African Journal of Biotechnology Vol. 8 (10), pp. 2265-2270, 18 May, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Dispersal distance of rice (*Oryza Sativa* L.) pollen at the Tana River delta in the coast province, Kenya

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Accepted 12 March, 2009

Rice is a staple food in Kenya and its production needs to be increased. Genetically modified (GM) rice may be a solution, but before it can be introduced, potential ecological impacts, such as pollen mediated gene flow from GM rice to non-GM rice or to its wild indigenous relatives, need to be understood. Pollen dispersal in rice (*Oryza sativa*) was studied in the Tana River district in the coast province of Kenya. *O. sativa* seedlings were planted in a 50 m diameter circular experimental design. Pollen traps (glass slides coated by vaseline attached to a board) were used to measure pollen flow at 2 heights and at increasing distances from the source plot. Pollen dispersal decreased rapidly with increasing distance from the pollen source up to 250 m, no pollen was found at 300 m. There was a significant ($P < 0.05$) difference in pollen dispersal in different directions, which correlated with the prevailing wind direction (south, occasionally east). Effect on wind speed and humidity could not be evaluated as they were relatively stable during the sampling period. No overall difference ($P > 0.05$) in pollen count between upper and lower pollen traps. The highest daily pollen count was observed between 11:00 am and 12:00 noon, and at a narrow range of temperatures 28 ± 2°C. On the basis of these data, an adequate isolation distance of more than 250 m should be considered to minimize chances of gene flow from transgenic rice to conventional or wild rices.

Key words: Pollen dispersal, gene flow, *Oryza sativa*, rice, genetically modified.

INTRODUCTION

Rice is one of the most important cereal crops worldwide and the staple food for nearly one-half of the global population (Song et al., 2003). It is therefore not surprising that rice is also one of the first crop species to which transgenic biotechnology has been effectively applied (Tyagi and Mohanty, 2000), although commercialization of genetically modified (GM) rice has not began. Many genetically modified rice varieties such as insect resistant (Bt) rice have been developed in confined environments (Rong, 2006).

However, cultivation of GM rice may have agronomic and environmental impacts that need to be evaluated and managed before their release and use (Lövei et al., 2007). Pollen mediated gene flow is foremost among the impacts that need to be addressed (Lu and Snow, 2005).

If the inserted genes, transgenes, spread to and are expressed in wild relatives of transgenic crop species, they may persist and be disseminated within the wild population through sexual or vegetative propagation. If the transgenes are coding for resistance to abiotic or biotic stress such as drought, salt tolerance, pests and diseases, this might significantly enhance the ecological fitness of these populations and possibly lead to problems such as invasion of novel plant types in natural ecosystems and production of aggressive weeds within agricultural fields (Ellstrand and Marshall, 1985; Crawley et al., 1993; Raybould and Gray, 1993; Snow and Moran-Palma, 1997). If the inserted transgenes instead spread to neighboring non-GM fields this may lead to contamination of the conventional rice seeds harvested here, which could affect marketing of these seeds due to a fall in seed quality and prices. This may especially be a problem in small-scale agriculture where distances between fields are small, and where seeds are recycled from year to year. Therefore, a better understanding of rice pollen dispersal, including its frequencies, distances

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and directions between crops such as rice and their wild relatives is required.

If transgenes are to be spread from a GM field and incorporated into seeds in a neighboring non GM crop, several biological processes have to be successful, pollen should disperse between GM pollen donors and the recipient non GM plants and successfully fertilize ovules. The resulting embryos further should survive and mature into full seeds. If transgenes are to be incorporated into the gene pool of a neighboring wild population, some of the seeds and offspring plants carrying the trans-gene further need to survive and reproduce successfully with other wild plants in the population. A first step in an impact assessment of gene flow from GM cultivars is therefore to evaluate the distances and directions traveled by pollen grains. Knowledge on pollen dispersal distances are further of utmost importance in order to determine adequate isolation distance between GM, non-GM and wild related populations. Isolation by distance is probably one of the most efficient ways of avoiding gene flow.

In this work, we present the results of a study of pollen dispersal distances from rice fields in Kenya where a minor part (5%; ministry of agriculture annual report, 2004) of rice cultivation is done by small-scale farming. The farming involves inter-village and farmer seed exchange and use of saved seeds. Further, the occurrence of wild *Oryza* species would favour gene flow between conventional and GM rice fields and between GM rice and wild related species, especially *Oryza longistaminata* Chev.et Roehr. *O. longistaminata* is an indigenous African rice species with a genome AA similar to that of *O. sativa,* which means that hybridization between the two species is possible (Vaughan, 1994). *O. longistaminata* grows in wild habitats and fields adjacent to rice farms and sometimes as a weed in rice fields, thus increasing chances of hybridization. Apart from the possible impacts of GM cultivation mentioned above, increased hybridization due to more widespread cultivation of rice could potentially result in the erosion of the wild *Oryza* genetic resources (Gao, 2004).

To get information on the possible isolation distance between the GM cultivated rice (*O. sativa*) and conventional rice fields or wild relatives, especially *O. longistaminata*, we set up a study in Tana Delta irrigation scheme. Here, we determined how far pollen travels from a source population to pollen traps at various distances and directions. We also examined the significance of time of the day and temperature on pollen dispersal. Since no transgenic plant materials were available, due to lack of biosafety laws for handling GM materials in Kenya, non-transgenic plant materials were used as the source of pollen.

MATERIALS AND METHODS

Study site

The Tana Delta irrigation scheme (40°10' 55 E and 2°16'35 S) is located in the Tana river Delta in the coastal province of Kenya, 250 km north-east of Mombassa. This site was selected partly because *O. longistaminata* is common in this region. The scheme has adequate supply of irrigation water and partly because large operations like this would be a likely first place to cultivate GM rice in Kenya.

Plant material and experimental design

Seeds of *O. sativa* (cv. Basmati 370) were obtained from the national irrigation board of Kenya and germinated after soaking them first in water for 48 h. The seedlings were transplanted to a 2.5 x 2.5 m nursery. After 22 days (15th January 2007) the plantlets were transplanted to the experimental field in a circular design with a diameter of 50 m. The spacing within and between rows within the circle was 15 x 20 cm. A total of about 8,000 seedlings were planted.

To estimate the amount and direction of pollen dispersal, we used the pollen trap method described by Kearns and Inouye (1993). Pollen was trapped on glass microscope slides (7.62 x 2.54 cm) coated with vaseline as bonding agent. Slides were attached horizontally to open-sided wooden plates (30 x 30 cm), with the adhesive surface facing towards the pollen source. 6 slides were placed on each plate (together called a "trap").

Traps were placed north (N), south (S), east (E) and west (W) at intervals of 2, 5, 10, 30, 60, 100 and 250 m from the pollen source. two additional pollen traps were placed 300 m away but only in the main wind direction, to the N and W. At each distance, 2 pollen traps (upper and lower) were attached to a shaft, one at 1.2 m (equivalent to the height of *O. sativa* plants) and another at 1.8 m (equivalent to the height of *O. longistaminata* plants) above the ground. One additional trap was placed at 2 m distance to the N and W to assess diurnal pollen dispersal. 64 pollen traps were used in total.

The pollen traps were placed daily from 8:00 (before flowering) to 17:00 (after most flowers had discharged) for 6 days during the peak flowering period (16 - 21 June 2007). Flowering within a panicle in rice lasts for about 7 days (Moldenhauer and Gibson, 2003). Temperature, humidity, wind speed and wind direction were measured during the pollen collection period using Microisis Systems-Agronic 4000-En 60947-1, England.

Estimation of dispersed pollen

Pollen traps were collected and taken to a confined space where pollen was counted using a light microscope. After pollen count, the glass slides were thoroughly cleaned and kept in a glass slide container ready for use the following day. Pollen traps for estimating daily pollen production were collected after every 1 h, pollen counted and then returned to the same position (2 m). To assess the number of pollen caught in each trap, 3 of the 6 glass slides were randomly selected from each pollen trap (upper and lower and at each distance) and pollen grains were stained with aniline blue in lactophenol (Kearns and Inouye, 1993). Two cover slips, with their edges clearly marked for easy identification of the field of view, were randomly placed over the glass slides. Pollen grains within each cover slip were counted under microscope and their numbers recorded. At each distance, sum total of pollen (caught at the upper and lower traps) were recorded per day. This was then converted to average number of pollen caught in each direction per distance for the whole sampling period. For the identification of the pollen grains of *O. sativa* from other *Oryza* and grass species, a microphotograph of *O. sativa* (Os) (Basmati 370) pollen grain was used. Morphologically, rice pollen grains are initially spherical but collapse about 5 min after shedding from the anther.

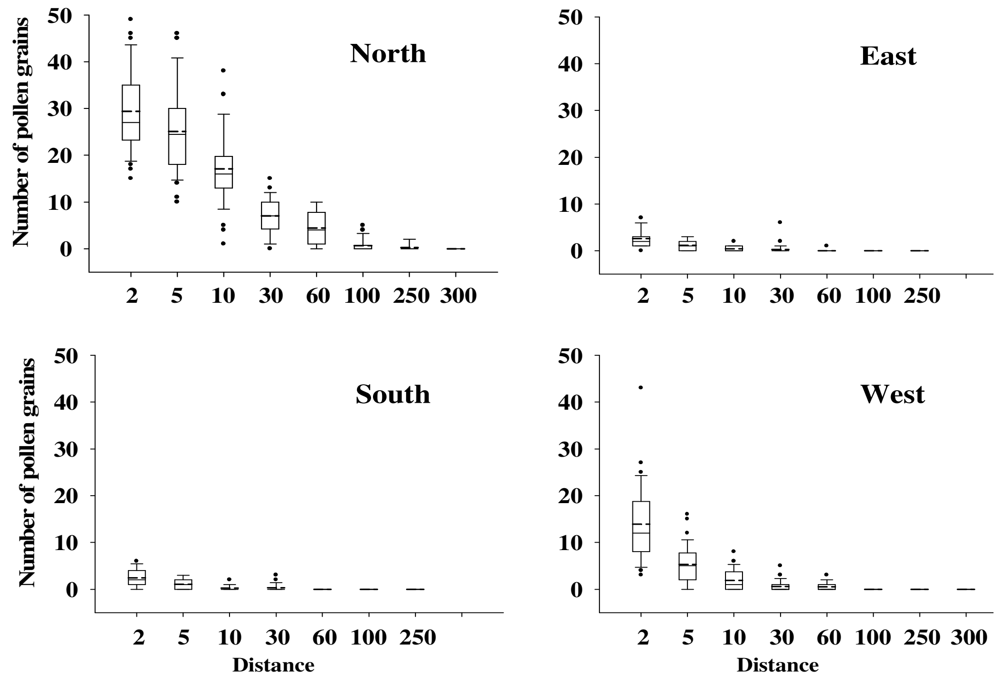

Figure 1. Average number of pollen grains caught daily in the pollen traps (upper and lower combined), placed in different directions and distances from the source plot. In the figure, 25, 50, and 75 percentiles are indicated by boxes, averages by broken lines, 10 and 90 percentiles by whiskers, and more extreme counts by dots, respectively.

Evaluation methods

The relation between pollen counts on distance and direction were analyzed by Poisson regression while the effect of trap height was analyzed by Wilcoxon signed-rank, using the R statistical software version 2.6.1 (http://www.R-project.org, 2006).

RESULTS

During the 6 days of sampling, wind was mostly from the south with occasional and brief changes to the east. Most pollen (average for 6 days) was accordingly recorded on traps placed to the north while the least pollen was recorded in the south (Figure 1 show significant differences between directions, poisson regression; $P < 0.05$). There was a general decrease in pollen count with increasing distance. Most pollen (over 80%) was recorded within a range of 30 m. The furthest pollen was caught at 250 m to the north and no pollen was recorded at distances 300 m in any direction.

There was no significant ($P > 0.05$) difference in pollen count between the upper pollen traps (1.8 m) and the lower traps (1.2 m) over the whole sampling period (Figure 2). Most pollen (over 80%) was recorded between mid-morning (10 - 11) and mid-day (12) for most of the days (Figure 3). The highest pollen count (average for 6 days) 27 was recorded at a mean temperature of 28°C and the lowest 2 at 26°C. Wind speed and relative humidity were relatively stable (Table 1) during the experiment (14 - 16 m/s and 5.6 - 6.9%, respectively) and did not influence pollen dispersal.

DISCUSSION

Wind direction had a great effect on pollen dispersal with more pollen being dispersed to the north along the prevailing wind direction. Our results are thus in line with a recent study by Hoyle et al. (2007) who showed that field to field windborne cross pollination varies greatly according to the wind direction. Although wind speed has been reported (Song et al., 2004) to have considerable influence on pollen dispersal, in our study wind speed was relatively stable and we could thus not determine if pollen dispersal was affected by this. Pollen dispersal decreased significantly with increasing distance from our pollen source and at 300 m distance to the north and west (the only directions in which pollen traps were placed at this distance) no pollen was detected at all despite relatively strong wind speed (Figure 1). Other studies of out-crossing frequencies support these findings, Wang et al. (2006) recorded 18% of outcrossing at 0 - 1 m and only 0.01% at 250 m, Rong et al. (2007) 0.28% at 2 m, while Wang et al. (2004) recorded 4.12 and 0.96% at 100 and 150 m respectively. Song et al. (2004) found the maximum

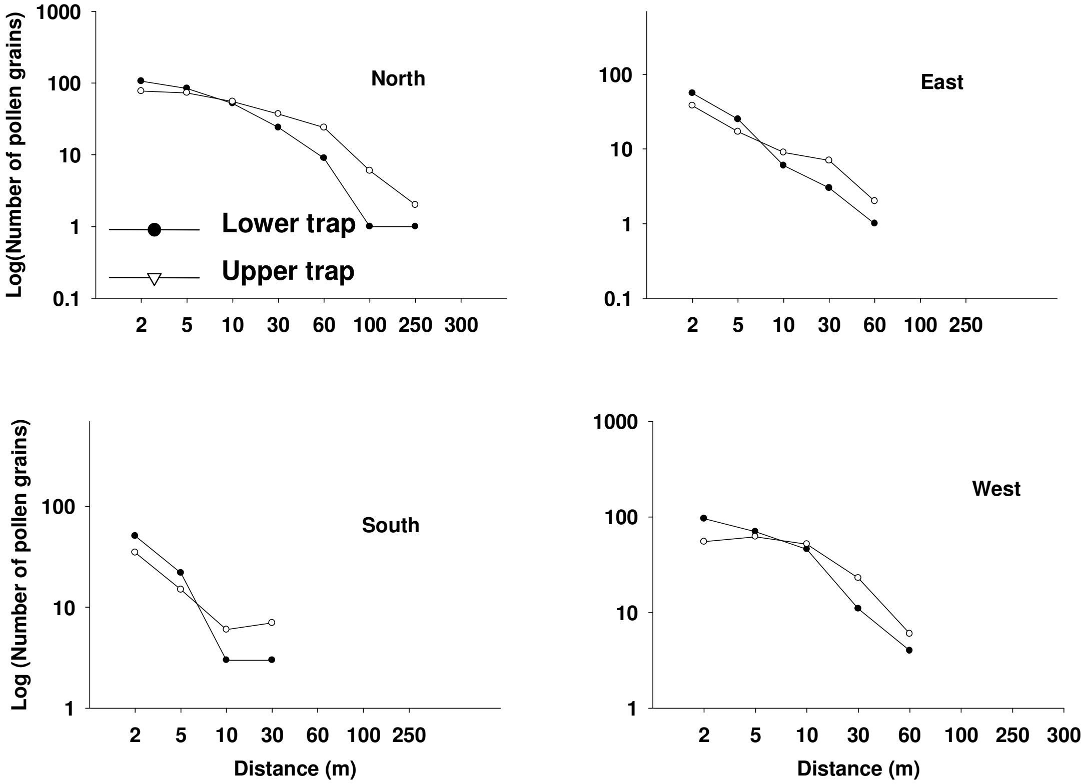

Figure 2. Sum of pollen grains trapped during 6 days in the upper and lower pollen traps, placed in different directions and distances from the source plot; notice log-transformation. No pollen was caught at distances without symbols.

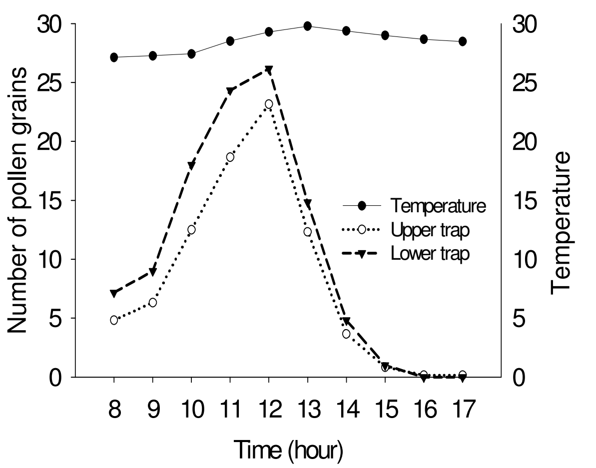

Figure 3. Average number of pollen grains caught in the upper and lower pollen traps from 8:00 to 17:00 h during the 6 days sampling. Traps were placed two meters from the source plot.

distance of rice pollen flow to be 110 m when the wind speed reached 10 m/s, indicating that rice pollen disperses for relatively short distances. This seems surprising, given that rice pollen grains are small (33 - 42 µm) and smaller than e.g. pollen of maize (58 - 99 µm (Oka, 1988), yet maize, which is wind pollinated like rice, disperses its pollen for longer distances (> 4000 m) in field experiments (Whitehead and Longman, 1965; Bannert and Stamp, 2007) and much further (32 km) in model studies (Doebley and Iltis, 1980). One explanation for the short dispersal distance detected in our study could be that the experimental field was relatively small. Song et al. (2004) suggested that pollen dispersal distance depends on pollen source size. Small fields would disseminate pollen for short distances while large fields would disseminate pollen longer distances, due to higher pollen density. However, according to Rong et al. (2007), pollen source size is not a significant determinant of pollen flow.

The difference in pollen counts at different trap heights indicates that there might be differential outcrossing at the same distance for cultivated (*O. sativa*) and the wild rice species (*O. longistaminata*). Variation in pollen dispersal between the upper and lower traps may have been caused by wind dynamics, lifting up the pollen near the pollen source and letting it fall before 300 m resulting in greater pollen density at the lower traps (1.2 m) close to the pollen source than at the upper traps (1.8 m). As distances increase from the pollen source, the wind gradient tends to favour the upper pollen traps (1.80 m). Although

Table 1. Hourly mean relative humidity and wind speed of 6 days, recorded from 8:00 to 17:00 h.

Time (h)	Relative humidity	Wind speed
8	6.9	14.2
9	6.9	14.1
10	6.9	14.8
11	6.5	15.2
12	6.0	15.8
13	5.9	16.3
14	5.8	16.0
15	5.7	15.4
16	5.6	15.7
17	5.6	14.8

no significant difference between the 2 heights, the results suggest that relatively short species such as *O. sativa* may receive more pollen when close to the pollen source and that the tall species such as *O. longistaminata* would capture far-traveling pollen.

Temporal factors had an influence on pollen dispersal. The high pollen counts between mid-morning and midday could be explained by the timing of floral anthesis. Rice florets are open from 9:00 to 15:00 depending on the rice cultivar and weather conditions (Datta, 1981; Khush, 1995). Although other authors (Horie et al., 1992; Matsui et al., 1999) have shown significant effect of temperature on pollen dispersal, in our study temperature remained relatively high (above 25℃) with minimal fluctuation of ± 2°C (Figure 3) and therefore it was not possible to relate its effect on pollen dispersal. Rice pollination is susceptible to temperature changes (Moon et al., 2006). High temperatures (> 35°C) at the time of flowering inhibit the swelling of the pollen grains, whereas low temperatures (below 16°C) impede pollen growth (Shimazaki et al., 1964; Horie et al., 1992). Since the driving force for anther dehiscence is the swelling of pollen grains at the time of floret opening (Matsui et al., 1999), temperature stress reduces the percentage of anther dehiscence at the time of flowering. However, in this study, most pollen dehiscence seems to occur between 27 and 29℃, below and above which (during the mornings and evenings) dehiscence significantly decreases. In this study the effect of humidity could not be assessed since it did not vary (5.6 - 6.9%) between days.

In Kenya, about 95% of rice is grown in large irrigation schemes; only 5% is rain-fed (Ministry of agriculture annual report, 2004). The irrigated fields are divided into blocks for easy supply of irrigation water. The blocks are further subdivided into sub-plots which are allotted to individual farmers. Planting is synchronized per block. If GM rice is introduced into this production system, there might be a large pollen source available at the same time which, according to some authors, could influence the probability of long distance pollen dispersal. However, this would depend on whether all farmers would plant GM rice at the same time. Rain-fed farming is practiced in small-scale farms (1 - 2 ha) in riparian habitats in the coastal region of Kenya. The type of farming coupled with inter-village seed exchange and the close proximity to wild rice populations (mainly *O. longistaminata*) would possibly encourage gene flow both between fields and to wild populations under the rain-fed production regime.

Typically the wild rice species are more outcrossing than the cultivated rice (Estorninos et al., 2006) and although various studies by Oka (1956), Oka and Chang (1961) and Song et al. (2003) indicate successful natural hybridization when *O. sativa* was the pollen donor, other studies by Noldin et al. (2002) indicate that pollen flow between cultivated rice and Oryza *punctata* can occur in either direction, often from the tall plants to the shorter plants than the reverse direction. Zhang et al. (2003) demonstrated that outcrossing rates between *O. punctata* and herbicide resistant rice (*O. sativa*) were higher (0.1 and 0.23%) with the former as the pollen donor than when cultivated rice was the pollen donor (0.0 and 0.14%) suggesting that gene flow will differ from crop to crop and from crop to wild. Equally, Messeguer et al. (2001) established outcrossing rate of 0.53% in a normal 1 m side-by-side plot design between transgenic rice and conventional rice, *O. sativa* in a direction of dominant wind.

In summary, isolation distance of more that 250 m between fields of GM rice and other rice fields and wild *Oryza* populations will minimize gene flow to a very low level. It can, however, not eliminate the probability of any dispersal of pollen from GM fields, as extreme conditions promoting pollen flow at longer distances may occur irregularly.

ACKNOWLEDGEMENTS

The authors thank the management of Tana Delta irrigation scheme with special gratitude to Buya Thiophilus for technical assistance, the University of Nairobi for providing facilities and the Danish agricultural council and Danida for additional support to James Kanya. We thank Jan Thiele for help with data analysis, as well as Gabor Lovei, Samuel Kiboi and Duncan Vaughan for discussions and comments on the manuscript. This work is in partial fulfillment of the requirements for the first author's PhD Thesis at the University of Nairobi, supported by the BiosafeTrain Project, and was financed by a DANIDA-ENRECA programme grant.

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