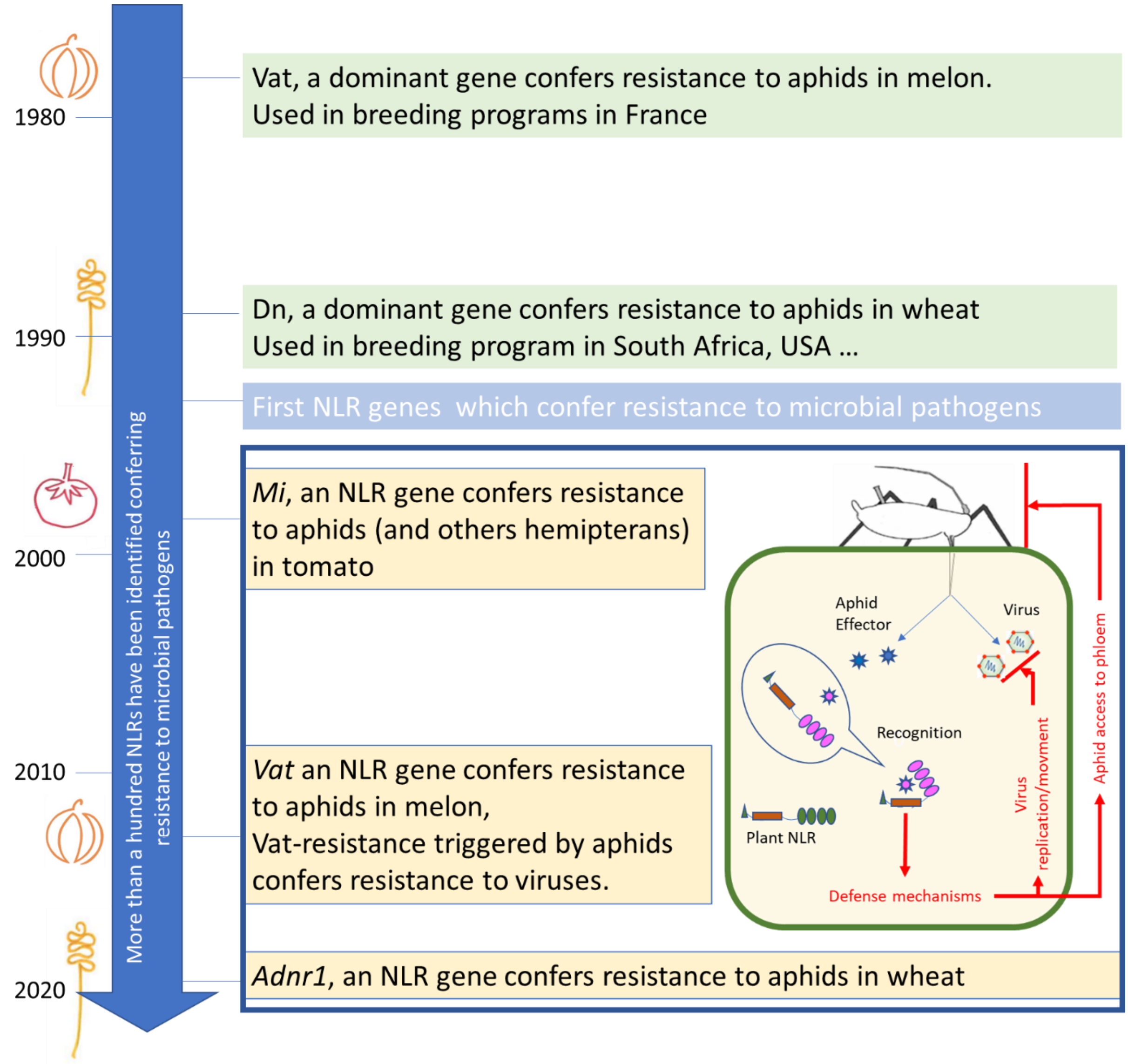
The breeding successes were achieved before plant resistance to aphids was understood, but substantial advances have been achieved over the last two decades, when the close links between plant-hemipteran interactions and plant-pathogen interactions were highlighted (Kaloshian and Walling 2005). In 2013, plant-aphid interactions were integrated in the PTI-ETI model (Dangl, Horvath, and Staskawicz 2013), which is a fruitful model for pathogen-plant interactions that has been developing since the 1990s. This model describes the molecular dialogue between pathogens and plants, which triggers immunity in plants (Yuan et al. 2021). This dialogue is initiated in plant apoplasts for PTI by pathogen-associated molecular patterns recognized by plant cell-surface receptors. Otherwise, for ETI, the dialogue is initiated within plant cells by effectors introduced by the pathogens and recognized by intracellular receptors belonging to the nucleotide-binding site leucine-reach repeat (LRR) receptor (NLR) gene family. This dialogue between plants and aphids is possible because the aphid mouthparts enter the apoplastic space and stealthily visit cells, contrary to chewing insects for which the molecular dialogue with the plant is short-lived since these insects swallow their leaf partners. As far as we currently know, no NLR gene has been found to be involved in resistance to chewing insects. From a plant breeding viewpoint, only the ETI part of the PTI-ETI model has clearly been effectively used for resistance to pathogens and aphids, probably because it can be associated with the Flor model according to which plant resistance is controlled by a dominant R-gene—this is the easiest heredity system for plant breeders to work with.

Yet only three NLRs have formally been shown to be involved in aphid resistance in plants. The first concerns the *Mi1-2* gene in tomato by serendipity (Rossi et al. 1998), this gene was initially described as conferring resistance to nematodes. Mi-transgenic tomato lines are resistant to *Macrosiphum euphorbiae*, a minor tomato pest, but this gene has also been widely used to enhance nematode resistance in tomato breeding programs. As expected in the ETI framework, this resistance is aphid clone-specific (Rossi et al. 1998). The second concerns the *Vat-1* gene in melon which confers resistance to *Aphis gossypii* (Dogimont et al. 2014), a major pest of cucurbits. Both *Mi1-2* and *Vat-1* are coiled coil (CC)-type NLR and belong to a cluster of several homologs. The number of *Vat*-like NLRs is highly variable in the melon diversity, apart from some being pseudo-*Vat*-like forms (Chovelon et al. 2021). Among these *Vat*-like NLRs, only the *Vat-1* gene in the Korean PI 161375 accession was formally shown to be involved in resistance in transgenic plants (Dogimont et al. 2014). *Vat*-1 resistance is thought to be triggered following recognition of an *A. gossypii* salivary effector and, remarkably, the triggered resistance is also effective against several viruses that are likely introduced at the same time as the aphid effector (Boissot et al. 2016) (Figure 1). This virus resistance was successfully used to reveal *Vat* resistance in several melon accessions, since it appears to be independent of the melon genetic background. This resistance was found to be aphid clone-specific, as expected in the ETI framework. In wheat, *Adnr1* is the third NLR gene characterized for its resistance to *Diuraphis noxia* aphids. Virus-induced gene silencing of *Adnr1* in a resistant wheat line abolished the resistance response and induced a highly susceptible phenotype (Nicolis and Venter 2018). *Adnr1* belongs to the widespread NLR-ID resistance gene family, which is thought to be an NLR diversification phenomenon to expand the pest and pathogen recognition range (Grund, Tremousaygue, and Deslandes 2019).

Several genetic studies have actually suggested that NRLs are candidates for resistance to aphids in a broad range of crops or related plant models. In peach, three major dominant resistance genes to *Myzus persicae*, a major peach tree pest, have been detected in three peach clones. These genes were mapped in the same genomic area containing two identical NLR genes with a Toll-interleukin 1 receptor (TIR). One was only identified in resistant genotypes while the second was overexpressed in resistant plants infected with *M. persicae* in comparison with mock plants (Duval et al. 2022; Pan et al. 2022). In sorghum, the *RMES1* locus conferring resistance to *Melanaphis sacchari* (which was formerly called the sugarcane aphid) contained five predicted genes, three of which are NLRs (Wang et al. 2013). The AIN locus in the *Medicago truncatula* model species occurs in a cluster of CC-NLR genes. The AIN locus was shown to be involved in resistance to *Acyrtosiphum kondoi* and, to a lesser extent, to *Acyrtosiphum pisum*. In apple, the *Dp-fl* locus, which confers resistance to *Dysaphis plantaginea*, contains 19 genes acting as R-genes, two of which are NLRs. *Sd-1* and *Sd-2* loci, which confer resistance to *Dysaphis devecta,* were mapped in the same genomic area hosting an NLR gene (Cevik and King 2002b, 2002a).

Moreover, molecular study findings have suggested that ETI is involved in plant resistance to aphids. No NLRs in *Arabidopsis thaliana* have been *Myzus persicae* or *Brevicoryne brassicae* resistance candidates. Nevertheless, in *A. thaliana* PTI/ETI is controlled by a family of three lipase-like proteins (EDS1, PAD4, and SAG101) (Ngou, Jones, and Ding 2022) and PAD4 was shown essential for the restriction of *Myzus persicae* infestation (Louis et al. 2012). Although this list is not exhaustive, based on the results obtained with *Mi1-2*, *Vat-1* and *Adnr1*, it strongly suggests that ETI is a widespread mechanism of plant resistance to aphids. As NLRs are not part of the core genome (Bayer et al. 2020), i.e. part of the genome shared by all genotypes within a species, some aphid resistance controlled by NLRs might have been missed in genetic studies due to their absence in the reference genome. Analyses using the pangenome, which harbors the NLRome diversity, should reveal many more NLRs involved in aphid resistance.

In aphids, i.e. the other partner of the interaction, no genes for any effectors interacting directly or indirectly with NLR genes involved in the ETI framework have been identified so far. The intracellular receptors are NLR proteins, and the effectors produced by aphids have to be introduced in the plant cell cytoplasm (Figure 1). This likely occurs via the aphid’s watery saliva, which is injected in cytoplasm when aphid stylets (special mandibular organs in Hemiptera insects that push through the plant apoplasts) puncture cells on the way to the phloem. Two difficulties currently have to be overcome. First, no experimental aphid lines encountering NLR resistance have been described to date, and this lack of availability of virulent and avirulent quasi-isogenic lines seriously complicates effector gene identification. The alternative could be to conduct studies using pools of virulent and avirulent clones. Yet, as shown in melon, some aphid clones are adapted to the NLR defenses they have triggered, thereby making the definition of virulent and avirulent aphid lines in reference to an NLR highly hypothetical. This has been noted in melon since *Vat*-1-triggered resistance offers another phenotype of resistance to viruses (Dogimont et al. 2014). On the basis of this virus-resistance phenotype triggered by aphid puncturing, the resistance was clearly aphid-clone-specific, so this phenotype was used to identify genes coding candidates for the effector (Boissot et al. 2016). Regarding the ETI model, the effector in aphid should be controlled by a dominant virulent allele in aphid genomes which are diploid and highly heterozygous. Mapping effector genes should be achieved in a plant-aphid couple for which the NLR phenotype is unambiguously defining avirulent vs virulent clones and, an high quality assembled aphid genome is available. Unfortunately, this is a further difficulty, given that the insect genome sequencing saga only started in the year 2000 and the assembly of aphid genomes is still problematic. New sequencing technologies using long DNA fragments have otherwise provided high-quality assemblies in terms of contiguity and completeness of repetitive regions. A series of high-quality aphid genome sequences have been recently released (Biello et al. 2021; Zhang et al. 2022)(Calevro, Tagu, and Callaerts 2019) (Calevro, Tagu, and Callaerts 2019; Mathers et al. 2021; Wei et al. 2022; Wenger et al. 2020) (Chen et al. 2019), and probably others will soon be made available. Remarkably, (Singh et al. 2021) produced sequences for 110 clones of *M. persicae*, making those clones of particular interest for mapping aphid effector interacting with NLR genes, in particular in peach species the only plant species for which NLRs triggering resistance to aphids has been described so far (Duval et al. 2022; Pan et al. 2022). Genetic/phenotype association approaches could be relevant to achieve this goal.



Vat, a dominant gene confers resistance to aphids in melon.
Used in breeding programs in France

Dn, a dominant gene confers resistance to aphids in wheat
Used in breeding program in South Africa, USA …

First NLR genes which confer resistance to microbial pathogens

Mi, an NLR gene confers resistance to aphids (and others hemipterans) in tomato

Vat an NLR gene confers resistance to aphids in melon,
Vat-resistance triggered by aphids confers resistance to viruses.

Adnr1, an NLR gene confers resistance to aphids in wheat

Figure 1 Timeline describing the main milestone of the 3 NLRs known conferring resistance to aphids. The scheme of molecular dialogue between aphid and plant cell, built according to ETI framework, is adapted from (Boualem, Dogimont, and Bendahmane 2016).

Actually, as noted in melon, the fact that aphids can adapt to the NLR defenses they triggered questions the use of NLRs for aphid resistance in breeding programs. In biotests in melon, this aphid adaptation pattern was partial to high depending on the aphid clone, but the *Vat*-resistance remained sufficiently effective in melon fields (Boissot, Schoeny, and Vanlerberghe-Masutti 2016) so let's not throw the baby out with the bathwater! Moreover, aphid-triggered NLR resistance is expected to be efficient against any viruses inoculated by these pests (Boualem, Dogimont, and Bendahmane 2016) (Figure 1). This was formally documented with regard to *Vat*-resistance in melon but it has not been sufficiently investigated in other crops even if it is highly suspected for *Rm*-resistance in peach and A-resistance in red raspberry. In melon, this resistance to viruses was shown to reduce some virus epidemics in the field when it was triggered by the main vector (Schoeny et al. 2017) and could pave the way to sustainable virus resistance.

The extent of NLR-related aphid resistance in crops highlights the relevance of integrating plant-aphid interactions in the ETI-PTI framework. The aphid resistance understanding will be fed by advances in this ETI-PTI framework, in particular for the role of certain NLRs as a hub for immunity (Ngou, Jones, and Ding 2022). Moreover, this provides an opportunity to discover such resistance genes in many plant species. But breeders would require complementary strategies to develop aphid-resistant cultivars in the future. Genes other than NLRs are also obviously involved in aphid-resistance. LRR-receptor-like kinase (LRR-RLK) gene analogs are candidates for resistance to *Myzus persicae* in pepper (Sun et al. 2020). Recessive resistance loci that have been identified in several plant species (Dogimont et al. 2010) but none genes identified. Moreover, QTLs for resistance to aphids have been identified in some plant species using bi-parental segregating populations. This strategy required large phenotypic datasets while the genetic architecture has only been described for one parent. Genome-wide association studies (GWAS) involving co-analysis of genotypic and phenotypic diversity in large panels of lines within a species provide a convincing way to map genomic regions that could enhance aphid resistance in crops. GWAS—as in the case of genetic studies on segregating populations—requires large phenotypic datasets, but unlike the findings of segregating population studies, the genetic architecture of resistance is obtained at the plant species level, not for a single line. Few GWAS focused on plant resistance to aphids have been implemented to date. In 240 pea lines, resistance was phenotyped using the fecundity of two *A. pisum* clones with controlled infestations (Ollivier et al. 2022), which represented a major phenotyping effort. To offset this substantial time constraint, resistance to *Melanaphis sorghi* in sorghum has been assessed in field trials by drone imaging or by crop damage rating (Poosapati et al. 2022; Punnuri et al. 2022), whereas available data of aphid population measures from 2,366 accessions were used in soybean (Hanson et al. 2018). These studies revealed both already known and new QTLs, thereby confirming that GWAS is an efficient way to study resistance to aphids. These studies relied on a single reference genome, whereas increasing the number of reference genomes could boost the number of genes detected by GWAS in particular NLR genes.

Declarations of interest: none

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