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ORIGINAL ARTICLE

Evaluation of A3 cytoplasmic male sterile forage sorghum lines for resistance to sugarcane aphid

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Abstract

Main conclusion **Three known sugarcane aphid-resistant pollinator parents were sterilized in A3 cytoplasmic male sterility and were confirmed in this study to be resistant to sugarcane aphid allowing for the development of sugarcane aphid-resistant forage hybrids.**

Abstract We utilized A3 cytoplasmic male sterility and converted known sugarcane aphid-resistant sorghum TX 2783, and newly released R. LBK1 (Reg. No. GP-865, PI 687244) and R. LBK2 (Reg. No. GP-866, PI 687245) into A3 sterility to determine if the sterile counterparts would also equally express tolerance and or antibiosis to sugarcane aphid. Free-choice flat screen trials and life-table demographic studies were utilized and compared to know susceptible/fertile entries KS 585, and TX 7000, and known resistant/fertile entries TX 2783 and DKS 37-07. The R. LBK1 fertile entry was more tolerant than the known susceptible entries KS 585 and TX 7000, but was not as resistant as the other resistant entries, sustaining a damage rating of 6.0 across two different screen trials. The sterile A3 R. LBK2 showed a greater tolerance and expressed higher levels of antibiosis during aphid reproductive studies when compared to the known resistant and fertile TX 2783. All other fertile (R. LBK2, TX2783) and the A3 male sterile counterparts (A3 R. LBK2, A3 TX2783) were very similar in expression of high levels of tolerance and exhibited statistically similar damage ratings of 3.3–4.3 when exposed to sugarcane aphids. No entry, either fertile or sterile, was as tolerant as DKS 37-07, a known resistant commercial hybrid. Other plant measurements including percent loss in chlorophyll content, difference in plant height, and number of true leaves for sugarcane aphid infested versus non-infested were very consistent and highly correlated with damage ratings. Antibiosis was also exhibited in both fertile and sterile versions of the resistant lines. There was a 2 × reduction in fecundity between the R. LBK1 fertile and its sterile A3 R. LBK1 when compared to the susceptible KS 585 and TX 7000; however, the remaining fertile and sterile entries had 3.8 × to 5.8 × decrease in fecundity when compared to the susceptible KS 585 and TX 7000. Other measurements in life-table statistics such as nymphs produced/female/d, and the intrinsic rates of increased were significantly lower for all fertile and sterile lines, showing that antibiosis significantly affected sugarcane aphid reproduction. In conclusion, the A3 cytoplasmic male sterility shows consistency for maintaining the single dominant trait SCA-resistant trait of TX 2783 for expressing both antibiosis and tolerance, and great utility in the development of sugarcane aphid-resistant forage sorghums.

Keywords Aphid · Plant resistance · Plant breeding · Back-crossing · Sorghum germplasm · Fecundity

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Abbreviations

SoSCA Preferred sorghum biotype of the sugarcane aphid

SuSCA Preferred sugarcane biotype of the sugarcane aphid

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the top five cereal crops produced worldwide (Mundia et al. 2019). The uses of sorghum range from feed and forage for livestock as a water-saving alternative to corn (Bean et al. 2013), a source for biofuel (Miron et al. 2007), syrup production as an alternative sweetener (Mercer et al 2011), alcohol fermentation (Mercer et al 2011; Maw et al. 2017), and grain for human consumption (Anjali et al. 2017; Mundia et al. 2019).

Grain sorghum production in the United States has been impacted by the sugarcane aphid (SCA), *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) across vast acreages beginning in the summer of 2013 (Armstrong et al. 2015; Bowling et al. 2016; Elliott et al. 2017), but was known to exist in Florida by 1977 (Denmark 1988) and identified on sugarcane in Louisiana in 1999 (White et al. 2001). In 2013, *M. sacchari* infested and reduced yield on sorghum crops in Liberty County South Texas (Bowling et al. 2016). Since the initial reports of damage in sorghum in 2013, the aphid has rapidly expanded its range (Kerns et al. 2015; Bayoumy et al. 2016) and it now colonizes 20 states annually across the sorghum belt.

Sugarcane aphids have been found colonizing and reproducing on Sudan grass, *Sorghum x drummondii),* Johnsongrass, (*Sorghum halepense* L.), Columbus grass, *Sorghum almum,* Parodi), Sugarcane (*Saccharun officinarum* L.) and Sorghum, *Sorghum bicolor* (L.) (Hall 1987; White et al. 2001; Armstrong et al. 2015; Harris-Schultz et al. 2021). Sugarcane aphids have also been observed on corn (*Zea maize* L.) and cotton (*Gossypium hirsitum* L.), but no survival and reproduction were observed (Bowling et al. 2016). The aphid overwinters in northern Mexico and south Texas on remnant sorghum and Johnsongrass (Bowling et al. 2016) with the lower and upper threshold temperature for fecundity estimated to be 9 and 32 °C (De Souza et al. 2019).

Sugarcane aphids collected from throughout the U.S. were phenotyped and genotyped and determined to be two biotypes; SoSCA, the sorghum preferred sugarcane aphid, and SuSCA, the sugarcane preferred sugarcane aphid (Paudyal et al. 2019). The two biotypes differ in genotype and differ in survival and reproduction when reared on a set of host plant differentials, namely, resistant and susceptible grain sorghums, Johnsongrass, *Sorghum halepense* (L.), Columbus grass, *Sorghum almum* (Parodi), and sugarcane *Saccharum officinarum* (L.). The two different biotypes (SoSCA, SuSCA) were easily differentiated by genotyping (Paudyal et al. 2019).

Within the U.S., the most damaging sugarcane aphid biotype found on sorghums is SoSCA; however, since its appearance into the U.S., a limited number of resistant grain sorghums have been developed that express resistance mechanisms including antibiosis, tolerance, combinations of the two, and antixenosis (Hayes et al. 2018; Mbulwe et al. 2016; Paudyal et al. 2018, 2020). Feeding by *M. sacchari* (SoSCA) to sorghum causes reduced plant height and plant biomass (Limaje et al. 2018; Backoulou et al. 2018), uneven growth of seed heads that may not produce grain from injury caused during anthesis (Rott et al. 2008), and, in some cases, death of the plant (Bowling et al. 2016).

The outbreak of *M. sacchari* in sorghum in 2013 initiated research to develop integrated pest management (IPM) options for the aphid. Host plant resistance to sugarcane aphid in sorghum germplasm has been identified in both commercial and parental breeding lines (Armstrong et al. 2015, 2017, 2018, Paudyal et al. 2018; Limaje et al. 2018; and Gonzales et al. 2019). Several sources were first identified from seedling screening in the greenhouse, followed by field evaluations and then breeding efforts for registration and release. In 2016, Tx3408 and Tx3409 were registered and released as seed parental lines developed and released by Texas AgriLife Research with sterile versions developed using the A1 cytoplasmic male sterility system (A1 CMS) (Mbulwe et al. 2016). In 2018, Peterson et al. (2018) continued with the release of nineteen lines RTx3410 through RTx3428 pollinator parents. Later in that same year, Hayes et al. (2018) registered and released an additional two lines R.LBK1 and R.LBK2 from the USDA-ARS Breeding program in Lubbock, TX. In terms of forage sorghum breeding, the industry commonly uses a small set of public seed parents (A/BTx623, A/BTx631, and A/BTx378) to produce forage and Sudangrass x sorghum hybrids (Rooney et al. 2011; Armstrong et al. 2017). These females are widely adapted and high yielding, but are not resistant to SoSCA. Therefore, unless the forage pollinator parent is SoSCA-resistant, the hybrid generated between the two inbreds will also be SoSCA susceptible, because SoSCA resistance is a dominant genetic trait (Hayes et al. 2018). One of the first resistant sources discovered for sugarcane aphid resistance was TX 2783, initially developed for greenbug C and E resistance with the dominant resistant trait originating from and SC110-9 a parent of TX 2783 (Peterson et al. 1984, 2018).

To broaden the genetic sources of SoSCA-resistant sterile sorghum, the USDA sorghum breeding program in Lubbock, TX recently sterilized three pollinator lines (TX 2783, R. LBK1, and R. LBK2) in the A3 cytoplasmic sterility system for the development of SoSCA-resistant forage sorghums using the dominant resistant gene originating from SC110-9

in TX 2783 (Hayes et al. 2018). The A3 cytoplasmic sterility system has been used in forage sorghum breeding for many years with the advantage of not having to worry about cross pollination in outdoor breeding nurseries (Worstell et al. 1984). The A3 system is uniquely different than the widely utilized A1 system in that many common A1 pollinator parents (i.e., RTx430, Tx2783) can be sterilized and used as seed parents in an A3 system. Many agronomic studies have shown no difference in forage hybrid yields utilizing A3 cytoplasm versus A1 cytoplasm (Pederson and Toy 1997; Hoffmann and Rooney 2013; Howad et al. 1999). Our research evaluated the sterile lines of A3TX2783, A3 R. LBK1, and A3 R. LBK2 to determine if the resistant trait to sugarcane aphid was maintained as it is in the fertile counterparts. The purpose of this research was to confirm the SoSCA dominant resistance trait from TX 2783 that expresses SC110-9 resistance found in the REMS1 (resistance to *Melanaphis sacchari*) region of chromosome 6 of sorghums, and if that dominant trait is carried over to sterile sorghums in the A3 sterilization backcrossing program (Wang et al. 2013).

Materials and methods

Sugarcane aphid resistance and sterile sorghum background

In 2017, two USDA sorghum lines R.LBK1 and R.LBK2 were identified as having tolerance and antibiosis to the sugarcane aphid (Limaje et al. 2018) and were registered and released (Hayes et al. 2018). Both R.LBK1 and R.LBK2 were developed using the pedigree method of plant breeding and are confirmed to be restorer lines. R.LBK1 has a pedigree of (SC56-14E/(86EO361/88BE2668)) and was originally tested as R.11259. SC56-14E is a fully converted caudatum landrace derived from IS12556 with good stay-green drought tolerance. 86EO361/88BE2668 is a line developed by and obtained from Texas AgriLife Research. The pedigree of 86EON361 is (R5646/SC326-6) and the pedigree of 88BE2668 is (Tx2783/(SC748/SC630)). R.LBK2 has a pedigree of (Tx2783/PI 567946) and was originally tested as R.11143. TX 2783 was released by Texas A&M AgriLife Research in 1984 (Peterson et al. 1984a, b). The pedigree of TX 2783 is complex (IS12610C/((((ROKY8/Tx2536)/SC110-9)/SC599)/SC110-14E)) and was originally selected for resistance to biotypes C and E greenbug, *Schizaphis graminum* (Rondani). TX 2783 has also been found to be cross-resistant to the sugarcane aphid (Armstrong et al. 2015, 2017, 2018). In 2017, Tx2783, R.LBK1, and R.LBK2 were crossed to a donor source of A3 cytoplasm (A3 RTx430) for the development of sterile A3 versions of the SoSCA-resistant pollinators. A total of four backcrosses were performed until the sterile line was phenotypically identical to the resistant pollinator parents. There was no SCA resistance screening included in the back-cross selection protocol. The results of the screening evaluations are presented here following the back-cross selections.

Aphid culture

A known biotype "SoSCA" of sugarcane aphid that were phenotyped and genotyped in 2019 (Paudyal et al. 2019) and maintained as parthenogenic female colony was collected from a post-harvested grain sorghum field near Bay City, Matagorda County Texas in August of 2013. This colony has been maintained at the USDA-ARS Stillwater, OK Laboratory by rearing them on susceptible TX 7000 sorghum seedlings in pots covered with sleeve cages in the greenhouse at temperatures ranging from 21 °C to 28 °C. The plants are grown under natural greenhouse light supplemented by two T-8 fluorescent lights. New sugarcane aphid colonies are transferred to new seedling plants every 2 weeks in the greenhouse to maintain viable colonies for experimentation.

Sorghum resistance trials for male sterile counterparts

Nine sorghum entries, including two known SoSCA-resistant sorghums TX2783 and DKS-3707 (Paudyal et al. 2018), and two known susceptibles TX 7000 and KS 585 (Paudyal et al. 2018), were evaluated in a free-choice flat screen trial. Also included were A3 sterile versions of TX 2783 labeled A3. TX 2783, R.LBK1, A3. R.LBK1, R.LBK2, A3 R.LBK2, and R.LBK2. The sorghum entries were planted in eight flats (plastic trays 60 cm × 90 cm with 128 individual cells, Growers Supply, Dyersville, IA 52042). Each entry was randomized and replicated 12 times using Research Randomizer (http://www.randomizer.org, 2020). Four of the eight flats were used for infesting, while a duplicate set of four flats were not infested for comparing plant growth characteristics. When the TX 7000 sorghum seedlings used for infesting were in the 4–5 leaf stage (approximately 20 cm in height), they were laid down each row and across each alley of the flats as reported by Starks and Burton (1977). By this procedure, all entries are placed under strong pressure from the infesting aphids, so that no ambiguity exists in the evaluation.

The measured variables for infested and non-infested sorghums were plant height (cm), number of true leaves excluding the lower cotyledon leaf, and difference in plant height between infested and non-infested plants. Difference in plant height is measured by subtracting an infested sorghum versus the same entry which is not infested and is more realistic in determining what the reduction in plant growth may have been due to aphid feeding. Total chlorophyll content

(chlorophyll a + b, Markwell et al. 1995) measured as µmol m^{-2} was estimated using an SPAD-502 chlorophyll meter (Minolta, Ramsey, NJ 07466). Three chlorophyll readings were taken from each entry that was infested and subtracted from the non-infested entries, so that the percent loss of total chlorophyll was calculated (C − T)/C × 100, where C is the SPAD measurement from the non-infested or control, and T is from infested plant. When the known susceptible TX 7000 was 90–100% dead based on the 16 replications of that entry, all plants in each flat were evaluated for damage using a rating of 1–9; where 1 is a completely healthy plant with no chlorotic tissue; 2 represents 1–5% chlorotic tissue; 3, 5–20%; 4, 21–35%; 5, 36–50%; 6, 51–65%; 7, 66–80%; 8, 81–95%; and 9 represents 95–100% chlorotic tissue (Burd et al. 1993). The variables of damage rating, plant height, difference in plant height, number of true leaves on a sorghum entry, and percent chlorophyll loss were subjected to PROC MIXED model analysis with sorghum entry means compared ($\alpha = 0.05$) using the least-squared means pair-wise comparisons at $P > |t| \leq 0.05$ level (SAS 9.4, SAS Institute 2016). This experiment was evaluated on December 19, 2020 and repeated on February 24, 2021 to check for consistency in results.

Sugarcane aphid demographics compared for male sterile sorghum counterparts

The reproductive life-table demographics of the SoSCA were compared for the male fertile TX 2783, R.LBK1, and R.LBK2 lines versus their A3 counterparts A3.Tx2783, A3.R.LBK1, and A3.R.LBK2. Also included for comparative purposes were the SoSCA-resistant DKS-3707, and the SoSCA known susceptibles TX 7000 and KS 585. A negative effect on the reproductive capacity of an aphid infesting a plant in a no-choice environment determines the expression level of antibiosis (Smith 2005).

For the evaluation of antibiosis, two seeds of each entry listed above were planted in cone-tainers™ (model SC10, S7S greenhouse supply, Tangent, Oregon 97389) in a three-layer media of potting soil, fritted clay, and sand from bottom to top, respectively. Each cone-tainer™ seeded with an individual entry was considered one of 12 replicates, representing a total of 108 individual containers. Each cone-tainer™ was fitted with an 8 cm-diameter Lexan sleeve, 45 cm in height and ventilated with organdy cloth. The cone-tainers™ were placed in a rack to hold them upright in a completely randomized design inside a growth chamber Conviron®, Winnipeg, Canada) set at 21 °C and 14:10 L:D photoperiod with lighting provided by seven TS 32 W Ecolux® daylight fluorescent lamps (Fairfield, Connecticut, USA) and four 60 W incandescent bulbs. This model of growth chamber is divided in two identical sections, wherein in one section, entries were challenged with SoSCA, while an identical set of entries that were not infested grew in the other section. When the sorghum entries reached the two-leaf stage or 4–6 cm in height, the most vigorous plant was kept, whereas the other was removed. Remaining seedlings were infested by a single viviparous female which was removed after 24 h. From these nymphs on each entry, a single, 24 h old, nymph per seedling was selected to remain on the nine different sorghum entries where the development time to reproductive adult (d) and net reproduction (Md), female longevity (L), and reproductive period (days in reproduction) was recorded. Intrinsic rate of increase (rm) was calculated using the formula: $rm = 0.0738(1oge M_d)/d$ (Wyatt and White 1977). All reproductive life parameters were analyzed using mixed model analysis (PROC MIXED, SAS Institute 2016) where mean comparisons were made by using the least significant differences method (LSD) at $P > |t| \leq 0.05$ level (SAS 9.4, SAS Institute 2016).

Results

Sorghum resistance trials for male sterile counterparts

Plant injury recorded as damage ratings from SoSCA feeding indicate that within the comparison of the fertile R. LBK1, R. LBK2, and TX 2783 with the sterile A3 counterparts, that in all instances, the dominant resistant expression carried through to the sterile counterpart A3. R. LBK1, A3. LBK2, and A3 TX 2783 (Table 1). The known resistant DKS-3707 was slightly more resistant than additional resistant TX 2783, but this result has been made confirmed in several other resistance trials (Armstrong et al. 2015; Paudyal et al. 2018; Lemaje et al. 2018). Loss in percent chlorophyll content for the infested entries closely followed damage ratings and presented evidence that the SoSCA dominant resistance trait in the fertile forms of R. LBK1, R. LBK2, and TX 2783 was carried through backcrossing to the sterile counterparts A3. R. LBK1, A3. LBK2, and A3. TX 2783 (Table 1). Differences for plant height within an entry for infested vs not infested were 2.6 cm shorter for the R. LBK1 compared to the A3. R. LBK1 and for the A3. TX 2783 sterile over the fertile TX 2783 (Table 1). Numbers of true leaves were similar for the fertile R. LBK1, R. LBK2, and TX 2783 versus the sterile counterparts. Leaf numbers for the susceptible KS 585 and TX 7000 were statistically alike to the fertile R. LBK1 and A3.R. LBK1.

Results from the second resistance evaluation presented in Table 2 were used to confirm the results of the first evaluation in Table 1. Damage ratings showed similar statistical separations in the second trial, and sterile vs fertile lines indicate that the dominant resistance factors carry over from the originating fertile sources to the sterile backcrosses just

Table 1 Mean (± S.E.) sorghum damage ratings, chlorophyll loss, and difference in plant height for sugarcane aphids reared on A3 cytoplasmic sterile lines compared to known fertile susceptible and resistant sorghums

Germplasm	Sterile/fertile	Damage rating (1–9) scale[a]	Percent loss chlorophyll content[b]	Difference in plant height (cm)[c]	Number of true leaves[d]
KS 585	Fertile	9.0 ± 0.0 a	100.0 ± 0.0 a	17.4 ± 1.3 a	2.1 ± 0.1 b
TX 7000	Fertile	8.5 ± 0.2 a	57.5 ± 2.2 a	18.3 ± 1.0 a	2.1 ± 0.3 b
R. LBK1	Fertile	6.8 ± 0.5 b	50.5 ± 4.0 c	12.0 ± 1.5 b	2.5 ± 0.2 b
A3. R. LBK1	Sterile	5.5 ± 0.8 bc	36.3 ± 5.7 cd	9.4 ± 1.7 de	2.6 ± 0.2 b
A3. TX 2783	Sterile	4.3 ± 0.8 c-d	34.0 ± 8.8 d	13.6 ± 2.8 bc	2.5 ± 0.3 b
A3. R. LBK2	Sterile	3.9 ± 0.7 d	30.9 ± 2.0 d	14.4 ± 1.4 bc	3.5 ± 0.1 a
R. LBK2	Fertile	3.4 ± 0.5 d	31.7 ± 5.3 d	12.8 ± 1.2 d	3.6 ± 0.2 a
TX 2783	Fertile	3.3 ± 0.4 d	32.4 ± 2.8 d	10.8 ± 0.9 d	3.5 ± 0.2 a
DKS-37-07	Fertile	2.9 ± 0.2 e	21.2 ± 3.0 d	9.0 ± 1.1 d	3.7 ± 0.1 a

Column means followed by the same lower case letters are not significantly different $P < 0.05$; LSD

[a]Damage ratings evaluated on a 1–9 scale, $df = 8, 103$; $F = 23.6$; $P > F = < 0.001$

[b]Chlorophyll loss index (C − T) /C × 100, where, C is the SPAD reading from the non-infested control, and T is from infested plant, $df = 8, 103$, $F = 8.1$, $P = < 0.001$

[c]Mean difference in plant height, (controls–infested), $df = 8, 103$, $F = 4.0$; $P = < 0.001$

[d]Mean number of true leaves per plant, $df = 8, 103$, $F = 12.9$; $P = < 0.001$

Table 2 Means (± S.E.) sorghum damage ratings, chlorophyll loss, and difference in plant height for sugarcane aphids reared on A3 cytoplasmic sterile lines and compared to fertile known susceptible and resistant sorghums

Germplasm	Sterile/fertile	Damage rating (1–9) scale[a]	Percent loss chlorophyll content[b]	Difference in plant height (cm)[c]	Number of true leaves[d]
KS 585	Fertile	9.0 ± 0.0 a	100.0 ± 0.0 a	22.2 ± 1.0 a	2.6 ± 0.1 cd
TX 7000	Fertile	9.0 ± 0.0 a	59.0 ± 1.6 b	21.2 ± 1.0 a	2.3 ± 0.1 d
R. LBK1	Fertile	6.5 ± 0.5 b	52.6 ± 4.0 b	8.7 ± 1.5 cd	2.6 ± 0.2 bc
A3. R.LBK1	Sterile	4.5 ± 0.6 bc	40.0 ± 5.7 c	11.2 ± 1.0 bc	2.9 ± 0.1 bc
A3. TX 2783	Sterile	3.8 ± 0.5 cd	27.4 ± 6.1 d	12.8 ± 1.5 b	3.3 ± 0.1 ab
A3. R.LBK2	Sterile	2.8 ± 0.3 de	24.8 ± 2.3 d	10.0 ± 0.7 bc	3.6 ± 0.1 a
R. LBK2	Fertile	3.2 ± 0.4 d	26.0 ± 4.1 d	18.5 ± 0.7 bc	3.6 ± 0.2 a
TX 2783	Fertile	3.5 ± 0.6 cd	32.0 ± 3.4 d	12.5 ± 1.9 b	3.6 ± 0.1 b
DKS-37-07	Fertile	2.8 ± 0.2 e	31.2 ± 2.2 d	5.8 ± 1.0 d	3.2 ± 0.1 a

Column means followed by the same lowercase letters are not significantly different, $P < 0.05$, LSD

[a]Damage rating evaluated on a 1–9 scale, $df = 8, 107$; $F = 41.9$; $P > F = < 0.001$

[b]Chlorophyll loss index (C − T)/C × 100, where C is the SPAD reading from the non-infested control, and T is from infested plant, df = 8, 99, $F = 42.2$, $P = < 0.001$

[c]Mean difference in plant height (controls–infested), $df = 8, 99$, $F = 20.4$; $P = < 0.001$

[d]Mean number of true leaves per plant, $df = 8, 107$, $F = 11.9$; $P = < 0.001$

as it did in the first evaluation in Table 1. Interestingly, the DKS-3707 damage rating was slightly lower than the other known resistant TX 2783 as was confirmed in the first trail. Percent chlorophyll loss in the second trial mirrored that of the first evaluation with the exception that the R. LBK1 was 12.6% higher than for the sterile counterpart A3. TX 2783 (Table 2). Differences in plant height (cm) for the fertile lines closely followed the sterile lines, indicating that the expression in plant height was also present in the counterparts for R. LBK1, R. LBK2, and TX 2783. Numbers of true leaves expressed the same for within entry comparisons for fertile versus sterile and in no instance were there statistical differences (Table 2).

Demographics of sugarcane aphid on fertile and sterile counterparts

There was a wide numerical difference in the reproductive response for fecundity, nymphs produced /d, and the intrinsic rate of increase when sugarcane aphids fed on fertile susceptibles, compared to when they fed on resistant fertile and the sterile counterparts of resistant entries (Table 3). The fertile susceptible KS 585 produced 152 ± 12.2 nymphs, which was significantly greater than TX 7000 which produced 131.9 ± 8.5 nymphs. A reduction in SoSCA fecundity was observed when the fertile R. LBK1 was compared to the sterile counterpart A3 R. LBK2 where 20 fewer nymphs

Table 3 Demographic statistics for sugarcane aphid reproduction when reared on A3 cytoplasmic sterile lines and compared to fertile known susceptible and resistant sorghums

Germplasm	Sterile/fertile	Fecundity (Md)[a]	Nymphs/♀/d[b]	Intrinsic rate of increase (rm)[c]
KS 585	Fertile	152.9 ± 12.2 a	5.1 ± 2.2 b	0.41 ± 0.01 a
TX 7000	Fertile	131.9 ± 8.5 b	5.5 ± 2.2 a	0.40 ± 0.01 a
R.LBK1	Fertile	60.0 ± 5.1c	2.8 ± 2.2 c	0.30 ± 0.01 b
A3. R.LBK1	Sterile	40.5 ± 3.6 d	2.3 ± 2.2 d	0.27 ± 0.01 c
A3. TX 2783	Sterile	34.5 ± 1.7 e	2.2 ± 2.2 de	0.26 ± 0.02 d
A3. R.LBK2	Sterile	29.5 ± 2.2 ef	2.1 ± 2.2 de	0.25 ± 0.03 de
R.LBK2	Sterile	26.4 ± 2.2 f	2.0 ± 2.2 e	0.24 ± 0.02 e
TX 2783	Fertile	26.4 ± 6.2 f	2.2 ± 2.2 de	0.24 ± 0.01 e
DKS-37-07	Fertile	13.0 ± 2.2 g	2.3 ± 2.2 de	0.19 ± 0.02 f

Column means followed by the same lowercase letters are not significantly different, $P > 0.05$, LSD

[a]Fecundity (Md) = sugarcane aphids/female, 12 replications, $df = 8, 106$, $F = 569.9$; $P > F = < 0.0001$

[b]Nymps/♀/d; = (Md/d), $df = 8, 106$, $F = 197.5$, $P > F = < 0.0001$

[c]rm = intrinsic rate of increase, rm = 0.738(ln Md/d); $df = 8, 106$, $F = 295.6$; $P > F = < 0.0001$

were produced, indicating that the sterile form was expressing greater antibiosis. The A3 TX 2783 averaged just over 34 nymphs across 12 replications and was significantly higher than the fertile TX 2783, whereas the sterile A3 R. LBK2 was not different from the R. LBK2 with 29.5 and 26.4 nymphs produced, respectively (Table 3). All other entries other than KS 585, TX 7000, R. LBK1A3, and R. LBK1 produced fewer than 35 nymphs, while DKS 37-07 had the lowest with on average 13 total nymphs produced. The expression of antibiosis was also evident in the number of nymphs produced per d, where the R. LBK1 was slightly higher than the A3 R. LBK1 and all other fertile and sterile counterparts were not different, indicating that the resistance trait was passed through from the A3 backcrossing. The nymphs produced per d were > 5.0 for the two fertile susceptibles KS 585 and TX 7000, and < 3.0 for the remainder of the entries. The intrinsic rate of increase (rm) was significantly higher for the KS 585 and TX 700 followed by decreases starting with the R. LBK1 at 0.30, down to 0.19 for the DKS 37-07 (Table 3). Although some differences in separation within fertile and sterile counterparts did occur for rm, it is notable that population increases, or decreases were within range of indication that the resistance trait was maintained in the fertile vs sterile comparisons.

The SoSCA founding female longevity was 28-d for the fertile susceptibles KS 585 and TX 7000, followed by a 6-d decrease in longevity for the R. LBK1, and reduced to 8.5-d for the known resistant DKS 37-07 (Table 4). The fertile R. LBK1 founding female was in reproduction approximately 4 d longer than the sterile A3. R. LBK1 signaling that antibiosis was more stringently expressed in the sterile form. The TX 2783 fertile female longevity was 13.2 d in length compared to 15.9 for the A3 R. TX 2783 which was significant for the comparison. However, the R. LBK2 was longevity was 13.8 and was not significantly different from the A3 R. LBK2 that lasted for 14.8 d. The reproductive period (d) followed the same pattern as longevity where the fertile susceptibles KS 585 and TX 7000 survived the longest at > 26 d, followed by a decline starting with R. LBK1 at 22 d, down to 5.5 d for DKS 37-07.

Table 4 Mean longevity and reproduction for sugarcane aphid when reared on A3 cytoplasmic sterile lines compared to fertile known susceptible and resistant sorghums

Germplasm	Sterile/fertile	♀ Longevity (d)[a]	Reproductive period (d)[b]
KS 585	Fertile	27.5 ± 0.44 a	26.1 ± 0.50 a
TX 7000	Fertile	28.5 ± 0.38 a	26.9 ± 0.41 a
R.LBK1	Fertile	22.5 ± 0.34 b	21.8 ± 1.01 b
A3. R.LBK1	Sterile	18.4 ± 0.24 d	15.7 ± 0.74 c
A3. TX 2783	Sterile	15.9 ± 0.49 e	13.4 ± 0.70 d
A3. R.LBK2	Sterile	14.8 ± 0.52 ef	12.5 ± 0.77 d
R.LBK2	Fertile	13.8 ± 0.34 fg	11.7 ± 0.51 d
TX 2783	Fertile	13.2 ± 0.24 g	11.8 ± 0.52 d
DKS-37-07	Fertile	8.5 ± 0.69 h	5.5 ± 0.53 e

Column means followed by the same lowercase letters are not significantly different, $P > 0.05$, LSD

[a]Female longevity (d), $df = 9, 110$, $F = 186.6$; $P > F = < 0.0001$

[b]Reproductive period (d) $df = 8, 106$, $F = 122.1$; $P > F = < 0.0001$

Discussion

These evaluations for SoSCA resistance showed from the free-choice flat screens that tolerance existed in all the fertile and sterile counterparts when compared to the known fertile/susceptible KS 585 and TX 7000. The fertile R. LBK1 (Hayes et al. 2018) was the moderately tolerant in terms of damage ratings and other plant measurement

factors such as chlorophyll loss, difference in plant height, and numbers of true leaves, and was duplicated in the previous results (Limaje et al. 2018). The fertile R. LBK2 (Hayes et al. 2018) was very similar in tolerance to TX 2783 with damage ratings in the 3.5's on the 9-point rating scale (Limaje et al. 2018). The sterile counterpart of A3 R. LBK2 was just as tolerant as the fertile R. LBK2 and appears suitable for use in development of SoSCA-resistant forage sorghums.

Antibiosis was also present and expressed in the fertile and sterile counterparts evaluated, and reduced fecundity by over twofold for the R. LBK1 to greater than 3.8-fold for all other entries.

In conclusion, the sterile counterparts developed using the A3 cytoplasmic male sterile system were as tolerant as known resistant varieties and expressed antibiosis that was comparable or better than their fertile counterparts TX 2783, R. LBK1, and R. LBK2. The forms of resistance were expressed in the fertile and their sterile counterparts by reductions in the reproductive capacity in the form of reduced fecundity, nymphs produced /female/d, and significant losses in the intrinsic rate increase (rm), longevity (d), and the reproductive period (d). For R.LBK1, the expression was not as pronounced as the all other fertile lines, but this is explained by the fact that it has TAM 430 resistance in its breeding background and has always been identified as an intermediate source of resistance to sugarcane aphids. The backcrossed sterile form A3 R. LBK1 showed an improvement in reducing SCA reproduction in the reduction of fecundity, nymphs produced/female/d and was an improvement in the expression of antibiosis. Plant responses used to determine if tolerance was a source of resistance were observed in the form of damage rating's, differences in plant height for the non-infested vs the infested heights, number of true leaves, and chlorophyll loss for the non-infested vs the sugarcane aphid infested plants. The A3 R.LBK1, A3 TX 2783, A3. R.LBK2, and R.LBK2 were as consistent in the expression of tolerance as were the known fertile and resistant sources TX 2783 but not quite to the level as DKS-37-07. The male sterility A3 cytoplasmic plant breeding is an efficient use of getting sugarcane aphid resistance into forage sorghums.

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