

## Hybridization Potential between Cultivated Rice *Oryza sativa* and African Wild Rice *Oryza longistaminata*

<sup>1</sup>J.I. Kanya, <sup>2</sup>T.P. Hauser, <sup>1</sup>J.I. Kinyamario and <sup>1</sup>N.O. Amugune

<sup>1</sup>School of Biological Sciences, University of Nairobi, P.O. Box 30197-GPO, Nairobi, Kenya

<sup>2</sup>Department of Agriculture and Ecology, University of Copenhagen, Rolighedsvej 21, DK-1958, Frederiksberg, Denmark

*Corresponding Author: J.I. Kanya, University of Nairobi, School of Biological Sciences, P.O. Box 30197-GPO, Nairobi, Kenya*

### ABSTRACT

Hybridization potential between cultivated rice (*Oryza sativa*) and wild rice (*Oryza longistaminata*) was studied in Kenya. At first seeds of the two parents were sown and their growth patterns established. At maturity, F<sub>1</sub> hybrid seeds were generated from manual crosses between the two *Oryza* species under screen house conditions. The F<sub>1</sub> hybrid seeds and seeds of the two parents were sown to compare the hybrids' growth patterns and seed production with those of the parents. Correlations of seed production with other and morphological discrimination of hybrids from the parents were also scored. This study showed that hybridization between the two species can occur resulting in 6% hybrids seeds. On growth patterns, *O. longistaminata* plants grew taller than F<sub>1</sub> plants which grew taller than *O. sativa* plants. The three types of plants continued to grow in height up to maturity but the gain in height in the hybrid and cultivar plants slowed down upon reaching the flowering stage, after the 10th week. It was also found that seed production in hybrids positively correlated with flag leaf length; while in *O. longistaminata*, seed produced positively correlated with plant height and panicle length. However, in *O. sativa*, number of seeds correlated with panicle exertion. The study also indicates that there were morphological differences (plant height, flag leaf length, panicle exertion and awn length) between the hybrids and the parents suggesting that these traits could be used as markers for identification of the hybrid plants from the parents.

**Key words:** Morphology, seed production, interspecific hybridization, *Oryza longistaminata*, *Oryza sativa*

### INTRODUCTION

Asian domesticated rice (*Oryza sativa*) was introduced to East Africa by Arab settlers about 600 years ago (Vaughan *et al.*, 2005). Cultivation of the species in Kenya was initially restricted to the coastal areas but currently it has spread into the interior regions. In Africa, the Asian rice got into contact with indigenous wild African rice species. Among these, *O. longistaminata* and *Oryza barthii* are likely to have hybridized with the introduced *Oryza sativa* species (Lu and Snow, 2005), as they share the same AA genome. Both *O. longistaminata* and *O. barthii* occur in natural populations outside rice cultivation regions and as weeds in rice fields (Kiambi *et al.*, 2005). Under field conditions, *O. longistaminata* seems to hybridize with the

cultivated rice (Oka and Chang, 1961; Chu and Oka, 1970; Ghesquiere, 1985; Kiambi *et al.*, 2005), although there is need for more studies to confirm this.

Hybridization between cultivated and wild rice may have a number of negative impacts. Foremost among these may be the development of aggressive weeds, where the wild recipients benefit from uptake of crop genes through repeated intercrossing and introgression (Barrett, 1983; Langevin *et al.*, 1990). This has received increasing attention recently due to the employment of transgenes in genetically modified crops which may potentially also benefit weedy and wild recipients (Spencer and Snow, 2001; Ellstrand, 2003; Lovei *et al.*, 2007).

In contrast, it has been demonstrated that wild relatives of rice can harbour favourable genes that are not present in the cultivated rice (Wu and Tanksley, 1993; Brondani *et al.*, 2002). For example, genes that enhance yield of cultivated rice can be introgressed from wild relatives through Genetic Engineering (GE). There is thus also a need to preserve the wild species germplasm for future plant improvement (Song *et al.*, 2005) which may include measure to avoid too much inflow of cultivar genes into the wild gene pool.

Thus, in order to manage gene flow for avoidance of weed evolution and loss of genetic resources, we need to understand the hybridization potential between the wild and cultivar species. The present study therefore aimed at enhancing this information. Our objectives were to investigate (1) the extent to which cultivated rice and the wild African rice are able to hybridize, (2) if hybrids differ in their vigour and seed set compared to their parents and (3) whether the growth and morphology of hybrids are distinct from the parents which would allow the detection of hybrids in the field.

## **MATERIALS AND METHODS**

**Study species and material:** The genus *Oryza* comprises 24 species, of which 22 are wild and two, *Oryza sativa* and *Oryza glaberrima*, are cultivated (Vaughan, 1994). *Oryza sativa* is grown worldwide while *O. glaberrima* is grown solely in West African countries. Cultivated rice has evolved into two eco-genetic types called *indica* and *japonica* (Oka, 1988). The majority of rice cultivars in the tropics are *indica* whereas *japonica* is generally grown in temperate regions or at higher altitudes in the tropics (Dally and Second, 1990).

*Oryza longistaminata* is a wild rice species that grows throughout tropical Africa and Madagascar (Gibbs-Russell *et al.*, 1989) at altitudes between sea level and 2,100 m (Lu, 1999; Kiambi *et al.*, 2005). In contrast to the cultigen, *O. longistaminata* is perennial with extensive, branched rhizomes. It is up to 2 m tall and grows in swampy areas, at edges of lakes and streams, in water down to a depth of 4 m (Gibbs-Russell *et al.*, 1989).

*Oryza sativa* seeds (cv. Basmati 370) were obtained from the National Irrigation Board Nairobi, Kenya while about 1 kg of *O. longistaminata* seeds were obtained from Tana River District in the Coast Province of Kenya, where the plant naturally grows in abandoned rice fields. The seeds of *O. longistaminata* were sun-dried and placed in porous paper bags and transported to Nairobi where they were stored at room temperature (23-27°C) for 6 months. The seeds were then transferred to Mwea Irrigation Agricultural Development (MIAD) Centre (37°20" E and 0°40" S; altitude 1,159 m above sea level) where confined field experiments were conducted.

**Controlled hybridization:** Two sets of experiments were conducted: the first experiment to generate F<sub>1</sub> hybrids (*O. sativa* × *O. longistaminata*) seeds and the second experiment to assess

vigour, seed production and morphological characteristics of the three plant types ( $F_1$  hybrids, *O. sativa* and *O. longistaminata*).

Ninety seeds of *O. sativa* and 90 seeds of *O. longistaminata* were germinated in 6 petri dishes (three petri dishes for seeds of each of the plant species). Seeds of *O. sativa* were planted in March 2007. To synchronize flowering of the two plant species, one set of *O. longistaminata* seeds was planted in December 2006 and a second and third set at the beginning of January and February 2007, respectively; the set from December later turned out to be synchronous with *O. sativa* and were used for the crossings. The resulting seedlings were transplanted into 6 plastic basins (3 basins for each species), with 30 cm diameter and 40 cm depth. Basins were filled with soil collected from the paddy fields. In each basin, 20 seedlings were planted (an equivalent density of 40 plants  $m^{-2}$ ) to make a total of 120 seedlings, 60 from each *Oryza* species. Basins were arranged in three blocks for each *Oryza* species.

From each basin, fourteen seedlings were randomly selected and marked for further measurements of morphological traits (plant height, flag leaf length and panicle exertion, grain length and awn length). At flowering, seven plants were randomly selected among the fourteen marked plants from each basin, for each species and were used as maternal plants. The other seven plants were used as paternal plants. From each of the maternal plants, all the florets (individual flowers) of a spike were emasculated by cutting off the tips of the florets, about one-third of the floret, as described by Kaushal and Raven (1998). Each plant (genet) has an average of nine tillers (ramets) which carry one panicle with an average of eight spikelets and an average of seven florets. Thus, in each genet, 504 florets were emasculated. Since seven genets (maternal parents) were selected for emasculation from each basin, 3,528 florets (7 genets $\times$ 504 florets) were emasculated per basin and in total approximately 10,584 florets (3,528 florets $\times$ 3 basins) were emasculated for each species in the entire study. The emasculated panicles were loosely covered in a porous paper to prevent contamination with unintended pollen.

The other seven plants in each basin were used as pollen donors. Panicles from these plants were cut off the day following emasculation of the female donors at 8.00 h and kept in separate flasks with warm water for about 2 h to facilitate opening of the florets, as described by Kaushal and Raven (1998). Once the maternal panicles opened (between 10.00-11.00 h), they were uncovered and pollen from *O. longistaminata* flowers in the flasks was immediately sprinkled over the maternal flowers. All tillers of an individual recipient (maternal plants) were pollinated with the pollen from the same donor and only one donor was used. The porous paper was immediately returned over the maternal panicles to prevent further pollination from other pollen sources. The process was repeated for *O. sativa* as the paternal and *O. longistaminata* as maternal and at the same time as that for reciprocal crosses. At maturity, the  $F_1$  seeds were harvested from each plant, counted and stored in separate porous paper bags. After two weeks, the seeds were incubated at 50°C for 7 days to break dormancy. After incubation, the  $F_1$  seeds were planted together with *O. sativa* and the *O. longistaminata* seeds for characterization of plant vigour, seed production and morphology.

To prevent unanticipated release of the wild rice species in this rice growing region (Mwea) of Kenya, where the wild species does not naturally occur, environmental safety issues were observed during controlled hybridization stage. All materials including pollen of the *O. longistaminata* were handled according to biosafety regulations in Kenya.

**Measurement of seed and morphological traits:** Twenty seeds of each of plant type, *O. sativa*, *O. longistaminata* and F<sub>1</sub> hybrids, were planted in the same experimental design as described above. Plant height was scored every two weeks from the time of radicle emergence as the distance from the base of the stem to the tip of the longest leaf (Yoshida, 1981). Flag leaf length, panicle length and panicle exertion (the protrusion of the flower head) were scored every two days from the time of panicle initiation to the end of complete panicle formation (which took about three weeks). The number of spikelets per plant, awn length (the terminal part of the bearded lemma), grain length and number of seeds were noted at harvesting. For each of the plant species self-pollination was facilitated by covering all the panicles of each plant.

**Statistical analysis:** Differences between cultivar, wild and F<sub>1</sub> plants were presented by box plots and statistically compared by analysis of variance (ANOVA), using R statistical software version 2.6.1 (R Development Core Team, 2006). Student-Newman-Keuls' (SNK) test was used to separate the means when ANOVA was significant.

## RESULTS

A total of 594 F<sub>1</sub> hybrid seeds were obtained from the controlled pollinations (i.e., approximately 6% of the pollinated flowers that developed into seeds; Table 1. All of the seeds were obtained with *O. sativa* as the recipient plant, despite seemingly successful emasculation and pollination. After the F<sub>1</sub> and parental seeds were sown, *O. longistaminata* plants grew taller than F<sub>1</sub> plants which grew taller than *O. sativa* plants ( $F_{2,288} = 34$ ;  $p < 0.05$ ; Fig. 1). The plants continued to grow in height up to maturity but the gain in height in the hybrid and cultivar plants slowed down upon reaching the flowering stage, after the 10th week (Fig. 1b).

F<sub>1</sub> hybrids developed the longest flag leaves and *O. longistaminata* the shortest (Fig. 2a, b;  $F_{2,321} = 29$ ;  $p < 0.05$ ). However, unlike in *O. sativa* and the F<sub>1</sub> hybrids, where growth in plant height decreased towards flowering period, growth of flag leaves continued to grow throughout the entire period (2b). A reverse growth pattern was however observed in panicle exertion; the exertion was

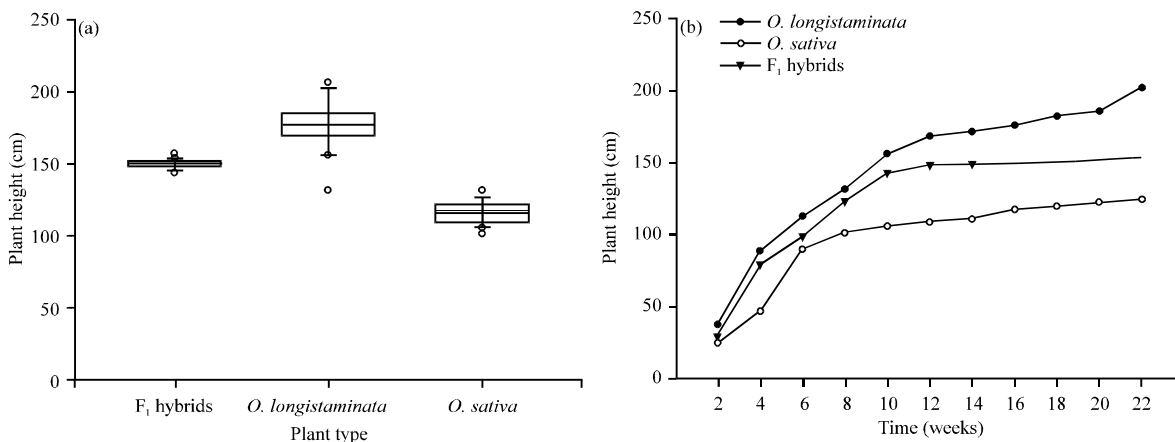


Fig. 1(a-b): (a) Final plant height at maturity and (b) Growth patterns in plant height of the three plant types. In the box plots the figures 25, 50 and 75 percentiles are indicated by boxes, averages by broken lines, 10 and 90 percentiles by whiskers and more extreme counts by dots, respectively

Table 1: F<sub>1</sub> hybrid seeds formed from crosses between *O. sativa* and *O. longistaminata*

Crosses				
Paternal	Maternal	No. of florets emasculated	No. of F <sub>1</sub> seeds formed	Crossing success (%)
<i>O. longistaminata</i>	<i>O. sativa</i>	10,584	594	6
<i>O. sativa</i>	<i>O. longistaminata</i>	10,584	0	0

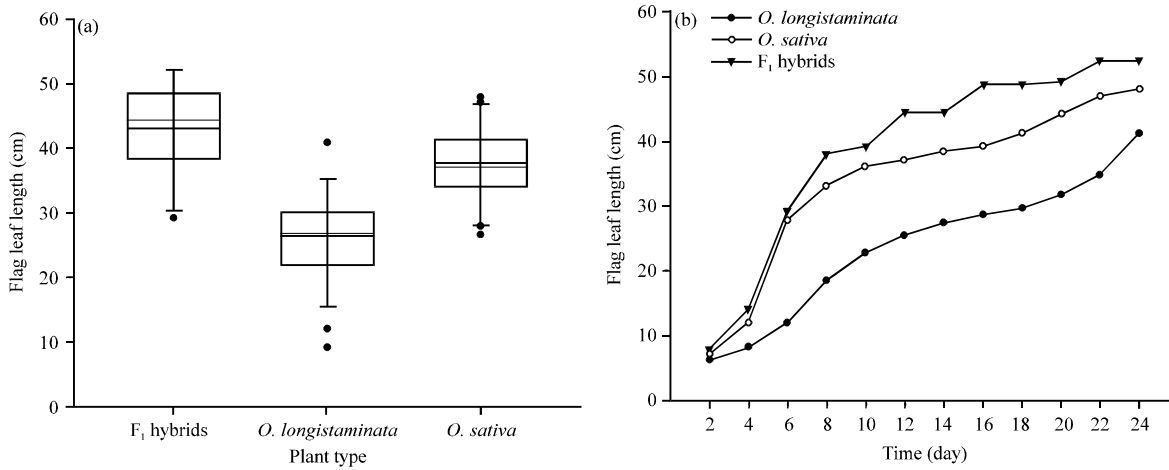


Fig. 2(a-b): (a) Mean flag leaf length at maturity and (b) Growth pattern of flag leaf in the three plant types

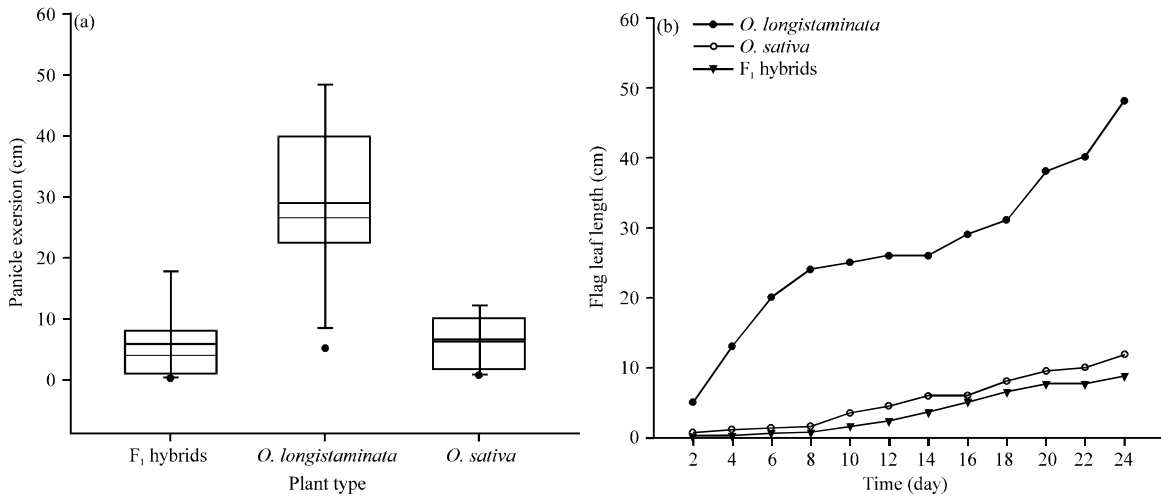


Fig. 3(a-b): (a) Mean panicle exertion at maturity and (b) Growth patterns in panicle exertion in the three plant types

greatest in *O. longistaminata* (29 cm) but lowest in hybrids (Fig. 3a; 5 cm;  $F_{2, 288} = 290$ ;  $p < 0.05$ ). Similarly, *O. longistaminata* displayed the greatest growth pattern in exertion while the hybrids had the least (Fig. 3b). Panicles were longest in F<sub>1</sub> hybrids (32 cm) and shortest in *O. sativa* (Fig. 4; 25 cm;  $F_{2, 417} = 126$ ;  $p < 0.05$ ). In contrast, awns were longest in *O. longistaminata* ( $F_{2, 388} = 258$ ;  $p < 0.05$ ; Fig. 5), with no substantial difference between F<sub>1</sub> hybrids and *O. sativa*.

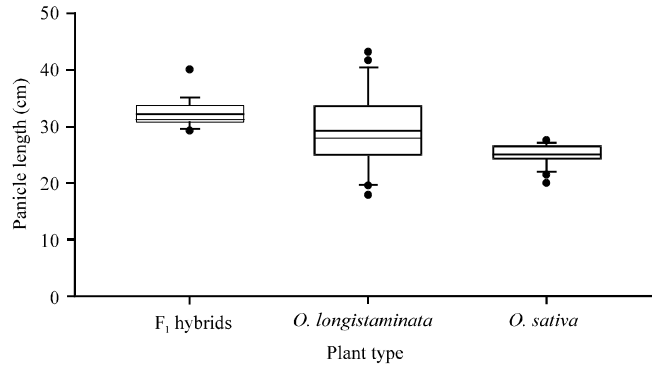


Fig. 4: Mean panicle length at maturity

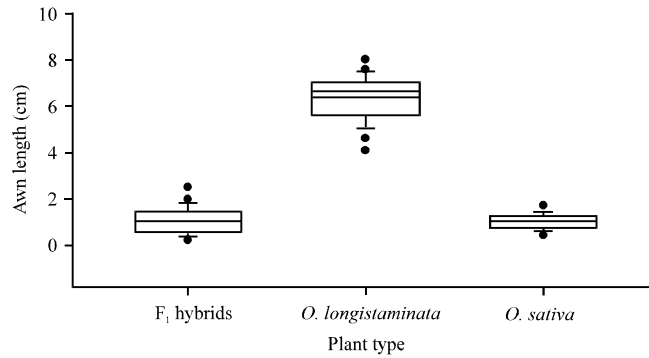


Fig. 5: Mean awn length between the F<sub>1</sub> hybrids and parents

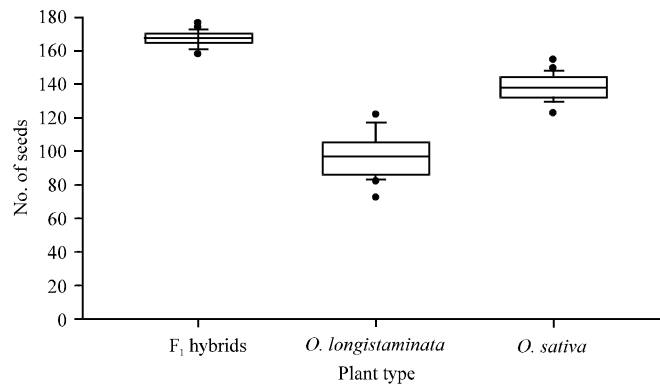


Fig. 6: Mean number of seeds produced by F<sub>1</sub> hybrids, *Oryza longistaminata* and *O. sativa*

At maturity, F<sub>1</sub> hybrids produced the highest number of seeds per plant while the wild plants (*O. longistaminata*) produced the least (Fig. 6;  $F_{2, 501} = 413$ ;  $p < 0.05$ ). Among the F<sub>1</sub> plants, seed production was positively correlated with flag leaf length ( $r = 0.843$ ;  $p < 0.05$ ; Fig. 7a), among *O. longistaminata* plants seed production was positively correlated with plant height ( $r = 0.767$ ;  $p < 0.05$ ) and panicle length ( $r = 0.664$ ;  $p < 0.05$ ; Fig. 7b) and among *O. sativa*, seed production was positively correlated with plant height ( $r = 0.741$ ;  $p < 0.05$ ) and panicle exertion ( $r = 0.854$ ;  $p < 0.05$ ; Fig. 7c).

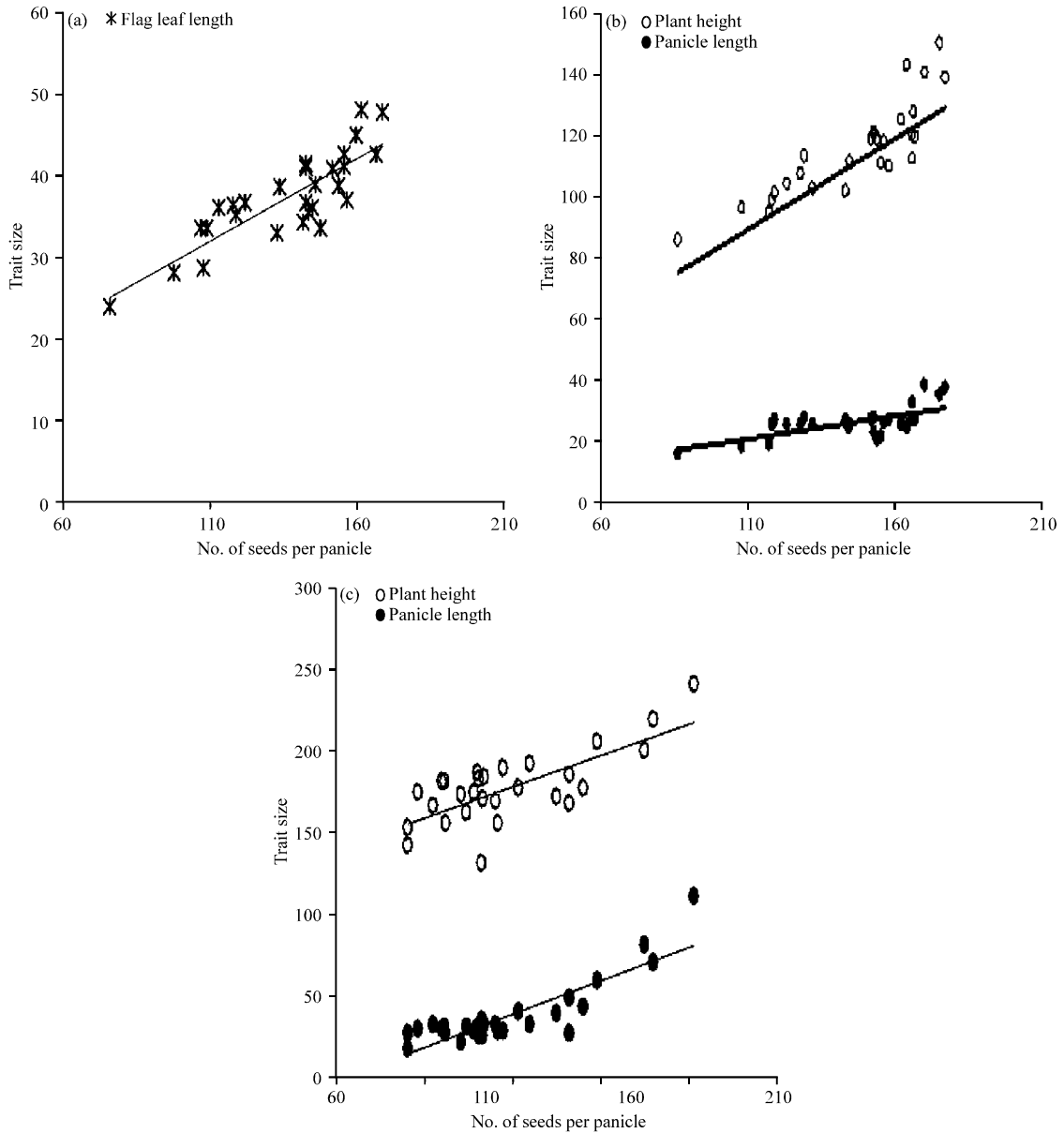


Fig. 7(a-c): Correlation of seed production with, (a) Flag leaf length in the F<sub>1</sub> hybrids, (b) Plant height and panicle exertion in *O. longistaminata* and (c) Plant height and panicle exertion in *O. sativa*

## DISCUSSION

Our study shows that hybridization between East African wild rice, *O. longistaminata* and Asian rice (*O. sativa*) is possible, although only when *O. sativa* is the maternal plant and even then with only low success (6% seeds/pollinations). The low hybridization success corresponds to the findings of Causse and Ghesquiere (1991) who reported a pollination success of 3% and 5% in crosses between the two species. The low success in producing F<sub>1</sub> hybrid seeds has been attributed to low pollen fertility of only 5% in *O. longistaminata* (Causse and Ghesquiere, 1991) and deterioration of hybrid embryos about 3 days after fertilization (Sano, 1989). It could also be attributed to possible errors in emasculation and the subsequent pollination processes.

Seed formation only with *O. longistaminata* as the pollen donor has been reported in other studies (Oka and Chang, 1961; Morishima *et al.*, 1992; Song *et al.*, 2003). Unidirectional hybridization has also been reported in crosses between *O. sativa* and other wild rice species (Oka, 1988); however, in hybridizations with *Oryza punctata*, Noldin *et al.* (2002) demonstrated that pollen can flow in either direction but often from the tall *O. punctata* to the short *O. sativa* plants. Studies by Oka (1988) and Morishima *et al.* (1992) indicate that floral morphological features can affect reciprocal seed set. Cultivated rice generally has short styles and stigmas (1.5-4.0 mm in combined length), short anthers, limited pollen viability and brief period between opening of florets and release of pollen (between half a minute and nine minutes). On the other hand, wild rice species differ in all of these characteristics, with longer styles, stigmas and anthers and pollen that remain viable for up to twice as long as in cultivated rice. This is likely the cause for the observed unidirectional hybridization in this study. Unidirectional hybridization has also been found in many other plant hybridizations (Arnold, 1997).

After germination of the F<sub>1</sub> seeds and seeds of *O. longistaminata* and *O. sativa*, growth patterns of the three plant types were similar at the early stages of development (2-8 weeks; the vegetative stage) but started developing differences in height from the 10th week onwards. This coincides with the stage of profuse tillering (WARDA, 1999), when rapid ground cover enables the rice crop to smother (rapid ground cover) and thus out-compete weeds. As the F<sub>1</sub> hybrids were intermediate between the tall wild *O. longistaminata* and the low *O. sativa*, this indicates that they may have less competitive advantage as weeds over the cultivar plants.

F<sub>1</sub> hybrids, in contrast, produced significantly more seeds than either their wild or cultivar parents, suggesting that this trait is affected by heterosis (Lu and Xu, 2010) and hybrid vigour, as has been found in other hybrids between cultivated and wild plants (Hauser *et al.*, 1998). This shows that the F<sub>1</sub> hybrids, once formed, may produce many offspring which will enhance chances of continued intercrossing and introgression of cultivar genes into the wild gene pool. However, the following generation, F<sub>2</sub> or backcrosses, may be negatively affected by the break-up of positive epistatic interaction of the pure species by recombination. Even though this will in the majority of cases have a negative effect on hybrid fitness, it may sometimes create plants with novel beneficial gene combinations e.g., enhancing weediness (Arnold *et al.*, 1999).

Seed production was thus not related to plant height. The wild species (*O. longistaminata*), despite having an outstanding height, produced the lowest number of seeds (mainly due to embryo breakdown) compared to the hybrids and cultivar parents. However, this observation is not in agreement with hybridization observed in other plants, where height improves access to light, hence increasing seed production (Kende *et al.*, 1998). However, in modern hybrid breeding, dwarf crops have been bred to avoid lodging i.e., falling of plants due to weak culms (Jackson, 1985; Kende *et al.*, 1998) and to improve translocation of resources from stems and leaves to seeds (WARDA, 1999). Therefore, short rice cultivars are preferred especially in ecological zones with strong winds. A possible explanation for the low yield in relation to height is that high investment in plant height incurs costs linked to construction and maintenance (Falster and Westoby, 2003). However, generation of tall rice hybrids that are capable of producing more seeds is an indication that the hybrids may have high incidence of lodging in the paddy fields. This can also be used as a good indicator for identification of hybrids, so that they are weeded out to avoid gene flow between crops and wild plants.

Seed production in the F<sub>1</sub> was instead correlated to their length of flag leaves. This is in line with findings of Dere and Yildirim (2006) that high seed production in rice correlated with well-



established flag leaves. Long and broad flag leaves have more surface area hence high productivity. Flag leaves, compared to other leaves; contribute most photosynthetic products that are important in the grain filling (Blake *et al.*, 2007). According to Dere and Yildirim (2006), flag leaves in cereals contribute about 41-43% of photosynthetic assimilates. Hasegawa and Horie (1996) found that distribution of assimilates from leaves varied depending on the position of the leaf. About 80% of assimilate from flag leaf was translocated to the panicle and ~5% of it came from the fifth leaf from the flag leaf. The influence of flag leaves on seed production is also observed in the two parental *Oryza* species. The wild plants (*O. longistaminata*) had the shortest flag leaves which corresponded with low seed production while the cultivar plants (*O. sativa*) had intermediate flag leaves that corresponded to intermediate seed production.

Although, awn length did not show any correlation with seed production among the three plant types in this study, in other studies it has been reported to play a major role post-harvest, as long awns reduces predatory effects of birds and other rice feeders, thus increasing their survival rate (Satoh *et al.*, 1990). Short awns in hybrids in this study imply that bird predation may be severe among the hybrids compared to parent plants. The effect of predation on rice grains is a serious problem in Kenya (Wanjogu and Mugambi, 2001) and therefore predation of hybrid seeds would reduce the potential of gene flow through seeds.

**Implications:** Our study clearly demonstrates that hybridization between cultivated and wild rice (*O. longistaminata*) is possible, despite a low experimental crossing success and that the resulting hybrids are vigorous and fertile. It is especially notable that hybrids are only (or preferentially) produced by *O. sativa* mother plants, suggesting a scenario where hybrids are first produced in by pollination from wild *O. longistaminata* donors into fields. Here the seeds may be spilled, or if used for resowing, hybrids could grow up and shatter their seeds within fields, as shattering is dominantly inherited (Lin *et al.*, 2007). Unless hybrids are weeded out, cycles of resowing and shattering may allow evolution of weedy types of hybrids, as is known to occur in other weedy rice types (Ferrero and Vidotto, 1998). The high seed production of F<sub>1</sub> hybrids may speed up this evolution. Hybrid descendants will carry a mosaic of segments of the *O. longistaminata* and genomes that are partly determined by selection on the segment genes and epistatic interactions. Presently it is not known whether *O. longistaminata* growing in and around rice fields in East Africa indeed originate from such hybridizations but it is likely. From weedy hybrids in fields, pollen and seeds may subsequently disperse into neighbouring wild populations, carrying with them cultivar genes.

Weeding may stop the cycle of shattering and resowing in fields. This is particularly possible if hybrids are easily distinguished from cultivar plants. Present study therefore shows that F<sub>1</sub> hybrids between the two *Oryza* species can be distinguished from their parents using morphological traits. In particular, they can be differentiated by their height (taller than cultivar plants), longer flag leaves and longer panicles. However, recognition of the descendants in subsequent generations may be more difficult due to segregation (Sorensen *et al.*, 2007). The findings of this study can be used in the assessment of gene flow between cultivated rice and Genetically Engineered (GE) rice in the event that the latter is introduced in the country. These findings will also argument similar studies to ascertain the stability of the hybrids.

## **ACKNOWLEDGMENTS**

The authors thank the management of Mwea Irrigation Agricultural Development (MIAD) Centre for availing land for the confined experiments, the University of Nairobi for providing

facilities and the Danish agricultural council and Danida for additional support to Dr. Kanya. We thank Buya Thiophilus for assisting in collection of wild seeds in Tana River District. This work was supported by the BiosafeTrain Project and was financed by a DANIDA-ENRECA programme grant.

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