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Diversity of genetic backgrounds modulating the durability of a major resistance gene. Analysis of a core collection of pepper landraces resistant to *Potato virus*

Y.

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## Summary (200 words max)

The evolution of resistance-breaking capacity in pathogen populations was shown to depend on the plant genetic background surrounding the resistance genes. We evaluated a core-collection of pepper (*Capsicum annuum*) landraces, representing the worldwide genetic diversity, for its ability to modulate the breakdown frequency by *Potato virus Y*, of major resistance alleles at the *pvr2* locus encoding the eukaryotic initiation factor 4E (eIF4E). Depending on pepper landrace, the breakdown frequency of a given resistance allele varied from 0% to 52.5%, attesting their diversity and the availability of genetic backgrounds favorable to resistance durability in the plant germplasm. The mutations in the virus genome involved in resistance breakdown also differed between plant genotypes, indicating differential selection effects exerted on the virus population by the different genetic backgrounds. The breakdown frequency was positively correlated to the level of virus accumulation, confirming the impact of quantitative resistance loci on resistance durability. Among these loci, *pvr6*, encoding an isoform of eIF4E, was associated with a major effect on virus accumulation and on the breakdown frequency of the *pvr2* mediated resistance. This exploration of plant genetic diversity delivered new resources for the control of pathogen evolution and the increase of resistance durability.

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The main limit to the use of resistant cultivars is the capacity of pathogens to counter-adapt and overcome plant resistances. In this context, many research efforts were devoted to handle adaptation of plant pathogen populations and maintain the durability of resistant cultivars (Kiyosawa, 1982; Wolfe, 1985; Mundt *et al.*, 2002; Pink, 2002; McDonald & Linde, 2002).

In several pathosystems involving virus, oomycete or nematode plant parasites, the durability of major resistance genes was experimentally shown to be dependent on the genetic background in which it was introgressed (Acosta-Leal and Xiong 2008, 2013; Palloix *et al.*, 2009; Brun *et al.*, 2010; Fournet *et al.*, 2012). In these studies, the breakdown frequency of the major resistance gene decreased when introgressed into a partially resistant cultivar, suggesting that the partially resistant genetic background was favourable to control pathogen evolution and enhance the durability of major resistance genes.

In the plant-virus system *Potato virus Y* (PVY)/pepper(*Capsicum annuum*), the protective effect of the genetic background on the breakdown frequency of the *pvr2*<sup>3</sup> gene, causing resistance to this virus, was mostly due to the additional level of quantitative resistance reducing the viral accumulation (Quenouille *et al.*, 2012). The breakdown frequency of the major gene was shown to be highly heritable (*i.e.* it was mainly under genetic control) and affected by four QTLs (Quenouille *et al.*, 2014). These results, obtained on a narrow plant genetic basis, *i.e.* in a progeny between two *Capsicum annuum* lines, opened new ways for sustainable resistance breeding, but little is known on the availability of such QTLs and the frequency of favorable backgrounds in the plant genetic resources.

In this context, the aim of our study was to estimate the availability and diversity of genetic backgrounds increasing the durability of the *pvr2*-mediated resistance on a broader genetic scale in pepper resources and to confirm (or not) the relationship with factors affecting quantitative resistance. Our strategy was to select a core-collection of pepper landraces from

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different origins but carrying two closely related *pvr2* alleles and to measure the resistance breakdown frequencies in these pepper accessions on the one hand, and the level of quantitative resistance controlled by their genetics backgrounds on the other hand.

### ***Selection of the core-collection.***

The pepper (*Capsicum* spp.) germplasm collection maintained at INRA *Unité de Génétique et Amélioration des Fruits et Légumes* was screened for resistance to PVY and 35.7% of the 862 tested accessions were found to be resistant (Sage-Palloix *et al.*, 2007). A subset of 107 resistant accessions was chosen while maximizing the diversity of their geographic origins, and were sequenced for the *pvr2* resistance allele. Twenty-one accessions carried the *pvr2*<sup>3</sup> allele, which was the most frequent allele, and five carried the *pvr2*<sup>4</sup> allele (Charron *et al.*, 2008 and personal communication). The sequence of the eIF4E protein (eukaryotic initiation factor 4E) encoded by these two *pvr2* alleles differs only by one amino-acid substitution at position 205 (a glycine for *pvr2*<sup>3</sup> and an aspartic acid for *pvr2*<sup>4</sup>). They displayed the same resistance specificity towards seven different PVY variants and were easily broken down compared to other *pvr2* alleles (Ayme *et al.*, 2007; Charron *et al.*, 2008; Moury *et al.*, 2014). To further test the durability of the *pvr2* alleles in these different genetic backgrounds, we selected the accessions which were homozygous for *pvr2*<sup>3</sup> (16 accessions) or *pvr2*<sup>4</sup> (4 accessions), while discarding the closely related accessions with identical genotypes at the tested SSR loci (Nicolai *et al.*, 2013), resulting in a final set of 20 *Capsicum annuum* inbred lines issued from American, African and Asian pepper landraces (table 1). The genetic diversity of this set of 20 *C. annuum* lines was compared to the genetic diversity of the entire *C. annuum* germplasm collection which includes 1063 accessions from 89 different countries, using 28 simple sequence repeat (SSR) loci (Nicolai *et al.*, 2013). The wide distribution of the 20 inbred lines retained for resistance analysis across the phylogenetic tree representing the

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whole collection attested for their high diversity and poor genetic structuration (Fig. 1). No shared origin was evidenced for the accessions carrying *pvr2*<sup>3</sup>, which were widespread in the different parts of the tree, corresponding to large sweet or small and pungent fruited pepper landraces from diverse geographic origins. The four accessions carrying *pvr2*<sup>4</sup> were loosely grouped in the distal part of the tree, corresponding to small fruited pepper landraces. The 20 accessions represented 36.7% of the allelic richness present in the entire *C. annuum* collection (mean of 4.6 alleles for the 20 accessions compared to 12.6 alleles for the whole *C.annuum* collection at the 28 SSR loci, Nicolai *et al.*, 2013). These results indicate that, in spite of the choice based on two related *pvr2* alleles, the retained 20 accessions carried diverse genetic backgrounds.

***Breakdown frequency of the resistance controlled by *pvr2*<sup>3</sup> or *pvr2*<sup>4</sup> in the core collection.***

To measure the resistance breakdown frequency (RB frequency) of *pvr2*<sup>3</sup> or *pvr2*<sup>4</sup> (*pvr2*<sup>3/4</sup>), a recombinant PVY, the “CI chimera” was mechanically inoculated to sixty seedlings per accession at the two expanded cotyledon stage in a climate-controlled room at 20-22°C and 12h light/day. The CI chimera is a derivative of the SON41p infectious cDNA clone where the CI-coding region was substituted with that of the LYE84.2 infectious cDNA clone, both clones corresponding to isolates of the C1 clade of PVY (Montarry *et al.*, 2011). This “CI chimera” was chosen because of its higher ability to break the *pvr2*<sup>3</sup> resistance down compared with the parental clones, allowing more precise estimations and comparisons of the resistance durability of different plant genotypes (Montarry *et al.*, 2011). The CI chimera is not infectious *per se* toward plants carrying *pvr2*<sup>3/4</sup> resistance alleles, *i.e.* in these plants, only mutants possessing single non-synonymous substitutions in the genome-linked viral protein (VPg) cistron could be detected but not the “CI chimera” itself. The susceptible line Yolo

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Wonder (carrying the *pvr2*<sup>+</sup> susceptibility allele) was used as virus infectivity control and the DH285 inbred line, carrying the *pvr2*<sup>3</sup> resistance allele and previously known for its high *pvr2*<sup>3</sup> RB frequency (Palloix et al. 2009) was used as a control for *pvr2*<sup>3</sup> RB capacity by the CI chimera. Fourteen days post inoculation (dpi), all Yolo Wonder plants showed mosaic symptoms in apical leaves and all plants carrying *pvr2*<sup>3/4</sup> were symptom free. Mosaic or necrotic symptoms further appeared along the testing period in apical leaves of part of the plants of some *pvr2*<sup>3/4</sup> accessions. At 35 dpi, three uninoculated leaves were sampled from each plant and pooled to test the presence of PVY by double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA). In these conditions, each case of systemic infection in plants carrying the *pvr2*<sup>3</sup> resistance allele was shown to result from the selection of at least one PVY variant carrying a RB mutation(s) in the VPg cistron (Montarry *et al.*, 2011). For each accession, the RB frequency was calculated by the frequency of systemically infected plants amongst inoculated plants. In this test, 80% of the DH285 resistant plants were broken down, attesting the high capacity of the CI chimera to break the *pvr2*<sup>3</sup> resistance down in the test conditions. Among the 20 accessions, the RB frequency of *pvr2*<sup>3/4</sup> varied from 0% to 52.5% (table 1), with highly significant differences between accessions (Pearson Chi<sup>2</sup> test, P<0.001). The 20 accessions were classified into two groups (pairwise Fisher exact tests at 5% type-I error threshold after Bonferroni correction). For 18 accessions, the *pvr2*<sup>3/4</sup> resistance was not, or rarely broken down (RB frequency ≤5%) and for the two accessions PI369940 and G4, the *pvr2*<sup>3/4</sup> resistance was frequently broken down (40% and 52.5%, respectively). For PI369940 and G4, all infected plants were checked for the presence of RB mutations in the PVY VPg cistron: total RNA was extracted and the PVY VPg cistron was amplified by reverse transcription-polymerase chain reaction (RT-PCR) and sequenced according to Moury *et al.* (2014). For one and four infected plants of G4 and PI369940, respectively, we failed to sequence the PVY VPg cistron. For the other 30 G4 and 20

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PI369940 infected plants, all VPg sequences obtained differed from the initial VPg sequence of CI chimera by only one amino acid substitution. Four of the mutations detected were previously known to result in the breakdown of *pvr2*<sup>3</sup> resistance (Ayme *et al.*, 2006), four were previously observed in *pvr2*<sup>3</sup> RB PVY populations and are strongly suspected to be responsible for RB (Montarry *et al.* 2011), and one was never observed before but located at the same codon position (119) as three other observed mutations, suggesting also its involvement in RB (supplementary data I). Five G4 plants and one PI369940 plant were infected by a mixture of two single VPg mutants. These results confirmed the correspondence between systemic infection of plants carrying *pvr2*<sup>3/4</sup> resistance allele after inoculation by CI chimera and occurrence of RB events. The high RB frequency was not associated with a particular allele at the *pvr2* locus since PI369940 carries *pvr2*<sup>3</sup> and G4 carries *pvr2*<sup>4</sup>, suggesting that the differences observed between accessions more probably result from the effect of the plant genetic background. The low RB frequencies observed in 18 landraces indicated that genetic backgrounds favorable to the durability of *pvr2*-mediated resistances are frequent among the genetic resources of pepper.

#### ***Differential selection pressure exerted by the plant genetic background***

In a previous study (Montarry *et al.* 2011), 67% of the *pvr2*<sup>3</sup> RB events in DH285 were associated to the selection of the aspartic acid to asparagine codon substitution at position 119 of the VPg cistron of PVY (named VPg-N mutation). In our test, we checked the presence of the VPg-N mutation in 25 infected DH285 plants by dCAPS analyses as described in Montarry *et al.* (2011). Results showed that 64% of RB events of DH285 plants were associated to the presence of the VPg-N mutation (table 2), which is not significantly different from the previous results (p-value = 0.13, Fisher exact test). In G4 and PI369940 plants, the percentages of RB events associated with the selection of the VPg-N mutation were equal to

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55% (17/31) and 29% (7/24) respectively (dCAPS analyses showed the absence of the VPg-N mutation in the five PVY populations from G4 or PI369940 plants for which the full sequence of the VPg cistron was not determined, Suppl. data 1). As a whole, this revealed a significant difference between PI369940 and DH285 ( $p=0.020$ , Fisher exact test) but not between PI369940 and G4 ( $p=0.10$ ) or G4 and DH285 ( $p=0.41$ ) for the proportion of RB events associated with selection of the VPg-N mutation. This variation cannot be associated to the effect of the *pvr2* allele, since both PI369940 and DH285 carry *pvr2*<sup>3</sup> (and differ in the selection of VPg-N mutation), whereas G4 (*pvr2*<sup>4</sup>) and DH285 (*pvr2*<sup>3</sup>) did not differ in their preferential selection of the VPg-N mutation. The difference observed between PI369940 and DH285 can be associated to the genetic background which selects more or less frequently the VPg-N mutation. This result showed that various plant genetic backgrounds can exert differential selection pressure on RB mutants, leading to differential frequencies of selection of the various virulent mutants toward the same resistance allele.

### ***Quantitative level of resistance controlled by the genetic background of the pepper accessions.***

It was previously shown that a large part of the variation of the RB frequency of *pvr2*<sup>3</sup> observed in a biparental progeny could be attributed to the level of quantitative resistance conferred by the genetic background (Quenouille *et al.* 2014). To explore this relationship in an enlarged germplasm, we estimated the level of quantitative resistance conferred by the genetic backgrounds of each of the 20 pepper accessions. The *pvr2*<sup>3/4</sup>-breaking mutant of the CI chimera carrying the VPg-N mutation was inoculated to 20 plants per accession. This RB mutant was chosen because it was the most frequently observed after RB of the *pvr2*<sup>3</sup> resistance by the CI chimera. It differed from the CI chimera by only one amino acid substitution (aspartic acid to asparagine) at position 119 of the VPg, allowing the breakdown

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of *pvr2*<sup>3</sup> and *pvr2*<sup>4</sup> and permitting to get rid of the effect of the *pvr2*-mediated resistance in order to reveal the effect of the genetic background. Symptoms were assessed every seven days until 35 dpi and for each plant the Area Under the Disease Progress Curve (AUDPC) was calculated according to Caranta & Palloix, (1996): symptom intensity from each plant was checked on the new developing leaves, with 0 (no symptom), 1 (weak mosaic) or 2 (severe mosaic or necrosis) at 7, 14, 21, 28 and 35 dpi. At 36 dpi, the relative virus accumulation (VA) was evaluated independently from 10 plants per accession by quantitative DAS-ELISA by comparing the absorbance at 405 nm – dilution factor curves of each plant sample to that of a common reference virus sample (Ayme *et al.* 2006). Significant differences of VA and AUDPC were observed between accessions ( $p < 0.001$ , Kruskal-Wallis test) with means of VA varying from 0.001 to 2.804 and means of AUDPC varying from 35 to 95 (table 1). Nine and eight groups of VA and AUDPC, respectively, were identified among the 20 peppers accessions (pairwise comparisons using Wilcoxon tests at 5% type-I error threshold after Benjamini & Yekutieli correction), showing a large diversity of the level of quantitative resistance conferred by the accession's genetic backgrounds. No significant correlation was observed between VA and AUDPC ( $p = 0.3$ , Spearman correlation test). This lack of correlation between VA and AUDPC (or plant damages) was previously observed for different plant-virus interactions (Sáenz *et al.*, 2000; Moury *et al.*, 2001; Pagán *et al.*, 2007; Araya *et al.*, 2011) and reveals different levels of tolerance between the 20 pepper accessions. Here, tolerance is defined as the capacity of the host plant to reduce the effect of an increase of virus accumulation on the plant fitness or damages (Råberg *et al.*, 2007). In order to evaluate if the resistance or tolerance level of the 20 accessions could affect the durability of the *pvr2*-mediated resistance, we looked at the relationship between VA or AUDPC and the frequency of *pvr2*<sup>3/4</sup> RB. Two cultivars (G4 and PI369940) presented a high relative VA ( $> 2.0$ ) and simultaneously a high RB frequency ( $\geq 40\%$ ), whereas all other cultivars had a low

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VA (<1.1) and a low RB frequency ( $\leq 5\%$ ) (Table 1). This resulted in a highly significant positive correlation between VA and the frequency of *pvr2*<sup>3/4</sup> RB ( $p=0.007$ , Spearman correlation test). A similar conclusion is reached if we consider only two categories of plant cultivars, those with high VA and high RB and those with low VA and low RB. The probability that the plants belonging to the two categories of VA coincide exactly with the plants belonging to the two categories of RB by chance is given by  $p=(2/20)^2 \times (18/20)^{18}=0.0015$ , a value quite similar to that obtained with the Spearman correlation test. In contrast, there was no significant correlation between AUDPC and the frequency of RB ( $p=0.38$ , Spearman correlation test). This reveals a strong relationship between viral accumulation and the breakdown frequency of the *pvr2*-mediated resistance. A causal relationship can be hypothesized, since an increase in virus census population size will lead to an increase in the probability of appearance of virus mutants and, hence, of mutant emergence. Our study broadens the results obtained by Quenouille *et al.* (2014), extending the relationship between the level of viral accumulation and the frequency of breakdown of *pvr2*-mediated resistance previously observed in a pepper progeny to a pepper core collection with diversified genetic backgrounds.

#### ***Association between a locus in the pepper genome and *pvr2*<sup>3/4</sup> RB frequency***

In the doubled haploid progeny issued from the F<sub>1</sub> hybrid between the two pepper inbred lines Yolo Wonder and Perennial, we showed that the *pvr6* gene encoding eIFiso4E, an isoform of the eIF4E encoded by the *pvr2* gene, co-located tightly with a major QTL ( $R^2=0.40$ ) affecting the *pvr2*<sup>3</sup> RB frequency and a major QTL ( $R^2=0.35$ ) affecting viral accumulation (Quenouille *et al.*, 2014). For these two QTLs located at the *pvr6* locus, the Perennial alleles strongly increased the frequency of the *pvr2*<sup>3</sup> RB and VA. However, this effect was shown to be compensated by additional QTL-alleles from the Perennial genetic

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background, so that Perennial always displayed an extremely low (or null) RB frequency. Compared to the Yolo Wonder *pvr6*<sup>+</sup> allele, the *pvr6* allele of Perennial carries a 82 nucleotide deletion (from nucleotide 89 to 170) which modifies the open reading frame (ORF), leading to a premature stop codon and a truncated, nonfunctional protein (Ruffel *et al.* 2006). To explore if this “natural” knockout (KO) of the *pvr6* allele was present among the 20 pepper accessions and if it was associated to the observed variations of VA and *pvr2*<sup>3/4</sup> RB frequency, we sequenced the eIFiso4E cDNA of the 20 pepper accessions as described in Ruffel *et al.* (2006). These sequences were aligned with the sequences of the Yolo Wonder *pvr6*<sup>+</sup> allele (Genbank accession DQ022080) and the Perennial *pvr6* allele (Genbank accession DQ022083). Alleles of the *pvr6* gene carried by each pepper accession are indicated in Table 2. Among the 20 pepper accessions, three carried the same KO *pvr6* allele: Perennial, PI369940 and G4. Interestingly, the two accessions PI369940 and G4 which showed a significantly higher RB frequency of *pvr2*<sup>3/4</sup> and the two highest VA values carried the KO *pvr6* allele. On the opposite, all plants carrying the *pvr6*<sup>+</sup> allele encoding a functional eIF(iso)4E protein displayed a low to very low RB frequency and a varying but lower VA. The *pvr6* allele is also present in the Perennial accession, which showed a very low RB frequency and this could invalidate the causal relationship between *pvr6* alleles and both RB frequency and VA. However, Quenouille *et al.* (2014) showed that in Perennial the effect of the QTL allele at the *pvr6* locus was compensated by three other QTLs that decreased the *pvr2*<sup>3</sup> RB frequency and virus accumulation. Taken together, these results show that the *pvr6* gene is a good candidate to explain the observed variation of *pvr2* RB frequency and VA. The high frequency of *pvr2*<sup>3/4</sup> RB observed in G4 and PI369940 could be interpreted as an effect of the *pvr6* KO allele which increased both VA and RB frequencies, without compensation effects from other QTLs in the genetic background as observed in Perennial. This simultaneous increase of VA and RB may result from a higher

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expression of the eIF4E encoded by *pvr2*<sup>3/4</sup> due to the absence of the eIFiso4E isoform encoded by *pvr6*, as shown in *Arabidopsis thaliana* (Duprat et al., 2002). Indeed, this higher eIF4E expression could increase its availability for PVY replication and/or translation, maybe by reducing competition effects between PVY VPg and the cap of plant mRNAs (Michon *et al.*, 2006). In turn, higher virus multiplication would lead to higher VA in case of a RB PVY mutant, and higher probability to generate RB mutants in case of a wild-type PVY. However, such hypothetical mechanism still has to be demonstrated and functional validation of the involvement of *pvr6* in RB and VA will help in this exploration. The compensatory effect by additional resistance QTLs from Perennial were shown to act at several levels (Quenouille *et al.* 2012): they were shown to directly reduce virus accumulation, but also to increase the number of viral mutations required for RB. Endly, they slowed down the selection and fixation of RB-mutations in the PVY population.

### **Conclusion**

This study provides the first evaluation of the availability, in plant genetic resources, of genetic backgrounds affecting the durability of major resistance genes. Among a core-collection of 20 pepper accessions, we observed a high diversity of quantitative resistance and of tolerance levels conferred by the genetic background. In term of durability of the *pvr2*<sup>3</sup> and *pvr2*<sup>4</sup> major resistance alleles, the pepper accessions were divided into two groups, either with no or rare breakdown or with frequent breakdown, the latter group including only two pepper accessions. The genetic backgrounds favorable to the durability of the *pvr2*-mediated resistance were carried by genetically distant landraces from diversified origins, indicating that such favorable genetic combinations are not only frequent but also widespread in the pepper genetic resources. The relationship between the *pvr2*<sup>3/4</sup> RB frequency and the level of quantitative resistance in a diversified core-collection extends the previous results which showed that the RB frequency of a major gene is reduced when introgressed into a partially

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resistant cultivar (Palloix *et al.*, 2009; Brun *et al.*, 2010; Fournet *et al.*, 2012) or combined with another major resistance gene (Acosta-Leal and Xiong 2008, 2013), and that the same QTLs or genes may affect quantitative resistance and major gene durability (Acosta-Leal and Xiong 2008; Quenouille *et al.* 2014). This former QTL analysis, together with the analysis of the eIFiso4E coding sequence in the core collection leads us to suggest that observed variations of RB frequency are strongly affected by the plant allele at the *pvr6* locus. However, the genetic background effect cannot be reduced to this single locus since this major effect can be compensated by additional quantitative resistance QTLs. This indicates that breeding “accidents” could happen when a major resistance gene is introgressed into a new genetic background. However, such accidents can be avoided if the major resistance gene is introgressed into a partially resistant cultivar, taking into account that quantitative resistance has to be evaluated through pathogen accumulation level since the symptom expression or tolerance level was not correlated to resistance breakdown frequency. Another interesting output was delivered by the distribution of the different RB mutants which differed between pepper landraces, suggesting that selection pressures could be modulated thanks to different plant genetic backgrounds. The availability of plant genetic backgrounds with contrasted differential effects on pathogen populations paves the way towards a durable management of resistance genes by breeding appropriate cultivars and deploying appropriate varietal mixtures to control the emergence of the different RB variants.

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*d'Azur* (PACA) and the *INRA-Meta Programme SMaCH* (Sustainable Management of Crop Health).

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### Figure legends:

Figure 1: Distribution of the accessions carrying *pvr2*<sup>3</sup> and *pvr2*<sup>4</sup> in the phylogenetic tree representing the genetic diversity of the *C. annuum* germplasm collection (core collection of 378 accessions representing the *C. annuum* genetic diversity at 28 SSR loci and structuration in 3 groups, Nicolai *et al.* 2013). Tree produced using the unweighted neighbor-joining method based on the dissimilarity matrix for the 28 SSR (software DarWin -6, Perrier and Jacquemoud-Collet, 2006). Scale: dissimilarity index based on simple matching method. Accessions are labelled by their PM code as in table 1. Non-italicized are *pvr2*<sup>3</sup> and italicized are *pvr2*<sup>4</sup> carrying accessions. Accessions in black boxes and white font are carrying the *pvr6*-KO allele.

### Table legends:

Table 1 : Frequency of resistance breakdown (RB), relative viral accumulation (VA) and area under the disease progress curve (AUDPC) for 20 pepper accessions carrying the *pvr2*<sup>3</sup> or *pvr2*<sup>4</sup> allele inoculated with *Potato virus Y*.

<sup>w</sup> Letters represent homogeneous groups identified by pairwise comparisons (Fisher exact tests at the 5% type-I error threshold corresponding to a 0.00027 threshold after Bonferroni correction for multiple comparisons). RB frequency evaluated after inoculation with PVY CI chimera.

<sup>x</sup> Letters represent homogeneous groups identified by pairwise comparisons (Wilcoxon tests at the 5% type-I error threshold with Benjamini & Yekutieli correction). VA and AUSPC evaluated after inoculation with PVY CI chimera carrying the VPg-N mutation.

<sup>y</sup> In three accessions a 82 nucleotide (nt) deletion (from nucleotide 89 to 170) resulted in a premature stop codon and a truncated nonfunctional eIFiso4E (Ruffel *et al.* 2006).

Table 2: Frequency of *pvr2*<sup>3/4</sup> resistance breakdown events associated to the selection of the aspartic acid to asparagine substitution at codon position 119 of PVY VPg cistron (VPg-N mutation).

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Supplementary data I: Distribution of VPg mutations in PVY populations infecting G4 (*pvr2<sup>4</sup>/pvr2<sup>4</sup>*) or PI369940 (*pvr2<sup>3</sup>/pvr2<sup>3</sup>*) pepper (*Capsicum annuum*) genotypes.

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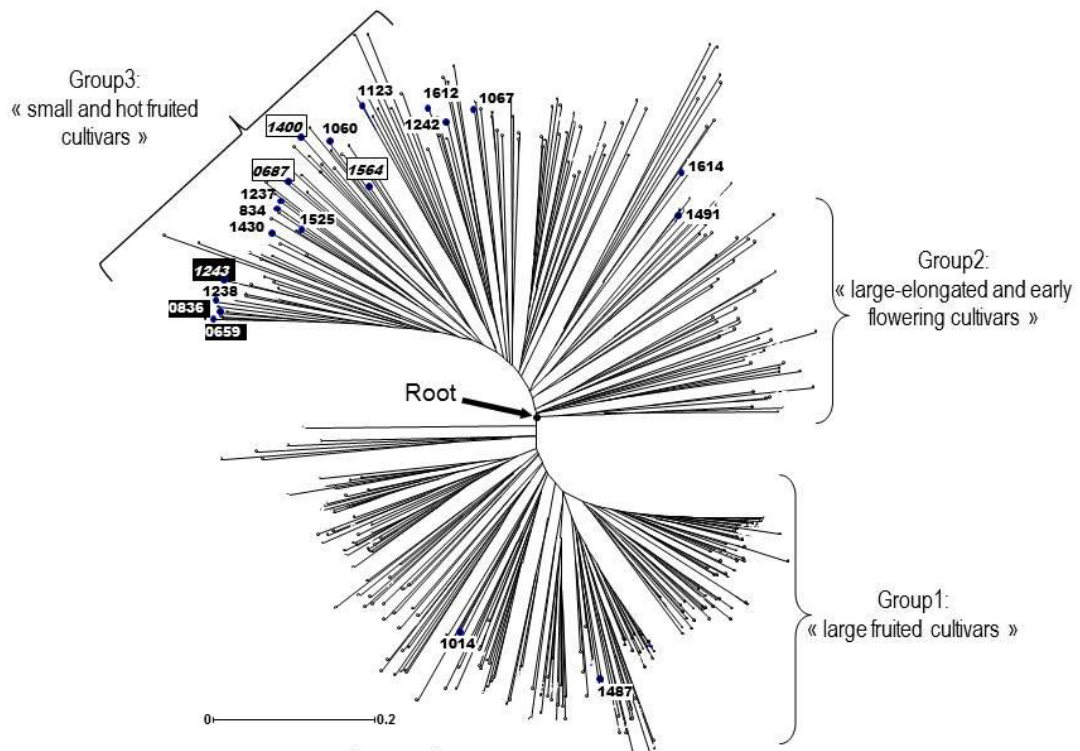


Fig.1: Distribution of the accessions carrying *pvr2<sup>3</sup>* and *pvr2<sup>4</sup>* in the phylogenetic tree representing the genetic diversity of the *C. annuum* germplasm collection (core collection of 378 accessions representing the *C. annuum* genetic diversity at 28 SSR loci and its structuration in 3 groups after Nicolai *et al.* 2013). The tree was produced using the unweighted neighbor-joining method based on the dissimilarity matrix for the 28 SSR (software DarWin -6, Perrier and Jacquemoud-Collet, 2006). Scale: dissimilarity index based on simple matching method for SSR alleles. Accessions are labelled by their PM code number as in table1. Non italicized are *pvr2<sup>3</sup>* carrying and italicized are *pvr2<sup>4</sup>* carrying accessions. Accessions in black boxes and white font are carrying the *pvr6*-KO allele.

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Table 1

Accession code	Accession name	<i>pvr2</i> allele	RB frequency (%) (n=60) <sup>w</sup>	VA (n=10) <sup>x</sup>	AUDPC (n=20) <sup>x</sup>	Deletion in the <i>pvr6</i> gene <sup>y</sup>
PM1237	Piment de Thaïlande	<i>pvr2</i> <sup>3</sup>	0 <sup>a</sup>	0.001 <sup>a</sup>	55 <sup>cd</sup>	No
PM1123	276F	<i>pvr2</i> <sup>3</sup>	0 <sup>a</sup>	0.006 <sup>ab</sup>	85 <sup>gh</sup>	No
PM1242	G1	<i>pvr2</i> <sup>3</sup>	0 <sup>a</sup>	0.010 <sup>bc</sup>	78 <sup>fg</sup>	No
PM1614	C69	<i>pvr2</i> <sup>3</sup>	0 <sup>a</sup>	0.063 <sup>cde</sup>	54 <sup>c</sup>	No
PM0834	AC 1448	<i>pvr2</i> <sup>3</sup>	0 <sup>a</sup>	0.103 <sup>def</sup>	67 <sup>de</sup>	No
PM1238	Piment Ile Maurice	<i>pvr2</i> <sup>3</sup>	0 <sup>a</sup>	0.166 <sup>efg</sup>	72 <sup>ef</sup>	No
PM1060	PI 123 474	<i>pvr2</i> <sup>3</sup>	0 <sup>a</sup>	0.173 <sup>efg</sup>	69 <sup>de</sup>	No
PM1430	Pikuti	<i>pvr2</i> <sup>3</sup>	0 <sup>a</sup>	0.202 <sup>efg</sup>	43 <sup>b</sup>	No
PM1525	Ubud Bali 2	<i>pvr2</i> <sup>3</sup>	0 <sup>a</sup>	0.215 <sup>bcdefg</sup>	43 <sup>b</sup>	No
PM1491	Copoya	<i>pvr2</i> <sup>3</sup>	0 <sup>a</sup>	0.250 <sup>efgh</sup>	95 <sup>h</sup>	No
PM1014	Bouso 2	<i>pvr2</i> <sup>3</sup>	0 <sup>a</sup>	0.269 <sup>bcdefgh</sup>	70 <sup>de</sup>	No
PM1564	Beijin	<i>pvr2</i> <sup>4</sup>	0 <sup>a</sup>	0.664 <sup>gh</sup>	61 <sup>cd</sup>	No
PM1400	P 709	<i>pvr2</i> <sup>4</sup>	1.6 <sup>a</sup>	0.014 <sup>cd</sup>	35 <sup>a</sup>	No
PM1612	Jaipur	<i>pvr2</i> <sup>3</sup>	1.6 <sup>a</sup>	0.398 <sup>efgh</sup>	88 <sup>gh</sup>	No
PM0659	Perennial	<i>pvr2</i> <sup>3</sup>	1.6 <sup>a</sup>	0.406 <sup>efgh</sup>	41 <sup>b</sup>	<b>Nt 89 to 170</b>
PM1067	Huixtan	<i>pvr2</i> <sup>3</sup>	1.6 <sup>a</sup>	0.416 <sup>gh</sup>	90 <sup>h</sup>	No
PM1487	Souman Boucoule 3	<i>pvr2</i> <sup>3</sup>	3.3 <sup>a</sup>	0.142 <sup>efg</sup>	71 <sup>e</sup>	No
PM0687	PI 322 719	<i>pvr2</i> <sup>4</sup>	5 <sup>a</sup>	1.010 <sup>hi</sup>	74 <sup>ef</sup>	No
PM0836	PI 369 940	<i>pvr2</i> <sup>3</sup>	40 <sup>b</sup>	2.141 <sup>i</sup>	74 <sup>ef</sup>	<b>Nt 89 to 170</b>
PM1243	G4	<i>pvr2</i> <sup>4</sup>	52.5 <sup>b</sup>	2.804 <sup>i</sup>	75 <sup>ef</sup>	<b>Nt 89 to 170</b>

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Table 2

Accession	% of plants with VPg-N mutation	Class <sup>a</sup>
PI369940	29 (7/24)	a
G4	55 (17/31)	ab
DH285	60 (16/25)	b

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