ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Biotechnology

ISSN 1682-296X
DOI: 10.3923/biotech.2022.30.38

Research Article
Comparative Effect of *Bacillus thuringiensis* Endotoxins on the Fitness of F_1 Sorghum×Weed Hybrids

[1,4]Titus O. Magomere, [1]Eliud K. Ngugi, [2]Solomon I. Shibairo, [1]Eunice Mutitu and [3]Silas D. Obukosia

[1]Department of Plant Science and Crop Protection, University of Nairobi, P.O. Box 29053-00625, Nairobi, Kenya
[2]Masinde Muliro University of Science and Technology, P.O. Box 1699-50200, Bungoma, Kenya
[3]African Union Development Agency-New Partnership for Africa's Development, Eastern Regional Office, Nairobi, Kenya
[4]Department of Biochemistry Microbiology and Biotechnology, School of Pure and Applied Sciences, Kenyatta University, P.O. Box 43844, Nairobi, Kenya

Abstract

Background and Objective: Disruptive selection has progressively maintained huge diversity among crop and weedy members of the sorghum genus. Crop improvement in the species has a greater impact on alleles that enhance cultivation and productivity in traditional cropping systems. Similarly, natural selection seems to reward wild sorghums with greater adaptive advantage in given environments. This study evaluated the differential effect of *Bacillus thuringiensis* (Bt) endotoxins sprays on plant performance, fitness and fecundity of two F_1 populations from *S. halepense*× *S. bicolor* and *S. sudanense* × *S. bicolor*, grown under competitive conditions. **Materials and Methods:** Parental lines of *S. bicolor*, *S. halepense*, *S. sudanense* and their F_1 hybrids obtained from weed to crop crosses were grown in close planted plots in varying plant mixtures. Replacement series assays were utilized to evaluate the competitiveness of the F_1 in the greenhouse, field and the presence or absence of (Bt) endotoxins spray. **Results:** The presence of Bt larvicide increased seed production in parental as well as in the F_1 population's between *S. halepense*× *S. bicolor* and *S. sudanense*× *S. bicolor* grown in competition at various parent: F_1 ratios by at least 17%. Bt larvicide sprayed populations showed increased total plant weight from 286-452 g in the hybrid between *S. halepense*× *S. bicolor*. However, there was no significant difference in total plant weight in sprayed or non-sprayed *S. sudanense*× *S. bicolor* F_1 populations. Total seed weight and the total number of seeds increased in *S. halepense*× *S. bicolor* F_1 populations exposed to Bt endotoxins. This significant difference was not seen in *S. sudanense*× *S. bicolor* F_1 populations. **Conclusion:** Results from the study indicate that the effect of Bt endotoxins varies with the genetic background of the parental groups of F_1 exposed to the Bt. Therefore, generalizations of the effect of transgene across all products of gene flow between crop and weedy sorghums may be insufficient. Assays of improved adaptive advantage in segregating populations from Interspecific crosses need to involve several wild and weedy progenitors before the release of improved transgenic varieties in the sorghum genus.

Key words: *Bacillus thuringiensis*, delta endotoxin, replacement series, *S. halepense*, *S. bicolor*, *S. sudanense*, relative fitness

Citation: Magomere, T.O., E.K. Ngugi, S.I. Shibairo, E. Mutitu and S.D. Obukosia, 2022. Comparative effect of *Bacillus thuringiensis* endotoxins on the fitness of F_1 sorghum×weed hybrids. Biotechnology, 21: 30-38.

Corresponding Author: Titus O. Magomere, Department of Plant Science and Crop Protection, University of Nairobi, P.O. Box 29053-00625, Nairobi, Kenya Tel: 254-20-631314 Fax: 254-20-632121

Copyright: © 2022 Titus O. Magomere *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The establishment and persistence of hybrid weeds in given crop ecosystems under varying biotic and abiotic conditions depend on their relative fitness and the levels of intraspecies and interspecies competition they may endure[1]. The interspecies flow of genes towards the weedy members of the species through pollen may enhance plant fitness traits that can condition levels of seed dormancy, enhance reproduction success and even influence success in specific habitats and the nature of competition[2]. Epistatic interactions between the gene of crop and feral origins residing in sorghum weeds may condition the selective advantage towards wild sorghums and further enhance the invasiveness of feral sorghum populations[3-5].

The presence of the transgenes in hybrids of crops and their wild and weedy relatives may boost their resistance to pest pressure. Bt endotoxins have proved to be important in the production of insect-resistant crops. Bt transgenes responsible for resistance to several biotypes of susceptible insect pests in effect confer an adaptive advantage to the crop predisposed to environments with high insect infestation. Studies conducted in *Brassica rapa* show that seedlings exposed to the Bt spray thus protected from Bt-susceptible herbivores lived 25% longer, were twice as likely to produce siliques and had 251% seed output when compared to those not exposed to the spray[6]. Studies using F_1 progenies obtained from herbicide-resistant crop×wild relatives in oilseed rape showed that the subsequent reciprocal backcrosses had a similar number of seeds per silique to non-crop *B. juncea*, germination percentages were also similar to the feral *B. juncea*[7]. A study in rice showed that the Bt Cry/AC transgene was successfully expressed in F_1 hybrids, obtained from crossing Bt crop rice with wild rice *O. rufipogon*. Expression of the Bt gene varied greatly among individuals of F_1 hybrids and F_2 progenies of crop×wild rice. Some wild rice hybrids had higher levels of Bt toxins than cultivated GM rice suggesting that there is potential for persistence of Bt transgenes in wild populations[8].

The most useful *Bacillus thuringiensis* strains applied in biopesticide formulations for Homopteran and lepidopteran pests include *B. thuringiensis* ssp. *tenebrionis*, *B. thuringiensis* ssp. *kurstaki*, *B. thuringiensis* ssp. *israelensis*, *B. thuringiensis* ssp. *aizawai*, *B. thuringiensis* ssp., *san diego*. *B. thuringiensis* ssp. *israelensis* is used for control of mosquitoes while *B. thuringiensis* ssp. *tenebrionis* for control of beetles. *Bacillus thuringiensis* products in Kenya are sold under several commercial names including Dipell®, Javelinl®, Thuricide® and Xen tari®. Javelin and Thuricide are *B. thuringiensis* ssp., Kurstaki strain SA-11 formulations that are active on Lepidopteran, *Helicoverpa* spp., *Heliothis* spp., Xen Tari is developed from *B. thuringiensis* ssp., aizawai, strain ABTS-1857, while DiPel is a formulation from *B. thuringiensis* ssp., kurtaki, strain ABTS-351. DELPHIN 6.4WG® is a biological insecticide based on the SA-11 strain of *Bacillus thuringiensis* (Bt) ssp., Kurstaki produced by Certis USA. The insecticide has crystals of delta-endotoxins proteins from the spore-forming bacterium equivalent to 53×10^9 su kg^{-1}. The endotoxin is toxic to Lepidopteran larvae (stem borers that are important at the site of the experiment) and pests in Heliothis/Helicoverpa. The susceptible insects using certain gut enzymes dissolve the crystal proteins[9] into active sub-fractions that attach themselves to receptor sites in the gut wall, resulting in pore formation causing gut paralysis in the insect leading to the cessation of feeding[10]. The biopesticide has specific insecticidal activity and lacks harmful effects on humans, domestic animals, honeybees, wildlife and fish (manufacturer information-Certis USA).

Bt larvicide have shown value in simulating Bt endotoxins genes in plants and thus in measuring the advantage that could be conferred to populations of crop/wild hybrids in Brassica[6]. The fitness of hybrids between crops and weedy genotypes showed a general enhancement of fitness traits due to the presence of Bt endotoxins in Brassica[11]. Studies using F_1s obtained from herbicide-resistant crops to wild relatives in oilseed rape showed that the third and fourth reciprocal backcrosses produced the similar number of seeds per silique to wild *B. juncea*, germination was also similar to the weedy *B. juncea*[7].

The diminishing variability on important insect pest resistance traits has led to the continuous evaluation of transgenic DNA technology methods that stack a combination of *Bacillus thuringiensis* endotoxins genes to improve production and reduce production associated costs in most major crops.

It is imperative to define ways of evaluating the effect of transgene on the fitness of crops, wild relatives and their hybrids. There is a need to build information on the relative competitiveness and fitness of crop×wild hybrids with Bt genes or other transgene, that may be useful in the judicious utilization of transgenic crops in traditional cropping systems. To evaluate this, the most important parameters to consider include, plant vigour, biomass production, seed production, seed dormancy and resistance or tolerance to given biotic pressures. The study determined the relative competitive ability, fitness and fecundity of the *S. halepense*×*S. bicolor* and *S. sudanense*×*S. bicolor* hybrids. In addition the study evaluated the effect of *Bacillus thuringiensis* (Bt) larvicide for the control of insect damage in the F_1 hybrids obtained from *S. halepense*× *S. bicolor* and *S. sudanense*× *S. bicolor* hybrids.

MATERIALS AND METHODS

Study area: The greenhouse and field experiment was carried out at the college of agriculture and veterinary sciences (CAVS) (-1°14' 59.72", +36°44' 30.79"). Seeds from *S. bicolor*, *S. halepense*, *S. sudanense* and their hybrids were germinated by subjecting them to temperatures of 5°C for 10 days then to 45°C for 24 hrs in a drying oven to break dormancy. Two weeks after germination, seedlings of uniform vigour were transplanted into the field plots. Transplanting was done with recommended rates of diammonium phosphate (DAP-18% N, 46% P_2O_5, 0% K_2O) to obtain 20-40 kg ha^{-1} of N.Top dressing was done with recommended rates of calcium ammonium nitrate (CAN-5Ca(NO_3)2•NH_4NO_3•10H_2O- 8% calcium and 21-27% nitrogen) to obtain 50-80 kg ha^{-1} of N. Bagging was done after natural pollination to keep off birds. Whole plants were harvested and stored in drying bags. The vegetative parts were air-dried in a greenhouse for 21 days. The seeds were dried separately, threshed and stored in labelled packets in greenhouse conditions.

Competitive study of F_1 hybrids and parents treated with *Bacillus thuringiensis* larvicide in the field: Parental lines of *S. bicolor*, *S. halepense*, *S. sudanense* and their F_1 hybrids obtained from weed to crop crosses were grown in close planted plots in varying plant mixtures. A biological insecticide based on the SA-11 strain of *Bacillus thuringiensis* (Bt) ssp. *kurstaki*-DELPHIN 6.4WG®-Certis USA spraying regime was applied on the plots to simulate the effect of Bt genes in crop plants. The relative competitive abilities of the crop, weed and F_1 hybrids were evaluated for significant fitness advantages that resulted from the presence of crop genes in weedy backgrounds.

Experimental design: The study genotypes were transplanted and grown at 0.3×0.3 m spacing in plant density competition plots under both monoculture and in mixed cultures. The genotypes included *S. bicolor*, *S. halepense*, *S. sudanense*, *S. halepense*× *S. bicolor* and *S. sudanense*× *S. bicolor* hybrids. A two-factor split-plot design consisting of two main plot effects and 5 levels of the subplot effects was used. The plots were randomized within the blocks as shown in Table 1. The design had two main plot effects, A1 represents plants growing in the presence of Bt larvicide and A2 represents plants growing in the absence of Bt Larvicide. The subplot effects were based on plant density ratios as follows, B1 represented parent to the hybrid ratio of 100:0, respectively, B2 represented parent to the hybrid ratio of 75:25, respectively, B3 represented parent to the hybrid ratio of 50:50, respectively, B4 represented parent to the hybrid ratio of 25:75, respectively while B5 represented parent to the hybrid ratio of 0:100, respectively. Twelve seedlings were planted in each of the twenty subplots in 4 plots totalling 960 plants.

Spraying and insect control: Due to the necessity to contain transgenes, a *Bacillus thuringiensis* based larvicide (DELPHIN 6.4WG®) was sprayed on the experimental plots on regular ten-day intervals under the manufacturer-recommended rates of 0.25-0.5 kg ha^{-1}. This was done to simulate a situation where the plants would posses Bt endotoxins genes and in addition measure the advantage that could be conferred to crop/wild hybrids by the Bt insect larva resistance genes[6,11]. The experiment was done in a plot where stem borers (*Chilo partellus*) are a common problem in sorghum fields. The types of insects that were expected to be controlled due to the application of the Bt. Larvicide included *Lepidopteran* spp. Larvae, *Heliothis*/*Helicoverpa* spp. and *Spodoptera* spp.

Data collection and analysis: The relative crowding coefficient (RCC) approach was used to measure the competitive ability of one genotype to obtain and utilize limiting resources of solar, nutrition and water when grown in mixtures with another genotype in the presence or absence of lepidopteran pests. This was compared with the ability of the genotypes to use those resources when grown in a pure stand[12]. Percent Homopteran and percent Homopteran/ Lepidopteran panicle and foliage pests were evaluated on the

Table 1: Competitive assay of parental and hybrid plants in the presence or absence of *Bacillus thuringiensis* (Bt) larvicide arranged in split-plot design

Bt larvicide sprayed	Bt larvicide not-sprayed
S. bicolor and (*S. halepense*× *S. bicolor*)	*S. bicolor* and (*S. halepense*× *S. bicolor*)
S. halepense and (*S. halepense*× *S. bicolor*)	*S. halepense* and (*S. halepense*× *S. bicolor*)
S. bicolor and (*S. sudanense*× *S. bicolor*)	*S. bicolor* and (*S. sudanense*× *S. bicolor*)
S. sudanense and (*S. sudanense*× *S. bicolor*)	*S. sudanense* and (*S. sudanense*× *S. bicolor*)

Bt sprayed and unsprayed plants across five plant ratio combinations. The number of panicles, total plant weight, the total number of seeds, total seed weight and the number of ratoons were analyzed. Analysis of variance and mean differences between the genotypes were compared at $p\leq 0.05$ in GEN STAT 14 (VSN 2012). The linear model used in the analysis had a main plot component and a subplot component as represented by Eq. 1:

$$Y_{ijk} = \mu+(\rho_i+\alpha_j+\delta_{ij})+\beta_k+(\alpha\beta)_{jk}+\varepsilon_{ijk} \quad (1)$$

Where:

ρ_i = Block effect
α_j = Effect of factor a
δ_{ij} = Random effect of the whole-plot units
β_k = Effects of factor b
$(\alpha\beta)_{jk}$ = Interaction effect for factors a and b
ε_{ijk} = Random effect of the sub-plot units

RESULTS

Effect of *Bacillus thuringiensis* delta-endotoxin proteins on panicle characteristics of *S. halepense*, *S. bicolor* and *S. halepense*× *S. bicolor* hybrids in competitive plant mixtures: The effect of the Bt sprays on genotypes in different plant competition mixtures was significant in Table 2. Monocultures of the *S. halepense*× *S. bicolor* F_1 that were sprayed produced 1025 seeds, had a total panicle weight of 57 g and had 21% plants with non-susceptible leaf and panicle Homopteran insects. In addition, populations of *S. halepense* × *S. bicolor* that were not sprayed had less seed (523), low panicle weight (35 g) and higher number of insects (22%) (Table 2). Monocultures of the parents *S. halepense* and *S. bicolor* that were sprayed with the Bt toxin had a mean of 559 seeds, total panicle weight of 35 g and 22% of the plants had insects (Table 2). Parental monocultures that were not sprayed produced 618 seeds had a mean panicle weight of 40 g and had 26% plants with insects on their leaves and panicles (Table 2). In the unsprayed plots there were both Homopteran and Lepidopteran larval and adult insects.

In the plant competition mixtures of 75:25-F_1: Parents, the sprayed plants had 679 seeds, panicle weight of 42 g and 26% had Homopteran pests. The plants that were not sprayed had 534 seeds, a mean panicle weight of 32 g and 17% of the plants had insects (Table 2). Genotypes planted in 50:50 F_1: Parents mixtures and sprayed with Bt endotoxins had 699 seeds, panicle weight of 45 g and 28% of the plants had aphids. Plants that were not sprayed had 580 seeds, with a mean panicle weight of 41 g and 28% of the plants had insects (Table 2). Pesticide (Bt) sprayed plant competition mixtures of 25:75-F_1: Parents had 510 seeds panicle weight of 37 g. Ten percent of the plants had insect pests. The plots that were not sprayed had 650 seeds per plant and panicle weights of 41 g. Up to 24% of the plants had both Homopteran and Lepidopteran pests on leaves, stems and panicles at maturity (Table 2).

Effect of *Bacillus thuringiensis* delta-endotoxin proteins on panicle characteristics of *S. sudanense*, *S. bicolor* and *S. sudanense*× *S. bicolor* hybrids in competitive plant mixtures: *Sorghum sudanense*× *S. bicolor* F_1 progenies grown in monoculture plots and sprayed with Bt endotoxins yielded 678 seeds, had a total panicle weight of 33 g and 10% of the plants had aphids. The two F_1 populations that were not sprayed but planted in monoculture produced 569 seeds and had a panicle weight of 23 g. In addition, 8% of the plants had Homopteran and Lepidopteran insect pests in Table 3.

The parental populations grown in Bt sprayed monoculture plots had 576 seeds and a panicle weight of 21 g. They also had 6% of their plants showing evidence of aphid attack. Parental populations that were not sprayed had 578 seeds with a panicle weight of 27 g and they had 12% of plants showing evidence of insect attack (Table 3). Bt sprayed plots with plant competition ratios of 75:25-parents: F_1 produced 561 seeds, gave panicle weights of 17 g and 6% of the plants had insects. On the contrary, the plots that were not sprayed had 472 seeds, panicle weights of 22 g and 6% insect attack (Table 3).

Plots with 50:50-parents: F_1 competition ratio and sprayed with Bt had 681 seed, produced 28 panicles and 5% of the plants had insects. The plots that were not sprayed produced 410 seeds and had a mean of 21 g per panicle. These plants had an 8% infestation with aphids and Lepidopteran larvae (Table 3). Plots maintained at 25:75-parents: F_1 plant competition ratio and sprayed with the Bt had 652 seeds per plant and yielded a mean of 27 panicles. Upto11% of these plants had evidence of Homopteran attacks. The plots that were not sprayed with Bt had a seed number of 627, produced 27 panicles and 14% of the plants had evidence of insect attacks in Table 4.

Effect of *Bacillus thuringiensis* delta-endotoxins proteins on tiller fitness of *S. halepense*, *S. bicolor* and *S. halepense* × *S. bicolor* hybrids in competitive plant mixture plots: The F_1 obtained by crossing *S. halepense* by *S. bicolor* showed a significant increase in the number of seeds produced (Table 4). The main stem in Bt sprayed plots produced 889 seeds, the

Table 2: Panicle characteristics of *S. halepense* (Sh), *S. bicolor* (Sb) and *S. halepense×S. bicolor* hybrids grown in five plant ratio combinations of Bt sprayed and unsprayed plants

Plant ratios	Spray	Total number of seeds main-stem	Total panicle weight (g)	Homopteran (%)	Homopteran/ lepidopteran (%)
Parents (100%)	+Bt	559±60	35±3	22±4	-
Parents (100%)	-Bt	618±70	40±4	-	26±5
F_1: Parents (75:25)	+Bt	679±49	42±3	26±3	-
F_1: Parents (75:25)	-Bt	534±55	32±3	-	17±4
F_1: Parents (50:50)	+Bt	699±64	45±3	28±4	-
F_1: Parents (50:50)	-Bt	580±68	41±4	-	28±5
F_1: Parents (25:75)	+Bt	510±56	37±3	10±4	-
F_1: Parents (25:75)	-Bt	650±92	41±5	-	24±6
Sh×Sb (F_1) (100%)	+Bt	1025±54	57±3	21±4	-
Sh×Sb (F_1) (100%)	-Bt	523±64	35±3	-	22±4

Means are followed with their respective, SE: Standard error values Ss: *S. sudanense*, Sb: *S. bicolour*, Bt: *Bacillus thuringiensis*, Sh: *Sorghum halepense*

Table 3: Panicle characteristics of *S. sudanense* (Ss), *S. bicolor* (Sb) and *S. sudanense× S. bicolor* hybrids grown in five plant ratio combinations of Bt sprayed and unsprayed plants

Plant ratios	Spray	Total number of seeds main-stem	Total panicle weight (g)	Homopteran (%)	Homopteran/ lepidopteran (%)
Parents (100%)	+ Bt	576±42	21±2	6±2	-
Parents (100%)	- Bt	578±41	27±1	-	12±2
Parents: F_1 (75:25)	+ Bt	561±40	17±1	6±2	-
Parents: F_1 (75:25)	- Bt	472±39	22±1	-	6±2
Parents: F_1 (50:50)	+ Bt	681±41	28±1	5±2	-
Parents: F_1 (50:50)	- Bt	410±49	21±2	-	8±2
Parents: F_1 (25:75)	+ Bt	652±38	27±1	11±2	-
Parents: F_1 (25:75)	- Bt	627±49	27±2	-	14±2
SsxSb (F_1) (100%)	+ Bt	678±41	33±2	10±2	-
SsxSb (F_1) (100%)	- Bt	569±50	23±2	-	8±2

Means are followed with their respective SE: Standard error values, SE: Standard error values Ss: *S. sudanense*, Sb: *S. bicolour*, Bt: *Bacillus thuringiensis*, Sh: *Sorghum halepense*

Table 4: Panicle characteristics of *S. halepense*, *S. bicolor and S. halepense×S. bicolor* F_1 tillers on Bt sprayed and unsprayed plants grown in competition

	Number of seeds			Panicle weight (g)			With panicle insects (%)		
Stems	Sb	Sh	F_1 (Sh×Sb)	Sb	Sh	F_1 (Sh×Sb)	Sb	Sh	F_1 (Sh×Sb)
Sprayed									
m+Bt	909±82	372±97	889±61	58±4	26±5	58±3	14±6	23±6	30±4
a+Bt	795±94	267±114	702.7±66.7	44±5	15±6	44±3	19±6	15±7	21±5
b+Bt	--	282±152	727.5±75.2	--	19±8	44±4	--	10±10	20±5
c+Bt	--	424±191	837.1±91.1	--	23±10	47±5	--	15±13	31±6
d+Bt	--	321±191	--	--	27±10	--	--	15±13	24±9
Unsprayed									
m-Bt	1070±85	293±120	622±69	61±4	25±6	41±4	46±6	10±7	31±5
a-Bt	862±102	198± 133	422±78	45±5	22±7	29±4	18±7	14±9	12±5
b-Bt	--	92±182	479±92	--	16±10	34±5	--	18±12	20±6
c-Bt	--	113±227	424±107	--	24±12	30±6	--	5±15	23±7
d-Bt	--	87±346	606±130	--	13±18	34±7	--	5±24	24±9

Means are followed with their respective, SE: Standard error values Ss: *S. sudanense*, Sb: *S. bicolour*, Bt: *Bacillus thuringiensis*, Sh: *Sorghum halepense* ,m: Main stem, a,b,c,d: Tillers 1 to 4 and Sb indicate lack of tillers b-d

first and second tillers produced 702.7 and 727.5 seeds respectively while the fourth tiller had 837.1 seeds. The unsprayed F_1 had a mean of 622 seeds on the main stem. There was a reduction of produced seeds to between 422 and 479 in the first to third tiller while the fourth tiller had 606 seeds produced. *Sorghum halepense* plants sprayed with Bt had higher seed numbers (424-267) while those without Bt had fewer seed numbers (293-87) on main stems and tillers. This was contrary to the situation in *S. bicolor* where there was an average of two tillers and Bt sprayed showed lower seed number values (795-909) than those without the spray (862-1070) (Table 4). *Sorghum halepense× S. bicolor* F_1 progenies had heavier panicles on the main stems than on tillers, in both Bt sprayed and unsprayed plots. However, the Bt sprayed plots panicles were heavier (58-44 g) and lighter (41-29 g) in unsprayed plots.

The heaviest panicles in *S. halepense* were in the Bt sprayed plots where they produced 26 g on the main stem and 27 g on the fourth tiller. The unsprayed *S. halepense* plots had lighter panicles with the fourth tiller giving 13 g. In *S. bicolor* panicles from the unsprayed plots had heavier panicles on the main stem (61 g) and the first tiller (45 g) than those from the sprayed plots (58 g) on the main stem and (44 g) on the tiller (Table 4).

Important insect pest species on the panicles were sorghum aphid (*Melanaphis sacchari*) and maize aphids (*Rhopalosiphum maidis*) belonging to the Homopteran family. The F_1 between *S. halepense* and *S. bicolor* had more insects on the unsprayed than the sprayed plants. *S. halepense* had more insects on the panicles of tillers on the sprayed plants than on the unsprayed plants. In this case, the two panicles from sprayed *S. bicolor* had fewer insects (14 and 19%) than those from the unsprayed plots (46 and 18%) (Table 4).

Effect of *Bacillus thuringiensis* delta endotoxins proteins on tiller fitness of *S. sudanense*, *S. bicolor* and *S. sudanense*× *S. bicolor* hybrids in competitive plant mixture plots: *Sorghum sudanense*× *S. bicolor* F_1 progenies grown in replacement series experimental competition plots and exposed to endotoxins showed an increase in the number of seeds on the tillers as compared to those not exposed to Bt endotoxins. The first to the fourth tillers among genotypes exposed to Bt had 525-421 seeds while those not exposed had between 440-361 seeds. The main stems of F_1 progenies of *S. sudanense*× *S. bicolor* that were not exposed to Bt spray had higher seed means (828) than those exposed (772 seeds). *Sorghum sudanense* showed a similar trend, where the main stem in Bt unsprayed plots had 748 seeds while the Bt sprayed plots had a mean of 593 seeds. However, the tillers in Bt sprayed plots had higher means of seed produced than those grown in Bt unsprayed plots. *S. bicolor* in Bt sprayed plots had higher seed number means of 1668 and 1266 on the main stem and tiller than *S. bicolor* in Bt unsprayed plots (973 and 727) in Table 5.

Panicle weight of *S. sudanense*× *S. bicolor* F_1 was higher (36 g) on the main stems of plants grown in absence of Bt than when grown in presence of Bt (27 g). The tillers from Bt sprayed plots had heavier panicles than those from Bt unsprayed plots. *Sorghum sudanense* gave 32 g on the main tiller in Bt unsprayed plots while in Bt sprayed plots it was lighter at 26g. The panicles from tillers on plants in Bt sprayed plots were generally heavier. In *S. bicolor*, both the main stem panicles (56 g) and the tiller (47 g) were heavier than those grown in Bt unsprayed plots, 41 g from the main stem and 33 g from the tiller (Table 5).

The number of Homopteran pests on *S. sudanense*× *S. bicolor* was significantly reduced on both tillers and the main stem as compared to those on *S. bicolor*. The F_1 had a reduced number of panicles with insects (3-9) in Bt sprayed plots than in Bt unsprayed plots (4-14). A similar trend was recorded in *S. sudanense* where there were no panicles with insects on tiller 4 but had a mean of 2-5 panicles with insects in plots sprayed with Bt. In plots not sprayed with Bt, there was an increase in the number of panicles with insects on the main stem and the tillers (0-11). *S. bicolor* showed reduction in the number of panicles with Homopteran pests (10-22) in Bt unsprayed plots (22-10) as compared to plants in Bt sprayed plots (37-22) (Table 5).

Effect of *Bacillus thuringiensis* delta endotoxin proteins on plant fitness traits of *S. halepense*, *S. sudanense*, *S. bicolor*, *S. sudanense*× *S. bicolor* and *S. halepense*× *S. bicolor* hybrids: *Bacillus thuringiensis* endotoxins sprays improved the fitness traits in the parents (*S. bicolor*,

Table 5: Panicle characteristics of *S. sudanense*, *S. bicolor* and *S. sudanense*×*S. bicolor* F_1 tillers on Bt sprayed and unsprayed plants are grown in competition

	Number of seeds			Panicle weight (g)			With panicle insects (%)		
Tillers	Sb	Ss	F_1 (Ss×Sb)	Sb	Ss	F_1 (Ss×Sb)	Sb	Ss	F_1 (Ss×Sb)
Sprayed									
m+Bt	1668±78	593±80	772±58	56±3	26±3	27±2	37±4	3±4	8±3
a+Bt	1266±96	393±84	525±61	47±4	21±3	21±2	22±5	2±4	9±3
b+Bt	--	421±89	542±64	--	22±3	25±2	--	4±4	7±3
c+Bt	--	441±93	562±70	--	21±4	21±3	--	5±4	9±3
d+Bt	--	447±100	421±74	--	21±4	19±3	--	0±5	3±3
Unsprayed									
m-Bt	973±90	748±83	828±61	41±3	32±3	36±2	22±4	11±4	14±3
a-Bt	727±128	408±86	485±62	33±5	18±3	24±2	10±6	2±4	11±3
b-Bt	--	342±88	478±65	--	17±3	23±2	--	2±4	8±3
c-Bt	--	361±95	483±68	--	16±4	22±3	--	0±4	4±3
d-Bt	--	440±110	439±66	--	20±4	21±3	--	7±5	10±4

Means are followed with their respective SE: Standard error values, Ss: *S. sudanense*, Sb: *S. bicolour*, Bt: *Bacillus thuringiensis*, Sh: *Sorghum halepense*, m: Main stem, a,b,c,d: Tillers 1 to 4 and Sb indicate lack of tillers b-d

Table 6: Fitness traits of species grown under Bt sprayed (+Bt) and unsprayed (-Bt) conditions

	Number of panicles		Total plant weight		Total number of seeds		Total seed weight		Number of ratoons	
Species	-Bt	+Bt	-Bt	+Bt	-Bt	+Bt	-Bt	+Bt	-Bt	+Bt
Sb	1.7±0.3	2.2±0.3	224±27	263±26	1730±247	2339±223	95±17	117±16	1.7±0.2	1.0±0.2
Sh	1.5±0.5	2.4±0.4	132±46	184±39	793±506	1234±379	60±36	93±27	3.0±0.4	2.8±0.3
Sh×Sb: F_1	3.1±0.3	3.5±0.3	286±30	452±28	1769±285	3006±248	138±20	212±18	2.4±0.2	1.5±0.2
Ss	5.4±0.4	6.4±0.4	254±38	292±39	2559±343	2835±336	117±24	141±24	1.3±0.3	1.7±0.3
Ss×Sb: F_1	6.0±0.3	6.6±0.3	427±29	410±28	3244±253	3756±240	188±18	171±17	1.3±0.2	2.1±0.2

Sh: *S. halepense*, Ss: *S. sudanense*, Sb: *S. bicolor* and means are followed with their respective, SE: Standard error values, Bt: *Bacillus thuringiensis*

S. halepense, *S. sudanense*) and the F_1 progenies from *S. halepense*× *S. bicolor* and *S. sudanense*× *S. bicolor* in Table 6. More panicles were recorded in *S. sudanense*× *S. bicolor* (6.6) grown in the presence of Bt. This was 0.6 points higher than when grown in Bt unsprayed plots. A similar increase was seen in *S. halepense*× *S. bicolor* grow in Bt sprayed plots (from 3.1-3.5). Total plant weight increased in all parents due to the presence of Bt endotoxins. The *S. halepense*× *S. bicolor* F_1 had a higher magnitude increase in total plant weight from 286-452 g due to the Bt spray. However, there was no significant change in total plant weight in *S. sudanense*× *S. bicolor* due to the presence of Bt.

Twofold increases on the total number of seeds were recorded in *S. bicolor*, *S. halepense* and *S. halepense*× *S. bicolor* due to the presence of Bt spray. The increase in seed number due to Bt was minimal in *S. sudanense* from (2559-2835) and *S. sudanense*× *S. bicolor* (3244-3756). Total seed weight was more enhanced in *S. bicolor* (95-117 g), *S. halepense* (60-93 g) and *S. halepense*× *S. bicolor* (138-212 g) due to Bt spraying. This increase was less in *S. sudanense* (117-141) but there was reduction in *S. sudanense*× *S. bicolor* (188-171) (Table 6). There was a reduction in the number of ratoons in plots that had Bt sprays in *S. bicolor* (1.7-1.0), *S. halepense* (3.0-2.8) and *S. halepense*× *S. bicolor* (2.4-1.5). However, there was an increase in post-harvest ratooning in *S. sudanense* (1.3-1.7) and *S. sudanense* × *S. bicolor* (1.3-2.1) (Table 6).

DISCUSSION

Bacillus thuringiensis (Bt) delta-endotoxins proteins were important in reducing susceptible insect (Lepidopteran) populations in the experimental plots. Non-susceptible insect pests (Homopteran) were not controlled in the replacement series. High aphid populations were observed on abaxial leaf surfaces and panicle structures. The delta endotoxins reduced the number of Lepidopteran insects on the panicle and therefore improved the panicle characteristics of main stems and tillers in competitive plant mixtures. The Bt spray also increased the fitness of the genotypes *S. halepense*, *S. sudanense*, *S. bicolor*, *S. halepense*× *S. bicolor* and *S. sudanense*× *S. bicolor*. The hybrids were heterotic and varied in their response to the Bt spray. The existence of significantly enhanced heterosis in interspecies hybrids has been previously reported in lineages of the crop-wild hybrids in lettuce under eld conditions[13,14] and in wild×cultivated Sorghum hybrids[15].

The presence of Bt delta-endotoxin proteins enhanced performance of F_1 hybrids obtained from *S. halepense*× *S. bicolor* and *S. sudanense*× *S. bicolor* hybridization. Presence of Bt larvicide increased seed production in the F_1 (*S. halepense*× *S. bicolor*) grown in competition with the parents. This increase was seen in all competition plant mixtures in replacement series except where the F_1 was grown in 25% ratios (Table 2). In such cases competition favoured the parental genotypes and the effect of Bt larvicide on seed number was not significant. The effect of the Bt larvicide was less in the *S. sudanense*× *S. bicolor* F_1 (Table 3). The number of seeds produced was not significantly different in F_1 by parent plant competition mixtures in the presence or absence of Bt endotoxins. This could be attributed to the presence of genes that confer partial resistance to Lepidopteran pests in the weedy parent. The large vegetative biomass in the weedy parent could partially mask the effect of the insect pests. An exception was observed in the 50:50 plant competition mixture where exposure to Bt endotoxins yielded higher seed numbers. However, enhanced phenotypic expression of fitness-related traits and subsequent competitive ability in interspecies hybrid in field conditions has been shown in crop-wild hybrid sunflower[16] and crop-wild hybrids lettuce under drought, salinity and nutrient deficiency[17].

The decrease of lepidopteran pests due to the Bt larvicides was accompanied by a substantial increase in leaf and panicle dwelling Homopteran pests. However, control of Lepidopteran pests including stem borers using Bt endotoxins exhibited a more pronounced effect on the total number of seeds and the total panicle weight. This situation is seen in previous studies on plant fitness consequences in *Brassica rapa* where seasonal variability was observed. Nevertheless, *B. rapa* seedlings protected from Bt-susceptible herbivores lived 25% longer, were twice as likely to produce siliques and had 251% seed output when compared to those not exposed to the spray[6].

Fitness and fecundity in F_1 hybrids growing in the presence of *Bacillus thuringiensis* (Bt) delta-endotoxin proteins were significantly altered. Spraying of Bt larvicides enhanced fitness and fecundity related traits in the F_1 progenies of *S. halepense*× *S. bicolor*, *S. sudanense*× *S. bicolor* and the parental genotypes *S. halepense*, *S. bicolor* and *S. sudanense*. There were more panicles and more seeds in the Bt sprayed populations. This general increase was seen in the number of ratoons, total plant weight and total seed weight. This was attributed to the reduction of Lepidopteran larvae that are important in sorghum production at the experimental site. First and second tillers of *S. halepense*× *S. bicolor* had more seed and higher panicle weight than *S. halepense* but less than *S. bicolor*, in both Bt sprayed and non sprayed plots. A similar increment was observed in the *S. sudanense*× *S. bicolor* hybrid and its parental populations grew in competition albeit of lower magnitude. The increase in total seed yield in some of the F_1 progenies is a consequence of the increase in the number of flowering tillers and the reduction of Lepidopteran pests due to the presence of Bt endotoxins proteins. Previous studies in rice show that *O. rufipogon*× GM rice individuals had higher expression of Bt toxins than cultivated GM rice[8]. The altered fecundity in favour of crop wild hybrids has been shown in *Raphanus* spp.[18], *Oryza* spp.[19] and in Cucurbitaceae[20]. Further to this, the fitness of hybrids between crops and weedy genotypes showed a general enhancement of fitness traits due to the presence of Bt endotoxins in Brassica[6].

CONCLUSION

The presence of Bt larvicides increased seed production in parental as well as the F_1 populations have grown in competition at different parent: F_1 ratios. This increase was seen in all competition plant mixtures in replacement series except where the F_1 was grown in 25% ratios. There was an increase in the numbers of panicles, number of seeds, number of ratoons, total plant weight and total seed weight in the Bt sprayed populations. This is especially important with the development and use of disease resistance, herbicide resistance, drought tolerance and crops with genes that boost their agronomic performance.

SIGNIFICANCE STATEMENT

This study discovered that there exists a significant change in fitness and fecundity of crop× weed hybrids in sorghums due to enhanced heterosis with a substantial conditioning effect on the invasiveness potential of the subsequent segregating populations. This effect is magnified differentially when the hybrids are grown in the presence of *Bacillus thuringiensis* (Bt) delta-endotoxin proteins under competitive growing conditions. This study will help the researchers to uncover the critical aspects of environmentally safe delta-endotoxin proteins transgenes in the Sorghum family. Thus there is a need to consider the interspecies geneflow capacity in crop ecologies, the pollination system in candidate crops for genetic modification and the potential of the transgene for altering the invasive features of the feral crop relatives in sorghum.

REFERENCES

1. Campbell, L.G., A.A. Snow and C.E. Ridley, 2006. Weed evolution after crop gene introgression: Greater survival and fecundity of hybrids in a new environment. Ecol. Lett., 9: 1198-1209.
2. Ellstrand, N.C., 2003. Dangerous liaisons? When cultivated plants mate with their wild relatives. 1st Edn., Johns Hopkins University Press, London, ISBN-13: 9780801881909 Pages: 244.
3. Hokanson, K.E., N.C. Ellstrand, J.T. Ouedraogo, P.A. Olweny, B.A. Schaal and A.F. Raybould, 2010. Biofortified sorghum in Africa: Using problem formulation to inform risk assessment. Nat. Biotechnol., 28: 900-903.
4. Guadagnuolo, R., J. Clegg and N.C. Ellstrand, 2006. Relative fitness of transgenic vs. non-transgenic maize× teosinte hybrids: A field evaluation. Ecol. Applic., 16: 1967-1974.
5. Morrell, P.L., T.D. Williams-Coplin, A.L. Lattu, J.E. Bowers, J.M. Chandler and A.H. Paterson, 2005. Crop-to-weed introgression has impacted allelic composition of johnsongrass populations with and without recent exposure to cultivated sorghum. Mol. Ecol., 14: 2143-2154.
6. Letourneau, D.K. and J.A. Hagen, 2009. Plant fitness assessment for wild relatives of insect resistant crops. Environ. Biosaf. Res., 8: 45-55.
7. Song, X., Z. Wang, J. Zuo, C. Huangfu and S. Qiang, 2010. Potential gene flow of two herbicide-tolerant transgenes from oilseed rape to wild *B. juncea* var. *gracilis*. Theor. Appl. Genet., 120: 1501-1510.
8. Xia, H., B.R. Lu, J. Su, R. Chen, J. Rong, Z. Song and F. Wang, 2009. Normal expression of insect-resistant transgene in progeny of common wild rice crossed with genetically modified rice: Its implication in ecological biosafety assessment. Theoret. Appl. Genet., 119: 635-644.
9. Karthikeyan, A., R. Valarmathi, S. Nandini and M.R. Nandhakumar, 2012. Genetically modified crops: Insect resistance. Biotechnology, 11 : 119-126.
10. CERA, 2011. A review of the environmental safety of the Cry1Ab protein. Environ. Biosaf. Res., 10: 51-71.

11. Letourneau, D.K., G.S. Robinson and J.A. Hagen, 2003. *Bt* crops: Predicting effects of escaped transgenes on the fitness of wild plants and their herbivores. Environ. Biosaf. Res., 2: 219-246.
12. Massinga, R.A., K. Al-Khatib, P. St. Amand and J.F. Miller, 2005. Relative fitness of imazamox-resistant common sunflower and prairie sunflower. Weed Sci., 53: 166-174.
13. Hooftman, D.A.P., Y. Hartman, J.G.B. Oostermeijer and H.J.C.M.D. Nijs, 2009. Existence of vigorous lineages of crop-wild hybrids in Lettuce under field conditions. Environ. Biosaf. Res., 8: 203-217.
14. Uwimana, B., M.J.M. Smulders, D.A.P. Hooftman, Y. Hartman and P.H. van Tienderen *et al*., 2012. Crop to wild introgression in lettuce: Following the fate of crop genome segments in backcross populations. BMC Plant Biol., Vol. 12. 10.1186/1471-2229-12-43.
15. Sahoo, L., J.J. Schmidt, J.F. Pedersen, D.J. Lee and J.L. Lindquist, 2010. Growth and fitness components of wild x cultivated *Sorghum bicolor* (Poaceae) hybrids in Nebraska. Am. J. Bot., 97: 1610-1617.
16. Mercer, K.L., D.L. Wyse and R.G. Shaw, 2006. Effects of competition on the fitness of wild and crop-wild hybrid sunflower from a diversity of wild populations and crop lines. Evolution, 60: 2044-2055.
17. Uwimana, B., M.J.M. Smulders, D.A.P. Hooftman, Y. Hartman and P.H. van Tienderen *et al*., 2012. Hybridization between crops and wild relatives: The contribution of cultivated lettuce to the vigour of crop-wild hybrids under drought, salinity and nutrient deficiency conditions. Theor. Appl. Genet., 125: 1097-1111.
18. Campbell, L.G. and A.A. Snow, 2007. Competition alters life history and increases the relative fecundity of crop-wild radish hybrids (*Raphanus* spp.). New Phytol., 173: 648-660.
19. Song, Z.P., B.R. Lu, B. Wang and J.K. Chen, 2004. Fitness estimation through performance comparison of F_1 hybrids with their parental species *Oryza rufipogon* and *O. sativa*. Ann. Bot., 93: 311-316.
20. Spencer, L.J. and A.A. Snow, 2001. Fecundity of transgenic wild-crop hybrids of *Cucurbita pepo* (Cucurbitaceae): Implications for crop-to-wild gene flow. Heredity, 86: 694-702.