

Adaptive values of wild × cultivated sorghum (*Sorghum bicolor* (L.) Moench) hybrids in generations F₁, F₂, and F₃

Moses M. Muraya · Hartwig H. Geiger · Fabrice Sagnard ·
Lassana Toure · Pierre C. S. Traore · Sabine Togola ·
Santie de Villiers · Heiko K. Parzies

Received: 8 September 2010 / Accepted: 21 February 2011 / Published online: 10 March 2011
© Springer Science+Business Media B.V. 2011

Abstract Gene flow between cultivated and their wild relatives is one of the main ecological concerns associated with the introduction genetically modified (GM) cultivars. GM sorghum cultivar has been developed and its commercial production may be possible in the near future. The rate of gene flow depends on the fitness of wild × cultivated sorghum hybrids. The study aimed at estimating adaptive values of wild × cultivated sorghum hybrids in generations F₁, F₂, and F₃ compared to their parents. Artificial crosses of four wild sorghums, five cultivated sorghums, and two male sterile lines were made to produce the F₁ generation, which were advanced to F₂ and F₃. Each hybrid generation and their respective parents were evaluated for their adaptive value at

two sites in a randomised complete block design with seven replicates. The resulting progenies did not show serious fitness penalties. Some hybrids were as fit as their respective wild parents and no consistent differences exist between the three generations studied. Thus, the resultant wild × cultivated hybrids may act as avenue for introgression.

Keywords Adaptive value · Ecological risk · Gene flow · Introgression · *Sorghum bicolor* · Wild × cultivated hybridisation · Wild-crop hybrids

Electronic supplementary material The online version of this article (doi:10.1007/s10722-011-9670-0) contains supplementary material, which is available to authorized users.

M. M. Muraya (✉) · H. H. Geiger · H. K. Parzies
Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, Fruwirthstrasse 21, 70599 Stuttgart, Germany
e-mail: mahugu2002@yahoo.com

M. M. Muraya
Leibniz Institute of Plant Genetics and Crop Plant Research, Corrensstrasse 3, 06466 Gatersleben, Germany

F. Sagnard
CIRAD, UMR Développement et Amélioration des Plantes, c/o ILRI, PO Box 30709, Nairobi, Kenya

Introduction

Sorghum has its centre of diversity in Africa, where the crop and wild sorghums co-exist (Doggett 1988).

F. Sagnard · S. de Villiers
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT-Nairobi), P.O Box 39063-00623, Nairobi, Kenya

P. C. S. Traore · S. Togola
International Crops Research Institute for the Semi-Arid Tropics, c/o LaboSEP, IER-Sotuba, POB 320, Bamako, Mali

L. Toure
Institut d'Economie Rurale C.R.R.A. de Sotuba, BP 258, Bamako, Mali

Majority of sorghums found in Africa are classified into the species *Sorghum bicolor* (L.) Moench (de Wet 1978). The species is further subdivided into *S. bicolor* ssp. *bicolor* (cultivated sorghum) and *S. bicolor* ssp. *verticilliflorum* (Steud.) De Wet; wild sorghum). The experimental evidence that cultivated sorghum and its wild relatives regularly exchange genes under natural conditions is well documented (Doggett 1988; Morrell et al. 2005). Wild-crop gene flow is a major potential risk in centres of crop origin and diversity, where cultivated and their wild relatives have sympatric ranges. Introgression of crop genes into their wild relatives may have important ecological consequences. It has already led to the loss of distinct wild relatives as well as the development of more aggressive weeds in the cases of sugarbeet and radish (Boudry et al. 1993; Ellstrand et al. 1999; Hedge et al. 2006).

Genetically modified bio-fortified sorghum cultivars are being developed in Africa (<http://www.biosorghum.org/project>) and their commercial production may start in foreseeable future. Ecological risks associated with the release of transgenic crops include non-target effects of the crop and escape of transgenes into wild populations (Pilson and Prendeville 2004). Non-target effects include unintended negative effects on species that do not reduce yield and greater persistence of the crop in feral populations. The escape of transgenes into wild populations, via hybridisation and introgression, could lead to increased weediness or to invasion of new habitats by the wild population. In addition, co-existing species could be negatively affected by transgenic-wild plants.

Hybridisation between cultivated plants and their wild relatives is a widespread phenomenon (Ellstrand et al. 1999; Lowe and Abbott 2004; Kadereit et al. 2006). Crop-wild introgression is mainly observed in allogamous species (Whitton et al. 1997). However, autogamy in the wild or cultivated populations does not totally preclude hybridisation (Arriola and Ellstrand 1996; Zemetra et al. 1998). Many autogamous species show outcrossing rates of up to 1, 2 or even more percent. Differences in pollination success may account for partial reproductive isolation between crops and their cross-compatible wild relatives (Rieseberg et al. 1995). For example, a higher proportion of hybrid seed was obtained on the diploid field mustard (*Brassica rapa* L.) and the allotetraploid oilseed rape (*B. napus* L. em. Metzg.) when a

mixture of pollen from the two species was applied (Hauser et al. 1997). If two species are cross-incompatible, no hybridisation is to be expected in nature.

Furthermore, the probability of introgression can be a property of individual genes or chromosomal segments (Lowe and Abbott 2004; Erickson and Fenster 2006). Since crossings-over are not randomly distributed along the chromosomes and the colinearity between homologous or orthologous chromosomes is rarely conserved along their full lengths (Lukaszewski 1995; Truco et al. 1996), the physical distribution of these genetically related regions should play a significant role in driving the amount and distribution of introgressed alleles, since recombination seems to occur preferentially between the most homoeologous regions (Delourme et al. 1998).

Recombination in wild \times cultivated hybrids can disrupt co-adapted gene complexes, thereby causing hybrid sterility and breakdown that interfere negatively with introgression (Li et al. 1997). Populations resulting from sib-mating or selfing hybrid plants generally display higher levels of sterility and weakness than those derived from backcrossing (Rieseberg et al. 1996). Chèvre et al. (1998) observed an increase of fertility in advanced backcross generations of hybrids between oilseed rape and wild radish.

Effective crop-to-wild gene flow depends on the completion of a number of successive steps: (1) existence of crops and its close relatives in sympatric ranges, (2) overlapping flowering window, and (3) production of viable and fertile hybrids remaining stable in successive generations. These conditions are met in sorghum. However, the ecological consequences of gene flow between cultivated sorghum and their wild relatives and the potential fate of introgressed crop genes and/or transgenes into the wild, are largely unknown. More information regarding the adaptive value of wild \times crop hybrids would in particular be useful for making predictions about the fate of engineered genes that may have escaped into wild populations.

Hybridisation and introgression between cultivated and wild sorghums have been explored and it has been shown that the frequency of hybridisation between adjacent cultivated and wild sorghum populations can be high (Arriola and Ellstrand 1996; Morrell et al. 2005). Morrell et al. (2005) found 32%

introgression in Johnsongrass populations sympatric to long-term sorghum production fields. The next step would be to assess the adaptive value of F_1 plants and successive hybrid derivatives relative to their parents.

Beside cross-compatibility, the fitness (adaptive value) of hybrid plants in generations F_1 , F_2 and F_3 determines how successful gene introgression is. Knowing these characteristics allows the population biologist to forecast the rate of gene introgression. This is of particular interest for transgene introgression. The objectives of this study were (1) to assess the relative adaptive value of wild \times cultivated sorghum hybrids in generations F_1 , F_2 and F_3 in comparison to their parents, and (2) to identify any unusual trait distribution in generations F_2 and F_3 .

Materials and Methods

Materials

Four wild sorghums and seven cultivated sorghums were used. The wild sorghums (BEN, DIOS, ETH, and PET) belong to two different botanical varieties of *S. bicolor* ssp. *verticilliflorum*. Wild sorghum are grouped into four basic botanical varieties (De Wet et al. 1970; De Wet 1978; Doggett 1988), corresponding to four of the Snowden's "species" (Snowden 1955), namely *S. arundinaceum* (Desv.) Stapf, *S. verticilliflorum* (Steud.) Stapf, *S. virgatum* (Hack.) Stapf and *S. aethiopicum* (Hackel) Rupr. ex Stapf. BEN collected in Benin, and DIOS, a wild-weedy sorghum found in the village of Dioulafoundo, southern Mali, were classified as *S. arundinaceum*. PET, a wild sorghum found in the village of Petaka, northern Mali, and ETH, collected from Ethiopia, were classified as *S. verticilliflorum*. The five (CSM63E, Hemenabana (Eme'na), Hemenabanu (Eme'banu), Nioba and V4) cultivated sorghums belong to different cultivars of *S. bicolor* ssp. *bicolor*. CSM63E, an improved variety disseminated by various local organisations, and Nioba, obtained from Siramana and Hemenabana in Petaka, were classified as cultivar guinea. Hemenabanu, and V4, both obtained from Petaka, were classified as cultivar guinea and cultivar caudatum, respectively. In addition, two cytoplasmic male sterile (cms) lines, MSP (male sterile Precoce) and MST (male sterile Tardif), were used as seed parents. Both belong to cultivar guinea and were developed by

ICRISAT, Mali. MSP is early maturing and MSTP is late maturing. Male sterile lines were used in addition to the cultivated sorghum in order to obtain enough seeds for testing in F_1 .

Mating scheme and seed production

Crosses using the four wild sorghums the five cultivated sorghums, and the two cms lines were made to produce F_1 's during June–Oct 2005 on the station of ICRISAT at Samanko near Bamako, Mali. Crossing was done as shown in Table 1 without reciprocal crosses. Two sets of F_1 's were produced, one set using wild and cultivated sorghums and the other set using ms inbred lines and wild sorghums. Eight F_1 's were produced by crossing the two cms lines with the four wild sorghums. In addition, 20 F_1 's were produced by crossing the five cultivated sorghums with the four wild sorghums. The 20 F_1 's were advanced to F_2 's and F_3 's (Fig. 1). All materials were then evaluated in a final comparative field experiment.

Evaluation of the hybrids and their parents, and trait assessment

Table S1 shows agro-ecological conditions of sites where wild and cultivated sorghums were collected and evaluated. Figure S2 indicate sorghum growing season in Mali. Four F_1 's (produced by crossing cms

Table 1 Production of wild \times cultivate sorghum hybrids in Mali (CMS = cytoplasmic male sterility), 2005 growing season

	Wild parents			
	BEN (♀)	DIOS (♀)	ETH (♀)	PET (♀)
Cultivated parents				
Hemenabanu (♂)	X	X	X	X
CSM63E (♂)	X	X	X	X
Hemenabana (♂)	X	X	X	X
Nioba (♂)	X	X	X	X
V4 (♂)	X	X	X	X
	BEN (♂)	DIOS (♂)	ETH (♂)	PET (♂)
CMS				
MSP (♀)	X	X	X	X
MST (♀)	X	X	X	X

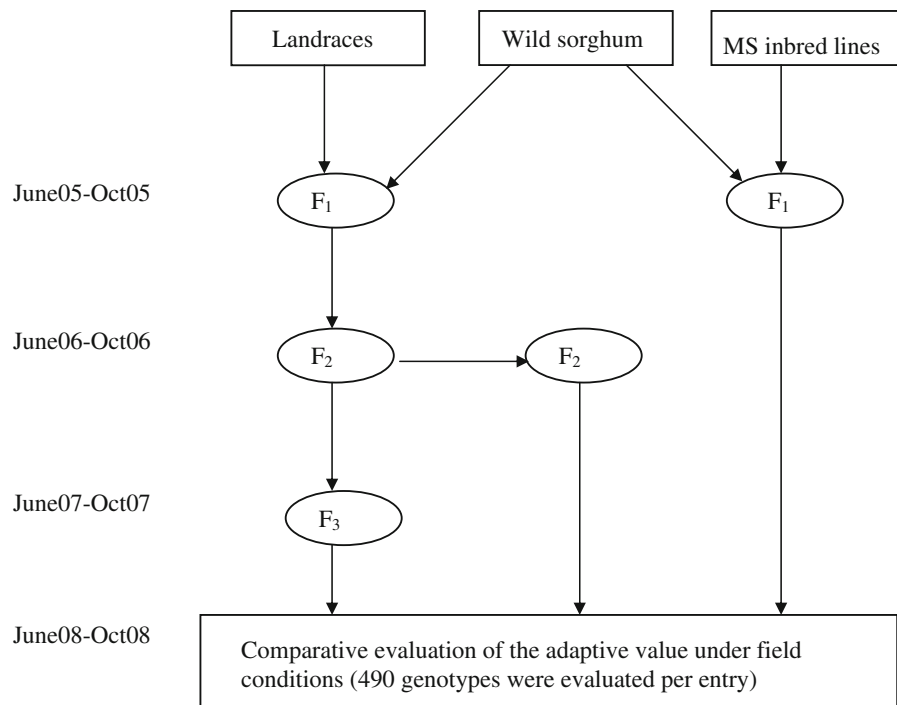


Fig. 1 Mating scheme of wild \times crop sorghum hybrids for producing various generations (ms = male sterile)

Table 2 Means of F_1 's and their parents at Samanko, Mali, during the 2008 growing season, for six quantitative traits

Genotype	Type of entry	Germ (%)	DTF (days)	NT (count)	PH (cm)	FLL (cm)	FLW (cm)
MSP	Cultivated parent	94.3a	62.7b	2.1a	321.5b	53.8b	6.7b
MST	Cultivated parent	92.0a	67.0a	2.2a	409.2a	66.0a	8.0a
	Average	93.1a	64.9a	2.2b	365.3b	59.9a	7.3a
ETH	Wild parent	88.6a	52.3b	3.5b	336.1a	41.3a	4.5ab
PET	Wild parent	58.6b	51.5b	12.8a	257.6b	39.4a	4.4b
DIOS	Wild parent	31.4c	63.0a	5.3b	327.9a	37.3a	5.8a
	Average	66.2b	53.9c	7.6a	302.3c	39.8c	4.7c
MSP \times ETH	F_1	88.6b	60.7b	4.8c	405.3b	43.0b	5.4c
MSP \times PET	F_1	97.1a	50.5c	10.1a	393.1b	48.6ab	5.9bc
MST \times PET	F_1	97.1a	58.4b	7.5b	419.5ab	53.0a	6.5b
MST \times DIOS	F_1	82.9c	66.7a	6.3bc	446.4a	50.6a	7.2a
	Average	91.4a	59.1b	7.2a	416.0a	48.8b	6.2b

Means followed by the same letter are not significantly different at $P = 0.05$ within each type of entry (cultivated parent, wild parent, F_1 , and average)

Germ Germination percentage, *DTF* days to flowering, *NT* number of tillers, *PH* plant height, *FLL* flag leaf length, *FLW* flag leaf width

lines with wild parents) and five F_2 's and F_3 's (advanced generations of F_1 's produced by crossing wild parents with cultivated parents) were evaluated

(Tables 2 and 3, respectively). We were not able to evaluate all the hybrids generated due to loss of seeds (due to storage problems). F_1 's produced by crossing

Table 3 Means of F_2 's, F_3 's, and their parents averaged across two sites (Samanko and Sotuba in Mali; 2008 growing season) for six quantitative

Genotype	Type of entry	Germ (%)	DFT (days)	NT (count)	PH (cm)	FLL (cm)	FLW (cm)
Hemenabanu	Cultivated parent	88.6a	53.1d	3.2ab	316.0b	56.8a	6.2a
CSM63E	Cultivated parent	88.7a	50.3d	4.0a	265.1c	54.5ab	6.1a
Hemena	Cultivated parent	90.4a	62.1c	2.4bc	293.4b	50.5b	5.9ab
Nioba	Cultivated parent	81.4b	86.2a	1.8c	383.4a	50.5b	5.4bc
V4	Cultivated parent	49.1c	67.9b	2.4bc	215.5d	45.6c	5.3c
	Average	79.6b	65.0c	2.7d	294.8a	51.2a	5.7a
PET	Wild parent	44.7c	61.5c	8.8a	256.2b	41.4ab	4.3c
BEN	Wild parent	84.3a	69.0b	7.9a	335.6a	43.2ab	5.0b
DIOS	Wild parent	19.8d	74.5a	8.7a	310.0a	37.4b	5.5a
ETH	Wild parent	69.3b	71.7ab	3.4b	304.5a	43.9a	4.9b
	Average	60.1c	68.7ab	6.9a	302.3a	42.1c	4.9b
Hemenabanu × PET	F_2	78.6b	64.0b	5.7a	305.6b	47.4a	4.8b
CSM63E × BEN	F_2	76.5c	58.7b	4.8ab	274.6c	49.1a	5.3ab
Hemena × DIOS	F_2	86.7a	61.1b	4.3b	284.6bc	48.2a	5.5a
Nioba × ETH	F_2	58.6e	78.3a	2.3c	357.0a	46.1a	4.8b
V4 × ETH	F_2	74.3d	63.1b	1.8c	203.6d	48.7a	5.0ab
	Average	75.0b	65.3bc	4.0c	294.1a	47.8b	5.1b
Hemenabanu × PET	F_3	80.7d	74.9a	8.7a	231.5c	45.9a	3.9c
CSM63E × BEN	F_3	89.7b	69.0b	6.3b	250.0b	45.4a	5.3b
Hemena × DIOS	F_3	88.6b	67.6b	5.9b	245.3b	46.0a	5.1b
Nioba × ETH	F_3	92.4a	66.9b	3.4c	237.0bc	48.2a	5.5ab
V4 × ETH	F_3	86.3c	72.9a	3.0c	305.3a	48.2a	5.7a
	Average	87.6a	70.3a	5.4b	253.80b	46.7b	5.1b

^a Means followed by the same letter are not significant different at $P = 0.05$ within each type of entry (cultivated parent, wild parent, F_2 , F_3 , and average)

Germ Germination percentage, *DFT* days to flowering, *NT* number of tillers, *PH* plant height, *FLL* flag leaf length, *FLW* flag leaf width

wild parents with cultivated parents could not be evaluated due to insufficient seeds.

Generations F_1 , F_2 , F_3 and their parental lines were evaluated in the field to compare for component traits of the adaptive value including germination percentage, number of panicles (plants) with seeds, number of tillers (number of reproductive axillary and basal tillers), days to flowering, flag leaf dimensions (length and width) and plant height and survivorship. The experiment was conducted during the 2008 growing season on the stations of ICRISAT at Samanko and at the Institut de Economie Rurale (IER) at Sotuba, both in the Bamako region of Mali. Due to insufficient numbers of seeds, F_1 's were only evaluated at one location (ICRISAT-Samanko). A randomised complete block (RCBD) design with seven replicates and one-row plots was used. Each

plot consisted of 70 plants but data were taken only from 10 random plants.

At planting three seeds were placed per hill and three weeks days after planting the stands were thinned to one plant per hill. Then density was adjusted transplanting and/or planting the missing hill. Planting was done at spacing of 75 cm between rows and 50 cm between plants within rows. During planting diammonium phosphate (DAP) at an equivalent rate of 150 kg/ha was applied. Plants were topdressed 45 days after planting urea at equivalent rate of 100 kg/ha. Weeding was carried out thrice.

Germination rate was determined using a laboratory test. Plant height, number of tillers per plant, number of panicles with seeds, flag leaf dimensions (diameter and width) was recorded from 10 middle plants per plot. Days to flowering were recorded after

50% of plants per plot have flowered. ‘Relative survivorship’ was scored as the proportion of germinated seeds of cross combination that survived to adult stage and set seeds relative to its wild parent.

Statistical analysis

The PROC GLM procedure of the software SAS (SAS Institute 2004) was used for statistical analyses. The effects of genotypes were assumed as fixed while the effects of replicates and sites as random. Counts were log transformed and percentage data transformed to the arcsine of their square roots, where necessary. Multiple F-tests were computed using SAS and a significance level of 5%.

Heterosis

Heterosis expressed as relative mid-parent heterosis (MPH) was estimated according to the formula:

$$\text{MPH} = \frac{F_1 - M_p}{M_p} \times 100$$

where F_1 is the F_1 performance and M_p is the mid-parent performance.

Results

Evaluation of F_1 's and their parents

On average the F_1 's had an intermediate value for days to flowering and flag leaf dimensions (Table 2). Cultivated parents had the highest germination percentage, days to flowering, and flag leaf dimensions, while wild parents scored the lowest for these traits. Cultivated parents and the F_1 's showed no significant difference for germination percentage. Wild parents and F_1 's did not differ significantly for the number of tillers. Generally, the F_1 's had the tallest plants.

Significant differences ($P = 0.05$) between genotypes within parents and the F_1 's, respectively, for most of the assessed quantitative traits (Table 2). Cultivated parents exhibited a higher germination percentage and flag leaf sizes compared to wild parents. Wild parents had higher number of tillers than cultivated parents. In the F_1 's, all traits differed significantly ($P = 0.05$) among genotypes (Table 2).

F_1 crosses with PET wild parent had higher germination percentage and number of tillers. All the F_1 hybrids displayed an increase in plant height and flag leaf dimensions compared to their wild parents.

Evaluation of F_2 's, F_3 's and their parents

On average most traits in the F_2 's and the F_3 's had values within the cultivated and wild parents values (Table 3). The F_3 's had the highest germination percentage and days to flowering and wild parent the lowest. There was no significant difference in days to flowering between wild parents and the F_2 's and the F_3 's. The wild parents had the biggest plants while the F_3 's the shortest. Wild parents had the highest number of tillers and cultivated the lowest. The cultivated parents had the biggest flag leaf dimensions and wild parents the smallest. There was no significant difference in flag leaf dimensions between the F_2 's and the F_3 's. However, the F_2 's and the F_3 's had bigger flag leaf dimensions compared to the wild parents.

Within all types of entry (cultivated and wild sorghums, the F_2 's and the F_3 's) genotypes generally differed significantly ($P = 0.05$) for all quantitative traits (Table 3). Most wild parents did not differ significantly in the number of tillers and plant height, but cultivated parents, the F_2 's and the F_3 's displayed a greater variability for these traits. In all cross combination, the F_2 's and the F_3 's had a higher number of tillers compared to their cultivated parent but lower than wild parent. However, there was an increase in number of tillers from the F_2 to the F_3 . In both the F_2 's and the F_3 's, crosses with PET gave the highest number of tillers and ETH the lowest. Most of the F_2 's and the F_3 's showed no significant difference for days to flowering and flag leaf dimensions. Generally, there was a decrease in plant height and flag leaf dimensions from the F_2 's to the F_3 's. In addition, the majority of the F_3 's did not differ significantly for germination percentage and plant height compared among them. However, there was an increase in germination percentage from the F_2 's to the F_3 's.

Genotype \times environment interaction was significant ($P = 0.05$) germination percentage, days to flowering number of tillers and plant height (Table S3). Significant genotype \times environment interaction for flag leaf dimensions were only observed in cultivated sorghums and the F_3 's.

Table 4 Relative deviations (%) of F₂'s and F₃'s from the wild parent for six quantitative traits; data were obtained from two sites, Samanko and Sotuba in Mali during the 2008 growing season

Cross combination	Generation	Germ (%)	DTF (days)	NT (count)	PH (cm)	FLL (cm)	FLW (cm)
Hemenabanu × PET	F ₂	75.88*	4.18	−35.20	19.26*	14.53*	10.44
Hemenabanu × PET	F ₃	80.67*	21.87*	−0.69	−9.67*	11.00	−9.28
CSM63E × BEN	F ₂	−9.29*	−14.88*	−39.70*	−18.17*	13.70*	7.44
CSM63E × BEN	F ₃	6.41*	−0.01	−20.99	−25.53*	5.05	5.84
Hemena × DIOS	F ₂	337.85*	−17.95*	−50.75*	−8.18	28.98	−0.36
Hemena × DIOS	F ₃	347.75*	−9.29*	−31.76	−20.87*	23.20	−7.78
Nioba × ETH	F ₂	−15.45*	9.20	−32.16	17.26*	5.15	−2.02
Nioba × ETH	F ₃	33.49*	−6.68*	−1.75	−22.15*	9.92	10.73*
V4 × ETH	F ₂	7.29	−12.02*	−47.95*	−33.12*	10.99	0.81
V4 × ETH	F ₃	24.58*	1.60	−13.45	0.29	9.78	14.98*
Average ²	F ₂	30.39	−6.63	−41.53	−5.65	14.23*	3.00
Average ²	F ₃	52.38*	0.82	−15.53	−16.00*	11.47*	2.96

Average = mean among generations F₂ and F₃; Deviation from wild parent (DWP) = 100 ((F_x−W)/W) where x denotes the generation and W wild parent

Germ Germination percentage, DTF days to flowering, NT number of tillers, PH plant height, FLL flag leaf length, FLW flag leaf width

*Significantly different from zero at $P = 0.05$

Deviation of generations F₂ and F₃ from the wild parent

Relative deviation of hybrid generations from wild parents was significant ($P = 0.05$) for majority of traits (Table 4). Generally, most of the F₂'s showed a greater deviation from the wild parent than the F₃'s. Most cross combinations displayed positive significant deviations from the wild parent for germination percentage. The F₃'s displayed greater deviation from the wild parent for germination percentage compared to the F₂'s. Moreover, some of the F₂'s displayed negative significant deviations from wild parent for germination percentage. Generally, all cross combinations displayed negative deviations from wild parent for number of tillers, but only three were significant. The F₂'s displayed greater deviation from the wild parent for number of tillers compared to the F₃'s. The majority of cross combinations expressed negative significant deviations from wild parent in plant height. The F₂'s generally displayed greater deviations from wild parent in plant height compared to the F₃'s. Most of cross combinations displayed positive deviation in flag leaf dimensions, but only a few were significant. The majority of the F₂'s

displayed a greater deviation from wild parent in flag leaf length than the F₃'s, while the majority of the F₃'s displayed greater deviation for flag leaf width than the F₂'s.

Survivorship in F₂ and F₃

'Relative survivorship' was significant ($P = 0.05$) in various cross combination for various genotypes (cultivated parent, the F₂ and the F₃) compared to the wild parent (Table 5). Most of cultivated and wild

Table 5 Relative survivorship¹ of five cross combinations in generations F₂ and F₃ and their parents; data were obtained from two sites, Samanko and Sotuba in Mali during the 2008 growing season

Cross combination	Cultivated	F ₂	F ₃	Wild
Hemenabanu × PET	1.61a	1.38a	1.08b	1.00b
CSM63E × BEN	0.98a	0.82b	0.99a	1.00a
Hemena × DIOS	3.58a	3.65a	3.74a	1.00b
Nioba × ETH	1.12a	0.69b	1.19a	1.00a
V4 × ETH	0.62b	0.92a	1.10a	1.00a

Means followed by the same letter within columns are not significantly different at $P = 0.05$

parents displayed an equal ‘relative survivorship’. Generally the majority of cultivated parents had a higher survivorship compared to wild parents. In comparison to their parents, many of the F_2 and the F_3 plants’ survived and set seeds. Generally the F_2 ’s and the F_3 ’s displayed a great range of survivorship compared to their parents, some displaying a higher survivorship compared to either of the parent. Most of the F_2 ’s differed significantly from their cultivated parents in respect to ‘relative survivorship’. The majority of the F_3 ’s did not differ significantly for ‘relative survivorship’ compared to their wild parents, while majority of the F_2 ’s differed. Most of the F_3 ’s had a higher ‘relative survivorship’ compared to the F_2 ’s.

Relative mid-parent heterosis of F_1 , F_2 and F_3

Relative mid-parent heterosis for number of tillers and plant height was significant ($P = 0.05$) in various cross F_1 cross combinations (Table 6). All cross

combination did not express a significant mid-parent heterosis for flag leaf length and width.

Generations F_2 and F_3 displayed a great range of mid-parent heterosis, ranging from negative to positive (Table 6). On average the F_3 ’s expressed greater mid-parent heterosis for number of tillers compared to the F_2 ’s. Generally in all cross combinations there was an increase in mid-parent heterosis for number of tillers from the F_2 to the F_3 . All the F_3 ’s displayed positive mid-parent heterosis for number of tillers, but only one cross combination was significant. Though not significant, the majority of the F_2 ’s had a negative relative mid-parent heterosis for number of tillers. Most of the F_2 ’s and the F_3 ’s cross combinations expressed significant mid-parent heterosis for plant height, but most were negative. Only the F_3 ’s expressed significant mid-parent heterosis for flag leaf length and width. Generally there was a decrease in mid-parent heterosis for flag leaf length and width from the F_2 to the F_3 .

Table 6 Relative mid-parent heterosis (%) of F_1 (data were obtained from one site, Samanko), and F_2 and F_3 (data were obtained from two sites, Samanko and Sotuba) crosses between

wild and cultivated sorghum accessions for four quantitative traits; conducted during the 2008 growing season in Mali

Cross combination	Generation	NT ¹	PH ³	FLL ⁴	FLW ⁵
MSP × ETH	F_1	69.54*	23.27*	-9.52	-2.65
MSP × PET	F_1	35.43*	35.76*	4.33	6.89
MST × DIOS	F_1	59.59*	20.08*	-1.09	-0.29
MST × PET	F_1	0.58	25.83*	0.51	4.50
	Average	38.82*	25.78*	-0.16	6.42
Hemenabanu × PET	F_2	-19.63	8.98*	-0.16	-6.23
CSM63E × BEN	F_2	-20.21	-8.57*	0.53	-3.59
Hemena × DIOS	F_2	5.13	-5.17	1.54	-5.32
Nioba × ETH	F_2	-10.18	3.79	-2.23	-6.64
V4 × ETH	F_2	-38.92	-23.16*	8.89	-1.75
	Average	-12.45	0.79	3.13	-2.06
Hemenabanu × PET	F_3	23.09	-17.46	-3.24	-23.03*
CSM63E × BEN	F_3	4.62	-16.79*	-7.13*	-5.04
Hemena × DIOS	F_3	45.14	-18.28*	-3.00	-12.40*
Nioba × ETH	F_3	30.06*	-31.09*	2.20	5.45
V4 × ETH	F_3	1.54	15.23*	7.68*	12.12*
	Average	15.75*	-14.65*	0.37	-3.55

Germ Germination percentage, *DTF* days to flowering, *NT* number of tillers, *PH* plant height, *FLL* flag leaf length, *FLW* flag leaf width

*Significantly different from zero at $P = 0.05$

Discussion

The study confirms that hybrids between wild and cultivated sorghums and subsequent offspring generations are able to reproduce, thereby allowing crop genes to introgress into natural populations. A great variability was observed in the hybrid generations for different traits studied, ranging from lower to higher values than their parents (Table 2 and 3). The F_1 's had bigger plants compared to wild and cultivated parents, indicating hybrid vigour.

The significant genotype \times environmental interaction observed in various traits (in the generations F_2 and F_3) imply that different genotypes resulting from wild \times cultivated sorghum hybridisation have different fitness in varying environment. This suggest that with a wide range of segregating genotypes resulting from wild \times cultivated sorghum hybridisation, a great potential exist that at least some hybrids would persist and survive in a given environment. Even in cases where the resultant hybrids are less fit on average, they may be viable and fertile enough to reproduce and thereby act as an avenue for introgression. Once they have the opportunity to establish in a given environment, natural selection may cause stepwise improvement of their adaptation. Since flowering in sorghum starts with terminal flowers and extends downwards in a fairly regular manner over 6–15 days (Doggett 1988), flowering in both hybrids and wild parents will overlap (Tables 2, 3) thus allowing hybrids to backcross with neighbouring wild plants.

Field observations in Mali and Kenya (Muraya et al., unpublished; Pierre C.S. Traore, Personal communication) confirmed that the various genotypes resulting from wild \times cultivated sorghum hybridisation can survive in variable environments'. In the field, hybrid plants were observed in sorghum fields, fallows, other cereal fields, along roads, river banks and water canals. They included plants showing introgression with cultivated sorghum in their phenotypic characters, e.g., plant height and vigour, fewer tillers, panicle shape and size, presence of awns, seed shattering, glume and seeds size.

The hybrids obtained in the present study had high germination scores suggesting that this trait is not affected by introgression barriers. The absence of such barriers can explain why many intermediate types were observed in fallow fields. Germination is

an important trait in plant evolution as it determines which plant will get a chance to reproduce.

Different wild \times cultivated cross combinations gave different germination scores. This implies that different parental combinations vary in their adaptive values. For circumventing unfavourable environmental conditions such as prolonged drought, dormancy is an important adaptive feature of wild sorghum. In the absence of dormancy, the seeds would germinate soon after shedding from the plant and the seedling would die during a drought. Hence, the reduced degree of dormancy in hybrid seeds means that under natural conditions drought might pose a significant selection pressure against them. Seed dormancy of hybrids and their wild parents need to be studied under natural conditions over several seasons for a more accurate interpretation of hybrid survival.

To assess the likelihood of crop to wild introgression, the adaptive traits in generation F_2 and F_3 were compared with those of their wild parents. In this study, the F_2 's displayed greater deviation from their wild parents for number of tillers, days to flowering and flag leaf dimensions than F_3 's (Table 4), indicating that F_3 plants were drifting towards wild sorghum. Generally, wild sorghum are characterised by abundant tillers and narrow flag leaf, while cultivated sorghum are characterise by less or no tillers and broad flag leaf. The fact that the majority of F_2 's and F_3 's did not deviate significantly from their wild parents for number of tillers, suggests that they may become as competitive as their wild parents. Traits in the F_2 and F_3 plants that did not deviate significantly from those in their wild parents indicate that they have a selective advantage, and thus will persist in natural habitats. The high significant (positive) deviation from wild parent for germination percentage in the majority of cross combination may have a selective disadvantage in case of little precipitation followed by a severe drought. On the other hand, the high significant (negative) deviation from wild parent on days to flowering displayed by most cross combinations may have selective advantage in cases of terminal drought stress. The majority of hybrids had a significant (negative) deviation from wild parent for plant height which may result in reduced lodging of plants, and thus enhance competitiveness in natural habitats. Normally, wild sorghum occurs in large aggregated populations and reduction in lodging would greatly increase their fitness.

Many of the F_2 and F_3 plants survived and set seed (Table 5), suggesting a good reproductive assurance, which in turn is an indication of their viability and fertility. The high number of hybrid plants that set seeds suggests that introgression of genes would not be restricted. Other fitness components, that were not measured in this study include pollen fertility, seed dormancy, and number of seeds per panicle. Reduction in pollen fertility has been reported for many hybrids (Hauser et al. 1998).

Both positive and negative heterosis was found in various cross combinations for the studied traits and some were significant (Table 6). Heterosis was high in the F_1 generation and significant heterosis was observed in plant height and number of tillers, but not in flag leaf dimensions. These high levels of heterosis for plant height and number of tillers suggest that the cross combinations in which there exists a relatively high amount of heterozygosity would provide a significant increase in vigour compared to nonhybrid plants. The general trend in F_2 and F_3 generations is hybrid breakdown and only a few traits expressed positive heterosis. Contribution of recessive genes caused by recombination between coadapted genes (i.e. epistasis) also may explain the general trend observed in F_2 and F_3 . The presence of hybrid breakdown has been observed (Alam et al. 2004) in the self-pollinated plant species, rice (*Oryza sativa* L.). In our study, the F_3 generation displayed high heterosis for number of tillers, indicating an increased performance for this trait. Though, the F_3 generation expressed significant (negative) heterosis for plant height, this do not necessary indicate poorer performance since a decrease in plant height may improve performance by reducing lodging. one hybrids (F_2 's and F_3 's) displayed significant (positive) heterosis for flag leaf dimensions, indicating a positive effect on yield as flag leaf dimensions have been related to yield potential (Hansen et al. 2005).

Variation for adaptive traits of genotypes derived from different cross combinations suggests that fitness of F_1 and subsequent generations plants will depend on wild and parent that goes into combination. Therefore, it can be predicted that the introgression success may vary between combinations of wild and cultivated sorghums, stressing that gene flow assessment experiments should cover a range of cultivated and wild sorghum parents (parents derived from different sorghum races and or ecotypes).

Comparing the relative fitness of wild \times cultivated hybrids and their progenies with that of their wild parents is essential for understanding the evolutionary significance of interspecific hybridisation. Fitness studies are therefore pivotal for assessing the ecological consequences caused by transgenes introgressed through gene flow.

Conclusion

All essential prerequisites for effective gene flow between cultivated and wild sorghum are met under natural and agricultural conditions as shown in this study. Genotypes resulting from wild \times cultivated sorghum hybridisation did not show serious fitness penalties in the evaluation environments and thus may act as an avenue for introgression. Moreover, the hybrids adaptive value and reproductive capacity data revealed that evolutionary consequences of cultivated and wild sorghum mating do not preclude introgression. The lack of effective introgression barriers needs to be considered in evaluating the risk of transgene transfer into wild sorghum. It is reasonable to infer that fitness neutral or advantageous genetically modified traits may persist in wild sorghum populations.

References

- Alam MF, Khan MR, Nuruzzaman M, Parvez S, Swaraz AM, Alam I, Ahsan N (2004) Genetic basis of heterosis and inbreeding depression in rice (*Oryza sativa* L.). *J Zhejiang Univ Sci* 5:406–411
- Arriola PE, Ellstrand NC (1996) Crop-to-weed gene flow in the genus *Sorghum* (*Poaceae*): spontaneous interspecific hybridization between Johnsongrass, *Sorghum halepense*, and crop sorghum, *S. bicolor*. *Am J Bot* 83:1153–1160
- Boudry P, Moerchen M, Saumitou-Laprade P, Vernet P, Van Dijk H (1993) The origin and evolution of weed beets: consequences for the breeding and release of herbicide-resistant transgenic sugar beets. *Theor Appl Genet* 87:471–478
- Chèvre AM, Eber F, Baranger A, Hureau G, Barret P, Picault H, Renard M (1998) Characterisation of backcross generations obtained under field conditions from oilseed rape-wild radish F_1 interspecific hybrids: an assessment of transgene dispersal. *Theor Appl Genet* 97:80–98
- De Wet MJM (1978) Systematics and evolution of sorghum sect sorghum (Gramineae). *Am J Bot* 65:477–484
- De Wet MJM, Harlan JR, Price EG (1970) Origin of variability in the spontanea complex of *Sorghum bicolor*. *Am J Bot* 57:704–707

- Delourme R, Foisset N, Horvais R, Barret P, Champagne G, Cheung WY, Landry BS, Renard M (1998) Characterisation of the radish introgression carrying the *Rfo* restorer gene for the Ogu-INRA cytoplasmic male sterility in rapeseed (*Brassica napus* L.). *Theor Appl Genet* 97:129–134
- Doggett H (1988) Sorghum, 2nd edn. Longman, London
- Ellstrand NC, Prentice HC, Hancock JF (1999) Gene flow and introgression from domesticated plants into their wild relatives. *Annu Rev Ecol Syst* 30:539–563
- Erickson DL, Fenster CB (2006) Intraspecific hybridization and the recovery of fitness in the native legume *Chamaecrista fasciculata*. *Evolution* 60:225–233
- Hansen KA, Martin JM, Lanning SP, Talbert LE (2005) Correlation of genotype performance for agronomic and physiological traits in space-planted versus densely-seeded conditions. *Crop Sci* 45:1023–1028
- Hauser TP, Jorgensen RB, Ostergard H (1997) Preferential exclusion of hybrids in mixed pollinations between oilseed rape (*Brassica napus*) and weedy *B. campestris*. *Am J Bot* 84:756–762
- Hauser TP, Shaw RG, Stergard H (1998) Fitness of F₁ hybrids between weedy *Brassica rapa* and oilseed rape (*B. napus*). *Heredity* 81:429–435
- Hedge SG, Nason JD, Clegg JM, Ellstrand NC (2006) The evolution of California's wild radish has resulted in the extinction of its progenitors. *Evolution* 60:1187–1197
- Kadereit JW, Uribe-Covers S, Westberg E, Comes HP (2006) Reciprocal hybridization at different times between *Senecio flavus* and *Senecio glaucus* gave rise to two polyploidy species in north Africa and south-west Asia. *New Phytol* 169:431–441
- Li Z, Pinson SRM, Paterson AH, Park WD, Stansel JW (1997) Genetics of hybrid sterility and hybrid breakdown in an intersubspecific rice (*Oryza sativa* L.) population. *Genetics* 145:1139–1148
- Lowe AJ, Abbott RJ (2004) Reproductive isolation of a new hybrid species, *Senecio eboracensis* Abbott et Lowe (Asteraceae). *Heredity* 92:386–395
- Lukaszewski AJ (1995) Physical distribution of translocation breakpoints in homeologous recombinants induced by the absence of the *Ph1* gene in wheat and triticale. *Theor Appl Genet* 90:714–719
- Morrell PL, Williams-Coplin D, Lattu AL, Bowers JE, Chandler JM, Paterson AH (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
- Pilson D, Prendeville HR (2004) Ecological effects of transgenic crops and the escape of transgenes into wild populations. *Ann Rev Ecol Evol Syst* 35:149–174
- Rieseberg LH, Desrochers AM, Youn SJ (1995) Interspecific pollen competition as reproductive barrier between sympatric species of *Helianthus* (Asteraceae). *Am J Bot* 82: 515–519
- Rieseberg LH, Arias DM, Ungerer MC, Linder CR, Sinervo B (1996) The effects of mating design on introgression between chromosomally divergent sunflower species. *Theor Appl Genet* 93:633–644
- SAS Institute (2004) The SAS System for Windows, Version 9.1. The SAS Institute, Cary
- Snowden JD (1955) The wild fodder Sorghum of the section Eusorghum. *J Linn Lond* 55:191–260
- Truco MJ, Hu J, Sadowski J, Quiros C (1996) Inter- and intra-genomic homology of the *Brassica* genomes: implications for their origin and evolution. *Theor Appl Genet* 93: 1225–1233
- Whitton J, Wolf DE, Arias DM, Snow AA, Rieseberg LH (1997) The persistence of cultivar alleles in wild populations of sunflowers five generations after hybridization. *Theor Appl Genet* 95:33–40
- Zemetra RS, Hansen J, Mallory-Smith CA (1998) Potential for gene transfer between wheat (*Triticum aestivum*) and jointed goatgrass (*Aegilops cylindrica*). *Weed Sci* 46:313–317