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Registration of four pest-resistant long bean germplasm lines

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Abstract

Long bean (*Vigna unguiculata* subsp. *sesquipedalis*, asparagus bean, Asian yardlong bean), the vegetable type of cowpea [*Vigna unguiculata* (L.) Walp], is a climate-resilient and nutritious food legume grown by Southeast Asian farmers in the Central Valley of California and marketed to Asian immigrant communities across the United States. Insect pests are major threats, reducing yield and quality of all current lines. Modern plant breeding protocols and extension activities were implemented to develop resistant lines using sources of natural resistance found in African cowpea germplasm. Three aphid-resistant long bean lines, Dark Green 1994 (Reg no. GP-320, PI 702995), Light Green 2055 (Reg. no. GP-321, PI 702996), and Purple 2056 (Reg. no. GP-322, PI 702997), were developed by introgression of two known quantitative trait loci (QTL) for aphid resistance into three local elite lines through marker-assisted backcrossing (MABC). One bush-type long bean line, Bush 2074 (Reg. no. GP-319, PI 702994), carrying two known QTL for root-knot nematode resistance, was also developed to enable scaling up production and to improve nematode management in large-scale commercial farming. These improved lines were evaluated in controlled experiments which also served in outreach activities to enable

Abbreviations: CB77, California Blackeye 77; CVARS, Coachella Valley Agricultural Research Station; GC, gas chromatography; KASP, Kompetitive allele-specific polymerase chain reaction; MABC, marker-assisted backcrossing; NIL, near-isogenic lines; QTL, quantitative trait loci; RKN, root-knot nematode; SNP, single nucleotide polymorphism; UC-KARE, University of California–Kearney Agricultural Research Center; UCR, University of California Riverside.

adoption. Each of these advanced lines, when forming a near-isogenic pair with its recurrent parent, can provide useful genetic materials for resistance gene discovery.

1 | INTRODUCTION

Long bean (*Vigna unguiculata* subsp. *sesquipedalis*), also known as Asian yard-long bean or asparagus bean, is grown traditionally across countries in Southeast Asia (Suma et al., 2021). It is also a popular crop in central California, where it is grown mostly on small-scale, diversified farms by Southeast Asian refugee Hmong farmers. Fresh market long beans are produced for both specialty wholesale distribution and farmers' markets, and are used traditionally in curries, soups, and stir-fry dishes. However, in a recent survey, long bean had the highest incidence of pesticide use of all specialty vegetables in Fresno County where a concentration of Hmong farmers live and grow Asian specialty crops (Thao et al., 2019). Through grower surveys, field observations, and at extension meetings led by the University of California Cooperative Extension small farms program in Fresno County, the cowpea aphid (*Aphis craccivora* Koch) was identified as an important pest limiting long bean production; re-infestation from adjacent fields is problematic, requiring frequent insecticide applications. Aphid pest pressure and/or concerns about high pesticide use have led to some farmers no longer growing long bean. Since all long bean lines currently grown on California small farms are susceptible to aphids, the availability of an aphid-resistant line would enable a dramatic reduction in insecticide use and allow a "no-spray" labeling for long beans sold at farmers markets.

The typical long bean lines grown on small-scale farms have an indeterminate, climbing growth habit (vine-type) that requires trellises to support plants and keep pods straight and clean, and are harvested by hand. In contrast, long bean lines used in large-scale commercial production ideally should have a determinate, erect growth habit (bush-type) to allow for mechanical harvesting. Root-knot nematodes (RKN; *Meloidogyne* spp.), which are commonly found in soils of commercial fields in California and the southern United States, cause serious crop losses. Soil fumigants are often used for nematode control (Koenning et al., 1999; Wang et al., 2009). Using available host plant resistance to RKN is thus vital and timely in the face of increasing restrictions on the use of currently registered pesticides. Several cowpea [*Vigna unguiculata* (L.) Walp] lines with RKN resistance exist, and their use as grain and/or cover crops in cropping systems can promote yield and suppress RKN populations in the soil (Roberts et al., 2005). However, to our knowledge, there is no RKN-resistant long bean line.

Here, we report on the development and release of one bush-type, Bush 2074 (Reg. no. GP-319, PI 702994), and three vine-type, Dark Green 1994 (Reg no. GP-320, PI 702995), Light Green 2055 (Reg. no. GP-321, PI 702996), and Purple 2056 (Reg. no. GP-322, PI 702997), long bean germplasm lines with RKN resistance and aphid resistance, respectively. Marker-assisted backcrossing (MABC) was performed in greenhouses at University of California Riverside (UCR) to expedite the breeding process. Existing knowledge of quantitative trait loci (QTL) for resistance to aphid (Huynh et al., 2015) and RKN (Huynh et al., 2016; Ndeve et al., 2019) was used for trait introgression into susceptible lines. Cowpea donor parents were 'California Blackeye 77' (CB77; Huynh et al., 2022) carrying two aphid-resistance QTL (*QAc-vu1.1* and *QAc-vu7.1*), and an African landrace FN2-9-04 (Ndeve et al., 2018) carrying two QTL (*QRk-vu1.1* and *QRk-vu4.1*) for broad-based RKN resistance.

2 | METHODS

2.1 | Germplasm development

Initial F_1 crosses were made in December 2018 between each of three vine-type recurrent parents (Dark Green Local, Light Green Local, and Purple Local) and the resistance donor, CB77. The recurrent parents are local long bean lines contributed by a Southeast Asian farmer (Fong Tchieng) in Fresno County, CA. They are market-preferred lines yet highly susceptible to aphids, and thus were selected for improvement. The seed provided was from lines that had been maintained by the farmer with seed saved from plants on the farm for over 10 years. Their original source is unknown. Local Hmong farmers in this region typically share seed and maintain them over generations. During each growing season, they save seed from plants with good-quality pods for future seasons.

The F_1s and recurrent parents were planted in June 2019 to make BC_1F_1 crosses. The BC_1F_1 progenies were planted in January 2020, and leaf disc samples of 10-day-old seedlings were collected (Figure 1) and sent to LGC Genomics (Hoddesdon, United Kingdom) for single nucleotide polymorphism (SNP) genotyping with the Kompetitive allele-specific polymerase chain reaction (KASP) assay (Semagn et al., 2014). For each cross, KASP markers that were polymorphic between the recurrent and donor parents and spaced at least 2 Mb apart on 11 cowpea pseudo-chromosomes (Lonardi

et al., 2017), were selected with the BreedIt SNP Selector program (http://breedit.org/) for genotyping their BC_1F_1 population. Based on SNP genotype data, one BC_1F_1 plant with two aphid-resistance QTL haplotypes from the donor parent (foreground selection) and highest recovery of the recurrent-parent's genome (background selection) was backcrossed to the recurrent parent to generate BC_2F_1 progenies. The BC_2F_1 progenies and recurrent parents were planted in September 2020 for genotyping using the same set of SNP markers, except those already homozygous for the recurrent-parent alleles. Foreground and background selection were applied to identify the best BC_2F_1 plants for use in further backcrosses to generate BC_3F_1 progenies. The selected BC_2F_1 plants were also allowed to self-pollinate to obtain BC_2F_2 seed. Both BC_2F_2 and BC_3F_1 seeds were SNP-genotyped in Spring 2021. Some BC_2F_2 lines with donor QTL haplotypes were tested for aphid resistance in Summer 2021 (see Section 2.2 Evaluation trials), while seed of selected BC_3F_1 plants were genotyped further for line development. Table 1 shows the timeline, population size, marker numbers, and percentage of the recurrent-parent genome in the selected plant of each backcross generation.

Core Ideas

- The aphid-resistant long bean lines can be substituted for current local lines to minimize insecticide use.
- The nematode-resistant long bean line can serve as climate-smart green beans used in mechanical harvest systems.
- The long bean near-isogenic lines and founder parents could be useful materials for resistance gene discovery.

Genotyping of 188 BC_3F_2 progenies carrying the Dark Green Local genome background enabled selection of three near-isogenic lines (NIL; 1992, 1993, and 2003) that were

FIGURE 1 Marker-assisted backcrossing (MABC) in long bean: (A) Backcross progenies and parents grown at high density in a University of California–Riverside (UCR) greenhouse for genotyping, and (B) selected desirable plants remain following removal of undesirable plants determined by foreground and background selection.

TABLE 1 Summary of marker-assisted backcrossing generations used in the development of pest-resistant long bean lines following the initial F_1 crosses in December 2018.

F_1 cross (recurrent × donor)	Backcross generation	Planting time	Population size	SNP numbers[a]	Recurrent-parent background (%)[b]
Dark Green Local × CB77	BC_1F_1	Jan 2020	90	116	60
	BC_2F_1	Sep 2020	90	49	95
	BC_3F_1	Mar 2021	90	16	98
Light Green Local × CB77	BC_1F_1	Jan 2020	90	108	63
	BC_2F_1	Sep 2020	90	48	94
	BC_3F_1	Mar 2021	90	19	98
Purple Local × CB77	BC_1F_1	Jan 2020	90	104	67
	BC_2F_1	Sep 2020	90	43	94
	BC_3F_1	Mar 2021	90	17	99
08KV-134-2b × FN2-9-04	BC_1F_1	Mar 2021	90	93	70
	BC_2F_1	Jun 2021	188	32	90

Abbreviations: CB77, California Blackeye 77; SNP; single nucleotide polymorphism.

[a]Number of polymorphic SNP markers genotyped with the Kompetitive allele-specific polymerase chain reaction (KASP) assay (LGC Genomics Ltd.).

[b]Percentage of the selected plant's genome fixed for the recurrent parent's alleles as revealed by KASP genotyping.

homozygous for donor haplotypes at two aphid-resistance QTL. The assigned four-digit line name is its unique DNA ID. Among them, line 1993 was heterozygous at two background loci. Further genotyping of its BC_3F_3 progenies identified two additional NIL (2047 and 2048) homozygous for the two resistance QTL. Meanwhile, genotyping of selfed seed derived from an aphid-resistant BC_2F_2 line grown in the field also identified two more NIL (2060 and 2061) that were homozygous for the resistance QTL.

Likewise, genotyping of 188 BC_3F_2 progenies carrying the Light Green Local genome background identified four NIL (1996, 1998, 2002, and 2005) that were homozygous for donor haplotypes at two aphid-resistance QTL. Among them, line 2002 was heterozygous at one background locus, and further genotyping of its BC_3F_3 progenies identified four additional NIL (2049, 2050, 2051, and 2055) that were homozygous for the two resistance QTL. Meanwhile, genotyping of selfed seed derived from an aphid-resistant BC_2F_2 line grown in the field also identified two NIL (2062 and 2063) that were homozygous for the two resistance QTL.

Genotyping of 188 BC_3F_2 progenies carrying the Purple Local genome background also identified three NIL (2012, 2014, and 2015) that were homozygous for donor haplotypes at two aphid-resistance QTLs. However, these NIL produced slightly green rather than purple pods. Association analysis of BC_2F_2 and BC_3F_2 populations revealed a linkage drag for the green-pod color trait from CB77, which is located in the same genome region affecting seed color next to an aphid-resistance QTL (*QAc-vu1.1*) reported in Huynh et al. (2022). The purple-pod trait was dominant; plants homozygous or heterozygous for the recurrent-parent allele at this locus produced purple pods. One BC_3F_2 plant (2007) with a critical recombination was allowed to self-pollinate to obtain BC_3F_3 seed. Genotyping of 186 BC_3F_3 progenies identified four NIL (2056, 2057, 2058, and 2059) with purple pods and homozygous for donor haplotypes at both QTL.

Similar MABC strategies were used to develop bush-type long bean lines with RKN resistance. The initial F_1 cross was made in March 2020 between the recurrent parent 08KV-134-2b and the resistance donor FN2-9-04. The parent, 08KV-134-2b, is a bush-type long bean line developed by UCR through a pedigree breeding approach starting with a biparental cross of an African cowpea cultivar, 'Big Buff' (Imrie, 1995). and an Asian vine-type long bean line. The F_1 and recurrent parent were planted in December 2020 for BC_1F_1 crosses. The BC_1F_1 progenies were planted in March 2021 for SNP genotyping and foreground and background selection for BC_2F_1 crosses. To accelerate the recurrent-parent genome recovery, a larger BC_2F_1 population, including 188 progeny, was planted in June 2021 and SNP genotyped (Table 1). One best BC_2F_1 plant was selected and allowed to self-pollinate to obtain BC_2F_2 seed. Genotyping of 183 BC_2F_2 progeny in Spring 2022 enabled selection of six NIL (2070, 2071, 2072, 2073, 2074, and 2075) that were homozygous for the donor haplotypes at two nematode-resistance QTL.

2.2 | Evaluation trials

2.2.1 | Aphid resistance phenotyping

A field trial was conducted in Summer 2021 to evaluate early backcross generations for aphid resistance. They included 15 vine-type BC_2F_2 lines that were homozygous or heterozygous at two aphid-resistance QTL. The recurrent parents Dark

FIGURE 2 A field-based evaluation trial for aphid resistance in long bean near-isogenic lines (NIL) in comparison with their recurrent parents (Dark Green Local, Light Green Local, and Purple Local) at the University of California–Kearney Agricultural Research Center, Parlier, CA, in 2022. The highly susceptible cultivar 'Big Buff' was planted as aphid spreader rows. No insecticide was applied during the course of experiment. Plants are shown at 56 days after planting when aphids had killed most Big Buff plants in spreader rows and had killed or stunted the growth of all local cultivars.

Green Local, Light Green Local, and Purple Local were also included for comparison. The trial was planted in a location known for high aphid pressure at the University of California–Kearney Agricultural Research Center (UC-KARE) in Parlier, CA. Each line or parent was planted in two rows of 0.76-m width and 7.6-m length at an average density of 1 seed every 10 cm using a tractor-mounted planter. A highly susceptible cowpea cultivar, Big Buff, was planted as aphid spreader rows to attract aphids and promote uniform infestation. The plots were scored for severity of aphid infestation using a rating scale combining estimates of aphid incidence and damage, from 0 (no aphids) to 10 (plant death) (Huynh et al., 2015). Plots were rated when aphids infested all spreader rows.

Twenty-two advanced lines (including four BC_2F_4, eight BC_3F_2, and 10 BC_3F_3 NILs fixed for two aphid-resistance QTL) were evaluated together with their recurrent parents under irrigated conditions at UC-KARE in Summer 2022. The trials were planted in two adjacent fields, with one receiving regular insecticide sprays (protected trial) and the other with no spray (unprotected trial). In each trial, each NIL and its recurrent parent were planted in two adjacent rows of 0.76-m width and 4.6-m length at an average density of one seed every 10 cm using a tractor-mounted planter. In the unprotected trial, the highly susceptible cultivar Big Buff was grown in alternate rows to attract natural aphid populations and promote heavy, uniform infestation levels in all plots. Plots were scored for aphid incidence and damage symptoms when aphids had killed most plants in spreader rows and caused distinct phenotypic variation among experimental plots (Figure 2). To measure yield, fresh pods were harvested twice per week when they reached commercial-maturity stage to obtain total fresh weight. Data from each NIL set and parent were compared using a paired sample *t*-test performed with the software GenStat (Payne et al., 2008).

2.2.2 | Nematode resistance phenotyping

The six bush-type long bean NIL fixed for two nematode-resistance QTL were evaluated together with their recurrent parent 08KV-134-2b at the Coachella Valley Agricultural Research Station (CVARS) in Thermal, California, in Autumn 2022. The trials were planted in two adjacent fields, one without RKN and the other infested with *M. incognita* isolate 77. The RKN infestation was established by injecting an inoculum of nematode eggs extracted from greenhouse-grown tomato (*Solanum lycopersicum*) plants into the root-zone of young susceptible tomato plants planted in previous years to provide high and uniform infestation levels. The RKN resistance donor parent FN2-9-04 and a susceptible check, CB46 Null (Huynh et al., 2016), were also planted in the RKN infested trial for comparison. Each trial was a randomized complete block design with three blocks. In each block, each line was planted in a row of 0.76-m width and 4.6-m length at an average density of 1 seed every 10 cm using a tractor-mounted planter. Fresh pods were harvested at 64 days after planting when they reached commercial-maturity stage to obtain total fresh weight. In the RKN infested trial, five root systems per plot were dug and scored for nematode root-galling symptoms using a rating scale from 0 (no symptoms) to 9 (severe galling) as adapted from Bridge and Page (1980).

The six bush-type NIL were also assayed for RKN resistance together with its parents and the susceptible check CB46 Null using a seedling growth-pouch method adapted from Atamian et al. (2012). The experiment was arranged

in a split-plot design with four blocks; each block was a file folder rack holding separate folders, each containing two pouches of the same line (whole plot), with one plant per pouch; one pouch was inoculated with *M. incognita* isolate 77 and the other with *M. javanica* isolate 811 (subplots). The isolate 77 was characterized previously to be avirulent on cowpeas with resistance conferred by the *Rk* gene (Huynh et al., 2016; Roberts et al., 1995), while the isolate 811 was virulent on plants with *Rk* resistance (Huynh et al., 2016; Ndeve et al., 2018; Santos et al., 2018). Seed from each line was scarified, two of which were then transferred into one pouch for germination. After 3 days, one healthy seedling was retained and the other discarded. The pouches were watered daily with distilled water and kept in a growth chamber with constant temperature (27˚C) and 16 h of light per day. After 14 days when adequate root systems had developed, each pouch was inoculated with approximately 1500 second-stage juveniles of either *M. incognita* isolate 77 or *M. javanica* isolate 811. The juvenile inoculum was prepared by hatching nematode eggs extracted from tomato roots. After inoculation, the plants were maintained in Hoagland's growth solution (Hoagland & Arnon, 1950) for 30 days and then treated with egg-mass-selective erioglaucine dye (Sigma Chemical Co.) overnight. Stained egg masses on plant roots were counted with the aid of a 10X illuminated magnifier. The mean number of egg masses per line was used to classify resistance levels, which were compared using analysis of variance and subsequent multi-comparison tests performed with the software GenStat (Payne et al., 2008).

2.2.3 | Pod quality measurement

Lines selected for registrations were assessed further for pod quality to confirm release decision. They included three vine-type lines with aphid resistance and one bush-type line with nematode resistance. Their four recurrent parents and two long bean samples purchased from local markets were also included for comparison. Fresh pods were harvested when they reached commercial-maturity stage from the aphid- and RKN-infested trials at UC-KARE and CVARS in 2022. Pod softness was calculated as the fresh weight divided by volume, which was determined by dipping 10 pods in a 1000-mL graduated cylinder containing deionized water and measuring its occupying water volume. The measurement was repeated three times on different sets of pods.

To measure sugar content which determines the sweet taste of long bean, three pods from different plants per line were sampled separately into three 50-mL Falcon tubes (three replications), then kept on dry ice and freeze-dried for 72 h. The dry samples were homogenized in a mortar and pestle, and 110 mg was aliquoted into a 2-mL screw cap tube with 3 ceramic beads for fine grinding with an Omni Bead Ruptor Elite (4 m s^{-1}, 4 cycles of 15 s, 10-s dwell time). Approximately 10 mg of dry sample powder was weighed into a 1.5-mL Eppendorf tube and extracted according to Pico et al. (2021) with minor modifications. In brief, 0.3 mL of 70% ethanol was added to each sample. The mixture was sonicated at 55˚C for 120 min and spun down for 10 min at 1399 ×g and 20˚C. The supernatant was collected into a fresh tube. A second extraction was repeated, and supernatants of the two extractions were combined. An extraction quality control sample was produced by pooling aliquots from all samples and used as matrix background measurement. A 2-µL aliquot of each experimental and quality control sample was dried down in a Centrivap Concentrator at 25˚C, then suspended in 50 µL of methoxyamine hydrochloride solution in gas chromatography (GC)-grade pyridine (20 mg mL^{-1}). The mixture was shaken at 800 rpm for 2 h at 37˚C before adding 50 µL of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (MSTFA-1% TMCS) reagent, then shaken again for 30 min as described in Bhatia et al. (2015). Sugar quantifications (glucose, fructose, galactose, sucrose, raffinose, and stachyose) were performed on a Thermo 1300 gas chromatographer coupled to a Thermo Fisher ISQ7000 mass spectrometer. Chromatographic separations of metabolites were carried out on a 30 m×0.25 mm×0.25 µm Thermo TG5SilMS column. For fructose, glucose, galactose, and sucrose analysis, the GC oven temperature was maintained at 60˚C for 4 min, then gradually raised at the rate of 20˚C min^{-1} to 200˚C, followed by 4˚C min^{-1} to 240˚C, 16˚C min^{-1} to 320˚C, and maintained for 4 min. For raffinose and stachyose, the GC oven temperature was maintained at 60˚C for 4 min, then gradually raised at the rate of 20˚C min^{-1} to 350˚C and maintained for 4 min. All samples were injected in split mode at a splitting ratio of 1:20. Helium was used as a carrier gas and set at a constant flow rate of 1 mL min^{-1}. The mass selective detector was run in the electron impact mode, with electron energy of 70 eV. Total ion chromatogram peak areas were extracted for each sugar after retention time and mass spectra confirmation with commercial standards. Quantification curves were obtained by subtracting peak areas from the extraction quality control sample from curve points in the extraction quality control background (Pico et al., 2021). Chromatographic and mass spectrometric data were analyzed with the Thermo Chromeleon 7.2 software.

3 | CHARACTERISTICS

In the 2021 field trial at UC-KARE, all parents were scored as 7–9 (aphids covering 70%–90% of plant surface area and causing severe crown damage), whereas lines homozygous or heterozygous at both resistance QTL were scored as 3–6 (aphids covering 30%–60% of plant surface area with minor crown damage). This result confirmed the effect of resistance

TABLE 2 Comparative performance of long bean near-isogenic lines (NIL) and their recurrent parents (Dark Green Local, Light Green Local, and Purple Local) under insect-unprotected and protected conditions at the University of California–Kearney Agricultural Research Center, Parlier, CA, in 2022.

Marker assisted backcrossing NIL and recurrent parent	Aphid damage in unprotected plots	Pod yield (kg ha^{-1})	
		Unprotected	Protected
Dark Green NIL	3.0 ± 0.3	5138 ± 506	5585 ± 294
Dark Green Local	10.0 ± 0.0	203 ± 101	9035 ± 500
P-value[a]	<0.001	<0.001	<0.001
Light Green NIL	2.3 ± 0.2	6991 ± 607	6106 ± 479
Light Green Local	7.1 ± 0.6	3359 ± 956	9817 ± 319
P-value	<0.001	0.030	<0.001
Purple NIL	2.4 ± 0.2	7149 ± 885	4858 ± 473
Purple Local	7.6 ± 0.2	1097 ± 334	5347 ± 410
P-value	<0.001	0.001	0.572

Note: Values are means ± standard error.

[a]Significance values of the paired sample *t*-test comparing each NIL set and recurrent parent grown side-by-side.

QTL in the local long bean genetic backgrounds and informed continuing efforts to develop advanced lines with fixed QTL for aphid resistance, as well as agronomic and marketing traits desired by growers.

All vine-type long bean NIL outperformed their recurrent parents under the insect-unprotected conditions at UC-KARE in 2022 (Figure 2 and Table 2). The parents were rated from 7.1–10 (aphids covering more than 70% of plant surface area and causing severe crown damage and plant death), whereas their NIL had ratings of 2.3–3 (aphids covering 23%–30% of plant surface area without causing any crown damage). The NIL also produced higher fresh-pod yields than their parents ($P < 0.05$); the yield gap between the NIL and parent was highest in the Purple group (6052 kg ha^{-1}), followed by the Dark Green (4935 kg ha^{-1}) and Light Green group (3632 kg ha^{-1}). Under the insect-protected condition, the Purple NIL yielded equivalently to its parent, while the Dark Green and Light Green NIL produced about 62% of the parental yield values ($P < 0.001$) (Table 2). Further replicated field trials would be needed to confirm whether this yield gap was caused by possible linkage drag in the Dark Green and Light Green NIL. If so, genotyping of additional BC_3F_2 progenies would help narrow down their introgressed QTL regions with reference to those observed in the Purple NIL.

The six bush-type long bean NILs yielded comparably to their recurrent parent 08KV-134-2b at CVARS in 2022, with average fresh-pod yield of 3390 kg ha^{-1} (with *M. incognita* infestation) and 3701 kg ha^{-1} (without RKN). In the *M. incognita* infested trial, based on a rating scale of root-galling symptoms from 0 to 9 (Bridge & Page, 1980), the susceptible check CB46-Null had a score of 9 (severe galling) whereas all NIL and parents had a score of 0 (no symptoms). The NILs and donor parent also produced zero to negligible amounts of egg masses compared to the susceptible check when assayed with *M. incognita* and *M. javanica* in growth pouches (Table 3).

Further observations for plant vigor and pod characteristics of individual lines within each NIL group enabled selecting three best-looking vine-type and one bush-type lines for registration. These advanced lines are similar to their respective recurrent parents with regards to botanical and morphological characteristics, including leaf shape (sub-hastate), flower color (purple), pod type, and growth habits. The vine-type line Dark Green 1994 derived from the parent Dark Green Local produces shiny, deep-green pods with pigmented tips (Figure 3A). The vine-type line Light Green 2055, derived from the parent Light Green Local, has shiny, light-green pods with pigmented tips (Figure 3B). The pods of Dark Green 1994 and Light Green 2055 also developed unique purple stains during cold weather, as observed at UC-KARE in Autumn 2022. The vine-type line Purple 2056, derived from the parent Purple Local, produces purple pods with a slightly green color at the tip and base of each pod (Figure 3C). All vine-type lines exhibit the indeterminate twining and climbing growth habit with long, pendant pods measuring up to 62 cm at maturity. In contrast, the bush-type line Bush 2074, derived from the parent 08KV-134-2b, has a determinate, erect growth habit, and thus does not require trellises; this line produces light-green pods which are also pendant and about 30-cm long at maturity (Figure 3D and 3E). All four lines produce kidney-shaped seed similar to their recurrent parents. With regards to mature seed color, Purple 2056 and Bush 2074 produce brown seed similar to their recurrent parents, whereas Dark Green 1994 and Light Green 2055 produce black seed.

Based on GC-mass spectrometry data, major sugars present in long bean samples were fructose, glucose, and sucrose; galactose and its oligo-saccharides, including raffinose and stachyose, showed minor chromatographic peaks and thus were omitted from the analysis. The total sugar concentration

TABLE 3 Mean egg-mass production by *M. incognita* isolate 77 and *M. javanica* isolate 811 on root systems of bush-type long bean near-isogenic lines (NIL) and checks tested using seedling growth-pouches in a growth chamber.

Line	Type	*M. incognita*	*M. javanica*	Mean
CB46-Null	Susceptible check	16.00	10.75	13.38a
08KV-134-2b	Recurrent parent	0.25	10.25	5.25b
FN2-9-04	Donor parent	0.00	1.00	0.50c
2070	BC_2F_2 NIL	0.00	2.25	1.13bc
2071	BC_2F_2 NIL	0.00	0.50	0.25c
2072	BC_2F_2 NIL	0.25	1.00	0.57bc
2073	BC_2F_2 NIL	0.00	0.00	0.00c
2074	BC_2F_2 NIL	0.00	0.25	0.14c
2075	BC_2F_2 NIL	0.00	2.25	1.13bc

Note: Means followed by the same letter are not significantly different (Tukey test, $P < 0.05$).

FIGURE 3 Pod samples of three vine-type long bean lines (A) Dark Green 1994, (B) Light Green 2055, (C) Purple 2056, (D) bush-type line Bush 2074, and (E) a bush-type long bean field trial in Thermal, CA, in 2022 demonstrating the determinate, erect growth habit of bush-type long bean plants

of advanced lines ranged from 8.18% to 10.85% of dry weight, which is equivalent to or higher than those measured in samples purchased from local markets ($P = 0.05$, Table 4). Except for Dark Green 1994, other advanced lines had total sugar concentration slightly higher than or comparable to their respective recurrent parents. All lines, parents, and one of the market samples also showed comparable pod softness values (0.92–0.97, Table 4).

Based on KASP genotyping, each of the three vine-type lines is near-isogenic to its recurrent parent and carries two

TABLE 4 Pod sugar concentrations and softness of four registered pest-resistant long bean lines (bold names) in comparison with their recurrent parents and long bean samples purchased from local markets.

Line	Fructose	Glucose	Sucrose	Total sugar	Softness
	% dry weight				g mL^{-1}
Dark Green 1994	3.96 ± 0.08	3.67 ± 0.13	0.55 ± 0.03	8.18 ± 0.24	0.95 ± 0.00
Dark Green Local	5.79 ± 0.49	6.37 ± 0.69	1.04 ± 0.19	13.20 ± 1.11	0.95 ± 0.02
Light Green 2055	4.32 ± 0.08	5.24 ± 0.02	0.56 ± 0.06	10.12 ± 0.14	0.96 ± 0.01
Light Green Local	3.98 ± 0.48	3.95 ± 0.50	0.51 ± 0.03	8.45 ± 0.95	0.97 ± 0.01
Purple 2056	3.33 ± 0.33	5.19 ± 0.73	1.02 ± 0.18	9.53 ± 0.79	0.94 ± 0.01
Purple Local	3.49 ± 0.48	3.72 ± 0.63	0.53 ± 0.06	7.74 ± 1.17	0.95 ± 0.01
Bush 2074	5.00 ± 0.43	4.10 ± 0.17	1.74 ± 0.15	10.85 ± 0.57	0.97 ± 0.01
08KV-134-2b	4.15 ± 0.50	4.53 ± 0.48	1.81 ± 0.28	10.49 ± 1.16	0.92 ± 0.01
Market sample 1	3.85 ± 0.15	4.84 ± 0.26	1.38 ± 0.35	10.06 ± 0.48	0.86 ± 0.03
Market sample 2	2.73 ± 0.17	2.08 ± 0.06	2.87 ± 0.33	7.68 ± 0.32	0.95 ± 0.01
LSD ($P = 0.05$)	1.07	1.32	0.60	2.33	0.04

Note: Values are means ± standard error of three replications.

aphid-resistance QTL haplotypes derived from the resistance donor CB77 (Huynh et al., 2022). The bush-type line is also near-isogenic to its recurrent parent and carries two nematode-resistance QTL haplotypes derived from the resistance donor FN2-9-04 (Ndeve et al., 2019). This explains the better performance of these lines compared to their recurrent parents in experiments infested with aphids (Table 2) and RKN (Table 3). The introgressed genome regions for aphid and RKN resistance do not overlap with those affecting pod length (Xu et al., 2017) or photoperiod sensitivity (Huynh et al., 2018). This explains the similar performance of each near-isogenic pair with respect to pod length and growth habit under the long day-length condition in California during summers. However, the introgressed region on chromosome 5 of Dark Green 1994 and Light Green 2055 contains a linkage drag for black seed from CB77 (Huynh et al., 2022). This black-seed haplotype contains a large insertion (up to 42 kb) compared to the non-black-seeded type (Herniter et al., 2018). While this black-seed haplotype did not affect the pod greenness, extra genes in the insertion might contribute to yield reduction (Table 2) and the increased pigmentation on the pods of these lines during cold weather. Removal of this linkage drag is underway at UCR.

Following future on-farm evaluation trials, the vine-type lines could be substituted for local lines in vegetable farms to minimize insecticide use, while the bush-type line could be grown in rotations in commercial fields to suppress RKN populations for following crops. The bush-type long beans could be sold as "climate-smart" green beans used in a mechanical harvest system. This represents a new concept for US vegetable producers and would be coupled with the bush-type line, hitting an earlier season market compared to the traditional vine-type long beans. In the short term, growing bush-type long beans may be an opportunity as a season extender for fresh green beans that are more heat tolerant. In the longer term, they could replace snap beans in frozen Asian vegetable products in the United States, where snap beans are used instead of long beans because it has been cheaper to use bush-type snap beans with mechanical harvesting.

4 | AVAILABILITY

Breeder seed of the three vine-type long bean lines Dark Green 1994, Light Green 2055, and Purple 2056 has been multiplied in UCR greenhouses and distributed to California growers. The growers can harvest their own seed for future growing seasons. Long bean is a self-pollinated crop and the released inbred lines would be genetically stable over generations. Breeder seed of the bush-type long bean line Bush 2074 has also been multiplied in UCR greenhouses. Some of these seeds will be provided to the University of California-Davis Foundation Seed Program for production of Foundation seed in 2024. The Foundation class seed would then be sold to seed producers, who would then produce Registered class seed in 2025 and Certified class seed in 2026 for commercial production. Breeder seed of the four lines is maintained by UCR. The bush-type long bean line Bush 2074 will be available for production under PVP license, and interested parties should contact the UCR Research and Economic Development. Breeder seed of the four lines has also been deposited into USDA-ARS National Laboratory for Genetic Resources. Small amounts of breeder seed accompanied with material transfer agreements for research purposes may be obtained from the corresponding authors through the project website https://uslongbeanbreeding.ucr.edu/ immediately upon publication.

AUTHOR CONTRIBUTIONS

Bao-Lam Huynh: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing—original draft; writing—review and editing. **Ruth M. Dahlquist-Willard**: Conceptualization; funding acquisition; investigation; methodology; project administration; supervision; validation; visualization; writing—review and editing. **Antoon T. Ploeg**: Conceptualization; funding acquisition; investigation; methodology; visualization; writing—review and editing. **Michael Yang**: Data curation; validation; visualization; writing—review and editing. **Lilian Thaoxaochay**: Data curation; validation; visualization; writing—review and editing; **Jessica Kanter**: validation; visualization. **Sukhmony Brar**: Validation; visualization. **Jose Paz**: Validation; visualization. **Sara Qaderi**: Validation; visualization. **Hardeep Singh**: Validation; visualization. **Tra Duong**: Data curation; methodology; supervision; validation; visualization; writing—review and editing. **Hoang Dinh**: Data curation; methodology; validation; visualization; writing—review and editing. **Hyun Park Kang**: Validation; visualization. **William C. Matthews**: Methodology; resources. **Amancio De Souza**: Data curation; methodology; writing—review and editing. **Anil Bhatia**: Data curation; methodology; writing—review and editing. **Haiyan Ke**: Methodology. **Jeffrey D. Ehlers**: Conceptualization; resources; supervision; visualization; writing—review and editing. **Philip A. Roberts**: Conceptualization; funding acquisition; investigation; methodology; resources; supervision; visualization; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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