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A series of eIF4E alleles at the *Bc-3* locus are associated with recessive resistance to *Clover yellow vein virus* in common bean

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Abstract *Clover yellow vein virus* (ClYVV) is capable of causing severe damage to common bean *(Phaseolus vulgaris* L.) production worldwide. The snap bean market class is particularly vulnerable because infection may lead to distortion and necrosis of the fresh green pods and rejection of the harvest. Three putatively independent recessive genes (*cyv*, *desc*, *bc-3*) have been reported to condition resistance to ClYVV; however, their allelic relationships have not been resolved. We identified, evaluated, and characterized the phenotypic and molecular genetic variation present in 21 informative common bean genotypes for resistance to ClYVV. Allelism testing phenotypes from multiple populations provided clear evidence that the three genes were a series of recessive alleles at the *Bc-3* locus that condition unique potyvirus strain- and species-specific resistance spectra. Candidate gene analysis revealed complete association between the recessive resistance alleles and unique patterns of predicted amino acid substitutions in *P. vulgaris* eukaryotic translation initiation factor 4E (PveIF4E). This led to the discovery and characterization of two novel *PveIF4E* alleles associated with resistance to ClYVV, *PveIF4E³*, and *PveIF4E⁴*. We developed KASPar allele-specific SNP genotyping assays and demonstrated their ability to accurately detect and differentiate all of the *PveIF4E* haplotypes present in the germplasm, allelism testing, and in three separate segregating populations. The results contribute to an enhanced understanding and accessibility of the important potyvirus resistance conditioned by recessive alleles at *Bc-3*. The KASPar assays should be useful to further enable germplasm exploration, allelic discrimination, and marker-assisted introgression of *bc-3* alleles in common bean.

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Introduction

Clover yellow vein virus (ClYVV) (family *Potyviridae*, genus *Potyvirus*) infection is capable of causing significant damage to leguminous crop and forage plants around the world (CABI/EPPO 2000). The impact of the disease can be particularly severe in certain interactions with the common bean (*Phaseolus vulgaris* L.) because symptom expression can include severe stunting, prominent mosaic, premature defoliation, systemic necrosis, and in some cases plant death (Provvidenti and Morales 2005). The impact can be even more acute in the snap bean market class where ClYVV has the potential to devastate marketable yield by causing twisting, distortion, and necrosis of the fresh green pods (Larsen and Myers 2006; Larsen et al. 2008; Provvidenti and Morales 2005). The threat of direct economic damage is compounded when pod-distorting strains of the virus appear in processing snap bean production regions as the presence of distorted or necrotic pods above a threshold may result in the rejection of the entire harvest by the processor.

ClYVV epidemics appear to occur sporadically in numerous regions where common beans are grown (CABI/EPPO 2000; Crnov and Gilbertson 2001; Dizadji

and Shahraeen 2011; Larsen and Myers 2006; Ortiz et al. 2009; Provvidenti and Schroeder 1973; Sasaya et al. 1997; Tu 1980, 1988), but the increased frequency of an aphid-transmitted virus disease complex of snap beans in the Great Lakes Region of the United States over the past decade has been cause for concern (Larsen et al. 2002, 2008; Shah et al. 2006). Processing snap bean is a major vegetable crop in this region where the farm-gate value alone exceeds $100 million (USDA-NASS 2011). A number of aphid species are present in the region that are potential vectors of ClYVV (Nault et al. 2004), but the increased incidence of ClYVV (and other viruses) in snap bean production has been associated with the accidental introduction of the soybean aphid (*Aphis glycines* Matsumura) to the United States in 2000 or earlier (Hill et al. 2001; Ragsdale et al. 2004). Aphid vectors transmit ClYVV in a nonpersistent manner where the virus may be acquired and transmitted within seconds of stylet penetration (Nault 1997; Nault et al. 2004). For this reason and others, nonpersistent aphid-transmitted viruses can be exceedingly difficult to control. Host plant resistance, if available, is the most effective, efficient, and ecologically sensitive means to reduce the potential for crop damage.

Genetic variation for resistance to ClYVV in common bean has been identified. The *by-3* gene was first identified in GN 1140 where it provided homozygous recessive resistance to a number of isolates of the severe (pod-distorting) strain of *Bean yellow mosaic virus* (BYMV-S) (Provvidenti and Schroeder 1973). The *by-3* gene symbol was subsequently revised to *cyv* by Provvidenti (1987) to reflect taxonomic revision of BYMV-S to ClYVV (as proposed by Bos et al. 1977, substantiated by Uyeda et al. 1991 and Tracy et al. 1992). A single gene in Kentwood that was donated by Clipper conditioned homozygous recessive resistance to a necrotic strain of ClYVV previously typed as BYMV-N from Ontario, Canada (Tu 1980, 1983). Genetic analysis of the resistance present in GN UI 31 to an isolate of BYMV-S from Oregon revealed that two recessive genes were required for resistance (Tatchell et al. 1985). Subsequent research demonstrated that the recessive *cyv* gene present in GN 1140 was allelic with the ClYVV resistance present in the cultivars Kentwood, Harokent, Imuna, and Amanda (Park and Tu 1991).

The mechanism of resistance present in Jolanda to the ClYVV no. 30 strain from Japan was characterized by employing a ClYVV vector that expressed green fluorescent protein (pClYVV/C3-S65T) (Sato et al. 2003). The results demonstrated that the resistance phenotype operated on the single-cell level by completely inhibiting ClYVV replication in healthy cells (Sato et al. 2003). A spontaneous resistance-breaking mutant of the virus (ClYVV-Br) was then utilized to develop chimeric clones and to map the avirulence determinant of the virus. The results

suggested that the viral genome-linked protein (VPg) was the avirulence determinant (Sato et al. 2003) similar to that observed in other plant-potyvirus pathosystems (Hjulsager et al. 2002; Keller et al. 1998; Nicolas et al. 1997). Genetic analysis revealed that the resistance was conditioned by a single recessive gene which was separate from the potyvirus resistance conferred by the *Bean common mosaic virus* (BCMV) resistance genes *I*, *bc-1*, and *bc-u* which were also present in Jolanda (Drijfhout 1978; Sato et al. 2003). The resistance gene was designated *desc* (determinant of susceptibility to ClYVV) to reflect the hypothesis that it was based on the absence of a factor necessary for viral infection and replication, and possibly analogous to the phenomenon of recessive resistance conditioned by mutations in eukaryotic translation initiation factor 4E (eIF4E) and its isoform (eIF(iso)4E) as elucidated in *Capsicum* spp. (Ruffel et al. 2002) and *Arabidopsis thaliana* (Lellis et al. 2002), respectively. Allelism testing with additional ClYVV no. 30 resistant bean cultivars Evolutie and Imuna revealed that they possessed the same *desc* resistance (Sato et al. 2003). The allelic relationship between *desc* and the previously identified *cyv* gene reported in Imuna (Park and Tu 1991) was not established. In addition, it appears the hypothesis that mutations in eIF4E or eIF(iso)4E present in Jolanda conferred resistance to ClYVV no. 30 was never tested.

A novel strain of ClYVV from Wisconsin (ClYVV-WI) associated with an emerging U.S. Great Lakes virus disease complex was recently discovered and characterized (Larsen et al. 2008). ClYVV-WI infection caused severe symptoms in a collection of 63 commercial snap bean cultivars and other common bean genotypes, including the previously reported resistant cultivars Imuna and Jolanda (Larsen et al. 2008). Complete resistance to ClYVV-WI, as well as the New York (ClYVV-NY) and Oregon (ClYVV-OR) strains, was identified in all common bean genotypes that possessed the recessive *bc-3* gene (USLK-1, USLK-2, USDK-4, USDK-5, USWK-6, USCR-7, USCR-8, USCR-9, and Raven) with the exception of IVT 7214 (Larsen et al. 2008). The potential involvement of *bc-3* is particularly interesting given that its combination with the *bc-u*, and/or *I* gene(s) (Ali 1950; Collmer et al. 2000) confers resistance to all known strains of the related potyviruses *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV) worldwide (Drijfhout 1978; Kelly et al. 1995; McKern et al. 1992), except the recombinant NL 3 K strain of BCMV/BCMNV (Larsen et al. 2005).

The *bc-3* gene is one of six recessive alleles (*bc-u*, *bc-1*, *bc-1^2*, *bc-2*, *bc-2^2*, and *bc*-3) at four independent loci, that along with the dominant allele of the *I* gene act in allele-specific combinations to condition resistance, and to define seven pathogenicity groups of BCMV and BCMNV (for reviews, see Drijfhout 1978; Drijfhout et al. 1978; Kelly et al. 1995; Morales 2005; Mukeshimana et al. 2005). Full

expression of the resistance conditioned by the five strain-specific alleles (*bc-1*, *bc-1²*, *bc-2*, *bc-2²*, and *bc-3*) requires the homozygous recessive state of the strain nonspecific *bc-u* gene when the dominant allele of the *I* gene is absent (Drijfhout 1978). When the *I* gene is present in *Bc-u* genotypes, all resistance alleles are fully expressed except for those at the *bc-2* locus (Kelly et al. 1995). The *i, Bc-u, bc-3* combination is known to condition resistance to all known strains of BCMNV, but not to all strains of BCMV (US1, US3, US7), although another uncharacterized gene that interacts with *bc-3* may be responsible for this resistance spectrum (Miklas et al. 1998). The allele combinations *I, bc-3* as well as *bc-u, bc-3,* condition resistance to all known strains of BCMV, BCMNV (except NL 3 K), and all known strains of ClYVV (Larsen et al. 2008). Due to the critical importance of the resistance spectrum conditioned by *bc-3,* and its recessive inheritance, common bean breeders and geneticists have invested in the development and employment of molecular markers to map *bc-3* to common bean chromosome 6, and to enable marker-assisted selection and introgression of *bc-3* (Johnson et al. 1997; Kelly et al. 2003; Mukeshimana et al. 2005; Naderpour et al. 2010; Pedrosa-Harand et al. 2008).

Revolutionary advances in the understanding of the molecular genetic and functional basis for resistance to the *Potyviridae* have led to the conclusion that naturally occurring amino acid substitutions in specific regions of eIF4E and eIF(iso)4E result in the numerous and effective recessive resistance genes common across many important monocot and dicot crop plant-potyvirus pathosystems (see reviews, Diaz-Pendon et al. 2004; Kang et al. 2005a; LeGall et al. 2011; Robaglia and Caranta 2006; Truniger and Aranda 2009; Wang and Krishnaswamy 2012). Recognizing this, eIF4E, eIF(iso)4E, and the novel cap binding protein (nCBP) were cloned and sequenced from a collection of nine common bean genotypes that represented eight different allele combinations of the *I* and *bc-* resistance genes (Naderpour et al. 2010). Predicted amino acid substitutions were discovered in a variant of *P. vulgaris* eIF4E (PveIF4E²), which was cloned exclusively from four genotypes that possessed the *bc-3* resistance allele. Alignment of PveIF4E² with eIF4E variants that have been confirmed to condition resistance to potyviruses in *Capsicum annuum* (Kang et al. 2005b; Ruffel et al. 2002), *Lactuca sativa* (Nicaise et al. 2003), and *Pisum sativum* (Gao et al. 2004) revealed analogous features of amino acid substitutions in the predicted cap binding pocket of the protein (Naderpour et al. 2010). A cleaved amplified polymorphic sequence (CAPS) marker diagnostic for *PveIF4E²* cosegregated with *bc-3* conditioned resistance to BCMV strain NL 1 (NL 1) in a segregating F₂ population of 96 individuals (Naderpour et al. 2010). Though not confirmed by direct complementation testing, these results provided the initial evidence towards mutated eIF4E alleles as the molecular basis for *bc-3*.

In light of the possibility that *bc-3* resistance to ClYVV may also be confirmed by a mutant eIF4E allele, and the immediate practical need to deploy resistance to ClYVV in the Great Lakes Region of the U.S., a more thorough understanding of the available genetic variation for resistance was needed. The objectives of this research were to evaluate, identify, and characterize the ClYVV resistance present in 21 informative common bean genotypes and establish the allelic relationships of all three previously reported recessive resistance genes to ClYVV (*cyv*, *desc*, and *bc-3*). Subsequent objectives were to further examine the potential association of predicted amino acid substitutions in PveIF4E with resistance to ClYVV and then to develop a set of single nucleotide polymorphism (SNP)-based markers for rapid allelic discrimination and marker-assisted selection of the recessive potyvirus resistance alleles at the *Bc-3* locus.

Materials and methods

Plant materials and population development

A panel of 21 common bean genotypes was assembled to include entries reported to possess the putatively independent recessive resistance genes to ClYVV (*cyv*, *desc*, or *bc-3*) as well as additional entries with known and/or demonstrated virus interaction phenotypes (Table 1). Five of the entries were snap bean genotypes (Baby Bop, Laureat, Paloma, Polder, Sonesta) that were identified to be resistant to ClYVV-NY as the result of a screen of cultivars (Hart and Griffiths, unpublished).

Populations were developed for cosegregation analysis and allelism testing. Midnight, the recurrent susceptible parent used in the development of the ClYVV resistant cultivar Black Knight was crossed to Black Knight to create F₁ and F₂ populations for cosegregation analysis. Black Knight is nearly isogenic with Midnight but possesses the *cyv* resistance allele (Halseth et al. 1998) from GN 1140 (Provvidenti 1987; Provvidenti and Schroeder 1973). Black Knight was also crossed to Raven (Kelly et al. 1994) that possesses the *I, bc-3* allele combination to develop F₁ and F₂ populations for cosegregation analysis and allelism testing. B/R RIL 105-25, a breeding line developed at Michigan State University (East Lansing, MI) that possesses *I, bc-3* from Raven was crossed to the ClYVV resistant cultivar Clipper that possesses *cyv* (Tu 1983) to develop F₁, F₂, and F₂:₃ populations for allelism testing. The breeding line CY-10 S₄ was crossed to USWK x H S₄ to develop F₁ and F₂ populations for allelism testing. CY-10 S₄ is a BC₈S₄ line developed at Cornell University (CU) New York state agricultural experiment station (NYSAES) (Geneva,

Table 1 Common bean genotypes, sources, market classes, previously reported resistance genes to *Clover yellow vein virus* (ClYVV), and their respective responses to the NY strain of ClYVV (ClYVV-NY) and the NL 3D strain of *Bean common mosaic necrosis virus* (NL 3D)

Genotype	Accession[a]	Market class	ClYVV resistance	Response to ClYVV-NY[j]	Response to NL 3D[j]
Dubbele Witte	PI 377736	Fresh snap	–	sS, VN, sM, TN	S, Ld, M
Midnight	PI 550032	Black	–	sS, VN, sM	VN, SN, D
Hystyle	PI 550288	Processing snap	–	sS, VN, sM, TN	VN, SN, D
GN 1140	PI 549667	Great northern	*cyv*[b]	NS	mM
Black Knight	CU	Black	*cyv*[c]	NS	VN, SN, D
Jolanda	G 7591	Fresh snap	*desc*[d]	NS	NLL, VN, Ld, M
Amanda	PI 599026	Fresh snap	*cyv*[e]	NS	lVN
Baby bop	PI 642144	Processing snap	–	NS	VN, SN, D
Laureat	PI 550261	Processing snap	–	NS	VN, SN, D
Paloma	CU	Fresh snap	–	NS	VN, SN, D
Polder	CU	Fresh snap	–	NS	VN, SN, D
Sonesta	CU	Fresh snap (wax)	–	NS	VN, SN, D
Imuna	PI 326420	Fresh snap	*cyv*[e], *desc*[d]	NS	mM
Evolutie	W6 42706	Fresh snap	*desc*[d]	NS	VN, SN, D
Clipper	PI 278776	Navy	*cyv*[f]	NS	S, Ld, M
CY-10 S$_4$	CU	Processing snap	*cyv*	NS	VN, SN, D
IVT 7214	PI 602987	Expt. line	*bc-3*[g]	NS	NS
Raven	MSU	Black	*bc-3*[h]	NS	NS
B/R RIL105-25	MSU	Navy	*bc-3*	NS	NS
USWK-6	PI 618815	White kidney	*bc-3*[i]	NS	NS
USWKH × H S$_4$	CU	Expt. line	*bc-3*	NS	NS

[a] Accessions that begin with 'PI' and 'W6′ were sourced from the U.S. National Plant Germplasm System, 'G' international center for tropical agriculture, 'CU' Cornell University, and 'MSU' Michigan State University

Resistance genes cited in the following references: [b] Provvidenti and Schroeder 1973, [c] Halseth et al. 1998, [d] Sato et al. 2003, [e] Park and Tu 1991, [f] Tu 1983, [g] Drijfhout 1978, [h] Kelly et al. 1994, [i] Miklas et al. 2002

[j] Symptom legend: *D* plant death, *Ld* leaf distortion, *M* mosaic, *mM* mild mosaic, *sM* severe mosaic, *NLL* necrotic local lesions, *NS* no symptoms, *S* stunting, *sS* severe stunting, *SN* systemic necrosis, *VN* vein necrosis, *lVN* localized vein necrosis

NY) through the introgression of *cyv* from Clipper into the susceptible recurrent parent Hystyle. USWKH x H S$_4$ is a BC$_2$S$_4$ line developed at CU through introgression of *bc-3* from USWK-6 into the susceptible recurrent parent Hystyle. USWK-6 is a breeding line developed by the USDA-ARS that possesses *I, bc-3* (Miklas et al. 2002).

Additional F$_1$ populations were developed to investigate allelic relationships through complementation testing. Amanda (*cyv*) was crossed to Imuna (*cyv/desc*) and Jolanda (*desc*) was crossed to Clipper (*cyv*) to develop F$_1$ populations. All F$_1$ hybrid plants were confirmed heterozygous by morphology, by allele-specific molecular marker assays, or both. The molecular marker assays are described in the 'sequence analysis and design of allele-specific assays' section of the methods below.

Seeds of all experimental material were sown in 'Cornell mix' (Boodley and Sheldrake 1972) in greenhouses at CU NYSAES, where routine watering, fertilization, and insect control measures were employed. Plant growth conditions were 24 °C day/21 °C night with a 14-h photoperiod.

Supplemental lighting was produced by 1,000 W metal halide bulbs (SunSystem III, Sunlight Supply, Inc., USA) to provide a rate of 300 μM m^2 s^1 of photosynthetically active radiation at bench level. All of the plants used in the experiments were grown in 14.6 cm^2 pots except for the F$_2$ derived F$_3$ families (F$_{2:3}$) which were grown in 18 cell flats (Speedling Inc., USA) with cells of 10 cm^2.

Virus isolates, inoculation, and resistance evaluation

ClYVV-NY was obtained from the Rosario Provvidenti collection at CU NYSAES and confirmed to be a pure isolate by host range and symptomatology, and RT-PCR (Provvidenti and Schroeder 1973; Shail et al. 2007). The reaction of the common bean BCMV/BCMNV host differential groups and genotypes to ClYVV-NY is presented in Online Resource 1, Table 1. ClYVV-NY is identical to the strain employed by Larsen et al. (2008). ClYVV-NY was maintained in the susceptible snap bean cultivar Hystyle. The NL 3D strain (Drijfhout 1978; Larsen et al. 2005) was obtained

from Dr. Phillip Miklas of the USDA-ARS (Prosser, WA) and was maintained in the cultivar Dubbele Witte. The virus strains were maintained by periodical transfer (~3 weeks) to the expanding primary leaves of seedlings of the susceptible cultivars by mechanical inoculation. Virus inoculum was prepared by homogenizing newly expanded, symptomatic, virus-infected trifoliate leaves (1:10 w/v) in cold 10 mM phosphate buffer (3 mM K_2PO_4, 7 mM Na_2HPO_4; pH 7.0) with a mortar and pestle. The homogenate was applied with the pestle by gently rubbing newly expanded primary leaves of seedlings (7–10 days after planting) that had been dusted with carborundum (silicon carbide, 400 mesh, Sigma-Aldrich Inc., USA). The inoculated plants were then lightly rinsed with water. All plants were inoculated again 2 days after the first inoculation, and plants that did not display virus symptoms 10 days post the first inoculation (dpi) were re-inoculated to prevent escape.

At least 20 inoculated plants of each of the 21 genotypes included in the panel were inoculated and evaluated in three separate experiments. The plants were examined daily for virus symptoms, and symptom expression was evaluated in comparison to non-inoculated, susceptible, and resistant controls, and recorded 10, 21, 30, and 45 dpi. Symptom expression in response to inoculation with ClYVV-NY and NL 3D was evaluated qualitatively following the descriptors defined in the caption of Table 1. At 21 dpi, leaf tissue from newly expanded trifoliate leaves was collected for virus detection by enzyme-linked immunosorbent assay (ELISA). The ELISA was performed according to the manufacturer's instructions (for ClYVV-NY:ClYVV-C81 and Pratt, AC Diagnostics Inc., USA; for NL 3D: Potyvirus Group, Agdia Inc., USA). The absorbance at OD_{405nm} was measured with a multi-mode microplate reader (Synergy 2, Biotek Instruments Inc., USA) following the final incubation and after two additional 1-h intervals. Absorbance reads that were at least two times greater than that of the healthy negative control were considered to be positive for presence of the virus.

Cloning and sequencing of *P. vulgaris* eIF4E

Coding sequences of *PveIF4E* for each genotype in Table 1 were obtained as follows. Total RNA was isolated with an Ambion RNAqueous-4PCR Kit (Life Technologies, USA) following the manufacturer's instructions. The RNA was eluted into 30 μL of elution buffer and quantified by spectrophotometry so that 1 μg of RNA could be used for cDNA synthesis. Reverse transcription was performed with an Ambion RETROscript First Strand Synthesis Kit following the manufacturer's instructions (Life Technologies, USA). Oligonucleotide primers capable of amplifying the entire *PveIF4E* coding sequence were designed prior to the release of the *P. vulgaris* genome sequence and thus were

designed to anneal to the extremities of the *eIF4E* coding sequences of pea (*Pisum sativum* L.) and soybean (*Glycine max* (L.) Merr.). The primer pair was designed using Primer 3 (Rozen and Skaletsky 2000); the forward primer was designated Leg4E-F (5′-ATG GTT GTA GAA GAT ACC C-3′) and the reverse primer, Leg4E-R (5′-TCA TAC AAC GTA TTT ATT TTT AGC-3′). PCR amplification was performed in 20 μL reactions containing 0.5 μL of cDNA, 5 pmol of each primer, and 1.5 units of Platinum® Taq DNA Polymerase High Fidelity (Life Technologies, USA) using an Eppendorf Gradient Master Cycler (Eppendorf North America, USA). The PCR program consisted of 1 denaturation cycle of 3 min at 95 °C followed by 40 cycles of 30 s each of 94, 53, and 72 °C. Aliquots of the PCR products were resolved by 1.5 % TAE agarose gel electrophoresis and visualized with ethidium bromide under UV-light to confirm that amplicons had the expected size of 693 bp. PCR products were then purified using a QIAquick PCR purification kit (Qiagen Inc., USA).

Two independently amplified PCR products from each genotype were cloned into the pCR4 vector using the TOPO TA Cloning Kit for Sequencing (Life Technologies, USA). DNA was isolated from liquid cultures of two single colony clones in selective media using the QIAprep Spin Miniprep kit (Qiagen Inc., USA). Nucleotide sequencing was performed with an Applied Biosystems Automated 3730 DNA Analyzer using Big Dye Terminator chemistry and Ampli-Taq-FS DNA Polymerase (Life Technologies, USA) at the CU Life Sciences Core Laboratories Center, Ithaca, NY.

Sequence analysis and design of allele-specific assays

The *PveIF4E* coding sequences and deduced proteins were aligned to detect non-synonymous SNPs and amino acid substitutions. Multiple sequence alignment by ClustalW was performed with the CLC Main Workbench (CLC Bio, USA) software. *PveIF4E* coding sequences were also aligned to the *Glycine max* (L.) Merr. genomic sequence (Schmutz et al. 2010) with the 'est2genome' model of the Exonerate multiple sequence alignment program (Slater and Birney 2005) to predict the intron–exon boundaries of *PveIF4E*. Non-synonymous SNPs that corresponded with defined virus resistance phenotypes were chosen for the design of KBiosciences Competitive Allele-Specific PCR (KASPar) SNP assays (LGC-KBiosciences, USA) (Fig. 1). At least 50 bp of sequence flanking both sides of the targeted SNPs was submitted to the KBiosciences KASPar By Design service and the primers in Table 2 were synthesized for the assays.

DNA extraction and KASPar assays

Genomic DNA for use in KASPar assays was isolated from ~50 mg of tissue from young trifoliate leaves according
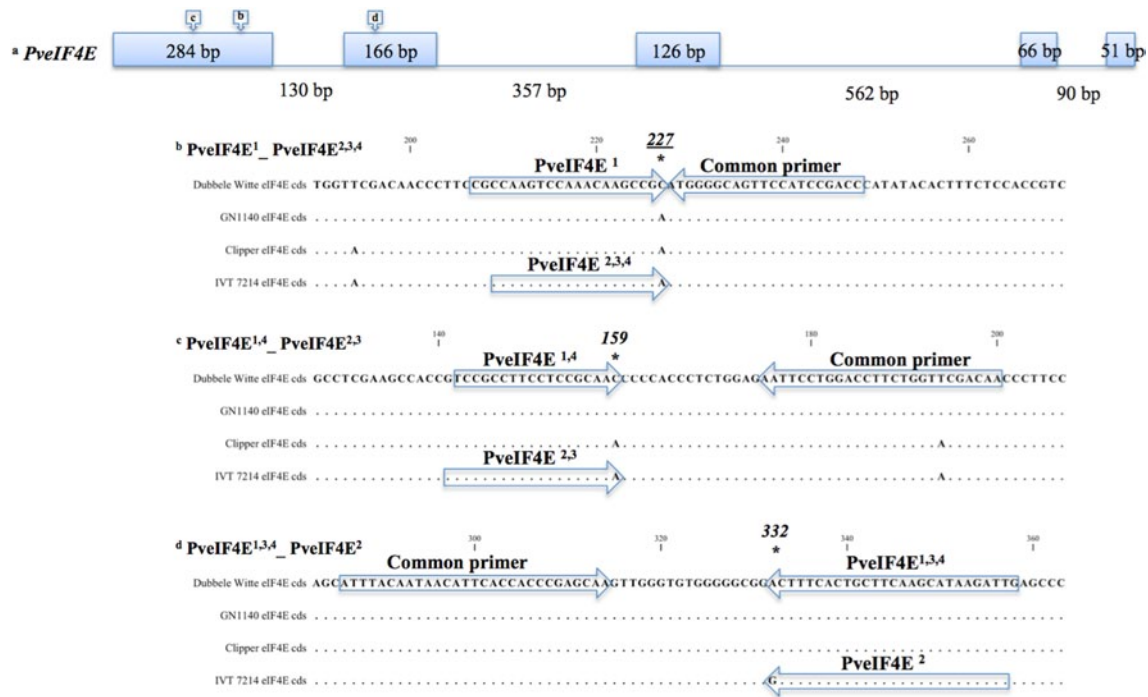
Fig. 1 Schematic of genomic *PveIF4E* where *boxes* correspond to exons, *lines* correspond to introns, and the relative positions of the non-synonymous SNPs targeted for the development of KAS-Par assays are indicated by *boxes* with *arrows*. The KASPar assays described in Table 2 are illustrated below

Table 2 Assay ID, target SNPs, and primer sequences of the KASPar assays employed

KASPar assay ID	SNP	Primer	Sequence
PveIF4E[1] __PveIF4E[2,3,4]	**C227A**	PveIF4E[1] Allele (**C**_VIC)	CGCCAAGTCCAAACAAGCCGC
		PveIF4E[2,3,4] Allele (**A**_FAM)	CGCCAAGTCCAAACAAGCCGA
		Common	GGTCGGATGGAACTGCCCCAT
PveIF4E[1,4]__PveIF4E[2,3]	**C159A**	PveIF4E[1,4] Allele (**C**_VIC)	GTCCGCCTTCCTCCGCAAC
		PveIF4E[2,3] Allele (**A**_FAM)	CGTCCGCCTTCCTCCGCAAA
		Common	TGTCGAACCAGAAGGTCCAGGAATT
PveIF4E[1,3,4] __PveIF4E[2]	**A332G**	PveIF4E[1,3,4] Allele (**A**_ VIC)	CAATCTTATGCTTGAAGCAGTGAAAGT
		PveIF4E[2] Allele (**G**_FAM)	AATCTTATGCTTGAAGCAGTGAAAGC
		Common	ATTTACAATAACATTCACCACCCGAGCAA

to Afanador et al. (1993). DNA was checked for purity by spectrophotometry (NanoDrop ND-1000, Thermo Scientific, USA) and was quantified using Quant-iT PicoGreen (Life Technologies, USA) and a multi-mode microplate reader (Synergy 2, Biotek Instruments Inc., USA). DNA was diluted with nuclease free water to 5 ng/µL. KASPar assays were performed according to the manufacturer's instructions (Robinson and Holmes, 2011) in 8 µL reaction volumes containing 4 µL of DNA, 4 µL of 2 × KASP reaction mix, and 0.11 µL of the assay mix containing the common primer and the fluor-labeled allele-specific primers. PCR amplification consisted of one 15 min cycle at 94 °C, followed by 10 cycles of 94 °C for 20 s, 65–57 °C for 60 s (dropping 0.8 °C per cycle), and then 30 cycles of 94 °C for 20 s, and 57 °C for 60 s using an Eppendorf Gradient Master Cycler PCR machine (Eppendorf, USA). Fluorescent endpoint analysis was performed with a ViiA7 Real-Time PCR system (Life Technologies, USA). Fluorescent endpoint analysis data were analyzed with the 'genotyping experiment' module of the ViiA7 Software package (Life Technologies, USA) and allele calls were assigned to the samples automatically by the software. In some cases, interaction with the software to infer an allele call, or to remove a failed reaction, was necessary.

Results

Allelism tests demonstrated that *cyv, desc,* and *bc-3* are allelic

The lack of symptoms (Table 1) and negative ELISA results (data not shown) confirmed that the genotypes included in the panel that possessed *cyv*, *desc*, and *bc-3* all conditioned resistance to ClYVV-NY. These results validated the use of ClYVV-NY to evaluate potential allelic relationships. All F$_1$, F$_2$, and F$_{2:3}$ populations derived from the crosses between ClYVV resistant parents possessing either *cyv*, *desc*, or *bc-3* were uniformly resistant based on the absence of symptoms (Table 3) and negative ELISA results. In total, 90 individuals from five separate F$_1$ populations, 289 individuals from two separate F$_2$ populations, and 132 F$_{2:3}$ lines (1,188 individuals) were resistant to ClYVV-NY. The failure of complementation to restore susceptibility provides clear evidence that *cyv*, *desc*, and *bc-3* are allelic and, therefore, map to the same genetic locus on chromosome 6.

Resistance to ClYVV-NY in *P. vulgaris* is associated with an allelic series at *PveIF4E*

The complete coding sequences of *PveIF4E* were obtained from each of the 21 genotypes in Table 1 and were examined for non-synonymous single nucleotide polymorphisms (Table 4; for full-length alignment see Online Resource 1). The Leg4E primer pair produced a single amplicon of the

expected size of 693 bp for all cDNA templates. BLASTn and BLASTp (http://blast.ncbi.nlm.nih.gov/) queries of the sequenced amplicons and the predicted amino acid sequences from Dubbele Witte and Raven confirmed identity with *PveIF4E^1* (EF571267) and *PveIF4E^2* (EF571273), respectively, previously cloned and sequenced from the same genotypes (Naderpour et al. 2010). This gene corresponds to the locus Phvul.006G168400 on chromosome six of the V1.0 release of the *P. vulgaris* genome (Phaseolus vulgaris V1.0 2013).

The complete *PveIF4E* coding sequences from Midnight and Hystyle, two genotypes (in addition to Dubbele Witte) that were susceptible to ClYVV-NY, and displayed the unprotected *I* gene response of systemic necrosis to NL 3D (Kelly et al. 1995), were identical to *PveIF4E^1*. The complete *PveIF4E* coding sequences from IVT 7214, B/R RIL 105-25, USWK-6, and USWKH x H S$_4$, four genotypes (in addition to Raven) that were resistant to ClYVV-NY and NL 3D as conditioned by *bc-3* were identical to *PveIF4E^2*. *PveIF4E^2* differed from *PveIF4E^1* by four SNPs that each resulted in predicted amino acid substitutions at positions 53 (N/K), 65 (F/Y), 76 (A/E), and 111 (D/G) of the predicted protein (Table 4). *PveIF4E^2* was associated with homozygous recessive resistance to NL 1 as conditioned by the *bc-3* gene in previous cosegregation analysis (Naderpour et al. 2010).

Two novel *PveIF4E* mutant alleles were identified, *PveIF4E^3*, and *PveIF4E^4*, that were identical for some, but not all of the SNPs and predicted amino acid substitutions

Table 3 Resistance to ClYVV-NY in common bean parental genotypes and populations tested for allelism

Genotype	ClYVV resistance	Population	No. plants/ lines	Phenotype (ClYVV-NY)
Clipper	*cyv*	Parent 1	18	Resistant
B/R RIL 105-25	*bc-3*	Parent 2	18	Resistant
Clipper × B/R RIL 105-25	*cyv bc-3*	F$_1$	18	Resistant
Clipper × B/R RIL 105-25		F$_2$	132	Resistant
Clipper × B/R RIL 105-25		F$_{2:3}$	132[a]	Resistant
CY-10 S$_4$	*cyv*	Parent 1	18	Resistant
USWKH × H S$_4$	*bc-3*	Parent 2	18	Resistant
CY-10 S$_4$ × USWKH × H S$_4$	*cyv bc-3*	F$_1$	18	Resistant
CY-10 S$_4$ × USWKH × H S$_4$		F$_2$	157	Resistant
Black Knight	*Cyv*	Parent 1	18	Resistant
Raven	*bc-3*	Parent 2	18	Resistant
Black Knight × Raven	*cyv bc-3*	F$_1$	18	Resistant
Black Knight × Raven		F$_2$	40	Resistant
Amanda	*cyv*	Parent 1	18	Resistant
Imuna	*cyv (desc)*	Parent 2	18	Resistant
Amanda × Imuna	*cyv cyv(desc)*	F$_1$	18	Resistant
Jolanda	*desc*	Parent 1	18	Resistant
Clipper	*cyv*	Parent 2	18	Resistant
Jolanda × Clipper	*desc cyv*	F$_1$	18	Resistant

[a] 9 plants per F2:3 line tested

Table 4 Resistance of 21 common bean genotypes to ClYVV-NY and NL 3D and their corresponding nucleotide polymorphisms and predicted amino acid substitutions in *PveIF4E*

Genotype	ClYVV-NY[a]	NL 3D[a]	Position of nucleotide (nt) polymorphism and amino acid (aa) substitutions[b]								*PveIF4E* alleles[c]
			nt	**aa**	nt	**aa**	nt	**aa**	nt	**aa**	
			159	**53**	194	**65**	227	**76**	332	**111**	
Dubbele Witte	S	S	C	**N**	T	**F**	C	**A**	A	**D**	*PveIF4E^1^*[(d)]
Midnight	S	S	–	–	–	–	–	–	–	–	
Hystyle	S	S	–	–	–	–	–	–	–	–	
GN 1140	r (cyv)	S	–	–	–	–	A	**E**	–	–	*PveIF4E^4^*
Black Knight	r (cyv)	SN	–	–	–	–	A	**E**	–	–	
Jolanda	r (desc)	VN	–	–	–	–	A	**E**	–	–	
Amanda	r (cyv)	VN	–	–	–	–	A	**E**	–	–	
Baby Bop	r	SN	–	–	–	–	A	**E**	–	–	
Laureat	r	SN	–	–	–	–	A	**E**	–	–	
Paloma	r	SN	–	–	–	–	A	**E**	–	–	
Polder	r	SN	–	–	–	–	A	**E**	–	–	
Sonesta	r	SN	–	–	–	–	A	**E**	–	–	
Imuna	r (cyv/desc)	S	A	**K**	A	**Y**	A	**E**	–	–	*PveIF4E^3^*
Evolutie	r (desc)	SN	A	**K**	A	**Y**	A	**E**	–	–	
Clipper	r (cyv)	S	A	**K**	A	**Y**	A	**E**	–	–	
CY-10 S₄	r (cyv)	SN	A	**K**	A	**Y**	A	**E**	–	–	
IVT 7214	r (bc-3)	r (bc-3)	A	**K**	A	**Y**	A	**E**	G	**G**	*PveIF4E^2^*[(e)]
Raven	r (bc-3)	r (bc-3)	A	**K**	A	**Y**	A	**E**	G	**G**	
B/R RIL105-25	r (bc-3)	r (bc-3)	A	**K**	A	**Y**	A	**E**	G	**G**	
USWK-6	r (bc-3)	r (bc-3)	A	**K**	A	**Y**	A	**E**	G	**G**	
USWKH × H S₄	r (bc-3)	r (bc-3)	A	**K**	A	**Y**	A	**E**	G	**G**	

[a] *S* susceptible, *SN* systemic necrois, *VN* vein necrosis, *r* resistant; confirmed by screening 5 to 10 plants of each cultivar in three independent experiments, with 'r' confirmed by ELISA

[b] A dash (–) in the table represents an identical nt or aa as *PveIF4E*1

[c] *PveIF4E* alleles are numbered by superscript in the order in which they were discovered

[d] *PveIF4E*1 first cloned and sequenced from Dubbele Witte and others in Naderpour et al. 2010

[e] *PveIF4E*2 first cloned and sequenced from Raven in Naderpour et al. 2010

of *PveIF4E^2^* (Table 4). These novel mutant alleles were exclusive to genotypes with resistance to ClYVV-NY and susceptibility to NL 3D. *PveIF4E^3^*, first cloned and sequenced from Clipper, also present in CY-10 S₄, Evolutie, and Imuna, possessed three of the four predicted amino acid substitutions present in *PveIF4E^2^* at positions 53 (N/K), 65 (F/Y), and 76 (A/E), respectively. *PveIF4E^4^*, first cloned and sequenced from GN 1140, also present in Black Knight, Jolanda, Amanda, Baby Bop, Laureat, Paloma, Polder, and Sonesta, possessed only one of the four predicted amino acid substitutions present in *PveIF4E^2^* at position 76 (A/E). This result strongly suggests that the A76E mutation is a determinant for resistance to ClYVV-NY.

Only genotypes that possessed *PveIF4E^2^* and the predicted D111G mutation were resistant to NL 3D (Table 4). Similarly, this result strongly suggests that the D111G mutation is a determinant for resistance to NL 3D. The pattern of exclusive non-synonymous nucleotide substitutions present in *PveIF4E^2^*, *PveIF4E^3^*, and *PveIF4E^4^* are analogous to the patterns of polymorphism in the critical area of eIF4E for cap-binding and recognition where amino acid substitutions have been associated with, or directly demonstrated to condition potyvirus resistance in an extensive range of crop plants (Diaz-Pendon et al. 2004; Kang et al. 2005a; LeGall et al. 2011; Robaglia and Caranta 2006; Truniger and Aranda 2009; Wang and Krishnaswamy 2012).

Allele-specific genotyping and cosegregation analysis

In order to progress from polymorphism discovery to allele-specific molecular markers as rapidly as possible,

three KASPar SNP genotyping assays were developed to differentiate and confirm the allelic state of all of the *PveIF4E* alleles (Table 2). The assays were employed to investigate genotype–phenotype relationships, to confirm allelism, and to validate the utility of the assays for allelic discrimination across the germplasm.

KASPar assay 'PveIF4E[1]__PveIF4E[2,3,4]' targets the SNP227 (C227A) mutation that corresponds to the predicted A76E amino acid substitution that is present in the *PveIF4E[2,3,4]* alleles, all associated with resistance to ClYVV-NY (Table 4). This assay was employed to investigate genotype–phenotype relationships in parental, F_1, and F_2 populations of the Midnight × Black Knight cross (Table 5). An example of the allele calls made by the software is illustrated in Online Resource 1, Fig. 4, for 88 Midnight × Black Knight F_2 individuals. The genotypic and phenotypic segregation ratios fit that expected of a single recessive locus, and the homozygous state of the mutant allele (AA) was in complete linkage with the resistance phenotype in 49 F_2 individuals (Table 5).

KASPar assay 'PveIF4E[1,3,4]__PveIF4E[2]' targets the SNP332 (A332G) mutation that corresponds to the predicted D111G amino acid substitution present only in *PveIF4E[2]* and that is associated with *bc-3* resistance to ClYVV-NY, ClYVV-WI (Larsen et al. 2008), NL 3D, and NL 1 (Naderpour et al. 2010) (Table 4). This assay was employed to investigate genotype–phenotype relationships in the parental, F_1, and F_2 populations of the Black Knight × Raven cross (Table 6). The genotypic and phenotypic segregation ratios fit that expected for a single recessive locus, and the homozygous state of the mutant allele (GG) was in complete linkage with the resistance phenotype in 50 F_2 individuals (Table 6).

KASPar assay 'PveIF4E[1,3,4]__PveIF4E[2]'(A332G) was also employed to demonstrate its ability to discriminate alleles at the molecular genetic level in the parental, F_1, and F_2 allelism testing populations of the Clipper x B/R RIL 105-25 cross where all phenotypes were identical (Table 7).

The genotypic segregation ratio fit the expectation of a single locus (Table 7). The phenotypic segregation ratio could not be ascertained because the population was inoculated and evaluated only for resistance to ClYVV-NY. KASPar assay 'PveIF4E[1,4]__PveIF4E[2,3]' (C159A) was designed for the purposes of allelic discrimination and haplotype analysis.

The KASPar assays were employed for haplotype analysis across the 21 common bean genotypes. Table 8 demonstrates the assays' ability to discriminate between the *PveIF4E* alleles and to ultimately predict the resistance allele and spectrum. The KASPar assays consistently and accurately identified the correct haplotypes for all of the *PveIF4E* alleles.

Discussion

The germplasm evaluation (Table 1), the allelism testing (Table 3), the complete association between unique non-synonymous SNPs in *PveIF4E* alleles and resistance to ClYVV and/or NL 3D (Table 4), and the KASPar allele-specific assays (Tables 5, 6, 7, 8) all contribute to the evidence that *cyv*, *desc*, and *bc-3* comprise a series of recessive resistance alleles at the *Bc-3* locus. This allelic series conditions strain- and species-specific resistance spectra to the potyviruses ClYVV, BCMV, and BCMNV in common bean. In light of this, the nomenclature for the *Bc-3* locus should be revised in accordance with the guidelines for gene nomenclature set forth by the Bean Improvement Cooperative (BIC) (Porch 2012).

The first genetic symbol designated to an allele at this locus was *by-3*, identified in GN1140 (Provvidenti and Schroeder 1973), although it was subsequently revised to *cyv* (Provvidenti 1987). It was previously demonstrated that *desc* from Jolanda was allelic with the ClYVV resistance present in the cultivar Imuna (previously reported as *cyv* (Park and Tu 1991)) (Sato et al. 2003). We also demonstrate

Table 5 Cosegregation of KASPar SNP PveIF4E[1] (**C**) allele with susceptibility and KASPar SNP PveIF4E[2,3,4] (**A**) allele with resistance to ClYVV-NY as conditioned by *cyvcyv* in the Midnight x Black Knight populations

Genotype	Population	ClYVV resistance	PveIF4E[1]__PveIF4E[2,3,4] SNP (C227A)	No.	Phenotype[b]	No.
Midnight	Parent 1	*CyvCyv*	PveIF4E[1] (**C**) PveIF4E[1] (**C**)	18	Susceptible	18
Black Knight	Parent 2	*cyvcyv*	PveIF4E[4] (**A**) PveIF4E[4] (**A**)	18	Resistant	18
Midnight × Black Knight	F_1	*Cyvcyv*	PveIF4E[1] (**C**) PveIF4E[4] (**A**)	18	Susceptible	18
Midnight × Black Knight	F_2	*cyvcyv*	PveIF4E[4] (**A**) PveIF4E[4] (**A**)	49	Resistant	49
		Cyvcyv	PveIF4E[1] (**C**) PveIF4E[4] (**A**)	97	Susceptible	144
		CyvCyv	PveIF4E[1] (**C**) PveIF4E[1] (**C**)	47		

[a] Expected genotypic ratio for F2 population of 1 AA: 2 AC: 1 CC; $\chi^2 = 0.046$, $P = 0.977$ ($df = 2$)

[b] Expected phenotypic ratio for F2 population of 1 resistant: 3 susceptible; $\chi^2 = 0.015$, $P = 0.902$ ($df = 1$)

Table 6 Cosegregation of KASPar SNP PveIF4E^{1,3,4} (**A**) allele with systemic necrosis and KASPar SNP PveIF4E^2 (**G**) allele with resistance to NL 3D as conditioned by *bc-3* in the Black Knight × Raven populations

Genotype	Population	NL 3D resistance	PveIF4E^{1,3,4}__PveIF4E^2 SNP (A332G)	No.[a]	Phenotype[b]	No.[b]
Black Knight	Parent 1	*cyvcyv*	PveIF4E^{1,3,4} (**A**) PveIF4E^{1,3,4} (**A**)	18	SN	18
Raven	Parent 2	*bc-3bc-3*	PveIF4E^2 (**G**) PveIF4E^2 (**G**)	18	Resistant	18
Black Knight × Raven	F$_1$	*cyv bc-3*	PveIF4E^{1,3,4} (**A**) PveIF4E^2 (**G**)	18	SN	18
Black Knight × Raven	F$_2$	*bc-3bc-3*	PveIF4E^2 (**G**) PveIF4E^2 (**G**)	50	Resistant	50
		cyv bc-3	PveIF4E^{1,3,4} (**A**) PveIF4E^2 (**G**)	84	SN	134
		cyvcyv	PveIF4E^{1,3,4} (**A**) PveIF4E^{1,3,4} (**A**)	50		

SN Systemic necrosis

[a] Expected genotypic ratio for F2 population of 1 AA: 2 AG: 1 GG; $\chi^2 = 1.392$, $P = 0.498$ ($df = 2$)

[b] Expected phenotypic ratio for F2 population of 1 resistant: 3 susceptible; $\chi^2 = 0.464$, $P = 0.496$ ($df = 1$)

Table 7 Allelic discrimination of KASPar SNP PveIF4E^3 (**A**) and PveIF4E^2(**G**) alleles in the Clipper × B/R RIL105-25 populations

Genotype	Population	ClYVV resistance	PveIF4E^{1,3,4}__PveIF4E^2 SNP (A332G)	No.[a]	Phenotype	No.
Clipper	Parent 1	*cyvcyv*	PveIF4E^{1,3,4} (**A**) PveIF4E^{1,3,4} (**A**)	18	Resistant	18
B/R RIL 105-25	Parent 2	*bc-3bc-3*	PveIF4E^2 (**G**) PveIF4E^2 (**G**)	18	Resistant	18
Clipper × B/R RIL 105-25	F$_1$	*cyvbc-3*	PveIF4E^{1,3,4} (**A**) PveIF4E^2 (**G**)	18	Resistant	18
		cyvcyv	PveIF4E^{1,3,4} (**A**) PveIF4E^{1,3,4} (**A**)	32		
Clipper × B/R RIL 105-25	F$_2$	*cyvbc-3*	PveIF4E^{1,3,4} (**A**) PveIF4E^{1,3,4} (**G**)	65	Resistant	132
		bc-3bc-3	PveIF4E^2 (**G**) PveIF4E^2 (**G**)	35		

[a] Expected genotypic ratio for F2 population of 1 AA: 2 AG: 1 GG; $\chi^2 = 0.166$, $P = 0.921$ ($df = 2$)

that the resistance from Jolanda is allelic with that possessed by Clipper and Amanda (previously reported as *cyv* by Tu 1983, and Park and Tu 1991, respectively). The assignment of the independent gene status and symbol *desc* was not warranted, nor does it appear to have ever been formally accepted by the BIC (Porch 2009). As the *bc-3* gene symbol, first identified in IVT 7214 (Drijfhout 1978) was assigned prior to the designation of *cyv*, it retains priority as the gene symbol for the locus. The original *bc-3* allele from IVT 7214 retains its designation as *bc-3*, and it conditions the widest spectrum of resistance to BCMV and BCMNV when in combination with *bc-u* or *I* (Kelly et al. 1995). The *cyv* allele first reported in GN 1140 should be designated *bc-3²* (Table 9) to reflect its recessive resistance to ClYVV-NY, its differential susceptibility to NL 3D, and the order in which it was discovered.

A causative relationship between mutations in PveIF4E and *bc-3* resistance is probable based on the clear precedent of recessive potyvirus resistance conferred by amino acid substitutions in eIF4E. This can be the result of as few as one amino acid substitution, or in many cases, an allelic series of eIF4E is present where various subtle signature amino acid substitutions in the surface loops of the eIF4E protein confer unique strain and species-specific resistance

spectra [the *pvr2* alleles in *Capsicum annuum* (Charron et al. 2008; Kang et al. 2005b; Ruffel et al. 2002), the *pot-1* allele in *Solanum lycopersicum* (Ruffel et al. 2005), the *mo1* allele in *Lactuca sativa* (Nicaise et al. 2003), the *sbm 1* alleles in *Pisum sativum* (Andrade et al. 2009; Bruun-Rasmussen et al. 2007; Gao et al. 2004), the *rym4* and *rym5* alleles in *Hordeum vulgare* (Hofinger et al. 2011; Stein et al. 2005), and the *zym*–FL allele in *Citrullus lanatus* (Ling et al. 2009)]. The pattern of predicted amino acid substitutions in the surface loops of PveIF4E and the complete association with differential resistance presented here closely resembles the numerous other pathosystems characterized. In addition, there is close alignment of mutations in PveIF4E with mutations in eIF4E that confer potyvirus resistance alleles present in the plant species *C. annuum* (Kang et al. 2005b; Ruffel et al. 2002), *L. sativa* (Nicaise et al. 2003), and *P. sativum* (Bruun-Rasmussen et al. 2007; Gao et al. 2004) (Naderpour et al. 2010). Here we provide strong evidence for an association between a single amino acid substitution at position 76 (A76E) and resistance to ClYVV-NY, and for a single amino acid substitution at position 111 (D111G) and differential resistance to NL 3D.

This result parallels previous research that has revealed a co-evolutionary 'arms race' where positive Darwinian

Table 8 *PveIF4E* haplotype analysis as enabled by the three KASPar assays employed in the research

Genotype	ClYVV[a] resistance	*PveIF4E* allele	KASPar SNP assays		
			PveIF4E[1]__PveIF4E[2,3,4]	PveIF4E[1,4]__PveIF4E[2,3]	PveIF4E[1,3,4]__PveIF4E[2]
			SNP (C227A)	SNP (C159A)	SNP (A332G)
Dubbele Witte	S	*PveIF4E[1]*	PveIF4E[1] (**C**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Midnight	S	*PveIF4E[1]*	PveIF4E[1] (**C**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Hystyle	S	*PveIF4E[1]*	PveIF4E[1] (**C**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
GN 1140	*cyv*	*PveIF4E[4]*	PveIF4E[2,3,4] (**A**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Black Knight	*cyv*	*PveIF4E[4]*	PveIF4E[2,3,4] (**A**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Jolanda	*desc*	*PveIF4E[4]*	PveIF4E[2,3,4] (**A**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Amanda	*cyv*	*PveIF4E[4]*	PveIF4E[2,3,4] (**A**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Baby Bop	*r*	*PveIF4E[4]*	PveIF4E[2,3,4] (**A**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Laureat	*r*	*PveIF4E[4]*	PveIF4E[2,3,4] (**A**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Paloma	*r*	*PveIF4E[4]*	PveIF4E[2,3,4] (**A**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Polder	*r*	*PveIF4E[4]*	PveIF4E[2,3,4] (**A**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Sonesta	*r*	*PveIF4E[4]*	PveIF4E[2,3,4] (**A**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Imuna	*cyv, desc*	*PveIF4E[3]*	PveIF4E[2,3,4] (**A**)	PveIF4E[2,3] (**A**)	PveIF4E[1,3,4] (**A**)
Evolutie	*desc*	*PveIF4E[3]*	PveIF4E[2,3,4] (**A**)	PveIF4E[2,3] (**A**)	PveIF4E[1,3,4] (**A**)
Clipper	*cyv*	*PveIF4E[3]*	PveIF4E[2,3,4] (**A**)	PveIF4E[2,3] (**A**)	PveIF4E[1,3,4] (**A**)
CY-10 S4	*cyv*	*PveIF4E[3]*	PveIF4E[2,3,4] (**A**)	PveIF4E[2,3] (**A**)	PveIF4E[1,3,4] (**A**)
IVT 7214	*bc-3*	*PveIF4E[2]*	PveIF4E[2,3,4] (**A**)	PveIF4E[2,3] (**A**)	PveIF4E[2] (**G**)
Raven	*bc-3*	*PveIF4E[2]*	PveIF4E[2,3,4] (**A**)	PveIF4E[2,3] (**A**)	PveIF4E[2] (**G**)
B/R RIL105-25	*bc-3*	*PveIF4E[2]*	PveIF4E[2,3,4] (**A**)	PveIF4E[2,3] (**A**)	PveIF4E[2] (**G**)
USWK-6	*bc-3*	*PveIF4E[2]*	PveIF4E[2,3,4] (**A**)	PveIF4E[2,3] (**A**)	PveIF4E[2] (**G**)
USWKH × H S4	*bc-3*	*PveIF4E[2]*	PveIF4E[2,3,4] (**A**)	PveIF4E[2,3] (**A**)	PveIF4E[2] (**G**)

[a] *S* susceptible, *r* resistant; confirmed by screening 5–10 plants of each cultivar in three independent experiments, with 'r' confirmed by ELISA

Table 9 Revised gene symbol nomenclature for the *Bc-3* locus

Genotype	Accession #	ClYVV resistance	*PveIF4E* allele[a]	Gene symbol
Dubbele Witte	PI 549695	Susceptible	*PveIF4E[1]*	*Bc-3*
IVT 7214	PI 602987	*bc-3*	*PveIF4E[2]*	*bc-3*
Clipper	PI 278776	*cyv*	*PveIF4E[3]*	*bc-3[2]*[b]
GN 1140	PI 549667	*cyv*	*PveIF4E[4]*	
Jolanda	G 7591	*desc*		

[a] In the absence of direct functional complementation, PveIF4E remains as the candidate gene for Bc-3

[b] Proposed here and under review by the Bean Improvement Cooperative Genetics Committee

selection has acted on single amino acid sites of eIF4E to create recessive virus resistance genes as the result of coevolution with the virus, where the viral-encoded VPg functions as the pathogenicity determinant and is also under positive selection (Cavatorta et al. 2008; Charron et al. 2008; Moury et al. 2004). Statistical methods for inferring positive selection in combination with the a priori data available from eIF4E resistance alleles in *C. annuum* (Charron et al. 2008), *S. lycopersicum* (Ruffel et al. 2005),

and *P. sativum* (Gao et al. 2004) demonstrated high precision and power to positively identify the single amino acid sites involved in potyvirus resistance (Cavatorta et al. 2008). Remarkably, out of the ten amino acid positions examined, positions 76 and 110 were identified with the highest posterior probabilities (Cavatorta et al. 2008). In *C. annuum*, *P. sativum*, and *P. vulgaris*, resistance to a potyvirus is either confirmed, or associated with the substitution of an alanine amino acid residue at position 76 with either an aspartic residue (*C. annuum*, *P. sativum*), proline residue (*P. sativum*), or a glutamic acid residue (A76E) (*P. vulgaris*). The D111G amino acid substitution in PveIF4E[2], and associated with differential resistance to NL 3D, is also remarkably close to position 110. Previous research has revealed that the pathogenicity determinant of the *cyv* resistance breaking strain (ClYVV-Br) maps to the central region of the VPg and is likely responsible for the restoration of pathogenicity (Sato et al. 2003).

Direct functional conferral of resistance and restoration of susceptibility is warranted, but common bean has lacked efficient transformation capabilities. A *Bean pod mottle virus* (BPMV) (family *Comoviridae*, genus *Comovirus*)-based virus induced gene-silencing (VIGS) vector for

common bean has recently been developed (Diaz-Camino et al. 2011) and may prove to be a useful tool for confirming the function of PveIF4E in potyvirus resistance in future research. Functional analysis of PveIF4E-potyvirus VPg interactions through protein–protein interaction experiments may also be warranted to better characterize, understand, and predict resistance spectra.

Establishment of the allelic relationships between the known sources of resistance to ClYVV in common bean directly informs the definition of the target genotype for the most effective resistance, at least in terms of the strains known in the United States. The possibility of pyramiding the previously identified putative independent genes is no longer possible, but it is clear that the original *bc-3* allele from IVT7214 conditions the widest spectrum of resistance. The *bc-3* allele is the only allele that conditioned resistance to all strains of ClYVV, including ClYVV-WI and ClYVV-OR, except when in the IVT 7214 background (Larsen et al. 2008), whereas *bc-3²* (present in Jolanda and Imuna) did not. It is difficult to speculate as to why IVT 7214 may be the exception, as it possesses the *bc-u* allele that should condition the full expression of *bc-3* (Drijfhout 1978). In addition, the genotype USCR-8 that possesses *bc-3* in the absence of both the *I* gene and *bc-u* (Miklas and Hang 1998) was resistant to ClYVV-WI and ClYVV-OR (Larsen et al. 2008). It is still somewhat unclear whether *bc-3* or *bc-3²* are effective for resistance to ClYVV in the absence of *bc-u* and/or *I* because all of the genotypes investigated in this research possessed one, or both genes. Another exception is GN 1140, as while it possesses *bc-3²*, and is mildly susceptible to NL 3D, it was resistant to ClYVV-WI and ClYVV-OR (Larsen et al. 2008). The genotype GN UI 31 may be an analogous case as it was the source of two recessive genes that conditioned resistance to the BYMV-S strain from Oregon (Tatchell et al. 1985), so it is feasible that GN 1140 and GN UI 31 possess an additional resistance gene, perhaps an alternate allele of *bc-u* (Strausbaugh et al. 2003) that widen their strain-specific resistance spectrum to include ClYVV-WI and ClYVV-OR where other *bc-3²* genotypes do not (Larsen et al. 2005). GN UI 31 has been demonstrated to have mixed reactions to specific BCMV/BCMNV strains potentially due to admixture or residual heterozygosity in previous research (Forster et al. 1994; Miklas et al. 2000). Additional effort is needed to obtain a more thorough characterization of the spectrum of resistance to ClYVV and BCMV/BCMNV pathogenicity groups provided by *bc-3²* alone, and in combination with *bc-u* and/or *I*.

The three KASPar assays developed here provide a suite of rapid, user-friendly, putatively functional molecular markers that have the capability to detect and differentiate all four of the *PveIF4E* alleles characterized thus far. These assays are an improvement over the RsaI CAPS marker

associated with *bc-3* resistance (Naderpour et al. 2010) because in addition to the *PveIF4E²* allele, the RsaI site at position 193 of the coding sequence is also present in the *PveIF4E³* allele (Online Resource 1, Fig. 1, Fig. 3), which is not associated with resistance to NL 3D. The PveIF4E KASPar assays are also more convenient to employ in marker-assisted selection (MAS) because they do not require enzymatic digestion or gel electrophoresis; however, they do require fluorescent endpoint detection capabilities. The RsaI CAPS marker, therefore, remains useful when this capability is absent, but the resistance to NL 3D of the donor parent must be known or confirmed. The discrimination capability of the KASPar assays developed here may also have the potential for rapid characterization of germplasm through the detection of mutant alleles, particularly for detecting predicted amino acid substitutions at positions 76 and 111. Table 8 summarizes the employment of the three assays for allelic discrimination in the 21 common bean genotypes studied and simultaneously provides a guide on how to use the KASPar assays for allelic discrimination and MAS.

This research contributes to an enhanced understanding of the gene-for-gene relationships between potyviruses and resistance alleles associated with mutations in *PveIF4E* at the *Bc-3* locus in common bean. This knowledge combined with the KASPar assays continues in the development of a platform to elucidate the role of eIF4E-mediated recessive resistance in common bean. Advanced research in this area in other crop plants has been rewarding, with new insights into the biology [e.g. complementation interactions with mutations in eIF(iso)4E for an enhanced resistance spectrum (Hwang et al. 2009; Rubio et al. 2009; Ruffel et al. 2006)], the demonstration of new tools for rapid identification of novel alleles by allele mining [e.g. High-resolution melting analysis (HRM) (Hofinger et al. 2009; Hofinger et al. 2011)], and to even create novel resistance alleles by mutagenesis (Piron et al. 2010). The research presented here provides a foundation on which to advance similar research in common bean. The KASPar assays developed here should also assist in the utilization of *bc-3* resistance, important to common bean growing regions around the world.

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