

## Experimental studies on pollen-mediated gene flow in *Sorghum bicolor* (L.) Moench using male-sterile bait plants

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### Abstract

Information on the potential of pollen mediated gene flow (PMGF) in sorghum is required for ensuring varietal purity and to mitigate risk transgenic gene flow. Replicated trials were conducted in Kenya using a local landrace, 'Ochuti' as pollen donor surrounded by male-sterile pollen baits. Frequency of PMGF decreased with the increase of distance from pollen sources and was significantly influenced by wind direction and speed. Anther dehiscence correlated with increase in vapour pressure deficit in the morning. A negative exponential regression model with logarithmic transformation of PMGF and square-root transformation of distance from source field best fitted the data. Up to 50% of female florets on a male sterile (MS) plant were pollinated at 1 m from pollen source and declined to 14% at 10 m. The maximum distance of PMGF using the PMGF model, based on a threshold of one seed per MS plant, was 203 m when data above the 95th percentile is considered. However, in the presence of self-produced pollen of male-fertile target plants, the possibility of long-distance cross-pollination may be very low.

**Key words:** sorghum — pollen dispersal — gene flow — flowering characteristics

Sorghum is one of the major food grains in the world covering about 50 million hectares worldwide (FAOSTAT 2009). Its production is constrained by biotic and abiotic factors especially in low input subsistence farming systems (Haussmann et al. 2000). To overcome some of these constraints, the crop is targeted for improvement through classical plant breeding and modern biotechnological approaches (Borrell et al. 1999, Haussmann et al. 2004). In sorghum, significant progress has recently been made in genetic transformation through both agro-bacterium and particle bombardment (Zhao et al. 2000, Howe et al. 2006). Various alien genes have been successfully engineered into sorghum to enhance resistance to diseases, pests and nutritional value of the crop (Zhu et al. 1998, Girijashankar et al. 2005, Tesso et al. 2008). Although biotechnology can provide solution to some intractable constraints in sorghum production, the potential of gene flow from genetically modified (GM) to non-GM or to weedy or wild relatives in centres of crop diversity needs to be assessed (Ellstrand 2001, Snow 2002, Gepts and Papa 2003, Snow et al. 2003).

The most debated environmental biosafety concerns with respect to deployment of GM crops include: (1) direct and

indirect effects of toxic transgenes to non-target organisms (O'Callaghan et al. 2005); (2) potential risks associated with the development of resistance to biotic-resistance transgenes in the target organisms (Li et al. 2007, Wu et al. 2007); and (3) transgene escape to crop landraces and wild relatives through gene flow and its potential ecological consequences (Ellstrand et al. 1999, Wilkinson et al. 2000, Ellstrand 2001, Lu and Snow 2005, Mercer et al. 2007). The main ecological concerns about GM crops are the potential weediness or invasiveness in the crop itself or in its wild or weedy relatives as a result of introgression of fitness-enhancing abiotic and biotic GM traits including herbicide, insect, and disease resistance, and drought-tolerance traits (Warwick et al. 2009). Transgene escape from a GM crop variety to its non-GM crop counterparts or wild relatives can easily occur via pollen mediated gene flow (Ellstrand et al. 1999, Ellstrand 2001, Ellstrand 2003, Lu and Snow 2005). So far, natural hybridization has been reported to occur in crop/wild ancestor complex in 22 of the world's most important crop species, including Sorghum (Ellstrand et al. 1999, Ellstrand 2003). The promiscuity of sorghum with its congeners is apparent from the numerous studies which show genetic and/or morphological evidence of crop-to-wild gene flow, especially where the interfertile wild and cultivated sorghums grow sympatrically (Doggett 1988, 1991, Aldrich and Doebley 1992, Aldrich et al. 1992, Morrell et al. 2005, Barnaud et al. 2009).

Besides GM gene flow, the potential of gene flow from non-improved to improved cultivars is an additional source of concern for ensuring purity of improved varieties during seed production. Contamination of improved varieties by pollen from non-improved cultivars in neighbouring fields may compromise their agronomic performance. Currently, farmer preferred landraces from various African countries are being improved using marker-assisted breeding for important traits such as resistance against striga, a parasitic weed (Folkertsma et al. 2005) and drought tolerance (Borrell et al. 1999). The future stability of these introgressed traits depends on minimizing intercrossing with non-modern cultivars that grow on farmers diverse fields. Intercrossing would result in recombination among genes conferring traits of interest, particularly those that are recessively expressed.

Risks associated with GM gene flow as well as erosion of purity of improved varieties are most relevant under

traditional farming systems as compared with commercial farming. In Africa, subsistence farmers often plant large varietal diversity to serve their diverse needs as well as to lower the risk of crop failure (Teshome *et al.* 1999, Barnaud *et al.* 2007). For example, up to 40 landraces in a single village and up to a dozen landraces per field have been reported (Barnaud *et al.* 2007). Mix-planting of different cultivars is also commonly practiced. Where land fragmentation is high, neighbouring fields may form a mosaic of different landraces that grow side-by-side. The likelihood of PMGF under such conditions is quite high. Indeed, landraces that are mix-planted have been shown to have little genetic differentiation among them (Barnaud *et al.* 2007, Rabbi *et al.* 2010). In order to maintain the identity of their landraces despite extensive PMGF, farmers mainly rely on visual selection of particular combination of morphological traits (Barnaud *et al.* 2007) rather physical or temporal isolation of their multiple landraces. This selection method may not be effective in traits that cannot be visually assessed by the farmers.

Moreover, subsistence farmers predominantly rely on traditional seed systems such farm-saved seeds or from relatives and neighbours (Almekinders *et al.* 1994). The free flow of genes through pollen and seed exchange substantially increases the potential of transgene escape as well as contamination of modern varieties. These complexities and lack of monitoring in most parts of Africa makes detecting and containing GM-spread difficult once it enters such complex system.

Previous attempts to measure gene flow *in situ* were based on the detection of diagnostic morphological markers when cultivated and wild-weedy sorghum grow sympatrically (Doggert and Majisu 1968, Aldrich and Doebley 1992, Aldrich *et al.* 1992). Arriola and Ellstrand (1996) used isozyme markers to measure PMGF between wild and weedy sorghum (*S. halepense*) in an experimental system. More recently, Schmidt and Bothma (2006) estimated PMGF using male sterile (MS) sorghum as pollen baits. However, the closest sampling distance to the pollen source of that study was 13 m. Thus no information is available on PMGF below that distance. Furthermore, as PMGF is dependent on environmental factors, more studies are required in order to come up with a reliable PMGF model. In the present study, PMGF was measured in two seasons from 1 to 100 m so as to provide a more realistic PMGF model. Furthermore, PMGF may vary widely among environments even for similar sampling distances and experimental designs in both inbred crops such as wheat (Gustafson *et al.* 2005) and rice (Chen *et al.* 2004, Messeguer *et al.* 2004, Song *et al.* 2004, Jia *et al.* 2007) as well as outbreeding crops such as maize (Gustafson *et al.* 2006). Main factors influencing PMGF include the reproductive biology (e.g. outcrossing rates), environmental or climatic conditions, wind speed and direction during flowering, and spatial relationship and phenology of pollen donor and recipient plants (Manasse 1992). Thus there is need for more studies on PMGF in sorghum in order to come up with generalized conclusions.

The objective of this study is to determine the extent and pattern of PMGF from the grain sorghum cultivar 'Ochuti' using MS lines as pollen traps. 'Ochuti' is a *durra*-type landrace being widely grown in Western Kenya by subsistence farmers. This region accounts for about 70% of sorghum production in Kenya (Ngugi *et al.* 2002) and it is characterized by highly fragmented agricultural landscape with average field size of less than 0.5 ha. To meet their subsistence needs, farmers in

this region also rely on a large varietal diversity that are often mix-planted (Mutegi *et al.* 2009, Rabbi *et al.* 2010). The landrace was selected for this study because: (1) it can be considered as a representative of the landraces grown in Western Kenya, (2) it has a low outcrossing rate of ~4% (I. Y. Rabbi, H. H. Geiger and H. K. Parzies, unpublished data). This should answer the question: do genotypes with low outcrossing contribute to pollen mediated gene flow and at what magnitude?

## Materials and Methods

**Field experiments:** Field experiments were conducted using Sorghum [*Sorghum bicolor* (L.) Moench], during the long rain (LR) and short rain (SR) seasons of 2006 at the ICRISAT Kiboko dry land field research station in Eastern Kenya (Latitude 2.39361 S and longitude 37.79194 E). The trial was conducted in a non-sorghum growing area. A diagrammatic representation of the experimental design is shown in Fig. 1. 'Ochuti' was planted in the centre of a field as a quadratic block of 100 m<sup>2</sup>. Serving as pollen receptors were rows of two MS-lines (ATX 623 and ICSA 88006) planted in eight canonical directions (N, NE, E, SE, S, SW, W and NW). Although they do not produce viable pollen, they display normal fertility. Thus, pollen flow can be estimated on the basis of seed set. In each direction, two rows per receptor-line were planted in an alternating fashion. Plant spacing was 30 × 75 cm for both pollen donor block and recipients strips. Pollen recipients were planted continuously up to 50 m and thereafter as blocks. The area between the pollen recipients were left as fallow.

In order to synchronize flowering between 'Ochuti' and the receptor lines, a pre-experiment was conducted in the previous SR season of 2005. The two MS lines, ATX 623, released by the Texas Agricultural Experimentation Station, US, and ICSA 88006 released by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) were chosen. The above mentioned lines flowered at 67 days after planting (d.a.p) and 70 d.a.p, respectively. As 'Ochuti' flowered at 74 d.a.p, it was planted 6 days earlier than the MS lines in the main experiment. The central pollinator plot was sown on March 8 in the LR season of 2006 and October 20 in the SR season. At maturity, five plants per MS line were harvested at 0.1 intervals from log<sub>10</sub>0 (=1 m) through to log<sub>10</sub>2 (=100 m). This enabled intense sampling at close distances to the source where pollen dispersal declines rapidly than at far distances. Actual distances in meters were 1, 1.3, 1.6, 2.0, 2.5, 3.2, 4.0, 5.0, 6.3, 7.9, 10.0, 12.6, 15.8, 20.0, 25.1, 31.6, 39.8, 50.1, 63.1, 79.4 and 100 m (Fig. 1).

An on-site weather station, Weather Monitor II® (Davis Instruments Corp), mounted on a 10 m post was used to record meteorological information, including wind speed and direction, temperature and relative humidity. Weather data was recorded in half-hour intervals from the beginning until the end of flowering.

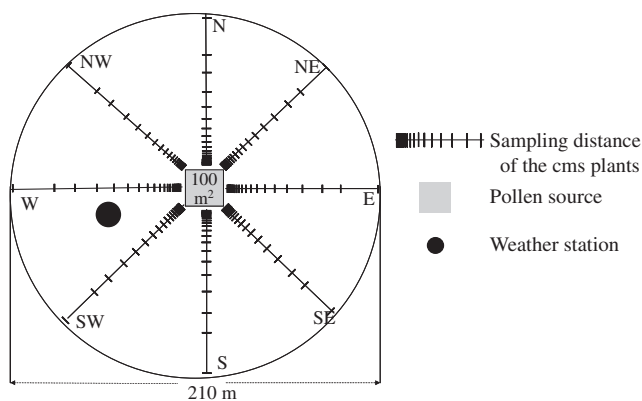


Fig. 1: Experimental set-up for measuring PMGF using male-sterile as baits

**Anther dehiscence of 'Ochuti':** Diurnal pollen release pattern of 'Ochuti' was monitored bi-hourly for 24 h per day during the SR season of 2006. Pollen grains were captured on a sticky transparent tape (Cellotape®) placed below the panicle canopy every 2 h from 6:00 am and 6:00 pm for 24 h, thus finishing at the same times the following day. This was done from January 15 to January 20, 2006. The pollen grains were photographed under a Wild Heerburg (M5) light microscope against a black background. Hourly vapour pressure deficit (VPD) values were calculated from air temperature and relative humidity using the formulae of Wang et al. (2004). Vapour pressure deficit is the difference between actual and saturation vapour pressure, expressed in Kilopascals (kPa).

**Statistical analysis:** Analysis of data and fitting of curvilinear models were carried out using the STATGRAPHICS Centurion XV software (<http://www.statgraphics.com>). Different regression models were fitted to the data and the model with the highest coefficient of determination ( $r^2$ ) was considered as the best model for the data. Three regression lines were fitted to the data of each season. The first line was calculated from all data-points. The second line represents the data above the 95th percentile, and the third line the 99th percentile (i.e. plants producing more seeds than the lower 95th and 99th percentile cut-off levels). Parameters of these lines were determined by the common non-linear least squares regression method (Gallant 1975).

## Results

### Diurnal pollen shedding pattern of landrace 'Ochuti'

The diurnal pattern of pollen release of 'Ochuti' was found to be dependent on VPD (Fig. S1). Peak pollen release time occurs between 8:00 am and 10:00 am, immediately following the rise in VPD caused by increase temperature and the corresponding decline in relative humidity in the morning. During clear days, the pollen release occurred around 8:00 am and during cloudy days, it occurred later than 8:00 am (data not shown).

### Weather data

The prevailing wind direction in the LR experiment was from SW in the morning hours (08:00–12:00 am) and from E in the afternoons (15:00–18:00 pm) (Fig. 2a). Wind speed in the study site generally increased as the day progresses and average wind speed was higher in the afternoon (7.41 km/h) compared with the morning period (5.34 km/h). More instances where wind speed exceeded 10 km/h were recorded in the afternoon compared with the morning. In the SR experiment, wind direction was more stable than in the LR season and mainly came from N and NE in the morning hours and E in the afternoon hours (Fig. 2b). Wind speed was generally lower during morning periods (4.43 km/h) than in the afternoon (9.26 km/h).

### Pollen mediated gene flow

The staggered planting dates of the pollen donor and recipients resulted in synchronization of flowering times. Materials started to flower in mid May 2006 in the LR and early January 2007 in the SR experiments. Pollen mediated gene flow was more frequent in the downwind direction in both seasons (Fig. 2c,d) but the SR experiment furnished substantially lower mean numbers of seeds in the up-wind directions. Percentage of seed set on MS plants at various distance from pollen source were estimated from overall data as well as the data above 95th percentile in the LR and SR

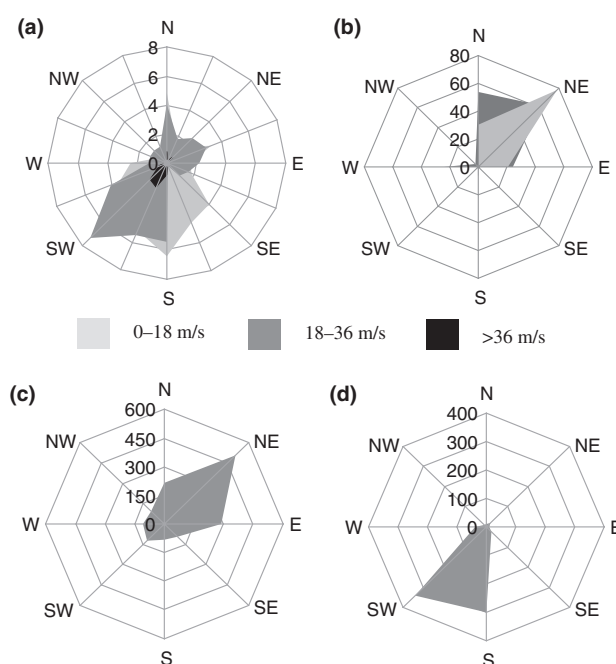


Fig. 2: Relative wind direction (values in %) recorded at 30-min intervals and number of seeds found on MS plants in the various wind direction. (a) and (c) represent LR while (b) and (d) represent SR seasons of 2006

experiments. These were calculated by dividing the number of seeds by the average number of florets per panicle (3385.3, SD = 615, N = 12). Using data above the 95th percentile, a rapid decline in seed setting rate on MS occurred between 1 m (50%) and 10 m (14%), and reached 1% at 60 m. On the contrary, estimates based on the overall data showed 6.6% and 1.8% seed setting rates at 1 and 10 m from pollen source, respectively. Furthermore, less than 1% seed setting rate can occur at 20 m from pollen source based on the overall data.

### Correlation between wind direction and cross-pollination patterns

Variation in seed set was compared with wind direction patterns, and correlation coefficients ( $r^2$ ) were calculated. Only morning data was used in the analysis. Wind speed was categorized into three classes (< 5, 5–10, and > 10 km/h). In the first experiment (LR), correlation between wind direction and seed set was moderate when unclassified wind direction frequencies were analysed ( $r^2 = 0.535$ ,  $P = 0.05$ ), but increased when wind speed of more than 18 m/s were used ( $r^2 = 0.66$ ,  $P = 0.01$ ). In the second experiment (SR), the wind was more stable in speed and direction. Thus a stronger correlation between wind direction and seed set was observed in this case ( $r^2 = 0.933$ ,  $P = 0.01$ ).

### Best fitting models of PMGF

Various transformations of the axes were implemented in order to determine a model describing relationship between PMGF and distance. The best fit was obtained by the equation  $y = \exp(a + bx^c)$  where  $y$  is the number of seeds at a particular distance,  $a$  is the number of seeds immediately

adjacent to the source,  $x$  is the distance from the source and  $b$  is a model parameter describing the steepness while  $c$  describes the curvature of the line. Parameter  $c$  was fixed at 0.5, that is, a square root transformation of distance because it closely fitted our data and it is well supported in the literature (Gustafson *et al.* 2005, 2006, Geines *et al.* 2007). Figure 3a demonstrates the relative advantage of the above model compared with an exponential model without square-root transformation of distance ( $c = 1$ ) (Fig. 3b). The model of PMGF appears as a concave-upward curve, thereby matching the scatter-plot trend of the data while the latter model appears as a straight line (Fig. 3b). As a result, it tends to underestimate PMGF at closer distance as well as the tail-end while it overestimates it in the middle distances. Similar pattern were obtained from the SR experiment (data not shown).

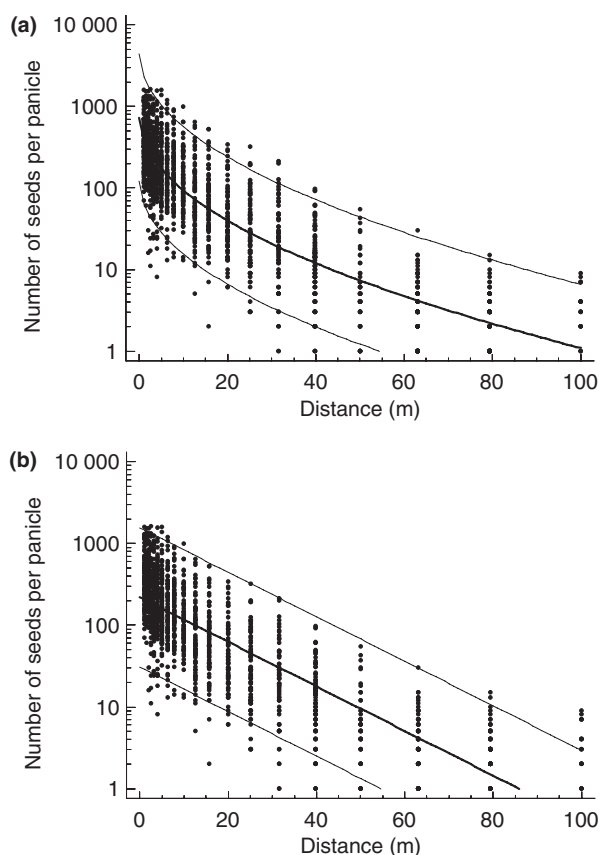


Fig. 3: Plots showing better fitting curvilinear PMGF model based on (a) square-root transformation of distance from pollen source compared with (b) non-transformed distance. Each point represents individual MS plants. Outer bounds indicate the 95% confidence interval of the model (inner line)

Coefficient of determination ( $r^2$ ) of the models, were high considering data above the 95th percentile and moderate for the total data set in each of the two seasons (Table 1). When the model was used to extrapolate distances for reaching a threshold of at least one seed per plant, in general when all plants are used for regression analysis, 103 m is obtained for the LR season and 111 m for the SR season experiments (Fig. 4). On the contrary, up to 203 m would be required to reach this threshold when the data above the 99th percentile is used (Table 1).

## Discussion

### Timing of anther dehiscence in 'Ochuti'

The diurnal pattern of anther dehiscence in cv. 'Ochuti' showed that pollen release reaches a peak during morning hours. This agrees with the viewpoint that weather factors have a strong effect on airborne pollen flow (Song *et al.* 2004, Schmidt and Bothma 2006). Anther dehiscence in angiosperms depends on two mechanisms: (1) environmentally-linked hygroscopic absorption of water by pollen (Keijzer 1999), and (2) disappearance of the anther's locular fluid and partial dehydration of the pollen (Pacini 2000). The latter mechanism seems to favour anther dehiscence in cv. 'Ochuti' as pollen shedding is correlated with an increase in VPD caused by increase in temperature and the accompanying decrease in relative humidity in the morning. Indeed, very little or no pollen was shed in the hours preceding 08:00 am. The former mechanism of anther dehiscence has been reported to occur in an Australian sorghum cultivar 'MR Buster®' (Ryley 2005). Ryley (2005) found that this cultivar's pollen release was favoured by highly humid air during the early hours of the morning (03:00 am) when VPD was at its lowest.

### Variation of gene flow with distance and wind direction

This study shows a sharp decrease of PMGF within the first 10 m of the source field. Beyond this distance, the pollination gradient declined less rapidly and PMGF was detected up to 100 m, the maximum distance investigated. It is difficult to compare the PMGF gradient of this study with that of Schmidt and Bothma (2006) because of differences in sampling strategies. In their study, the authors started sampling at 13 m from source. In rice, a predominantly self-pollinating crop, gene flow gradients show rapid decline within the first 5 m (Yuan *et al.* 2007). PMGF in maize, a highly outcrossing species, generally decreases rapidly over short distances of up to 30 m, with low cross-pollination detected over few hundred meters (Ma *et al.* 2004, Goggi *et al.* 2007). Luna *et al.* (2001) reported that cross-fertilization in maize could occur at a maximum distance of 200 m from the source. PMGF between

| Season  | Data used                  | $a$   | $b$   | $r^2$ | P       | Distance (m) <sup>1</sup> |
|---------|----------------------------|-------|-------|-------|---------|---------------------------|
| LR 2006 | All plants                 | 6.597 | -0.65 | 75.9  | <0.0001 | 103                       |
|         | Data above 95th percentile | 8.263 | -0.64 | 96.0  | <0.0001 | 168                       |
|         | Data above 99th percentile | 8.608 | -0.67 | 97.1  | <0.0001 | 165                       |
| SR 2006 | All plants                 | 4.603 | -0.44 | 27.7  | <0.0001 | 111                       |
|         | Data above 95th percentile | 7.771 | -0.55 | 95.6  | <0.0001 | 200                       |
|         | Data above 99th percentile | 8.033 | -0.56 | 96.6  | <0.0001 | 203                       |

Table 1: Estimates of regression parameters, coefficient of determination ( $r^2$ ), and significance levels (P) three pollen dispersal models in the LR and SR seasons of 2006

<sup>1</sup>Estimated distance at which at least one seed per plant is expected.

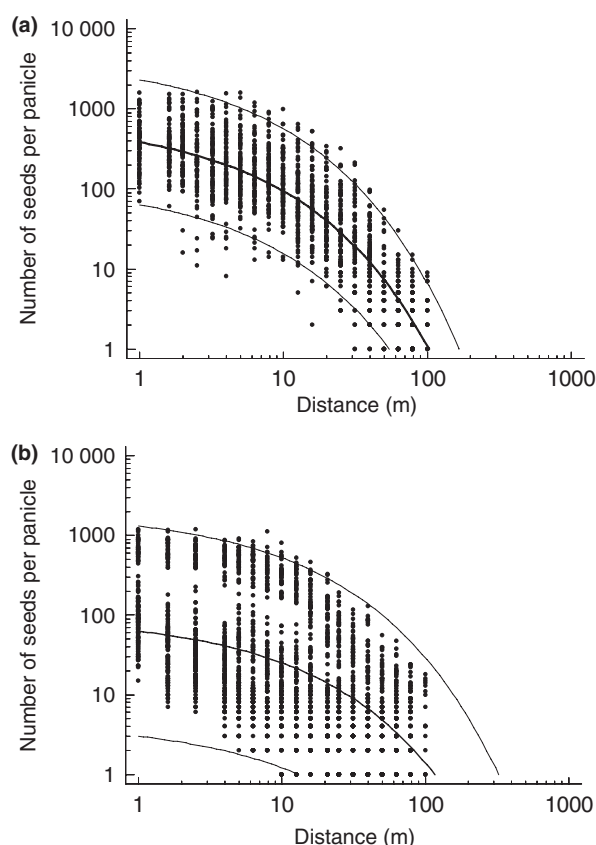


Fig. 4: Plots showing the amount of seeds produced per MS-plant and fitted model of PMGF based on square-root transformation of distance and  $\log_{10}$  of seed set in the (a) long rain and (b) short rain season experiments

GM and non-GM bentgrass, a highly outcrossing perennial species was reported to occur at great distances of 20 km (Watrud et al. 2004).

Average PMGF was higher in the LR than in the SR experiment (Fig. 2c,d). Two factors could have led to the observed differences: Firstly, frequent changes of the wind direction in the LR experiment led to substantial amount of seed formation in N, NE and E directions whereas the stable wind directionality in the SR resulted in most of the seeds to be formed in S and SW directions. The remaining four directions contributed very little seeds and this resulted in comparatively lower average seed set. Secondly, higher average wind speed observed in the morning hours of the LR season resulted in more pollen transportation and deposition on the MS bait plants. Other factors that would have led to seasonal difference in the pattern of PMGF include base temperature and day length (Quinby 1973). As 'Ochuti' and the MS plants generally flowered at the same times, the minor difference in day length could have had little influence on the results. The effect of base temperature could not be ascertained as it was not measured.

The estimated seed setting rates on MS pollen recipients in this study were comparable to that of Schmidt and Bothma (2006), who reported an average seed setting rate of 2.5% at 13 m. In the present study, average seed setting rate at the same distance were 2.1% in the LR and 0.6% in the SR. When the upper 5th percentile data is used, seed setting rates was found to be high close to the pollen source, whereby up to 50%

of florets on MS plants were pollinated. As pollen flow is unidirectional, only the side of a MS recipient facing pollen source is pollinated, thus explaining the 50% pollination rate at the closest distance sampled.

#### Model of gene flow

The model of PMGF as a function of distance from the source plants can be used to determine the isolation distance required to reduce the probability of hybridization between adjacent genotypes to below preset limits. The negative exponential model with a logarithmic- and square root-transformation of the seed numbers per MS plant and distance from pollen source, respectively, that was fitted to our data also performed well in other cereal crops such as wheat (Gustafson et al. 2005, Geines et al. 2007) and maize (Gustafson et al. 2006, Porta et al. 2008). A previous model of sorghum crop-to-crop PMGF proposed by Schmidt and Bothma (2006) did not fit our data. In the latter study, the authors employed exponential and power functions on double log-transformation. The exponential model underestimated PMGF at closer distances as well as the tail-end of the distribution in our data (Fig. 3b). The power model did not fit our data at all and had the opposite effect of overestimating gene flow at close as well as far distances (not shown).

Based on our model, the maximum distance required to reach a threshold of one seed per plant ( $\approx 0.03\%$  cross-pollination rate on male-sterile plants) based on data above the 99th percentile was determined to be 203 m in the SR season and 165 m in the LR season. This distance is slightly lower than that of Schmidt and Bothma (2006) which was estimated at 238 m. However, this prediction is only valid under 'average recorded' weather conditions. Extreme wind conditions can significantly alter the cross-pollination levels and result in long-distance PMGF.

#### Implications of this study to crop-to-crop and crop-to-wild PMGF

Measurement of PMGF is an essential component of ecological risk assessment of GM crops as pollen is the main vehicle to disseminate alien genes (Rong et al. 2004). However, the transfer of the findings of the present study to male-fertile cultivated and wild sorghum plants needs careful consideration as the frequency of the PMGF is mainly determined by the outcrossing rates of a pollen recipient (Ellstrand et al. 1999, Lu and Yang 2009). The likelihood of crop-to-crop PMGF is expected to be low as foreign pollen has to compete with pollen from the receptor plants, which is normally responsible for between 60% and 95% autogamy in cultivated sorghum (Ellstrand and Foster 1983, Pedersen and Toy 1998, Djè et al. 1999, Barnaud et al. 2008). For instance in a concurrent estimation of PMGF using male-sterile and male-fertile baits in maize, Wang et al. (2006) reported a 17-fold decrease in outcrossing when MF baits was used instead of MS ones. In addition, the extended stigma receptivity in the absence of self-pollination of up to 16 days (Shertz and Dalton 1980, cited in Schmidt and Bothma 2006) substantially increases the amount of PMGF when male-sterile plants are used as receptors.

On the contrary, the extent of PMGF from cultivated to wild sorghum such as Johnsongrass (*Sorghum halepense* (L.) Pers.) is expected to be significantly higher than that of crop-

to-crop because of the highly outcrossing nature (Snow and Ejeta 2007). Indeed, Morrell *et al.* (2005) found 32% frequencies of crop-specific alleles in Johnsongrass populations that were adjacent to sorghum fields in Texas and Nebraska, USA. Furthermore, Arriola (1995) detected hybridization between sorghum and Johnsongrass at 100 m from the crop, the maximum distance examined. This suggests that long-distance cross-pollination should not be excluded when cultivated and wild sorghum occurs sympatrically.

In the current study, 'Ochuti' was shown to release adequate amounts of pollen despite having low outcrossing rate of about 5% (Rabbi *et al.* unpublished). An important implication of this observation is that cultivars with low outcrossing rates may still have high potential of pollinating wild relatives of sorghum that are usually characterized by high outcrossing rates. An asymmetric gene flow that is predominantly from crop-to-wild is expected when wild and cultivated grow sympatrically. However, this hypothesis needs to be experimentally verified as other factors such as pollen competition may play a significant role.

## Conclusions

The occurrence of PMGF can be restricted to preset limits with use of appropriate isolation distance. This is feasible in commercial farming but can be difficult in subsistence agriculture. In situations where agricultural land is highly fragmented and farmers grow multiple varieties in their small fields, strong pollen flow over short distance (0–10 m) as detected in this study can lead to substantial inter-cultivar hybridization, thus posing a challenge in the maintenance of cultivar purity. Similarly, if transgenic varieties are introduced into this system, it is almost inevitable that transgenes will introgress into the local cultivars. In addition, once transgenes enter these systems, it may be difficult or even be impossible to control as the system is based on the free flow of genes through pollen and seeds. Sympatric occurrences of diverse wild and cultivated sorghums have been reported in many of the sorghum-growing regions of Africa (de Wet 1978, Tesso *et al.* 2008, Barnaud *et al.* 2009, Mutegi *et al.* 2009). Furthermore, wild and cultivated sorghum commonly flower together (Tesso *et al.* 2008). In Kenya, intermediate forms between wild and cultivated sorghum belonging to the *S. bicolor* ssp. *drummondii* (sudangrass) are frequently found in predominantly sorghum growing areas (Mutegi *et al.* 2009). Careful consideration of these factors in the light of PMGF in sorghum is required before deployment of GM sorghum in the subsistence farming systems. As PMGF mainly depends on the outcrossing rate of the target variety, further studies using male-fertile plants as pollen baits is required.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Diurnal variation of pollen release of Sorghum cv. 'Ochuti' with temperature (T), relative humidity (RH) and vapour pressure deficit (VPD, kPa) averaged over 5 days period.

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