BLACKEYE VARIETAL IMPROVEMENT - 2014 PROGRESS REPORT

P. A. Roberts, B. L. Huynh, W. C. Matthews and C. A. Frate[1]

ABSTRACT

Our breeding program is developing improved blackeye beans for California growers. In 2014 work supported by the California Dry Bean Advisory Board was conducted under the following research objectives. ***Evaluating experimental breeding lines for grain yield, grain quality and agronomic characteristics:*** Trials comparing yield and grain quality of nine new blackeye breeding lines together with CB46Rk^2, CB46, and CB50 were conducted under early-planted, double-flush production conditions at the Kearney Research and Extension Center (KREC) and a Tulare County commercial blackeye field. Overall the yields were higher than in 2013. All nine advanced lines had higher grain yield than CB46 at KREC, and seven lines had equivalent yield to CB46 at the Tulare Co. location. Some lines also combine the advantage of stronger, broad-based resistance to root-knot nematodes and resistance to Fusarium wilt Race 4. They have seed size that is consistently the same or larger than CB46 but less than CB50. CB46Rk^2, a new version of CB46 with improved resistance to root-knot nematodes, performed well again in 2014 and may be useful as a 'canner' due to smaller seed than CB46. ***Advanced lygus resistant blackeye lines****:* In 2014 three lines first selected in 2007-2009 were evaluated under insect protected and unprotected conditions at Kearney. These lines resulted from a long-term breeding effort to combine lygus resistance with high quality grain and high production. They were selected based on their performance in similar trials conducted in 2010 to 2013. Lygus pressure was heavy but late in 2014, resulting in grain yield loss of between 10% and 30% in comparisons between protected and unprotected conditions. The experimental lines had significantly higher protected yield than CB46 indicating they have high innate yield potential, although CB46 yield was affected by late season lygus attack. The unprotected yields were significantly higher than CB46 for all three advanced lines, confirming strong yield ability under lygus pressure. ***Modern breeding of blackeyes:*** Funding from other projects continued to support development and application of molecular marker breeding tools to improve efficiency and accuracy of plant selection decisions in the blackeye breeding program. These included application of a high-throughput genotyping capability and markers linked to resistance to Fusarium wilt, root-knot nematodes and cowpea aphid. Marker-assisted backcrossing of aphid resistance into CB46, CB50 and CB27 was taken to the BC5 stage.

Key Words: blackeye beans, cowpea, lygus bug, aphids, grain quality, yield potential

INTRODUCTION

This project targets development of improved blackeye bean varieties for California growers. Our short term goal is to develop blackeye varieties that have increased yield potential and yield stability, large 'split-free' grain, resistance to 'early cut-out' and Fusarium wilt (races 3 and 4), and broad-based resistance to root-knot nematodes. In the medium to longer term, we aim to develop blackeye varieties with resistance to aphids and lygus bugs.

[1]Philip A. Roberts, Bao Lam Huynh, and William Matthews, Dept. of Nematology, University of California, Riverside, CA 92521; Carol Frate, UCCE, Tulare Co. CA. Email: philip.roberts@ucr.edu, baolam.huynh@ucr.edu ***In:*** University of California Dry Bean Research 2014 Progress Report published by the California Dry Bean Advisory Board, 531-D North Alta Avenue, Dinuba CA 93618, and available at http://beans.ucanr.org/.

PROJECT OBJECTIVES AND PROGRESS

Evaluating advanced blackeye breeding lines for grain yield and quality, and agronomic characteristics at two locations: At Kearney Research and Extension Center (KREC) and in a Tulare County commercial blackeye field, identical four-replicate trials were conducted in the 2014 main season comparing yield and grain quality of 9 new blackeye breeding lines, together with varietal candidate line CB46Rk^2 and checks CB46 and CB50. The new breeding lines included 4 lines that had been tested in 2011, 2012 and 2013, and 5 near-isogenic lines of CB46 (N lines) with better nematode resistance and grain size. Separate root-knot nematode evaluations of some of these lines were previously conducted at South Coast REC in 2012 and 2013 in specially managed plots infested with either *Meloidogyne incognita*, *M. javanica*, or *M. incognita* Muller root-knot nematodes. Yield trials were also conducted at KREC in 2014 for the 5 N lines and checks in two specially managed plots infested with either *M. incognita* or *M. javanica*.

The selection history of two of these advanced blackeye lines (Table 1) started with single-pod F_3 bulks made from ten populations at Shafter REC in 2005, from which 64 selections were made after advancing one generation at the Coachella Valley Agricultural Research Station in 2005. The 64 F_4 lines were evaluated for agronomic and grain quality characteristics at Shafter in 2006, and 19 best lines were single pod bulked from selected rows. These bulks were planted in eight-row plots at Coachella in fall 2006 from which 300 F_5 lines were harvested. The F_5 lines were planted at Riverside and Shafter REC in May 2007, and 200 selections were made based on visual assessment of grain yield and quality. These were planted at Shafter in 2008, 100 selections made and then evaluated in 2009 at Shafter in two-row plots with two replications. Bulks were composited from selected lines by harvesting a single pod from each of 20 plants in the plot, from which ten seeds per bulk were planted in the greenhouse to multiply 10 pure sub-lines of each of the selections. In 2010, a breeding nursery with these advanced generation blackeye breeding lines was conducted at KREC. Twelve breeding lines looked particularly promising and the seed of one row of each selection was hand-harvested to provide seed for replicated trials that were conducted in 2011-2013.

The other two lines (10K-77, 10K-115; Table 1) were developed through backcrossing to resemble CB5, but also with the addition of resistance to Fusarium wilt derived from CB27. The recurrent backcrossing procedure was used to transfer into CB5 the Fusarium wilt resistance from CB27, which is effective against both wilt races 3 and 4. Through line development and testing, these two lines were chosen for advanced testing based on yield and seed-size performance in tests with sister lines over three years from 2011-2013, with excellent seed size and yield potential over locations and years relative to CB46, CB5 and CB50 check plots. DNA markers linked the Fusarium resistance genes were used to check for the resistance traits in advanced generations.

The nematode-resistant N lines originated from a cross between two near-isogenic lines of CB46, one carrying the CB3 galling resistance gene but not resistance gene *Rk*, and the other carrying both *Rk* and *Rk2*. F2 plants were planted in a nematode infested field (*M. incognita* Muller) at South Coast REC, Tustin, in 2008. F3 seeds from resistant plants were tested in pouches and pots in UC-R greenhouses in 2009 to identify plants that had the CB3 and *Rk* genes. F4 seeds from resistant plants were planted to produce F5 seed in 2010 for further testing. The F5 was greenhouse pot-tested again with ‘Muller’ and ‘Beltran’ *M. incognita* isolates in early 2011 to confirm presence of the CB3 gene and *Rk* genes. The F5 was also tested in a non-nematode field in 2011 for single plant selection. F6 seeds from selected plants were tested in inoculated growth pouches to select for broad-based nematode resistance. The resistant plants were transferred to greenhouse pots for production of F7 seeds, from which 20 N lines were planted in single rows at KREC in 2012. Seven N lines with good seed size were re-tested at KREC and UC-R in 2013. The best five of these were evaluated in the 2014 KREC and Tulare Co. advanced yield trials.

Table 1. Advanced blackeye breeding lines and their selection history that were tested in replicated trials in 2011 - 2013 and re-tested in 2014 at Kearney REC.

2010 Selection	2009 Origin	2008 Origin	2007 Origin	Pedigree
10K-19	09Sh-13-9	08Sh-39-3	07Sh-60-2	CB27/CB50
10K-29	09Sh-31-9	08Sh-73 (DLS)	07Sh-107-3	Sh 65/UCR53
10K-77	Greenhouse	Greenhouse	Greenhouse	CB27/CB5//CB5^2-7-2-3
10K-115	Greenhouse	Greenhouse	Greenhouse	CB27/CB5//CB5^2-70-1-1

Table 2 lists the grain yield, 100-seed weight and root-galling reactions to two species of root-knot nematodes garnered from the KREC and Tulare Co. 2014 trials. The KREC trial was planted on May 29, machine-cut on October 6 and harvested using a modified CB Hay Junior on October 30. The Tulare Co. trial was planted on June 6 and hand-harvested on October 2. The trials had a higher Coefficient of Variation for grain yield than for 100-seed weight (9-11% for grain yield and 3-5% for seed weight). Yield was higher than observed in 2013: CB46 yielded 3789 and 4243 lb/ac at KREC and Tulare Co., respectively. Yield of the four advanced lines described in Table 1 ranged from 4433 to 5146 lb/ac at KREC and 3519 to 4172 at Tulare Co., with 10K-77 being the top performer. Grain size of these lines were significantly larger than CB46 (Table 2). Yield of the N lines ranged from 4499 to 5212 at KREC and from 3910 to 4523 lb/ac at KREC and Tulare Co., respectively. Grain size of the N lines was similar to or slightly larger than CB46 across both field trials (Table 2). These data indicate that the advanced lines have good performance potential across locations. Line 10K-77 had the second highest yield at KREC (5146 lb/ac) and equivalent yield to CB46 in Tulare (4172 lb/ac), coupled with significantly larger grain size than CB46. Among the N lines, N17 was the highest yielding line at KREC (5212 lb/ac) and similar in yield to CB46 in the Tulare trial (4121 lb/ac). N2 and N5 also had high yields, with N2 having the highest yield (4523 lb/ac) in the Tulare commercial field trial and grain size the same as CB46. These top-performing lines also ranked higher for yield than CB50, but with not as large grain size as CB50. These top lines will be tested in multi-location strip trials in 2015.

The assays for Fusarium wilt Race 4 resistance indicated that lines K10-29, N2, N5, and CB46Rk2 have Race 4 resistance while the other lines are Race 4 susceptible. The assays for root-knot nematode resistance indicated that the five N lines, 10K-77 and 10K-115 hade stronger broad-based nematode resistance than CB46 (Table 2). In the 2014 yield trials conducted on the KREC nematode-infested field sites (Table 3), these lines also showed the broader nematode resistance effective against the *M. javanica* population. In those trials the CB46NIL-Null line is a CB46 type without the *Rk* gene, representing a fully susceptible control for response to nematode infection. CB46 contains resistance gene *Rk*, which is effective against the *M. incognita* ‘Project 77’ isolate but less effective in suppressing *M. javanica* (Table 3). The grain yields of the N lines were equivalent or higher than the CB46 yield in both nematode trials as expected, with similar grain size to CB46. The much lower yield of the susceptible CB46NIL-Null line indicated the dramatic effects of root-knot nematode infection in the absence of resistance genes (Table 3).

Selecting new advanced and early generation blackeye breeding lines in nurseries:

Seed from 75 F_2 lygus-resistant plants selected in 2012 was planted in 75 single rows in a nursery at KREC under unprotected (no insecticide) conditions in 2013. From these, 17 rows with high pod abundance were hand-harvested. Seed bulks (F2-derived F3 family seed) from each single row were grown at KREC in 2014 in double-row nursery evaluations under lygus pressure. Two families (2014-447-05 and 2014-447-06 from the 2011 cross 07KLN-74-4 x 07KLN-46-4) with high pod abundance and best quality grain were selected. Seed bulks (F2-derived F4 family seeds) will be grown under lygus pressure for single plant selection in 2015.

Developing lygus resistant blackeyes:

Restrictions on the use of currently available insecticides are likely to increase, and few new insecticides for minor crops such as blackeyes may be available due to the high cost of pesticide registration. Therefore, insect-resistant blackeye varieties may become very important in the near future to maintain high grain quality and yield levels. The Lygus bug damages blackeye crops in two ways. First, feeding on young floral buds causes these buds to drop (‘bud-blasting’) which can drastically reduce pod set and grain yield. Second, feeding by lygus during grain/pod development leads to pitted and discolored grains. Therefore, we use measures of pod-set and grain yield to evaluate the ‘bud-blasting’ resistance of genotypes and direct measures of grain damage to evaluate resistance to pod feeding. Screening begins with growing single- or two-row plots of new accessions and breeding lines in unprotected nurseries in locations such as the Kearney REC (see previous section), where strong attacks from lygus often occur. Selections are made and then tested under unprotected conditions in larger, replicated plots, and from further selections smaller numbers of lines are evaluated for grain yield in unprotected and protected plots with at least four replications.

From four lines evaluated under protected and unprotected plots in 2010 to 2013, we re-tested the best three lines in comparison with checks (CB46, CB50 and CB27) in 2014 at KREC under protected and unprotected conditions. These lines resulted from a long-term breeding effort to combine lygus resistance with high quality grain and high yield potential. The 2014 KREC trial was planted on May 29, and harvested 96 days later (September 2) by hand-picking pods from one middle row of a four-row plot. Lygus pressure was heavy but arrived late in 2014, resulting in grain yield loss of between 10% and 30%, in comparisons between protected and unprotected conditions (Table 4). The experimental lines had significantly higher protected yield than CB46 indicating they have high innate yield potential. However, the insecticide protection was unable to keep up with the late season lygus attack and this also contributed to the relatively lower yield of CB46 in protected plots. The unprotected yields were significantly higher than CB46 for all three advanced lines, indicating strong yield ability under lygus pressure (Table 4). Some of the variability in results from year to year reflects differences in the amount and timing of lygus infestation, as well as variations in seasonal growing conditions, and emphasizes the need for multiple year and location testing for the more promising advanced lines. Line 07KN-74 has performed well over multiple trials and in 2014 had the highest protected and unprotected plot yields and a low lygus damage loss in unprotected plots at KREC (Table 4). However, this line cuts out early (single flush only) and thus yield is not directly comparable to CB46 which was full season. The Lygus ‘sting’ damage to harvested grain was not significantly different among the test lines and the CB46 and CB50 checks, ranging from 13 to 17% (Table 4).

Six crosses between blackeye varieties CB46, CB50 and CB27 and lygus resistant breeding lines (07KN-74 and 09KLN-1-9) were made in the greenhouse during late 2013 and the F1s grown out during the 2013-2014 winter to produce F2 seed. F2 seed from each cross was planted under protected and unprotected conditions at Kearney in 4-row plots. Among these, the CB27 x 09KLN-1-9 F2 population showed a clear difference between the protected and unprotected plots and thus was selected for further investigation. Leaf samples for SNP genotyping assays and seeds from 136 F2 plants with clear symptoms (susceptible or resistant) were collected for single-row evaluations in 2015.

Developing aphid resistant blackeyes:

Cowpea aphids are prevalent in the Central Valley and cause severe damage on cowpea plants from the early vegetative stage to flowering and pod development stages. All current California blackeyes are susceptible to this pest, so development of resistant varieties will help increase on-farm yield with reduced insecticide application. During the last few years, efforts have been made to identify sources of aphid resistance and two cowpea genome regions conferring resistance were discovered using a genetic mapping population derived from the cross between a susceptible blackeye CB27 and a resistant African breeding line IT97K-556-6. Since African resistant cowpeas are non-blackeyes and flower poorly in the Central Valley during summer, marker-assisted backcrossing (MABC) was initiated in 2013 to introduce the aphid resistance genes into California blackeyes (CB46, CB50 and CB27). Up to five cycles of MABC were made in UCR greenhouses during 2013-2014 to generate new BC5 breeding lines that resemble California blackeyes and carry aphid-resistance genes from IT97K-556-6. These plants will be marker-genotyped in early 2015, and seeds from selected plants identified as fixed (homozygous) for resistance alleles at both resistance loci will be tested at KREC in 2015 for line development.

Table 2. New blackeye breeding lines and checks tested at Kearney REC and Tulare in 2014: grain yield, 100-seed weight, galling ratings from 2014 field screening with root-knot nematodes *M. incognita, M. javanica,* and *M. incognita* Muller, and 2014 greenhouse screening with Fusarium Race 4.

Entry	KREC Yield (lb/ac)	Tulare Yield (lb/ac)	KREC 100 seed wt (g)	Tulare 100 seed wt (g)	Galling M. incognita	Galling M. javanica	Galling M. incognita Muller	Fusarium Race 4 index
N17	5212	4121	20.4	23.0	1.1	1.2	3.1	4.8
10K-77	5146	4172	22.6	25.3	0.9	1.7	3.7	5.0
N2	5106	4523	19.5	22.7	1.2	1.2	3.8	0.2
N5	4979	4132	19.5	22.4	0.8	1.5	3.5	0.8
N20	4974	4069	19.5	23.1	0.9	1.0	3.5	5.0
CB50	4589	3840	23.4	25.1	-	-	-	0.0
10K-29	4548	4072	22.2	23.3	2.4	2.9	4.2	0.0
N16	4499	3910	20.1	23.2	0.8	0.8	3.4	4.5
10K-19	4439	4144	20.8	25.0	1.3	2.9	4.1	5.0
10K-115	4433	3519	22.4	25.4	1.0	1.6	2.5	5.0
CB46Rk2	4288	4329	18.3	20.6	1.0	2.7	2.9	0.0
CB46	3789	4243	19.2	22.1	1.6	3.4	4.2	4.9
Mean	4667	4105	20.7	23.4				
CV(%)	11	9	5	3				
LSD(0.05)	754	530	1.4	1.0				

Kearney REC trial planted on May 29 and cut on October 6 (130 days).
Tulare trial planted on June 6 and hand-harvested on October 2 (118 days).
Root-galling score on scale of 0 (no galling) to 8 (severe galling).
Fusraium wilt disease index (0 to 5; where 0 = no wilt symptoms and 5 = plant death).

[1]Philip A. Roberts, Bao Lam Huynh, and William Matthews, Dept. of Nematology, University of California, Riverside, CA 92521; Carol Frate, UCCE, Tulare Co. CA. Email: philip.roberts@ucr.edu, baolam.huynh@ucr.edu ***In:*** University of California Dry Bean Research 2014 Progress Report published by the California Dry Bean Advisory Board, 531-D North Alta Avenue, Dinuba CA 93618, and available at http://beans.ucanr.org/.

Table 3. New blackeye lines and checks evaluated in specially managed plots infested with either *Meloidogyne incognita* (Field 77) or *M. javanica* (Field 811) root-knot nematodes at KREC in 2014.

Entry	Field77 Yield (lb/ac)	Field811 Yield (lb/ac)	Field 77 100 seed wt (g)	Field 811 100 seed wt (g)	Galling *M. incognita*	Galling *M. javanica*
CB46NIL-RkRk2CB3	4935	3428	19.4	19.8	3.0	3.8
N16	4800	3206	21.0	21.2	3.2	4.0
N5	4773	3160	20.5	20.1	3.0	4.2
N2	4768	3229	20.0	20.4	3.0	3.9
N17	4737	3163	20.8	21.0	3.1	4.1
N20	4511	3030	20.5	20.7	3.2	3.9
CB46	4480	2969	20.6	21.2	3.6	5.2
CB46-Rk2	4420	3297	19.5	19.5	3.2	3.9
CB46NIL-RkCB3	4377	2727	20.7	21.0	3.9	5.6
CB46NIL-Null	3406	1784	19.4	19.4	6.4	7.8
Mean	4521	2999	20.2	20.4	3.5	4.6
CV(%)	14	11	3	3	13	18
LSD(0.05)	812	418	0.7	0.7	0.6	1

Trial planted on May 29 and cut on October 6 (130 days). Root-galling score on scale of 0 (no galling) to 8 (severe galling).

Table 4. Grain yield, 100-seed weight, and lygus grain damage of 3 advanced blackeye lines, CB46, CB50 and CB27 when grown under insect-protected and unprotected conditions at Kearney REC in 2014.

Line	Yield (lbs/ac)			100-seed weight (g)		*Lygus damage (%)
	Protected	Unprotected	Loss (%)	Protected	Unprotected	
07KN-74	3537	3083	13	20.2	21.6	17
09KLN-1-9	3359	2767	18	18.3	19.0	15
09KLN-2-30	3340	3015	10	18.8	18.8	13
CB27	3499	2764	21	22.0	22.6	14
CB46	2127	1549	27	19.8	20.0	16
CB50	2298	1612	30	23.0	24.4	14
Mean	3026	2465	19	20.4	21.1	15
CV(%)	9	15	80	6	5	28
LSD(0.05)	309	425	18	1.4	1.3	4.9

Kearney trial planted on May 29 and hand-harvested on September 2 (96 days). *Lygus damage on grain measured in the unprotected plots.

[1]Philip A. Roberts, Bao Lam Huynh, and William Matthews, Dept. of Nematology, University of California, Riverside, CA 92521; Carol Frate, UCCE, Tulare Co. CA. Email: philip.roberts@ucr.edu, baolam.huynh@ucr.edu ***In:*** University of California Dry Bean Research 2014 Progress Report published by the California Dry Bean Advisory Board, 531-D North Alta Avenue, Dinuba CA 93618, and available at http://beans.ucanr.org/.

Recent Publications:

1. Lucas MR, Ehlers JD, Roberts PA, Close TJ. 2012. Markers for quantitative inheritance of resistance to foliar thrips in cowpea. *Crop Science* 52:2075-2081.
2. Pottorff M, Li G, Ehlers JD, Roberts PA, and Close TJ. 2012. Genetic mapping, synteny, and physical location of two loci for *Fusarium oxysporum* f.sp. *tracheiphilum* race 4 resistance in cowpea [*Vigna unguiculata* (L.) Walp]. *Molecular Breeding*: DOI 10.1007/s11032-013-9991-0. Pp 1-13.
3. Lucas MR, Huynh BL, Ehlers JD, Roberts PA, Close TJ. 2013. High-resolution SNP genotyping reveals a significant problem among breeder resources. *The Plant Genome* 6(1):1-5.
4. Muchero W, Roberts PA, Diop NN, Drabo I, Cisse N, Close TJ, Muranaka S, Boukar O, Ehlers JD. 2013. Genetic architecture of delayed senescence, biomass, and grain yield under drought stress in cowpea. *PLoS ONE* 8(7): 1-10. e70041. doi:10.1371/journal.pone.0070041
5. Huynh BL, Close TJ, Roberts PA, Cissé N, Drabo I, Boukar O, Lucas MR, Wanamaker S, Pottorff M, Ehlers, JD. 2013. Gene pools and the genetic architecture of domesticated cowpea. *The Plant Genome* 6:1-8.
6. Huynh BL, Ehlers, JD, Close TJ, Cissé N, Drabo I, Boukar O, Lucas MR, Wanamaker S, Pottorff M, Roberts PA. 2013. Enabling tools for modern breeding of cowpea for biotic stress resistance. Pp. 183-200 *in* Translational Genomics for Crop Breeding, Vol. I: Biotic Stress. Varshney R, Tuberosa R, eds. Wiley-Blackwell, USA. 368 pp.
7. Huynh BL, Ehlers JD, Ndeve A, Wanamaker S, Lucas MR, Close TJ, Roberts PA (2015) Genetic mapping and legume synteny of aphid resistance in African cowpea (*Vigna unguiculata* L. Walp.) grown in California. *Molecular Breeding* 35:1-9

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