A Novel Root-Knot Nematode Resistance QTL on Chromosome Vu01 in Cowpea

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ABSTRACT The root-knot nematode (RKN) species *Meloidogyne incognita* and *M. javanica* cause substantial root system damage and suppress yield of susceptible cowpea cultivars. The narrow-based genetic resistance conferred by the *Rk* gene, present in some commercial cultivars, is not effective against *Rk*-virulent populations found in several cowpea production areas. The dynamics of virulence within RKN populations require a broadening of the genetic base of resistance in elite cowpea cultivars. As part of this goal, F_1 and F_2 populations from the cross CB46-Null (susceptible) x FN-2-9-04 (resistant) were phenotyped for *M. javanica* induced root-galling (RG) and egg-mass production (EM) in controlled growth chamber and greenhouse infection assays. In addition, $F_{2:3}$ families of the same cross were phenotyped for RG on field sites infested with *Rk*-avirulent *M. incognita* and *M. javanica*. The response of F_1 to RG and EM indicated that resistance to RKN in FN-2-9-04 is partially dominant, as supported by the degree of dominance in the F_2 and $F_{2:3}$ populations. Two QTLs associated with both RG and EM resistance were detected on chromosomes Vu01 and Vu04. The QTL on Vu01 was most effective against aggressive *M. javanica*, whereas both QTLs were effective against avirulent *M. incognita*. Allelism tests with CB46 x FN-2-9-04 progeny indicated that these parents share the same RKN resistance locus on Vu04, but the strong, broad-based resistance in FN-2-9-04 is conferred by the additive effect of the novel resistance QTL on Vu01. This novel resistance in FN-2-9-04 is an important resource for broadening RKN resistance in elite cowpea cultivars.

INTRODUCTION

Root-knot nematode (RKN) species, particularly *Meloidogyne incognita* and *M. javanica*, cause substantial damage to root systems and suppress yield of susceptible cowpea (*Vigna unguiculata* L. Walp) cultivars by impairing water and nutrient uptake, and the partitioning and translocation of photo-assimilates (Bird and Loveys 1975; McClure 1977; Taylor and Sasser 1978; Williamson and Hussey 1996; Sikora *et al*. 2005). Host-plant resistance is an important strategy to mitigate the impact of nematode infestation (Hall and Frate 1996; Roberts 1992; Ehlers *et al*. 2000; Castagnone-Sereno 2002; National Research Council 2006), both in Africa where access to agronomic inputs including nematicides is limited (Sasser 1980; Luc *et al*. 2005), and in developed agriculture where resistant varieties are the best option economically (Ehlers *et al*. 2000).

Narrow-based resistance to conferred by gene *Rk* has provided protection against RKN in cowpea agricultural systems worldwide (Amosu and Franckowiak 1974; Singh and Reddy 1986; Helms *et al.* 1991; Fery *et al.* 1994; Roberts *et al.* 1995; Roberts *et al.* 1996; Roberts *et al*. 1997; Ehlers and Hall 1997; Ehlers *et al.* 2009). The resistance conferred by gene *Rk* is highly effective against avirulent forms of RKN populations (Roberts *et al*. 1995; Hall and Frate 1996; Roberts *et al*. 1997; Ehlers *et al*. 2000; Roberts *et al*. 2013), but *Rk*-virulent and aggressive forms of common RKN species have been identified (Swanson and Van Gundy 1984; Roberts *et al*. 1995; Hall and Frate 1996; Roberts *et al*. 1997; Petrillo *et al*. 2006). Selection for virulence to *Rk* (Roberts *et al*. 1997; Petrillo and Roberts 2005; Petrillo *et al*. 2006) has prompted efforts to broaden the genetic base of resistance in elite cowpea cultivars (Hall and Frate 1996; Roberts *et al*. 1996; Roberts *et al*. 1997; Ehlers *et al*. 2000; Roberts *et al*. 2013). The threat imposed by virulence in RKN populations led to the discovery of new

resistance genes, *Rk*^{2} and *rk*^{3} to broaden the genetic base of resistance, and advanced breeding materials with one or more of these genes have shown promising performance under RKN infestation (Roberts *et al*. 1996; Roberts *et al*. 1997; Ehlers *et al*. 2000; Ehlers *et al*. 2002). Broad-based genetic resistance can be developed through effective gene pyramiding of independent sets of resistance genes from distinct genetic sources (Ehlers *et al.* 2002).

The RKN resistance currently deployed in many cowpea cultivars is governed by a single dominant gene, *Rk* (Fery *et al.* 1994; Singh and Reddy 1986), but additional resistance genes *Rk*^{2}, with a dominant effect, (Roberts *et al*. 1996; Roberts *et al*. 1997; Ehlers *et al*. 2000), and *rk*^{3}, with a recessive and additive effect, (Roberts *et al*. 1996; Ehlers *et al*. 2000), have been identified in cowpea backgrounds (Roberts *et al*. 1997; Ehlers *et al*. 2000). The action of gene *Rk*^{2} alone is not clearly understood, but in breeding line IT84S-2049 (which also carries gene *Rk*) its additive effect contributes substantially to an enhanced resistance to *Rk*-virulent populations of *M. incognita* and to *M. javanica* compared to gene *Rk* alone (Roberts *et al*. 1996; Roberts *et al*. 1997; Roberts *et al*. 2005). The *rk*^{3} locus was characterized as a modifier which improves resistance of cowpea cultivars carrying *Rk* when challenged with *Rk*-virulent RKN isolates (Ehlers *et al*. 2000b) and was bred into cowpea cv. CB27 (Ehlers *et al*. 2000a).

The *Rk* locus has been mapped on chromosome Vu04 (Huynh *et al*. 2016) previous cowpea linkage group 11 of the cowpea consensus genetic map (Lucas *et al*. 2011; Muñoz-Amatriaín *et al*. 2017). This genomic region and flanking markers associated with RKN resistance within this region are important resources for introgressing this resistance into elite cowpea cultivars. Also, markers flanking the resistance in this genomic region can be utilized as a reference to decipher the genetic relationship

between the resistance conferred by gene *Rk* and potential novel sources of resistance to RKN.

A broad-based resistance to RKN has been identified through a series of field, greenhouse and seedling growth pouch tests in a cowpea accession FN-2-9-04 from Mozambique (Ndeve *et al*. 2018). This accession carries higher levels of resistance to avirulent *M. incognita* and *M. javanica* than that conferred by the *Rk* gene alone. The performance of FN-2-9-04 under *M. javanica* infestation was contrasted to cowpea breeding lines and cowpea cultivars carrying sets of RKN resistance genes, including *RkRk/Rk²Rk²*, *RkRk/rk³rk³*, *RkRk/Rk²Rk²/gg* and IT84S-2049 which indicated that the RKN resistance in accession FN-2-9-04 is unique. Therefore, to characterize the resistance in FN-2-9-04, genetic analyses were conducted to determine its genomic architecture and localization through genetic linkage analysis and QTL mapping.

Data Availability

All F_2 and $F_{2:3}$ populations and root-knot nematode isolates are available upon request. Phenotypic and genotypic date are included in data (D) files 1 - 5. These data files and supplementary tables and figures are available at Figureshare.

MATERIALS AND METHODS

Plant materials

Four F_1, three F_2 and one $F_{2:3}$ populations (Table 1) were developed under greenhouse conditions at the University of California Riverside (UCR). Accession FN-2-9-04 was crossed with CB46-Null, CB46, Ecute and INIA-41. A single F_1 seed from each of the crosses CB46-Null x FN-2-9-04, CB46 x FN-2-9-04 and INIA-41 x FN-2-9-04 was grown to derive three independent F_2 populations, and 150 F_2 lines of population

CB46-Null x FN-2-9-04 were advanced to generate 150 $F_{2:3}$ families (Table 1). Four F_1 populations (CB46-Null x FN-2-9-04, CB46 x FN-2-9-04, INIA-41 x FN-2-9-04, Ecute x FN-2-9-04) and subsets of their F_2 populations were phenotyped for root-galling and egg-mass production in greenhouse and seedling growth-pouch screens, respectively, following infection with nematode isolates listed in Table 1. Five to ten seeds per F_1 population were also screened in each test. The subsets of F_2 populations and $F_{2:3}$ families (Table 1) also were phenotyped for root-galling in field experiments.

CB46 is a California blackeye cultivar carrying gene *Rk* (Helms *et al*., 1991), and the CB46-Null genotype is a near-isogenic breeding line (NIL) derived from CB46. This breeding line has the CB46 background, but it is susceptible (minus *Rk* via backcrossing) (Huynh *et al*., 2016). Ecute and INIA-41 are landraces and FN-2-9-04 is an accession from Mozambique. FN-2-9-04 is resistant to both the avirulent *M. incognita* isolates and *M. javanica* isolate used in this study, whereas CB46-Null, CB46, Ecute and INIA-41 are all susceptible to *M. javanica*. In addition, CB46-Null and Ecute are susceptible to the avirulent *M. incognita* isolates (Beltran and Project 77), whereas INIA-41 is resistant.

Table 1. Cowpea populations used for inheritance studies and QTL mapping, their size, phenotyping conditions, target trait, nematode isolate used and year of testing.

Exp	Population	Size	Environment	Trait	Nematode isolate	Year
1	[a]CB46-Null/FN-2-9-04 (F_2)	163	SGP-UCR	EM	*M.j*	2015
2	[a]CB46/FN-2-9-04 (F_2)	172	SGP-UCR	EM	*M.j*	2015
3	[a]INIA-41/FN-2-9-04 (F_2)	126	GH-UCR	RG	*M.j*	2015
4	[a]CB46-Null/FN-2-9-04 (F_2)	177	GH-UCR	RG	*M.j*	2015
5	[a]CB46/FN-2-9-04 (F_2)	197	GH-UCR	RG	*M.j*	2015
6	CB46/ FN-2-9-04 (F_2)	400	CVARS	RG	Avr-*M.i*	2015
7	CB46/FN-2-9-04 (F_2)	162	KARE	RG	Avr-*M.i*	2015
8	CB46-Null/FN-2-9-04 ($F_{2:3}$)	150	SCREC	RG	*M.j*	2016
9	CB46-Null/FN-2-9-04 ($F_{2:3}$)	150	SCREC	RG	Avr-*M.i*	2016

Exp. = experiment; SGP = seedling growth-pouches; GH = greenhouse; RG = root-galling; EM = egg masses; Avr-*M.i* = avirulent *M. incognita* and *M.j* – *M. javanica* Project 811; UCR = University of California Riverside; CVARS = University of California Coachella Valley

Agricultural Research Station; KARE = University of California Kearney Agricultural Research and Extension Center; [a]Experiment included the F_1 plus Ecute x FN-2-9-04 F1 plants.

Root-knot nematode isolates

Four RKN isolates were used to phenotype plant materials for response to infection. Three *M. incognita* isolates, Beltran, Project 77 and an equivalent isolate indigenous to CVARS are avirulent to the *Rk* gene, with little or no galling and EM production on root systems of plants carrying gene *Rk* (Roberts *et al.,* 1995; Roberts *et al.,* 1996; Roberts *et al.,* 1997), whereas *M. javanica* isolate Project 811 is an aggressive isolate due to its enhanced parasitic ability (Ehlers *et al*., 2000; Ehlers *et al*., 2009), inducing galling and reproducing successfully on roots of plants carrying *Rk* (Thomason and Mckinney, 1960; Roberts *et al.,* 1997; Ehlers *et al*., 2009).

Resistance phenotyping: egg-mass production

The F_1 and F_2 populations (Table 1) plus parental genotypes were phenotyped for *M. javanica* EM production in seedling growth-pouches according to Ehlers *et al*. 2000 and Atamian *et al*., 2012. Briefly, a single seed of each F_1 and F_2 was planted per plastic pouch, and the plants were grown in a controlled environment chamber with day/night temperatures set at 28/22 ^oC under 16 h day-length. Plants were inoculated two weeks after germination with 1500 freshly hatched second-stage juveniles (J_2) of *M. javanica*. Two days after inoculation, plants were supplied daily with fertilizer for 3-5 days using half-strength Hoagland's solution (Hoagland and Arnon, 1950). Thirty-five days after inoculation, the pouches were irrigated with erioglaucine dye (Sigma Chemical Co., St. Louis, MO, USA) to stain egg-masses, which were counted under 10X magnification.

Resistance phenotyping: root-galling

Phenotyping for resistance to root-galling was conducted under greenhouse and field conditions in 2015 and 2016 (Table 1). In the greenhouse, the F_1 and F_2 populations and parental genotypes phenotyped for response to *M. javanica* egg-mass production in seedling growth-pouches (in growth chamber conditions) were then transplanted into 4L pots containing soil UC-mix 3 and maintained at 28/22 ^{o}C day/night temperatures. After 21 days, each plant was inoculated with 10 ml of *M. javanica* egg suspension in water adjusted to 1000 egg/ml. All greenhouse-grown plants were irrigated twice per day by drip-irrigation for about 90 days to allow seed production, and $F_{2:3}$ seeds were collected from each F_2 plant. After seed collection, the plant tops were cut at 2 – 3 cm above the soil line, and the roots were washed and scored for root-galling response under 10X magnification, using a 0 - 9 gall index (GI) modified from Bridge and Page (1980): 0 = no galls on root system; 1 = very few, small galls and hard to see; 5 = generally large galls can be seen on the root system and the taproot slightly bumped, with bumps of different sizes; 9 = large galls on the root system, and most lateral roots lost.

Field experiments were conducted in 2015 and 2016 at three sites (Table 1). At CVARS and KARE, 400 and 162 CB46 x FN-2-9-04 F_2 lines, respectively, were phenotyped for root-galling response to avirulent *M. incognita* (isolate Project 77 at KARE and an equivalent to it at CVARS). In 2016 at SCREC parental genotypes, F_2 and $F_{2:3}$ populations were phenotyped for root-galling response in separate fields infested with avirulent *M. incognita* isolate Beltran or *M. javanica* (Table 3). In both experiments (Exps. 8 and 9), $F_{2:3}$ families with 25 – 30 plants/family were planted in single plots. The *M. javanica* isolate used in the pot and seedling growth-pouch screens was the same isolate used to infest field sites. For both F_2 and $F_{2:3}$

generations, 25 - 30 seeds were planted on a 1.5 m-long single row plot, and 60 days after plant emergence plant tops were cut at 2 – 3 cm above the soil line, and the root systems dug and evaluated for root-galling using the same root-galling index described for the pot tests (Bridge and Page 1980).

Inheritance of resistance and allelism test

Segregation for the FN-2-9-04 resistance to root-galling and reproduction by *M. javanica* and root-galling by avirulent *M. incognita* isolates was determined using both phenotypic (root-galling and egg-masses) and genotypic data. In addition, phenotypic data of F_1, F_2 and $F_{2:3}$ populations, and SNP marker genotypes of F_2 populations at mapped QTL regions were processed for goodness-of-fit analysis to determine the genetic model underlying resistance to RKN in FN-2-9-04. Analysis of goodness-of-fit of segregation ratio between resistant-susceptible lines in the F_2 was performed through marker-trait association analysis using marker genotypes within mapped QTL regions (see Table 2) and phenotypic response of F_2 and $F_{2:3}$ populations. Each F_2 line was scored for presence of parental alleles at each locus within the mapped QTL, and scores 2, 1 and 0 were assigned to homozygous favorable allele (BB = resistant parent), heterozygous (AB) and homozygous non-favorable allele (AA = susceptible parent), respectively. The genotype of each F_2 line, within the QTL region, was determined as the mean score across all marker loci, and it was associated with its RG or EM phenotypic response determined at the F_2 and $F_{2:3}$ generations. The data for frequency distribution of genotypes (BB, AB and AA) (Table 3) were processed for goodness-of-fit analysis, and the chi-square values were determined following Yates correction for continuity (Little and Hills 1978). The numbers of genetic determinants associated with resistance were estimated using the Castle-Wright (1921) estimator of gene number, $= \frac{(\boldsymbol{P1}-\boldsymbol{P2})^2}{\boldsymbol{8Vg}}$, where *n* is the estimated number of genes influencing

the trait, *P1* and *P2* are the mean phenotypic values of the parents of the population and *Vg* is the genetic variance of the trait. To estimate the number of genes governing response to root-galling and egg-mass production, the *Vg* influencing these traits was derived as the genetic variance in the mapped QTL regions, flanked by known SNP markers.

Broad-sense heritability ($H^2 = V_g/V_p$) of resistance was estimated using two methods, midparent-offspring regression analysis (Fernandez and Miller 1985; Falconer and Mackay 1996) and the phenotypic variation among F_2 lines and among $F_{2:3}$ families accounted for by V_g^* at the QTL regions associated with resistance. The phenotypic variance, $V_{\dot{p}}$, in root-galling or egg-masses attributed to genetic factors, V_g^*, was estimated using SNP marker genotype scores (V_{gs}) and SNP marker effects (SNP_{eff}) at the mapped QTL regions plus the observed root-galling or egg-masses phenotypes using the algorithm: $V\dot{p} = \frac{Vgs\ x\ (\mathrm{SNPeff})^2}{Vp}\ x\ 100$. In this algorithm (adapted from Xu 2013), the product $Vgs\ x\ (SNPeff)^2$ is the V_g^* associated with the variation in root-galling or egg-masses phenotypes in tested F_2 and 2 $F_{2:3}$ populations. To estimate the narrow-sense heritability ($h^2 = V_a/V_p$), the genetic variance ($V_g^* = V_a + V_d$) was partitioned into additive and dominance variances, and the V_a component was used to compute the h^2 of the trait. Root-galling data of seven F_2 populations (populations in Table 1 plus their subsets) and parental genotypes were used to perform midparent-offspring regression analysis, and four mapping populations (two F_2 and two $F_{2:3}$, Exps. 1, 4, 8 and 9, Table 1) were used to derive genetic variances (V_g^*) within the QTL regions, influencing the response to galling and egg-mass production. Allelic relationships between the *Rk* locus present in cv. CB46 (Roberts *et al*. 1995; Hall and Frate1996; Roberts *et al*. 1996; Roberts *et al*. 1997; Ehlers *et al*. 2009; Huynh *et al*. 2016) and the genetic determinants of resistance in FN-2-9-04 were determined using

the four F_2 population sets of CB46 x FN-2-9-04 phenotyped with *M. incognita* isolate Project 77 and *M. javanica* infestation (Table 1).

Genotyping and QTL mapping

Leaf samples were collected from parents and each of 119 and 137 F_2 lines of populations CB46-Null x FN-2-9-04 and CB46 x FN-2-9-04, respectively (Exp. 1, 5, Table 1) 30 days after transplanting and dried in plastic ziploc bags containing silica gel packs. Genomic DNA was extracted from dried leaves using Plant DNeasy (Qiagen protocol) and quantified using Quant-iTTM dsDNA Assay Kit and fluorescence measured using a microplate reader. In addition, each F_2 plant of population CB46-Null x FN-2-9-04 was selfed to generate $F_{2:3}$ seeds for field phenotyping (Table 1). The 119 F_2 lines are part of the 163 lines tested for egg-mass production (Exp. 1) and transplanted for root-galling assay (Exp. 4, Table 1).

Each DNA sample was assayed for single nucleotide polymorphism (SNP) using the Cowpea iSelect Consortium Array containing 51128 SNPs (Muñoz-Amatriaín *et al*. 2017). The SNP data were filtered for quality as follows: (i) elimination of SNPs with > 20% missing data; (ii) elimination of monomorphic SNPs; (iii) elimination of SNPs with minor allele frequency (MAF) < 0.4 and < 0.3 for populations CB46-Null x FN-2-9-04 and CB46 x FN-2-9-04, respectively; iv) and elimination of duplicated lines. No loci were detected with non-parental alleles.

Linkage-maps of the CB46-Null x FN-2-9-04 and CB46 x FN-2-9-04 F_2 populations were constructed with MSTmap (Wu *et al*., 2015), and linkage groups were determined at LOD threshold = 10 and marker placement followed the Kosambi mapping function. The options “no mapping size threshold” and “no mapping distance threshold” were fixed at 2 units and 10 cM, respectively. In addition, the no mapping distance threshold option was set at 15 cM and the detection of genotyping errors was not solicited. The

linkage groups of the final genetic map were numbered and ordered following the cowpea consensus genetic map order (Muñoz-Amatriaín *et al.* 2017) and the cowpea pseudomolecules (Lonardi *et al*. 2017 in preparation; https://phytozome.jgi.doe.gov/). Also, the cowpea reference genome was used to determine the physical positions of the SNPs and to identify candidate genes on mapped QTLs associated with the traits (Lonardi *et al*. 2017 in preparation; https://phytozome.jgi.doe.gov/). Using physical position, candidate genes were retrieved from the Joint Genome Institute cowpea genome portal (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Vunguiculata_er).

QTL mapping was performed using five phenotypic data sets comprising two F_2 populations of crosses CB46-Null x FN-2-9-04 and CB46 x FN-2-9-04, and two $F_{2:3}$ populations of cross CB46-Null x FN-2-9-04 (Exps. 1, 4, 5, 8 and 9, Table 1). QTL analysis was performed following the mixed-model for QTL mapping described by Xu (2013) using RStudio v1.1.442, and significant QTLs were declared using Bonferroni adjusted threshold value -log (*P*-value) *at* $P < 0.05$. Reported QTL regions associated with resistance were based on the SNP markers with the most significant threshold values.

Candidate genes within QTL regions

Single nucleotide polymorphism markers flanking mapped QTL regions on Vu01 and Vu04 were used to determine physical locations of the QTLs and associated candidate genes on the cowpea reference genome v1.0 (Lonardi *et al*. 2017), and a list of gene models and corresponding annotation within each QTL region was generated from the Joint Genome Institute cowpea genome portal (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Vunguiculata_er)

Results

Linkage and QTL mapping

The linkage map of the F_2 population CB46-Null x FN-2-9-04 (n = 119) contained 17208 polymorphic SNP markers distributed on 11 chromosomes and spanned 985.89 cM (Supplementary file S1A). Of the total SNPs, 90.79% (15624 SNPs) were mapped on the cowpea consensus genetic map (Muñoz-Amatriaín *et al*. 2017), while 9.21% (1585 SNPs) were unique to this population, and this portion corresponds to 2.5% of SNPs not mapped to the cowpea pseudomolecules. The linkage map comprised 1392 bins distributed at an average density of 1 bin per 0.71 cM. The linkage map of the F_2 population CB46 x FN-2-9-04 (n = 137 lines) contained a total of 17903 polymorphic SNPs and spanned 1158.68 cM (Supplementary file S1B). Of these SNPs, 97.6% (17465 SNPs) mapped to the cowpea consensus genetic map, while 9.4% (1675 SNPs) are not part of the cowpea consensus genetic map, and this portion makes 2.4% of the total SNPs not mapped on the cowpea pseudomolecules (Lonardi *et al*. 2017 in preparation; https://phytozome.jgi.doe.gov/).

Table 2. Chromosome locations of root-knot nematode (RKN) resistance determinants in cowpea accession FN-2-9-04, mapped using F_2 and $F_{2:3}$ populations of the cross CB46-Null x FN-2-9-04 and the F_2 population of the cross CB46 x FN-2-9-04.

Pop	Trait	RKN	Vu	Position	Flanking markers	-log*p*	PVE (%)	A	D/A
		Avr-	1	34.4	2_04038-2_26991	5.4	33.0	-1.3	0.5
$F_{2:3}$	RG	*M.i*	4	24.7-27.6	2_44685-2_10583	20	73.3	-2.0	0.5
	RG	*M.j*	1	27.7-42.0	2_47796-1_0027	20	95.1	-2.3	0.3
F_2	RG	*M.j*	1	30.3-38.7	2_32677-2_19840	20	47.3	-2.8	0.4
F_2 [a]	RG	*M.j*	1	19.2-72.9	2_53036-2_18359	20	65.9	2.7	0.8
F_2	EM	*M.j*	1	31.5-36.9	2_21671-2_07103	10.9	34.1	-17.0	0.5
F_2 [a]	EM	*M.j*	1	47.1-52.1	2_21671-2_12209	8.8	24.7	-16.4	0.4

Pop = mapping population; the $F_{2:3}$ were phenotyped in the field whereas the F_2 were phenotyped in greenhouse and growth chamber (seedling-growth pouches) screens; RG = root-galling; EM = egg-masses per root system; Avr-*M.i* = avirulent *M. incognita* isolate Beltran; *M.j* = *M. javanica*; [a]mapping population CB46 x FN-2-9-04 phenotyped for RG and EM; Vu = cowpea chromosome pseudomolecule numbering (Lonardi *et al*. 2017); -log*p* =

329 level of significance of the detected QTL ($P < 0.05$); PVE = percent of total phenotypic variation
330 explained; A = additive effect of favorable alleles from the resistant parent (negative values
331 indicate the extent of average reduction in RG or EM production due to the presence of
332 favorable alleles; D = dominance effect due to substitution of favorable allele; and D/A =
333 degree of dominance.
334

335 QTL analysis revealed two major QTLs associated with resistance to root-galling (RG)
336 and egg-mass (EM) production in FN-2-9-04 (Table 2; Figs. 1 and 2); these QTLs were
337 mapped on chromosomes Vu01 and Vu04 of the CB46-Null x FN-2-9-04 population
338 and chromosome Vu04 of the CB46 x FN-2-9-04 population. The QTL region on Vu01
339 consistently mapped almost within the same genomic location using F_2 and $F_{2:3}$
340 populations phenotyped under greenhouse, seedling-growth pouch and field
341 conditions using two RKN isolates (Table 2; Supplementary file S1C).
342 Two QTLs controlling resistance to RG by avirulent *M. incognita* Beltran were detected
343 and mapped on Vu01 and Vu04 ($P < 0.05$, threshold value $-\log(p) = 4.8$) (Fig. 1A) of
344 the CB46-Null x FN-2-9-04 $F_{2:3}$ population. The resistance QTL on Vu01 mapped to
345 position 34.4 cM which spanned 0.1 Mb (28855569 - 28960128 bp) on the cowpea
346 pseudomolecules (Supplementary file S1C) between flanking markers 2_04038 and
347 2_04039; it accounted for 33% of the total phenotypic variation (V_p) of the RG
348 resistance response and had a likelihood of occurrence expressed by $-\log_{10}(p) = 5.4$
349 (Table 2).
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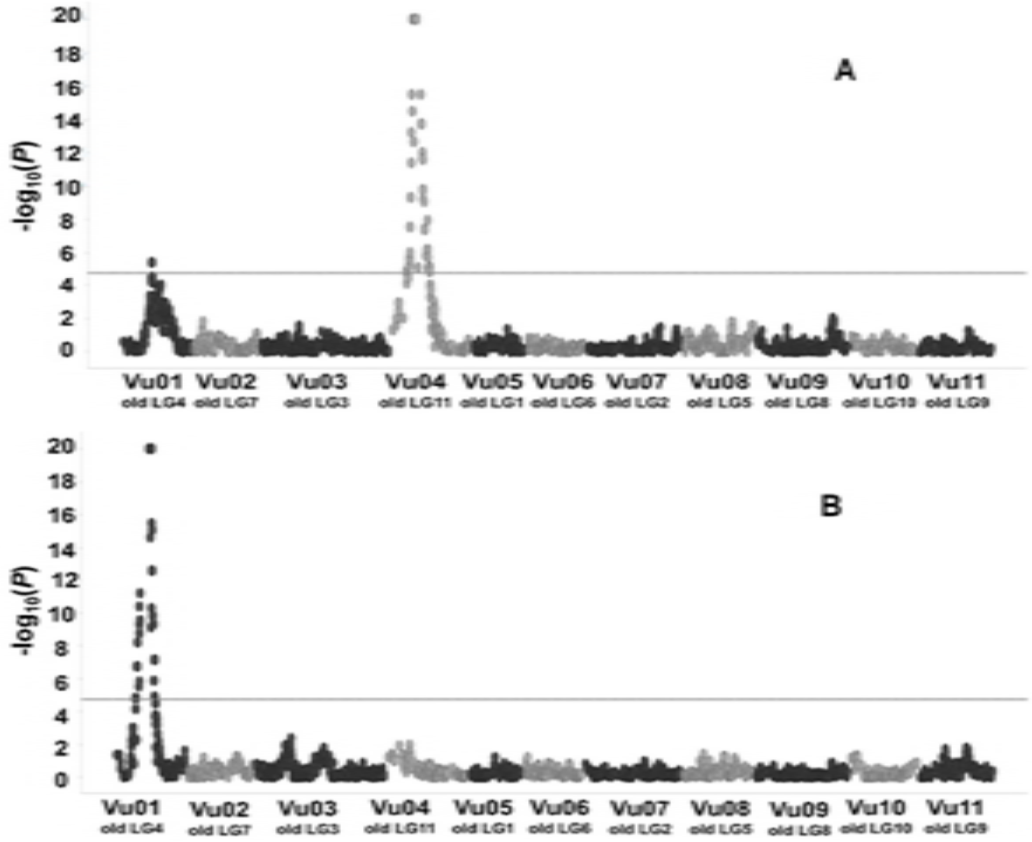

Fig. 1. Genomic localization of QTLs associated with resistance to root-galling (RG) by: **A,** avirulent *M. incognita* and **B,** aggressive *M. javanica*. The QTLs were detected in the CB46-Null x FN-2-9-04 $F_{2:3}$ population phenotyped for RG under field infestation. Horizontal dashed line represents the Bonferroni threshold of significance at $P < 0.05$ [-log(p) = 4.8]. Old LG represents former cowpea linkage group numbering and Vu indicates the new cowpea linkage group numbering based on the cowpea pseudomolecules (Lonardi *et al.* 2017).

The resistance QTL on Vu01 (Fig. 1A) detected under plant infection by avirulent *M. incognita*, exhibited additive and dominance effects of -1.3 and -0.6, respectively, and the degree of dominance, measured as a ratio between dominance and additive effects (D/A), indicated that the resistance in this QTL has partial dominant effect (D/A = 0.5) (Table 2). A second resistance QTL associated with response to the avirulent *M. incognita* was detected on Vu04 (Fig. 1A, Table 2) at chromosome position 24.7 - 27.6 cM of the CB46-Null x FN-2-9-04 $F_{2:3}$ population and spanned 2.9 cM which corresponds to approximately 1 Mb (3141521 – 4138458 bp) on the cowpea

366 pseudomolecules (Supplementary file S1C), and it was flanked by SNP markers
367 2_44685and 2_10583 (Table 2). This QTL explained 73.3% of the total V_p of the
368 resistance response, and it had an infinite likelihood of occurrence which was
369 represented by $-\log_{10}(p)$ = 20 (Table 2). In addition, the additive (A = -2) and
370 dominance (D = -1) effects of the QTL on Vu04 were slightly higher than those of the
371 QTL on Vu01, but both QTLs showed the same degree of dominance (D/A = 0.5).

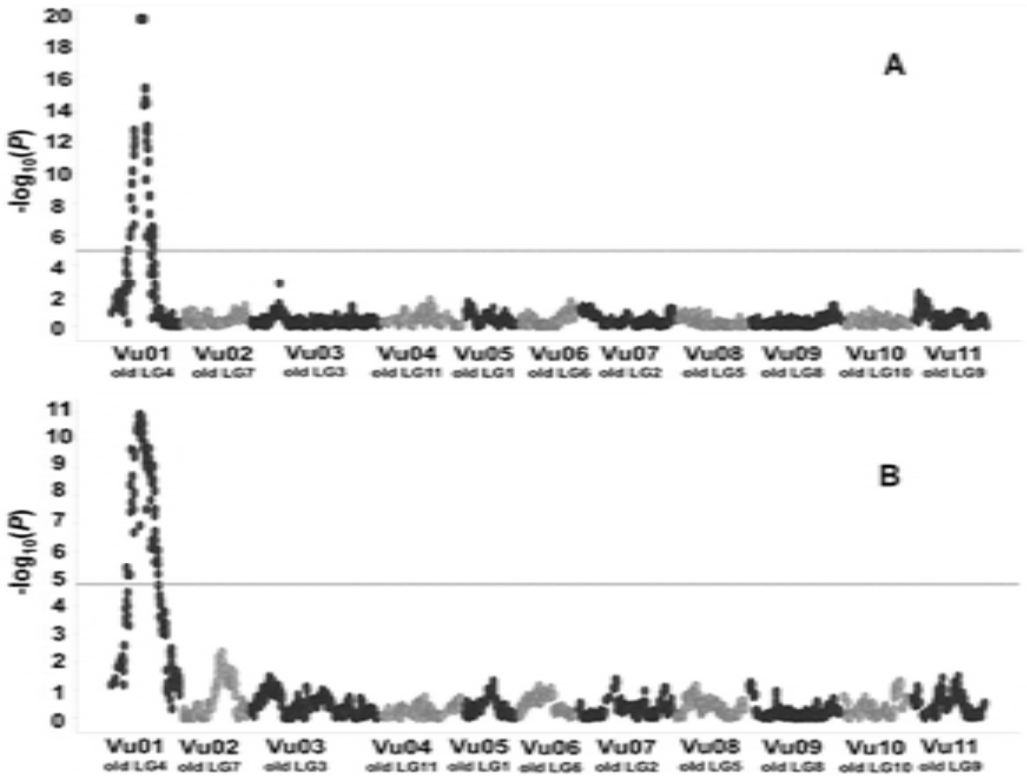

Fig. 2. Genomic localization of QTL associated with resistance to **A**, root-galling (RG) and **B**, egg-mass production (EM) by aggressive *M. javanica*. The QTLs were detected in the F_2 population CB46-Null x FN-2-9-04 phenotyped for RG in the greenhouse and for EM in seedling growth-pouch inoculations, respectively. Horizontal dashed-line represents the Bonferroni threshold of significance at $P < 0.05$ [-log(*p*) (**A** and **B** = 4.9 and 4.8, respectively]. Old LG represents former cowpea linkage group numbering and Vu indicates the new cowpea linkage group numbering based on the cowpea pseudomolecules (Lonardi *et al.* 2017).

372

373

374

375 On Vu01, an additional genomic region controlling resistance to *M. javanica* RG (Figs.
376 1B; 2A) and EM production (Fig. 2B) was consistently mapped on the same
377 chromosomal region of the CB46-Null x FN-2-9-04 F_2 and $F_{2:3}$ populations using RG
378 and EM phenotypic data from field, greenhouse and seedling-growth pouch
379 experiments (Table 2). The *M. javanica* root-galling resistance QTL mapped to
380 positions 30.3 - 38.7 cM and 27.7 - 42.0 cM on Vu01 using F_2 (greenhouse experiment)
381 and $F_{2:3}$ (Field experiment) populations from the CB46-Null x FN-2-9-04 cross,
382 respectively. These genomic regions spanned 8.4 and 14.3 cM, which correspond to
383 4.4 (26617356 - 31070755 bp) and 6.2 Mb (25784028 - 31953708 bp) on the cowpea
384 pseudomolecules (Supplementary file S1C) and were flanked by SNP markers
385 2_32677 - 2_19840 and 2_47796 - 1_0027, respectively (Table 2). In both F_2 and $F_{2:3}$
386 populations, the RG resistance QTL was detected with infinite likelihood represented
387 by $-\log_{10}(p) = 20$ (Figs. 1B, 2A, Table 2). The percent of total phenotypic variation in
388 RG explained by the QTL effect in the $F_{2:3}$ (PVE = 95.1%) was higher than in the F_2
389 (PVE = 47.2%), while the contributions of the additive and dominance effects in the
390 total phenotypic variation in the F_2 and $F_{2:3}$ were similar (Table 2). Also, the degree of
391 dominance in both generations were comparable, D/A = 0.4 and 0.3, respectively,
392 indicating resistance with partial dominance.
393 The QTL on Vu01 associated with resistance to *M. javanica* reproduction (EM)
394 mapped to position 31.5-36.9 cM of the CB46-Null x FN-2-9-04 F_2 population (Fig. 2B;
395 Table 2). This QTL spanned 5.5 cM which corresponds to 2.7Mb (27254299 -
396 29984745 bp) on the cowpea pseudomolecules (Supplementary file S1C), and it was
397 flanked by SNP markers 2_21671 and 2_07103. This QTL accounted for 34.1% of the
398 total phenotypic variation in EM production with additive and dominance effects of 17.1
399 and 7.8, respectively; the gene action measured within the same QTL region indicated

400 resistance with partial dominance (D/A = 0.5). Although this QTL was detected with
401 high likelihood, -log$_{10}$(p) = 10.9 (critical threshold = 4.8) (Fig. 2B), it was lower than
402 that observed for the RG QTL (Table 2).
403 QTL mapping using the F_2 population of CB46 x FN-2-9-04 validated that the genomic
404 region on Vu01 is associated with resistance to *M. javanica* RG (Fig.3; Table 2).

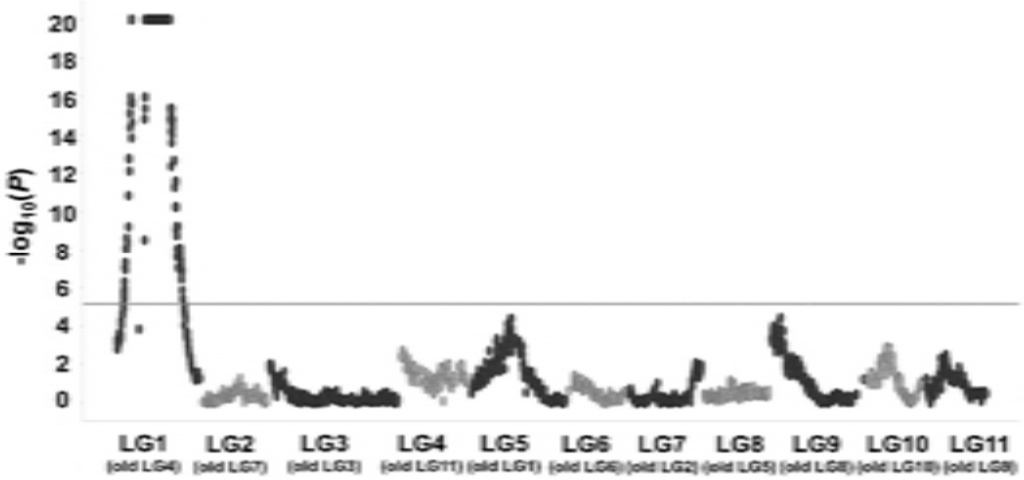

Fig. 3. Genomic localization of QTL associated with resistance to root-galling induced by aggressive *M. javanica*. The QTL was detected in the CB46 x FN-2-9-04 F_2 population phenotyped for RG in the greenhouse. Horizontal dashed-line represents the Bonferroni threshold of significance at $P < 0.05$ [-log(p) = 5.1. Old LG represents former cowpea linkage group numbering and Vu indicates the new cowpea linkage group numbering based on the cowpea pseudomolecules (Lonardi et al., 2017).

405
406 This Vu01 genomic region was mapped to position 19.2-72.9 cM in the CB46 x FN-2-
407 9-04 F_2 population, and it spanned 53.7 cM which corresponds to 13.5 Mb (20889089
408 - 34401992 bp) on the cowpea pseudomolecules with flanking SNP markers 2_53036
409 - 2_18359 (Table 2; Supplementary file S1C). The QTL on Vu01 explained 65.9% of
410 the total phenotypic variation in *M. javanica* root-galling, and the contribution of the
411 additive and dominance effects were 2.7 and 2.1, respectively. The estimated gene
412 action within this region indicated resistance with partial dominance (D/A = 0.8) (Table
413 2). This QTL was detected with high likelihood, -log$_{10}$(p) = 20 (critical threshold = 5.1)

(Fig. 3). In addition, a genomic region associated with resistance to *M. javanica* EM production was mapped on Vu01 of the CB46 x FN-2-9-04 F_2 at position 46.7 – 53.5 cM, and it spanned 6.8 cM corresponding to 3.2 Mb (27254299 - 30434421 bp) on the cowpea pseudomolecules flanked by SNP markers 2_21671 – 2_12209. This QTL explained 24.7% of the total phenotypic variation in *M. javanica* EM production. (Table 2; Supplementary file S1C).

Candidate genes within mapped QTL regions

Candidate gene analysis identified a total 316 genes within the genomic region associated with RKN resistance on Vu04 (Supplementary file S2B). Of these, three encode for disease resistance family proteins belonging to leucine rich repeat (LRR) family protein; two genes encode for LRR transmembrane protein kinase; eight encode for disease resistance proteins belonging to toll-interleukin-1-receptor (TIR-NBS_LRR); thirteen genes are putatively considered to also encode for TIR-NBS-LRR class of resistance proteins; one gene encodes for MAP kinase 9; seven genes encode for protein kinase superfamily proteins; three genes encode for receptor-like protein kinase; one gene encodes for pathogenesis-related thaumatin superfamily protein; and two genes encode for TIR-like proteins. Most of these classes of *R* genes were found in adjacent physical positions on the cowpea pseudomolecules.

Within the resistance QTL region on Vu01 a total of 466 genes were identified (Supplementary file S2A). Of these, three encode for LRR family resistance proteins; one gene encodes for TIR-NBS-LRR resistance proteins; eight genes encode for disease resistance-responsive proteins; one gene encodes for hypersensitive-like lesion inducing protein; two genes encode for kinase interaction protein; three encode for LRR protein kinase family protein; one genes encodes for LRR receptor-like protein kinase; three genes encode for LRR and NB-ARC domains-containing disease

439 resistance proteins; fourteen genes encode for NB-ARC domain-containing disease
440 resistance proteins; and four genes encode for protein kinase family proteins.

441 **Inheritance of resistance in FN-2-9-04**

442 Figures 4A and 4B show the response of four F_1 populations and their parental
443 genotypes to root-galling (RG) and egg-mass (EM) production, respectively by *M.*
444 *javanica*. All recurrent parents (Ecute, CB46, INIA-41 and CB46-Null) exhibited
445 susceptible phenotypes for RG and EM, and their mean RG scores and EM scores
446 ranged from 5.8 to 7.7 and 41 to 82, respectively, whereas the resistant parent, FN-2-
447 9-04 had mean RG and EM scores of 0.4 and 4, respectively.

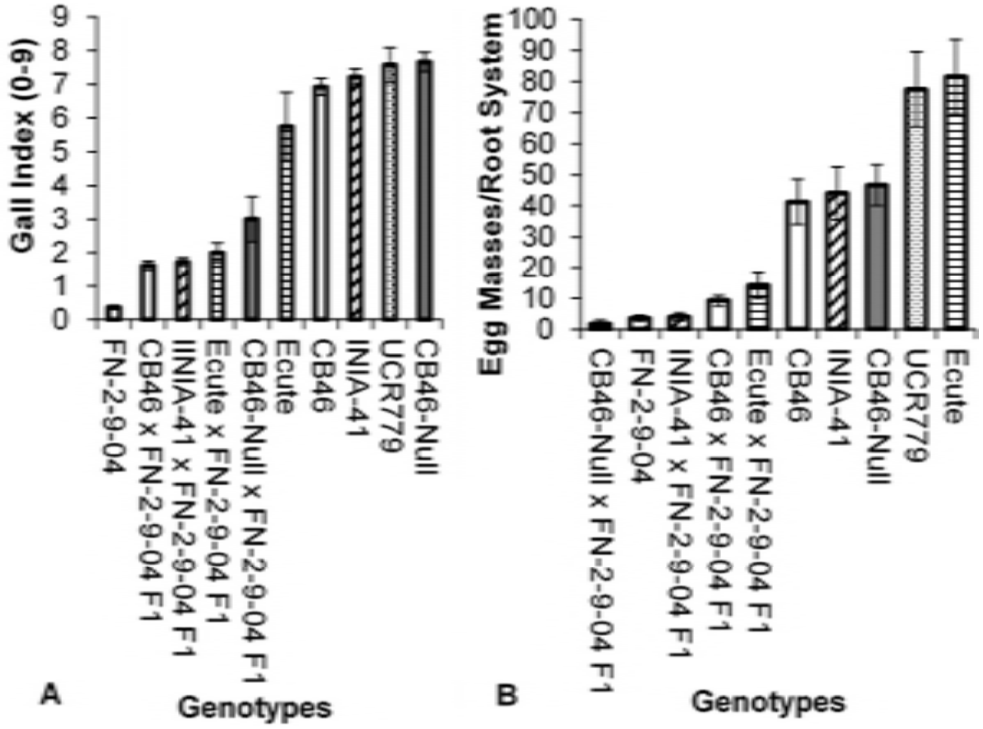

Fig. 4. Mean response of F_1 populations and their parents to: **A,** root-galling and **B,** egg-mass production by *M. javanica* in greenhouse-pot and seedling growth-pouch inoculations, respectively. Bars represent +/- SE.

448

449 All F_1 populations were resistant to *M. javanica* (Fig. 4), with mean RG and EM scores
450 below the mid-parent RG and EM score (GI = 6.9 and EM = 53). The CB46-Null x FN-
451 2-9-04 F_1 had the highest mean RG (GI = 3) of the four F_1 populations. The observed

differences in RG and EM between the resistant and susceptible parents were significant ($P < 0.05$), but the RG phenotype of the resistant parent was only different from F_1 populations CB46-Null x FN-2-9-04 and Ecute x FN-2-9-04. The EM phenotypes of the resistant parent and F_1 were not different. Significant differences among the genotypes were detected at GI = 1.3 and EM = 31.4 (Fig. 4A and 4B).

The segregation of F_2 (Fig. 5A) and $F_{2:3}$ (Fig. 5B) populations for *M. javanica* RG response appeared to follow a bimodal distribution, skewed toward lower RG phenotype. Also, a bimodal segregation pattern was observed for *M. javanica* EM production in the CB46-Null x FN-2-9-04 and CB46 x FN-2-9-04 F_2 populations (Fig. 5C). In these same experiments, the average RG observed for parents CB46-Null,

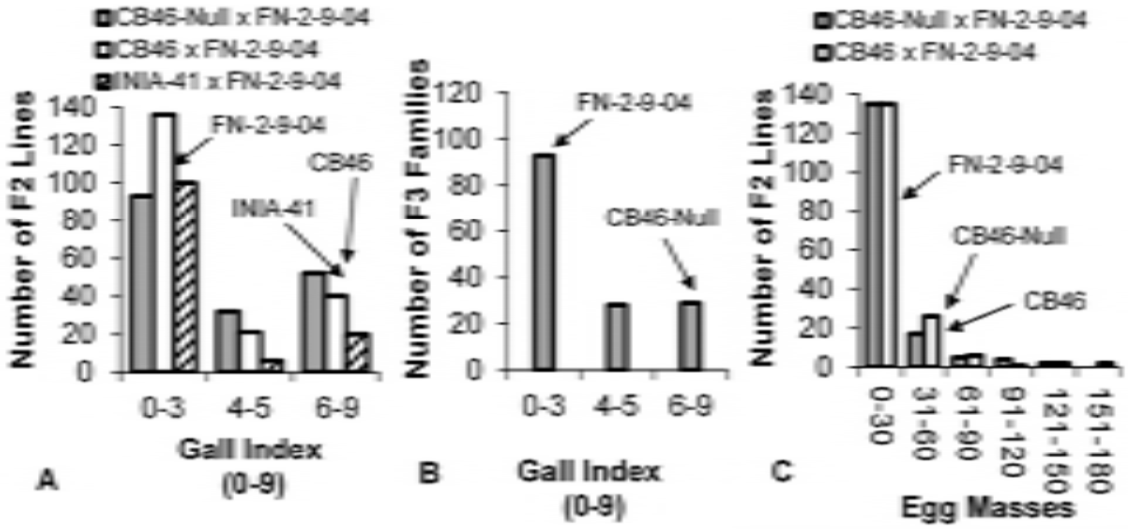

Fig. 5. Distribution of root-galling responses in **A**, F_2 populations (greenhouse), **B**, $F_{2:3}$ population CB46-Null x FN-2-9-04 (field), and **C**, egg-mass production in F_2 populations CB46-Null x FN-2-9-04 and CB46 x FN-2-9-04 (seedling growth-pouch) under *M. javanica* infestation.

CB46, INIA-41 and FN-2-9-09 in greenhouse pots was 7.7, 6.9, 7.2 and 0.4, respectively. In the field experiment (Fig. 5B), RG of 6.7 and 0.1 were observed for parents CB46-Null and FN-2-9-09, respectively, while egg-mass counts per root system equal to 46.7, 45 and 1.8 were observed for parents CB46-Null, CB46 and FN-2-9-09, respectively (seedling-growth pouches).

A similar pattern of root-galling distribution was observed in F_2 (Fig. 6A) and $F_{2:3}$ (Fig. 6B) populations of CB46-Null x FN-2-9-04 under field infestation by avirulent *M. incognita* Beltran. This segregation pattern was consistent across all phenotyping environments (greenhouse, field and seedling growth-pouches) and traits (RG and EM). Egg-mass phenotypes ranged from 0 – 180 (Fig. 5C), and RG across environments and generations ranged from 0 – 9 (Figs. 5 and 6). The resistant parent FN-2-9-04 had consistently lower ($P < 0.05$) RG compared to all susceptible parents. The average *M. incognita* root-galling indices for parents CB46-Null and FN-2-9-04 in the field experiment were 6.4 and 0, respectively.

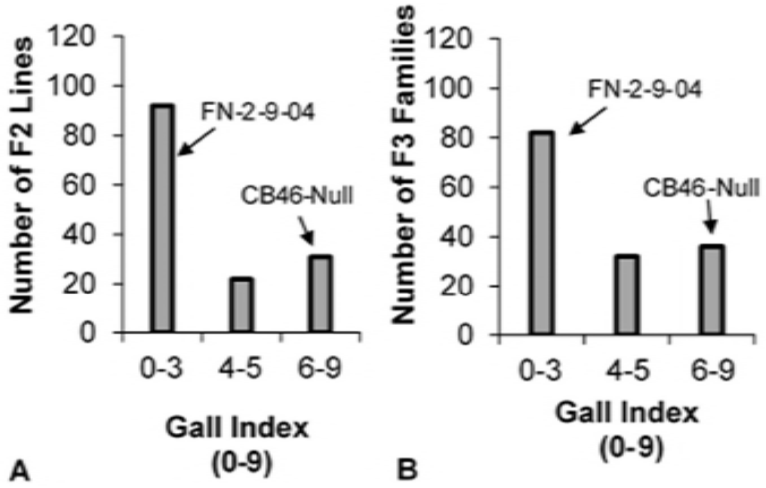

Fig. 6. Distribution of root-galling response in the F_2 (**A**) and $F_{2:3}$ (**B**) populations of CB46-Null x FN-2-9-04 under field infestation with avirulent *M. incognita* isolate Beltran.

The broad-sense heritability (H^2) of resistance to *M. javanica* root-galling estimated through regression of 7 field phenotyped F_2 populations to the mean performance of their parents (CB46-Null, CB46, FN-2-9-04 and INIA-41,) was high ($b = 0.76 \pm 0.07$, $P = 0.00004$) (Fig. 7), while estimates of H^2 for the same trait computed using the genetic variance (V_g^*) directly derived from the QTL region located on Vu01 were moderate

(0.47) and high (0.95) for greenhouse and field phenotyped F_2 and $F_{2:3}$ populations, respectively. For these populations, the estimates of narrow-sense heritability (h^2) of RG were 0.33 and 0.71, respectively. Egg mass production (EM) response in the F_2 had low H^2 (0.34) (Table 2) and h^2 (0.23). The estimated H^2 and h^2 for resistance to avirulent *M. incognita* RG were 0.33 and 0.23 on Vu01 and 0.73 and 0.49 on Vu04, respectively.

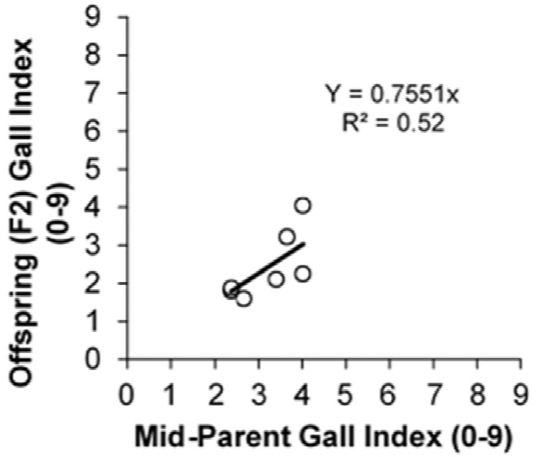

Fig 7. Midparent – offspring regression for F_2 population means regressed on the midparent root-galling values.

Because the *M. javanica* RG and EM resistance QTLs were co-located (Figs. 1B, 2A and 2B), analysis of correlation between RG and EM responses was performed using RG and EM data of F_2 populations CB46 x FN-2-9-04 and CB46-Null x FN-2-9-04. These traits were highly correlated in both populations, CB46 x FN-2-9-04 and CB46-Null x FN-2-9-04 ($r = 0.78$, $P = 0.008$ and $r = 0.62$, $P = 0.06$, respectively), although the correlation in the F_2 population CB46-Null x FN-2-9-04 was not significant ($P = 0.06$) (Fig. 8). The relationship between RG and EM in populations CB46 x FN-2-9-04

497 and CB46-Null x FN-2-9-04 was explained at 60.3% and 38.1%, respectively, based
498 on the estimated coefficient of determination.

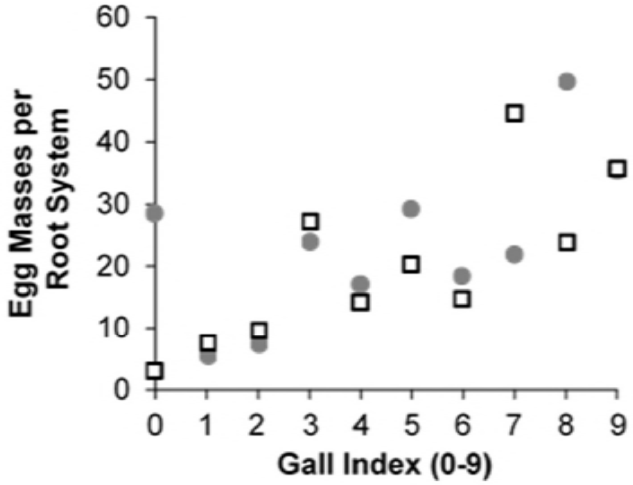

Fig. 8. Correlation between *M. javanica* root-galling (greenhouse) and egg-mass production, (seedling growth-pouch) in F_2 populations ● = CB46-Null x FN-2-9-04 (*r = 0.62*) and □ = CB46 x FN-2-9-04 (*r = 0.78*).

499

500 The 119 and 137 F_2 lines of populations CB46-Null x FN-2-9-04 and CB46 x FN-2-9-
501 04, respectively, assayed for 51128 SNP markers segregated for resistance-
502 susceptibility to RG and EM within each mapped QTL, and it fit closely a ratio of 13:3
503 for phenotypic traits (Table 3). Also, a 3:1 ratio was significant, suggesting that the
504 resistance at both QTL regions is mainly governed by one dominant gene or a
505 combination of genes acting under dominant-recessive interaction. The fit to a 13:3
506 ratio could also indicate genetic distortion for a single dominant gene.

507

Table 3. Best fit segregation ratios (resistant:susceptible) in 119 and 141 F_2 plants from crosses CB46-Null x FN-2-9-04 and CB46 x FN-2-9-04, respectively, determined using SNP marker loci at the two nematode resistance QTL regions.

F_2 Population	Genotypes (Observed) BB + AB	AA	Exp	X^2	P value	Trait	Vu	Isolate
	96	23	13:3[a]	0.002	0.95-0.99	RG	1	
CB46-NullxFN-2-9-04	93	26	13:3[a]	0.56	0.25-0.50	RG	4	Avr-*M.i*
CB46-NullxFN-2-9-04	97	22	13:3[a]	0.002	0.95-0.99	RG	1	*M.j*
CB46-NullxFN-2-9-04	98	21	13:3[a]	0.04	0.75-0.90	EM	1	*M.j*
CB46xFN-2-9-04	111	30	13:3[a]	0.44	0.50-0.75	RG	1	*Mj*
CB46xFN-2-9-04	109	32	13:3[a]	1.19	0.25-0.50	EM	1	*Mj*

BB = alleles from resistant parent, AB = heterozygous, AA = alleles from susceptible parent; Exp. = expected ratio; RG = root galling, EM = egg masses per root system; Vu = cowpea chromosome naming (Lonardi *et al*., 2017); Isolate = Nematode isolate; Avr = avirulent *M. incognita* Beltran, *M.j* = *M. javanica*; [a]also fit a 3:1 ratio.

To validate the genetic models of segregation for resistance-susceptibility to avirulent *M. incognita* and *M. javanica*, gene enumerations were estimated at the mapped QTL regions associated with resistance to RG (Vu01 and Vu04) and EM production (Vu01) following the Castle-Wright (1921) algorithm. The estimates indicated that the resistance to avirulent *M. incognita* RG is under control primarily by 2 and 5 genes residing in QTL regions mapped on Vu04 and Vu01, respectively; whereas, the responses to *M. javanica* RG and EM production mapped on Vu01 are governed mainly by 2 genes each (Supplementary file S3).

Because two QTLs, on Vu01 and Vu04, were associated with resistance to avirulent *M. incognita* RG, analysis of QTLs allele combinations were performed to understand the interaction of both QTLs. Through SNP marker-trait association, the genotype (AA, AB and BB) of each of the 119 F_2 lines was determined at the QTL regions on Vu01 and Vu04 associated with resistance to avirulent *M. incognita* RG, and each genotype was associated with the average RG phenotypic response of the

corresponding $F_{2:3}$. Based on this association, nine QTL combinations (Vu01/Vu04) (Fig. 9) were derived by combining all possible haplotypes on Vu01 and Vu04 contributed from resistant (FN-2-9-04 – favorable allele donor) and susceptible (CB46-Null – non-favorable allele donor) parents.

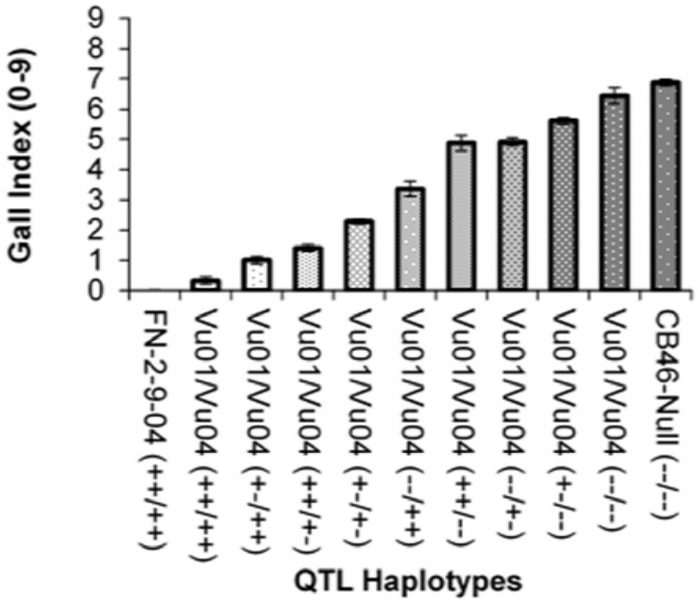

Fig. 9. Avirulent *M. incognita* root-galling values for QTL allele combinations for the resistance traits in accession FN-2-9-04 mapped to Vu01 and Vu04 of the cowpea consensus genetic map. The zygosity status within each QTL is indicated by ++, +- and --, representing homozygous favorable, heterozygous and homozygous un-favorable, respectively, in each QTL. Bars are standard errors.

Analysis of variance showed significant effect ($P < 0.05$) of combining QTLs on avirulent *M. incognita* RG response; significant mean differences in RG phenotypes between genotypes carrying combined QTLs were detected at gall index (GI) = 0.88. The resistant parent FN-2-9-04 [Vu01/Vu04(++/++)] did not show any root-galling (Fig. 9), and its response was different ($P < 0.05$) from all genotypes carrying QTL haplotypes with favorable allele dosage different from this parent. Any of the genotypes carrying at least a single favorable allele on at least one of the chromosome regions had less galling than the susceptible parent CB46-Null [Vu01/Vu04(--/--)].

542 Absence of a single favorable allele in either chromosome predisposed the plants to
543 root-galling, and substantial allele effect was observed for Vu04 [Vu01/Vu04(++/+-)]
544 (Fig. 9). At both loci the favorable alleles must be in the homozygous condition for fully
545 effective *M. incognita* RG resistance.

546 **Resistance relationship between CB46 and FN-2-9-04**

547 The relationship between the root-galling and nematode reproduction resistance in
548 accession FN-2-9-04 and resistance conferred by the *Rk* gene in CB46 (Huynh *et al*.
549 2016) was determined through allelism tests using F_2 populations of CB46 x FN-2-9-
550 04. In addition, analysis of similarity was performed between FN-2-09-04, CB46 and
551 breeding line CB46-Null within the mapped QTL regions to identify putative haplotypes
552 associated with resistance in FN-2-9-04. In 2015 (Table 1), 400 and 162 F_2 plants plus
553 parents were phenotyped for avirulent *M. incognita* root-galling under field infestation
554 at CVARS and KARE, respectively. At both sites (Fig. 10), all F_2 plants were resistant
555 with no obvious segregation for root-galling response between plants, indicating that
556 FN-2-9-04 carries a resistance locus allelic to or equivalent to the *Rk* gene found in

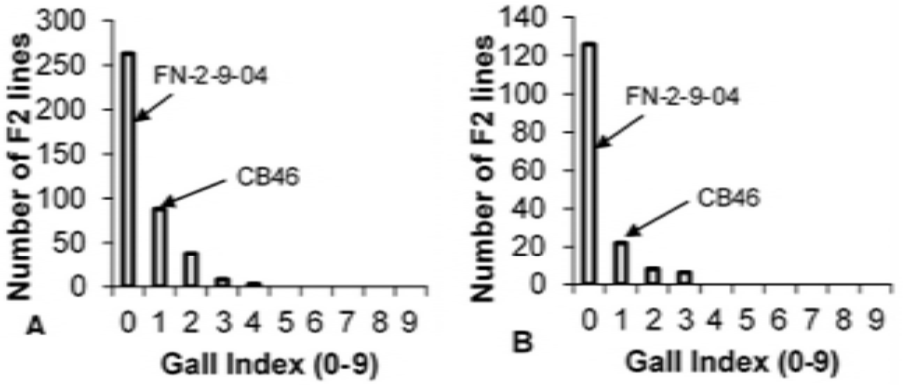

Fig. 10. Distribution of root-galling response in the F_2 population CB46 x FN-2-9-04 under field infestation by avirulent *M. incognita*(**A**): Coachella Valley Agricultural Research Station and (**B**): Kearney Agricultural Research and Extension Center, respectively.

CB46. The average root-gall indexes for CB46 and FN-2-9-04 were 0.7 and 0.2, respectively.

To validate the allelic relationship between resistance determinants conferring resistance to RKN in CB46 and FN-2-9-04, F_2 population subsets of CB46 x FN-2-9-04 were also phenotyped for resistance to *M. javanica* RG and EM, since these parents exhibited significant differences in *M. javanica* RG and EM production responses (Fig. 4). Using 197 and 172 F_2 lines for RG and EM phenotyping, respectively (Table 1), segregation occurred for *M. javanica* RG and EM in these F_2 populations as shown in Figs. 5A and 5C.

Analysis of similarity between FN-2-09-04, CB46 and CB46-Null within the Vu04 genomic region associated with avirulent *M. incognita* RG resistance (Table 2; Fig. 1A) revealed a putative haplotype associated with the resistance (Supplementary file S4). The location of the *Rk* locus on Vu04 identified in CB46 (Huynh *et al*. 2016) overlapped with the resistance region on the same chromosome in FN-2-9-04 within 2.9 cM of the CB46-Null x FN-2-9-04 F_2 population and within 1.59 cM on the cowpea consensus genetic map (Muñoz-Amatriaín *et al*. 2017), corresponding to approximately 1 Mb on the cowpea pseudomolecules. Within this region, based on SNP marker haplotypes, FN-2-9-04 is 39% identical to CB46 and completely different from CB46-Null (identity = 0%) which is 60% identical to CB46.

Conversely, in the region on Vu01 where an additional resistance QTL was detected in FN-2-09-04 (Table 2; Figs. 1B, 2A, 2B), this resistant parent shares no SNP haplotype similarity with either CB46 or CB46-Null (identity = 0%), whereas CB46 and CB46-Null are 100% identical.

DISCUSSION

Characterization of the resistance to avirulent *M. incognita* and aggressive *M. javanica* present in cowpea accession FN-2-9-04 from Mozambique revealed that the resistance is determined by two major QTLs which were mapped on chromosomes Vu01 (old LG4) and Vu04 (old LG11) in the CB46-Null x FN-2-9-04 populations and on Vu01 in the CB46 x FN-2-9-04 population.

The QTL mapped on Vu04 overlaps with the previously mapped genomic region which harbors the *Rk* resistance locus (Huynh *et al*. 2016), suggesting that the *Rk* locus is also present in FN-2-9-04. In our previous RKN resistance QTL mapping of *QRk-vu4.1* (old *QRk-vu11.1*) (Huynh *et al*. 2016), this region associated with the *Rk* resistance spanned about 8.35 cM compared to 2.9 cM in this study. This difference in mapping resolution is attributed in part to the current availability of the high-density SNP genotyping platform and high-density cowpea consensus genetic map (Muñoz-Amatriaín *et al*. 2017). If the genomic region harboring the *Rk* locus is a multi-allelic or multi-gene locus, the overlap between *QRk-vu4.1* and the QTL mapped in this study on Vu04 indicates that the resistance alleles are within 2.9 cM interval of the CB46-Null x FN-2-9-04 population corresponding to approximately 1 Mb on the cowpea pseudomolecules. This locus provides effective resistance against avirulent *M. incognita* populations. The resistance to avirulent *M. incognita* present on Vu01 in FN-2-9-04 is confined to 0.1 Mb of the cowpea pseudomolecules, and its relative low contribution to the total phenotypic variation in root-galling response (33%) compared to the resistance in Vu04 (73.3%) supports that the resistance in Vu04 is the main resistance for this nematode although both are required in the FN-2-9-04 background for fully effective resistance. The estimated values of contribution of each resistance QTL to the total phenotypic variance (Vu01 + Vou4; 33% + 73.3%) give a reliable

607 indication of activity of each resistance QTL to the observed root-galling phenotypic
608 response, with the excess in estimation attributed to error.
609 The resistance to *M. javanica* in FN-2-9-04 consistently mapped to Vu01 using root-
610 galling and egg-mass production phenotypic data from F_2 and $F_{2:3}$ populations
611 phenotyped under distinct environmental conditions (greenhouse, growth chamber
612 and field). The QTL associated with resistance to *M. javanica* egg-mass production
613 was collocated with the QTL controlling root-galling response, and based on the
614 physical positions, on the cowpea pseudomolecules, of the mapped resistance QTLs,
615 the resistance to *M. javanica* root-galling and egg-mass production are confined within
616 6.2 Mb. The resistance QTL on Vu01 is distinct from the *Rk* locus (*QRk-vu4.1*, Huynh
617 *et al*. 2016) which was mapped on Vu04, also it is distinct from the recently mapped
618 RKN resistance locus on Vu11 (Old LG9) which also confers resistance to *M. javanica*
619 (Santos *et al*. 2018). Therefore, it represents a novel RKN resistance QTL in cowpea
620 designated here as *QRk-vu1.1*.
621 The response of four F_1 populations to root-galling and egg-mass production relative
622 to the resistant parent, and the skewed segregation of these nematode-induced
623 phenotypes in the F_2 and $F_{2:3}$ populations indicated that these responses are under
624 control by major genes with partial dominance effects, as also indicated by the
625 estimated degrees of dominance (D/A). Resistance to RKN under control by major
626 genes with partial dominance effect has been reported in several studies (Ali *et al*.
627 2014; Huynh *et al*. 2016).
628 Analysis of segregation for resistance against *M. javanica* and avirulent *M. incognita*
629 through marker-trait association better fit a 13:3 ratio expected for a genetic control
630 under a single dominant gene plus a recessive gene on both Vu01 and Vu04, also
631 suggesting that the major genes controlling resistance are putatively aided by

632 minor/recessive genes, and collectively in a dominant-recessive interaction to confer
633 substantially stronger, broad-based resistance than that conferred by the *Rk* gene
634 alone. A similar genetic phenomenon of major gene and minor/recessive gene
635 interaction was described in cowpea cultivar CB27, where gene *Rk* acts together with
636 a recessive gene to enhance and broaden root-knot nematode resistance (Ehlers *et*
637 *al*. 2000). The data also fit a 3:1 ratio expected for a single major gene, and the better
638 fit to the 13:3 of the SNP haplotypes could represent genetic distortion within each
639 locus. However, using the Castle-Wright (1921) algorithm for gene enumeration, the
640 estimates also supported that two genes on Vu01 and two genes on Vu04, may be
641 responsible for the resistance against *M. javanica* and avirulent *M. incognita*,
642 respectively, but the estimates of genes involved in resistance against avirulent *M.*
643 *incognita* on Vu01 did not support the observed segregation for resistance. The extent
644 of genetic distortion in these regions or multi-allelic effects require further study.
645 Analysis of candidate genes within QTL regions harboring resistance to root-knot
646 nematode revealed several classes of *R* genes known to be associated with plant
647 disease resistance (Ellis and Jones 2003; Takken and Tameling 2009; Gururania *et*
648 *al*. 2012); for example, genes encoding for LRR resistance proteins, LRR
649 transmembrane protein kinase, TIR-NBS-LRR resistance proteins, hypersensitive-like
650 lesion inducing protein, and NB-ARC domains-containing disease resistance proteins.
651 The composition and arrangement of these classes of candidate *R* genes identified on
652 QTL regions housed on chromosomes Vu04 and Vu01 were substantially distinct; this
653 phenomenon may explain the specificity and the structure of resistance to root-knot
654 nematode reported in this study. The resistance QTL on Vu04 was specific to *M.*
655 *incognita* although effective resistance to this nematode was guaranteed by additive
656 effect of the resistance QTL on Vu01, which was specific to *M. javanica*. In both QTL

657 regions, the candidate *R* genes were arranged in tandem. The candidate *R* genes
658 identified in the QTL on Vu04 matched those reported by Santos *et al*. (2018), further
659 supporting that the *Rk* resistance locus is also present in cowpea accession FN-2-9-
660 04. How many of these identified candidate *R* genes are directly involved in
661 determining the RKN resistance phenotypes requires further investigation. The current
662 lack of a functional analysis system in cowpea hampers the determination of which
663 genes are directly involved in the resistance reported here. Therefore, further analysis
664 and testing of function of candidate genes within QTLs associated with resistance to
665 root-knot nematode is a pertinent research goal.
666 Estimates of heritability of resistance in FN-2-9-04 to avirulent *M. incognita* and
667 aggressive *M. javanica* in the F_2 generation using greenhouse phenotypic data were
668 lower than those estimated in the $F_{2:3}$ generation using phenotypic data from field
669 experiments. This can be accounted for by the segregation in both populations and
670 because greenhouse phenotyping is less variable compared to field testing. The
671 estimates of narrow-sense heritability of resistance to root-galling induced by both
672 RKN species were in the range 0.23 – 0.71, indicating that the resistance in FN-2-9-
673 04 can be transferred successfully into elite cowpea cultivars to broaden the genetic
674 base of root-knot resistance which currently relies on the *Rk* gene. The resistance
675 response to *M. javanica* reproduction had lower heritability estimates (H^2 = 0.25 and
676 0.34; h^2 = 0.17 and 0.24) compared to those for *M. javanica* induced root-galling (H^2
677 = 0.47 - 0.95; h^2 = 0.33 - 0.71), which could be due to egg-mass production data being
678 generally more variable compared to root-galling data. High correlation between root-
679 galling and nematode reproduction responses, and the co-location of resistance QTLs
680 associated with both phenotypes suggests that both traits may be governed by the
681 same genes determining resistance. Similarly, significant correlation between root-

682 galling and reproduction phenotypes in cowpea recombinant inbred populations was
683 reported by Huynh *et al* (2016) for the *Rk* locus on Vu04. In contrast, in lima bean
684 (*Phaseolus lunatus* L.) the responses to root-galling and nematode reproduction were
685 reported to be under control by independent genetic factors (Roberts *et al*. 2008).
686 Since genetic factors explained 38.1 and 60.3 % of the association between root-
687 galling and egg-mass production in this study, these data suggest that although the
688 genomic regions governing both traits are co-located, these traits may be under
689 distinct regulatory mechanisms, or that the resistance to both traits may reside within
690 a multi-allelic locus or tandemly arranged loci.
691 The heritability of resistance to avirulent *M. incognita* root-galling comprised two
692 components, one on Vu01 (H^2 = 0.33; h^2 = 0.23,) and the other on Vu04 (H^2 = 0.73*;* h^2
693 = *0.49*) indicating that the major locus for this resistance in FN-2-9-04 is housed on
694 Vu04, and it is aided by the additional locus on Vu01 with low resistance heritability.
695 Also, the differential activity between the resistance loci on Vu01 and Vu04 points to
696 specificity of resistance to avirulent *M. incognita* and *M. javanica*. Huynh *et al* (2016)
697 reported that, although the QTL harboring the *Rk* locus had a significant effect on
698 controlling both avirulent *M. incognita* and *M. javanica*, its resistance activity was lower
699 against *M. javanica*. Marker-trait association analysis in the current study indicated
700 that resistances on both Vu01 and Vu04 are required for effective resistance under
701 avirulent *M. incognita* infestation.
702 The allelism test between CB46 and FN-2-9-04 revealed a lack of resistance
703 segregation in the CB46 x FN-2-9-04 F_2 population under avirulent *M. incognita*
704 infestation, indicating that both parents carry the same major gene *Rk* locus previously
705 mapped by Huynh *et al* (2016) on Vu04 (old LG11) of the cowpea consensus genetic
706 map (Munoz-Amatriain *et al*. 2017), also supporting that the resistance mapped in this

707 study on Vu04 corresponds to the *Rk* locus. *Rk* was the first identified RKN resistance
708 locus in cowpea, and it has been bred into many commercial cowpea cultivars (Fery
709 and Dukes 1980; Helms *et al*. 1991; Ehlers *et al*. 2009). In contrast, the segregation
710 found in F_2 population CB46 x FN-2-9-04 for *M. javanica* root-galling and reproduction
711 responses, and the mapping of resistance QTLs for root-galling and egg-mass
712 production confirmed that the heightened and broad-based resistance response in FN-
713 2-9-04 relative to CB46 is conferred by novel resistance determinants located on
714 Vu01.
715 Flanking markers associated with the mapped genomic regions on Vu01 and Vu04
716 can be used to assist the introgression of the resistance into elite cowpea cultivars. In
717 particular, the novel resistance detected on Vu01 confers the most effective *M.*
718 *javanica* resistance known to date in cowpea. The resistance on Vu01 appears to be
719 more specifically effective against aggressive *M. javanica*, while both the Vu01 and
720 Vu04 QTLs have activity against avirulent *M. incognita*, but with the QTL on Vu04
721 playing the major role in resistance. This was also demonstrated by QTL pyramiding
722 of resistance on Vu01 and Vu04. Thus, both resistance QTLs on Vu01 and Vu04 are
723 responsible for the strong and broad-based resistance observed in FN-2-9-04, which
724 is more effective than the narrow-based resistance provided by the *Rk* gene alone.
725 The mechanism of resistance displayed by this novel broad-based resistance is yet to
726 be determined.
727 The genetic linkage maps of the F_2 populations CB46-Null x FN-2-9-04 and CB46 x
728 FN-2-9-04 are additional valuable genetic resources, especially because they are the
729 first cowpea linkage maps constructed using a genotype from the cowpea gene-pool
730 II from southeastern Africa (Huynh *et al*. 2013), and because 9.2% of the 17209 SNP
731 markers on the CB46-Null x FN-2-9-04 map were unique to this population and were

732 not mapped on the most recent version of the cowpea consensus genetic map
733 (Munoz-Amatriain *et al*. 2017).
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