

BLACKEYE VARIETAL IMPROVEMENT - 2020 PROGRESS REPORT

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ABSTRACT

Our breeding program, supported by the California Dry Bean Advisory Board, is developing improved blackeye cowpea bean varieties for California growers. In 2020, work was conducted under the following objectives:

Evaluating experimental breeding lines for grain yield, grain quality and agronomic characteristics: A trial comparing yield and grain quality of eight new blackeye breeding lines together with CB46Rk2 and CB46 was conducted under long-season, double-flush production conditions at the Kearney Research and Extension Center (KREC). Some promising lines were evaluated together with CB46 and CB5 in large strip plots in commercial blackeye fields in Tulare, Yolo, and Colusa Counties, respectively. The varietal candidate, 2014-008-51-77, a new version of CB46 with improved aphid resistance, had higher or equivalent grain yield and quality to CB46 across two field locations in 2020 and eleven other field locations during 2016-2019. It also showed less lygus damage than CB46 and CB5 in the 2020 trials in Tulare, Yolo (UC Davis), and Colusa Counties. This line is being registered as a new cultivar “California Blackeye 77” (CB77).

Advanced lygus resistant blackeye lines: Lygus-tolerant lines first selected in 2007-2009 were evaluated under insect protected and unprotected conditions at Kearney during 2010 to 2019. These lines resulted from a long-term breeding effort to combine lygus resistance with high quality grain and high production. Selection and testing over this period resulted in choosing a best line, 07KN-74, for larger scale testing. This varietal candidate yielded equivalently to CB46 under single-flush production in East Tulare Co., and outperformed CB5 and CB46 in Colusa Co. and at UC Davis, respectively. One new promising line derived from single plant selection was tested in 2020 under long-season double-flush production at KREC in comparison with 07KN-74. Lygus pressure was not heavy at KREC in 2020, resulting in grain yield loss up to 5% in the checks CB46 and CB46Rk2 in comparison with the protected conditions in the same field. At the nursery level, single plants of the CB46 x 07KN-74 F4:8 bulk were selected for line development.

Fusarium wilt resistance breeding: Aphid resistant advanced lines derived from CB46 (resistant to Fusarium wilt race 3) and CB50 (resistant to Fusarium wilt races 3 and 4) were developed through marker-assisted backcrossing (MABC) in 2012-2014 and yield-tested in 2015-2017. They were intercrossed in 2016 to recombine resistance to both Fusarium wilt races 3 and 4. F2-derived F3 seeds were harvested at UCR in 2017 for single-seed descent phenotyping in 2018 until F5. The F5 lines were phenotyped in spring 2019, and resistant lines were tested in summer 2019 and 2020. Likewise, crosses were made in 2017 between CB5 (susceptible to Fusarium wilt and aphid) and CB27 and CB77 as donors for Fusarium wilt races 3 and 4 and aphid resistance, for use in MABC starting in 2018. Their third backcross cycle was made, and BC3F1 plants were intercrossed to stack all QTLs, followed by SNP genotyping, for line development using markers in 2021.

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

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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.

PROJECT OBJECTIVES AND PROGRESS

Evaluating advanced blackeye breeding lines for grain yield and quality, and agronomic characteristics:

At Kearney Research and Extension Center (KREC), a four-replicate trial was conducted in the 2020 main season comparing yield and grain quality of 8 new blackeye breeding lines, together with varietal candidate line CB46Rk2 and check CB46. The new breeding lines included one near-isogenic line (NIL) of CB46 with better nematode resistance and grain size, one NIL of CB46 carrying 2 aphid-resistance quantitative trait loci (QTL), 2 lygus-resistant lines including 07KN-74 and one selected from the 2016 nursery plots, and 4 other lines with resistance to aphid and fusarium wilt races 3 and 4. Each experimental plot was 4 rows x 16-feet long. Separate root-knot nematode evaluations of some of these lines were previously conducted at South Coast REC in 2012 - 2016 in specially managed plots infested with either *Meloidogyne incognita*, *M. javanica*, or *M. incognita* Muller (*Rk* virulent) root-knot nematodes. In 2019 greenhouse screening with Fusarium Race 4 was conducted. Two strip trials were conducted in Tulare County commercial blackeye fields. In the first trial (cooperating grower Rick Borges), one CB46-backcrossed NIL with aphid-resistance QTLs (2014-008-51-77) and check CB46 were arranged in a randomized complete block design with 3 replications; each line was planted in a plot of 6 rows on 38-inch row-spacing and 1267 feet in length. In the second trial (cooperating grower Darren DeCraemer), 2014-008-51-77, one lygus-resistant line (07KN-74), and one CB46-backcrossed NIL with nematode-resistance (N2) were planted together with check CB46; each experimental line was planted in a plot of 6 rows on 30-inch row-spacing and 1070-1448 feet in length. Two other strip trials were conducted in Colusa Co. a commercial blackeye field (cooperating grower Jeff Miller) and at UC Davis. In Colusa Co., 2014-008-51-77, 07KN-74, N2 and checks CB46 and CB5 were planted in one 30-inch row of 1200 feet in length. At UC Davis, 2014-008-51-77, 07KN-74, N2 and check CB46 each was planted in a block of two 30-inch rows on a 5-foot bed and 300 feet in length.

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 growth-pouches and pots in UCR 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 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 UCR in 2013, five lines re-tested in 2014, and the best three lines re-tested in 2015.

The best two of these were evaluated in the 2016-2019 KREC performance trials, and one of these lines (N2) was tested in the 2020 KREC trials (see Tables 1 and 3).

The aphid-resistant NILs were developed through marker-assisted backcrossing (MABC). 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 an aphid 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, 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. In early 2015, 180 BC5F2 plants per population were marker-genotyped, and seeds from 18 selected plants identified as fixed (homozygous) for resistance alleles at both resistance loci were tested at KREC in 2015 for line development. Three lines from crosses with CB46 (2014-008 lines) and three from crosses with CB50 (2014-010 lines) with the highest pod abundance and best quality grain selected in 2015 were evaluated in the 2016-2018 KREC replicated trials. Two best performing lines (2014-008 lines) were evaluated in the KREC replicated trial and in grower fields in Tulare, Colusa and Sutter Counties in 2019. The best performing line (2014-008-51-77) was evaluated in the KREC replicated trial and in grower fields in Tulare and Colusa Counties in 2020.

Table 1 lists the grain yield and 100-seed weight of the lines in the KREC 2020 trial grown under full-season, insect-protected conditions. The trial was planted on June 24, machine-cut on October 23 and harvested using a combine harvester on November 17. CB46 yielded 2387 lb/ac, which was lower than that in 2019 (3827 lb/ac) in part due to heat waves during the flowering stage. Yield of the entries ranged from 1685 to 2973 lb/ac at KREC, with nematode-resistant lines (CB46rk2 and N2), the CB46 aphid-resistant NIL (2014-008-51-77), and two fusarium and aphid -resistant lines (2016-017-036-4-3 and 2016-017-055-5-1) being the top performers or equivalent to CB46 ($P < 0.05$). Four lines evaluated at KREC were also tested in strip trials in production fields in Tulare and Colusa counties and at UC Davis. One of the Tulare Co. strip trials was planted in Darren DeCreamer's field on June 18, machine-cut on September 24 and harvested on October 7 (single-flush production). The other trial in Tulare Co. was planted in Rick Borges' field on June 16, machine-cut on October 15 and harvested on November 2 (double-flush production). The Colusa Co. strip trial was planted in Jeff Miller's field on May 30, machine-cut on August 24 and harvested on September 22 (single-flush production). The strip trial at UC Davis was planted on June 10, machine-cut on August 31 and harvested on September 22 (single-flush production). In the Tulare Co. production field strip trials, the advanced aphid-resistant line 2014-008-51-77 performed comparably to the CB46 check under both single and double-flush conditions (Table 2). In the Colusa Co. strip trial, 2014-008-51-77 and 07KN-74 out-performed the check CB5 in grain yield (Table 2). They showed resistance to aphids and less lygus stings compared to CB5.

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

Reciprocal crosses were made in 2016 between 2014-008-51-77 and 2014-010-41-43, both of which are homozygous for two aphid-resistance QTLs from the African cowpea line IT97K-556-6. The line 2014-008-51-77 has the CB46 background, which has resistance to Fusarium wilt race 3 but not Fusarium wilt race 4. The line 2014-010-41-43 carries the CB50 background, which has resistance to both Fusarium wilt races 3 and 4. F2 seeds were planted in the field at UCR in 2017. Seeds of the F2-derived F3 generation were harvested and phenotyped for resistance to Fusarium wilt race 4 in a UCR greenhouse during spring 2018. F4 seeds collected

from the resistant F3 plants were further phenotyped for Fusarium wilt race 4 resistance during fall 2018. F5 seeds derived from resistant F4 plants were phenotyped in spring 2019. F6 seeds collected from 30 resistant F5 plants were tested in single rows together checks CB46 at KREC in 2019, from which a subset of four lines with higher or equivalent grain yield and quality to CB46 were selected for replicated trials in 2020. Two better performing lines in 2020, 2016-017-036-4-3 and 2016-017-055-5-1 (Table 1), are being seed increased for replicated trials at KREC in 2021 and in grower production fields in 2022.

Developing lygus resistant blackeyes:

Restrictions and issues of pesticide resistance 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 (no insecticide) 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.

One top-performing line from the 2016 single-plant selections was tested together with one aphid-resistant line (2014-008-51-77) and two nematode-resistant lines (N2 and CB46Rk2) in comparison with checks (CB46 and 07KN-74) in 2020 at KREC under insect-protected and unprotected conditions. Each experimental plot was 4 rows x 16-feet long. The trial was planted on June 24, machine-cut on October 23, and harvested on November 17 by a combine harvester from the two middle rows of each four-row plot. Lygus pressure was not heavy, resulting in grain yield loss of only 6% or less in the check varieties, in comparisons between protected and unprotected conditions; there were no significant differences ($P < 0.05$) in yield losses between varieties (Table 3). Except for 16KN-06-2-1 and 07KN-74, most other experimental lines had equivalent or higher yield than CB46 under both protected and unprotected conditions. The lygus 'sting' damage to harvested grain ranged from 4.9 to 6.8%, with no significant difference ($P < 0.05$) between varieties (Table 3). 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 these promising advanced lines.

For marker-assisted breeding, 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 KREC in 2014 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. At KREC in 2015, ten F2:3 plants showing contrasting symptoms (5 with very low pod set, 5 with very high pod set) were leaf-sampled and genotyped with the 60K SNP iSelect marker platform to identify polymorphic

markers that may be associated with lygus resistance. However, we did not find any SNP loci that differentiated the two phenotypic groups, indicating that lygus resistance is not simply inherited and may involve multiple genes with minor effects. Therefore, we are applying a genomic-selection approach instead of MABC. F3-bulked seeds from the cross between CB46 and 07KN-74 were planted under unprotected conditions in 2017. One pod from each plant with high pod-abundance was hand-harvested and bulked. These seeds were planted in UCR greenhouses in early 2018. F5 seeds from 136 single F4 plants were collected, and leaf tissues collected for DNA extraction for use in future genotyping and marker development. These lines and an additional set of 100 F2 and F5 lines from the CB46 x 07KN-74 cross were grown in single row plots under insect-protected and unprotected conditions at KREC in 2020. Aerial canopy images of these plots were taken by a UAV (drone) and are being subjected to analysis of vegetation index (as an indirect measure of lygus resistance) in collaboration with Alireza Pourreza and German Zuniga-Ramirez based at KREC. For each plot, one pod per plant was collected and seeds were bulked for further screening in 2021.

Adding Fusarium wilt resistance to CB5 and aphid resistant advanced lines:

Foundation seeds of CB5 were genotyped with the 60K SNP iSelect assay in 2017. F1 crosses were then made between CB5 and both CB27 and CB50 as resistance donors for Fusarium wilt races 3 and 4. The aphid resistant lines 2014-008-51-77 and 2014-008-51-82 were also crossed with CB27 and CB5. F1 seeds were grown for marker-assisted backcrossing (MABC) to their recurrent parents (CB5 and aphid-resistant lines) during spring 2018. Polymorphic SNP markers flanking two resistance QTLs (to races 3 and 4) and at least 2-cM intervals genome-wide were used for selecting donor QTL haplotypes (foreground selection) and the recurrent-parent genome (background selection). The second MABC cycle was made during fall 2018. The third MABC cycle was made during spring 2019, and BC3F1 plants were intercrossed in 2020 to stack aphid and fusarium resistance QTLs, followed by selfing and SNP genotyping to select for near-isogenic lines with homozygous resistance QTLs, for nursery screening at KREC in 2021.

Table 1. Grain yield and seed size of new blackeye breeding lines and checks tested in a replicated uniform trial at Kearney REC in 2020.

Entry	Yield (lb/ac)	Seed size (g/100 seeds)
CB46rk2	2973	22.2
N2	2812	21.4
2016-017-036-4-3	2697	22.0
2016-017-055-5-1	2572	23.5
2014-008-51-77 (CB77)	2515	21.8
CB46	2387	22.2
2016-016-042-1-5	2017	22.4
16KN-06-2-1	1827	21.0
2016-016-012-2-4	1794	22.9
07KN-74	1685	22.5
<i>Mean</i>	2326	22.2
<i>CV%</i>	11.0	2.5
<i>LSD (0.05)</i>	371	0.8

Trial planted on June 24, cut on October 23 (121 days), and machine-harvested on November 17.

Table 2. New blackeye lines and checks CB46 and CB5 tested in large strip trials in grower production fields (Trials I, II and III) and at UC Davis (Trial IV) in 2020.

Parameter	Trial I	Trial II	Trial III	Trial IV	Mean ± SE
Location	Tulare	Farmersville	Colusa	Davis	
Area (ac)	3.3	1.9	0.28	0.12	
Yield (lbs/ac)					
2014-008-51-77	4433	2545	3031	NI	3336 ± 566
07KN-74	NI ^a	2665	2732	2100	2499 ± 200
N2	NI	2368	1626	556	1517 ± 526
CB5	NI	NI	2340	NI	2340
CB46	4182	2697	NI	1471	2783 ± 784
100-seed wt (g)					
2014-008-51-77	20.8	20.4	20.0	NI	20.4 ± 0.23
07KN-74	NI	22.4	20.6	22.5	21.8 ± 0.62
N2	NI	19.9	21.7	20.7	20.8 ± 0.52
CB5	NI	NI	27.7	NI	27.7 ± 0.00
CB46	20.9	21.2	NI	21.1	21.1 ± 0.09
Lygus damage (%)					
2014-008-51-77	2.8	1.5	2.0	NI	2.1 ± 0.38
07KN-74	NI	1.4	4.0	34.7	13.4 ± 10.69
N2	NI	3.6	7.3	53.0	21.3 ± 15.89
CB5	NI	NI	7.0	NI	7.0
CB46	5.1	3.2	NI	38.3	15.5 ± 11.40

Trial I planted on June 16, cut on October 15 (121 days), and machine-harvested on November 2.

Trial II planted on June 18, cut on September 24 (98 days), and machine-harvested on October 7.

Trial III planted on May 30, cut on August 24 (86 days), and machine-harvested on September 22.

Trial IV planted on June 10, cut on August 31 (82 days), and machine-harvested on September 22.

^a NI, not included because the variety was either in outer rows or not planted due to insufficient seed.

Table 3. Grain yield, 100-seed weight, and lygus grain damage of advanced blackeye lines and checks CB46 and CB46rk2 grown under insect-protected and unprotected conditions at Kearney REC in 2020.

Line	Yield (lbs/ac)			100-seed weight (g)		Lygus damage ^a (%)
	Protected	Unprotected	Loss (%)	Protected	Unprotected	
CB46rk2	2987	2808	5	22.3	22.6	5.8
N2	2437	2270	6	22.3	22.3	4.9
CB46	2424	2296	4	22.5	22.6	5.9
2014-008-51-77	2406	2286	7	22.3	22.0	6.7
07KN-74	1828	1868	-6	22.7	23.7	5.7
16KN-06-2-1	1664	1560	6	22.4	22.2	6.8
Mean	2249	2140	4	22.4	22.6	5.9
CV%	15	19	94	2.3	2.0	28
LSD(0.05)	568	666	NS ^β	NS	0.7	NS

Trial planted on June 24, cut on October 23 (121 days), and machine-harvested on November 17.

^a Lygus damage on grain measured in the unprotected plots.

^β NS, not significant according to LSD test of significance with $P \leq 0.05$.

Recent Publications (2017-2020)

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- Long RF, Light S (2020) New California blackeye varieties show resistance to cowpea aphid, UC Dry Bean Blog <https://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=43376>
- Light S, Long RF (2020) Why aren't my crops drying down? UC Dry Bean Blog <https://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=43708>
- Long RF, Vinchesi-Vahl A (2020.) Managing root-knot nematodes in crop production. UC Dry Bean Blog, <http://beans.ucanr.org/?blogpost=43735&blogasset=91063>
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