Available online at http://ajol.info/index.php/ijbcs

Int. J. Biol. Chem. Sci. 7(5): 2040-2049, October 2013

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper

http://indexmedicus.afro.who.int

Issues concerning reproductive isolation in a rice hybrid swarm involving *Oryza sativa* Linn., *O. longistaminata* A. Chev. et Roehr. and *Oryza glaberrima* Steud. located in Jebba Nigeria

Abolade Oluremi BOLAJI [1*] and Chinyere Constance NWOKEOCHA[2]

[1]*Obafemi Awolowo University, Department of Botany, Ile-Ife, Nigeria.*
[2]*Obafemi Awolowo University, Museum of Natural History, Ile-Ife, Nigeria.*
Corresponding author; E-mail: *jesusflower1@yahoo.co.uk*

ABSTRACT

A hybrid swarm involving *O. sativa*, *O. glaberrima* and *O. longistaminata* occuring in Jebba, Nigeria was studied to investigate the processes involved in the population dynamics. Reproductive parameters such as pollen stainability, pollen size, anther structure, seed set were investigated. The chromosomes of the hybrid population were also studied. The study reveals post-zygotic mechanism involving segregational as well as developmental hybrid sterility to be the major isolating mechanism involved the reproductive isolation existing among the parent populations in the swarm. Hybridization and introgression have played significant roles in creating this hybrid swarm which holds a lot of promise for speciation in the genus.

Keywords*:* Rice swarm, population dynamics, hybrid sterility, isolating mechanism, introgression.

INTRODUCTION

A hybrid swarm is a population characterized by a continuum of morphological forms arising from hybridization between two related species occurring in sympatry. Because a hybrid swarm is in a dynamic state of gene exchange with the parents, it usually consists of populations of various hybrid forms that are characterized by species with intermediate characters or forms that may appear to look like off-types of the original parent populations. They tend to possess great amount of genetic diversity that could actually serve as good genetic resource for plant breeding programs and high quality seeds.

The occurrences of rice hybrid swarms have been reported by many researchers (Majumder et al., 1997; Delouche et al., 2007; Bolaji et al., 2012) in different parts of the world. Oka and Chu (1970) discovered a rice hybrid swarm in China that seemed to demonstrate that reproductively-isolated species can hybridize over time when they occur in sympatry. Sano et al. (1984) noted that the two cultivated species of rice, *O. sativa* and *O. glaberrima*, usually occur in sympatry on farmers' fields in West Africa and as a result, their natural hybrids are occasionally encountered on these fields. Ghesquiere (1986) also observed that introgressive hybridization has been going on between *O. sativa* and *O. glaberrima* and

DOI : http://dx.doi.org/10.4314/ijbcs.v7i5.21

between *O. sativa* and *O. longistaminata* in Africa.

Over the years, researchers have observed that *Oryza* hybrids were usually faced by the problem of reproductive isolation such as hybrid sterility caused by F_1 sterility barriers or a complex of disharmonious gene interactions, phylogenetic remoteness such as hybrid non-viability and hybrid breakdown (Long et al., 2008; Kubo et al., 2011; Yang et al., 2012). Reproductive isolation is the inability of two populations to interbreed because they are geographically isolated, or isolated from each other by differences in behavior, mating time or genital morphology (Eleanor, 2000). According to Sano (1986) isolation promotes genetic differentiation and permits different populations to co-exist without losing their identity. Oka and Chu (1970) however suggested that a balance between isolation and hybridization among species could aid their evolutionary dynamics. A hybrid swarm involving *O. sativa*, *O. glaberrima* and *O. longistaminata* was discovered in Jebba, Nigeria (Bolaji et al., 2012). In this swarm, *O. longistaminata* grows as weed around the cultivated rice, *O. sativa*. Although *O. glaberrima* used to be the local rice cultivated by farmers in this region, it is now encountered as small populations as was reported by Aladejana et al. (2007) for the Jebba population. Aladejana et al. (2007) also reported the occurrence of hybridization and introgression among the rice species in this swarm.

Although Aladejana et al. (2007) reported the meiotic and population dynamics of Oryza species in the Jebba hybrid swarm, no attempt has been made so far to investigate the processes and mechanisms involved in their population dynamics. The main objective of this work is to investigate the processes and mechanisms of the reproductive isolation involved in this rice hybrid swarm.

MATERIALS AND METHODS

Plant source

The different species of rice as well as their putative hybrids investigated in this study were collected from Jebba Nigeria (Table 1). The selections were made on the basis of intermediate characters involving plant form, idiotype, awning, spikelet form, panicle form and pigmentation patterns. Morphological assessment of the various accessions was based on the IBPGR-IRRI Rice Advisory Committee (1980). Seeds, rootstocks and whole plant selections made were raised as sole plants in 10-litre buckets at the rate of one plant/bucket. Seeds harvested from the putative hybrids were raised as F_1 plants labeled PUTATIVE 1…..PUTATIVE9. The other collections include TOL (*O. longistaminata*), TOS JEBBA BIG IMP, TOS JEBBA BIG IMP OPEN PANICLE, TOS JEBBA LC2, TOS JEBBA LC3, TOS JEBBA TYPICAL and TOS JEBBA PSS which were mainly *O. sativa*. Backcross 1 F_2 (BC_1F_2) populations were raised from crosses between the putative hybrids and the TOS.

Accessions and these were labeled $PUT2B_1F_2.1$ to $PUT2B_1F_2.32$, $PUT7B_1F_2.1$ to $PUT7B_1F_2.5$ and $PUT9B_1F_2.1$. Seeds collected on Putative Hybrid 2 are considered as Backcross$1F_1$ seeds from which Backcross$1F_1$ plants and then Backcross$1F_2$ plants were raised. This also applies for seeds of Putative Hybrid 7 and Putative Hybrid 9.

Pollen fertility and seed set (spikelet sterility) study

Pollen grains from freshly-dehisced anthers were harvested onto microscope slides. They were stained with Cotton-Blue-in-Lactophenol and examined under the light microscope for stainability. Well-formed (round), intact and uniformly-stained pollen grains were considered fertile while those that were only partially stained or not stained at all and those with collapsed outline were scored as non-viable (Faluyi, 1985). Pollen grain stainability and size as well as seed set were recorded for all the accessions studied. The seed set (spikelet sterility) was calculated based on the IBPGR-IRRI Rice Advisory Committee (1980). Mean pollen grain size was based on measurements of the diameters

of 100 pollen grains at ×400 in ocular units and converted to micrometer.

Anther anatomical studies

Transverse sections of mature anthers of some of the putative hybrids were made inorder to investigate their anatomical structure. They were fixed in freshly-prepared 1:3 acetic alcohol and made to go through the process of dehydration, infiltration, embedding, microtome sectioning and mounting following the Paraffin Method (Vijendra Das, 2005).

Chromosome studies

Young flower buds of all the accessions and their backcrosses were harvested before maturity and fixed on the spot in 1:3 acetic-alcohols. Slides were prepared by squash technique and stained in FLP Orcein (2gm of Orcein in 100cm^3 of solution containing equal parts of Formic acid, Lactic acid, Propionic acid and distilled water (Olorode et al., 2011). Pollen mother cells were examined and scored for associations. Cells with good chromosome spread were photographed under the oil immersion phase contrast objective of LEITZ DIALUX research microscope equipped with a camera unit. Meiotic behavior was studied in all the accessions and their backcrosses.

RESULTS

Pollen fertility and seed set study

It was observed during the stainability study that the anthers of the putative hybrids could not dehisce such that the pollens shed were very scanty. This was not the case with the pure strains. The pure strains had copious pollens that were shed quite readily.

The accessions of *Oryza longistaminata* studied were very fertile having 92%-99% pollen stainability and 84.80%-90.23% seed set. The pollen grain size ranged between 47.06 µm and 47.93 µm. All the TOS accessions studied were very fertile with 97%-100% pollen stainability, 90.00%-96.10% seed set and pollen sizes ranging between 44.94 µm and 50.56 µm.

Most of the putative hybrids were completely sterile having a seed set of 0%, pollen stainability that ranged between 1% and 12% and pollen size ranging between 33.36 µm and 46.87 µm. The backcrosses were generally highly sterile having a spikelet sterility of 0.00%-52.74%, pollen stainability of 6.35%-67.89% and pollen size of 35.59 µm-46.55 µm (Table 2).

Anther anatomical study

The anther structure of the putative hybrids studied show a thick outer wall (epidermis) that is single-layered, uniseriate and cutinized. The epidermal cells are rectangular, varying in sizes, with cuticle forming an undulating layer around it. The endothecium consists of some oval-shaped and some irregularly shaped cells forming 2-3 layers with no particular arrangement.

The middle layer consists of several layers of tetragonal cells that are elongated, having no definite arrangement and extending to the walls of the tapetal cells. The tapetum is single-layered and uniseriate, forming a continuous layer of cells that completely surrounds the sporogenous tissue. In some of the mature anthers, the tapetal cells completely envelop the pollen grains, many of which were poorly formed with most being wrinkled, unfilled and also poorly stained. The cells of the tapetum were also poorly formed (Figure 1A).

Chromosome study

All the TOS Jebba and TOL accessions studied showed a modal chromosome association of 12II (Table 3) with regular metaphase and anaphase. The putative hybrids showed a modal chromosome association of 12II (Table 3 and Figure 1B). Most of the putative hybrids showed paucity of pollen mother cells (PMC). Also chromosomal aberrations such as precocious movements at metaphase I, non-congression and laggards were observed (Figure 1C).

Table 1: Rice accessions used in the study.

Accessions	**Collector/Source/Location**	**Ploidy level**	**Comment**
TOL1	Faluyi and Adekunle; Jebba, 9˚8.599' N; 4˚48.834'E; Elevation:89m a.s.l Nigeria	2n=24	Wild; whole plant selection.
TOL2	”	”	”
TOS JEBBA BIG IMP	”	”	Cultivated, raised from seeds
TOS JEBBA BIG IMP	”	”	”
OPEN PANICLE	”	”	”
TOS JE BBA LC2	”	”	”
TOS JEBBA LC3	”	”	”
TOS JEBBA PSS	”	”	’’
TOS JEBBA TYPICAL	”	”	”
JEBBA TOL X TOS	”	”	Putative Hybrid
PUTATIVE 2	”	”	F_1 plant of the putative hybrid
PUTATIVE 3	”	”	”
PUTATIVE 4	”	”	”
PUTATIVE 5	”	”	”
PUTATIVE 6	”	”	”
PUTATIVE 7	”	”	”
PUTATIVE 8	”	”	”
PUTATIVE 9	”	”	”

Abbreviations: TOL=Tropical *Oryza longistaminata*; TOS=Tropical *Oryza Sativa*; IMP=Impoundment; LC2=Left Corner 2; LC3=Left Corner 3; PSS=Poor Seed Set

Table 2: Pollen fertility and seed set (percentage sterility) of accessions studied.

Accessions	**Pollen stainability (%)**	**Mean pollen size* (µm)**	**Seed set** (%)**
TOL1	92.00	47.06±0.33	84.80(3)
TOL2	99.28	47.93±0.20	90.23(1)
TOS JEBBA BIG IMP	99.00	48.41±0.32	94.60(1)
TOS JEBBA BIG IMP OPEN PANICLE	98.00	44.94±0.39	90.20(1)
TOS JEBBA LC2	97.00	45.63±0.31	90.00(1)
TOS JEBBA LC3	100.00	50.56±0.41	96.10(1)
TOS JEBBA PSS	98.00	45.85±0.23	90.60(1)
TOS JEBBA TYPICAL	100.00	48.56±0.28	95.50(1)
JEBBA TOL X TOS	N/A	N/A	7.50(7)
PUTATIVE 2	12.00	37.96±0.41	34.65(7)
PUTATIVE 3	16.00	33.36±0.17	0.00 (9)
PUTATIVE 4	8.00	35.62±0.28	0.00 (9)
PUTATIVE 5	1.00	35.33±0.23	0.00 (9)
PUTATIVE 6	1.00	36.86±0.35	0.00 (9)

Table 2: Continued

Accessions	**Pollen stainability (%)**	**Mean pollen size* (µm)**	**Seed set** (%)**
PUTATIVE 7	13.00	41.28±0.30	4.06(7)
PUTATIVE 8	16.00	36.87±0.34	0.00 (9)
PUTATIVE 9	3.00	35.44±0.34	1.33(7)
Backcrosses			
$PUT2B_1F_2$.1	10.56	40.74±0.29	0.00(9)
$PUT2B_1F_2$.2	16.89	41.10±0.38	0.00(9)
$PUT2B_1F_2$.3	43.45	40.63±0.33	1.25(7)
$PUT2B_1F_2$.4	7.90	45.30±0.35	0.00(9)
$PUT2B_1F_2$.5	39.67	42.78±0.47	1.01(7)
$PUT2B_1F_2$.6	45.89	43.37±0.33	3.13(7)
$PUT2B_1F_2$.7	67.89	44.32±0.47	52.74(5)
$PUT2B_1F_2$.8	12.34	44.13±0.49	0.00(9)
$PUT2B_1F_2$.9	26.67	40.88±0.36	2.63(7)
$PUT2B_1F_2$.10	23.45	45.12±0.36	0.85(7)
$PUT2B_1F_2$.11	47.12	42.60±0.29	8.69(7)

Table 2 Continued

Accessions	**Pollen stainability (%)**	**Mean pollen size* (µm)**	**Seed set** (%)**
$PUT2B_1F_2$.12	12.34	40.74±0.38	0.00(9)
$PUT2B_1F_2$.13	16.35	40.70±0.40	0.00(9)
$PUT2B_1F_2$.14	45.78	40.55±0.33	7.69(7)
$PUT2B_1F_2$.15	34.67	45.60±0.31	0.00(9)
$PUT2B_1F_2$.16	45.45	42.78±0.48	0.00(9)
$PUT2B_1F_2$.17	53.92	43.37±0.33	4.84(7)
$PUT2B_1F_2$.18	12.88	44.32±0.47	0.00(9)
$PUT2B_1F_2$.19	9.56	40.77±0.35	0.00(9)

PUT2B$_1$F$_2$.20	38.45	45.67±0.34	10.90(7)
PUT2B$_1$F$_2$.21	32.67	42.96±0.49	4.94(7)
PUT2B$_1$F$_2$.22	57.14	44.10±0.46	0.00(9)
PUT2B$_1$F$_2$.23	40.50	40.48±0.34	30.56(7)
PUT2B$_1$F$_2$.24	54.29	45.85±0.32	4.55(7)
PUT2B$_1$F$_2$.25	15.45	43.04±0.49	0.00(9)
PUT2B$_1$F$_2$.26	13.89	42.42±0.34	0.00(9)

Table 2 Continued

Accessions	**Pollen stainability (%)**	**Mean pollen size* (µm)**	**Seed set** (%)**
PUT2B1F$_2$.27	11.11	43.08±0.52	0.00(9)
PUT2B$_1$F$_2$.28	9.91	44.46±0.48	0.00(9)
PUT2B$_1$F$_2$.29	24.90	40.74±0.35	0.00(9)
PUT2B$_1$F$_2$.30	18.29	45.45±0.35	0.00(9)
PUT2B$_1$F$_2$.31	23.67	42.75±0.49	0.00(9)
PUT2B$_1$F$_2$.32	14.58	42.53±0.35	0.00(9)
PUT7B$_1$F$_2$.1	6.35	35.59±0.39	0.00(9)
PUT7B$_1$F$_2$.2	51.41	40.81±0.29	24.03(7)
PUT7B$_1$F$_2$.3	54.09	43.00±0.37	3.48(7)
PUT7B$_1$F$_2$.4	59.46	41.91±0.48	3.45(7)
PUT7B$_1$F$_2$.5	62.17	43.08±0.37	16.67(7)
PUT9B$_1$F$_2$.1	58.25	46.55±0.38	21.9(7)

Abbreviations: PUT2B$_1$F$_2$=Putative 2 backcross1 F$_2$; PUT7B$_1$F$_2$=Putative7 backcross1 F2; PUT9B$_1$F$_2$= Putative 9 backcross1 F2.

* Tabulated values are Mean ± Standard Error of 100 determinations

**Seed set (Spikelet sterility) categorization

(1) Highly fertile (>90%) (3) Fertile (75-90%) (5) Partly sterile (50-74%) (7) Highly sterile (trace - <50%) (9) Completely sterile (0%).

Table 3: Chromosome configuration of accessions studied.

Accessions	**Genome class**	**Chromosome No.**	**Chromosome configuration (modal)**	**Comment**
TOL1	A	24	12II	
TOL2	A	24	12II	
TOS JEBBA BIG IMP	A	24	12II	
TOS JEBBA BIG IMP OPEN PANICLE	A	24	12II	
TOS JEBBA LC2	A	24	12II	
TOS JEBBA LC3	A	24	12II	
TOS JEBBA PSS	A	24	12II	
TOS JEBBA TYPICAL	A	24	12II	
JEBBA TOL X TOS	A	24	12II	Paucity of PMC

Putatives				
PUTATIVE 2	A	24	12II	Paucity of PMC
PUTATIVE 3	A	24	12II	,,
PUTATIVE 4	A	24	12II	,,
PUTATIVE 5	A	24	12II	,,
PUTATIVE 6	A	24	12II	,,
PUTATIVE 7	A	24	12II	,,
PUTATIVE 8	A	24	12II	,,
PUTATIVE 9	A	24	12II	,,

Table 3
Continued

Accessions (Backcrosses)	**Genome class**	**Chromosome No.**	**Chromosome configuration (modal)**	**Comment**
$PUT2B_1F_2.1$	A	24	12II	,,
$PUT2B_1F_2.2$	A	24	12II	,,
$PUT2B_1F_2.3$	A	24	12II	,,
$PUT2B_1F_2.4$	A	24	12II	,,
$PUT2B_1F_2.5$	A	24	12II	Paucity of PMC
$PUT2B_1F_2.6$	A	24	12II	,,
$PUT2B_1F_2.7$	A	24	12II	,,
$PUT2B_1F_2.8$	A	24	12II	Showed a laggard at anaphase II
$PUT2B_1F_2.9$	A	24	12II	Paucity of PMC
$PUT2B_1F_2.10$	A	24	12II	,,
$PUT2B_1F_2.11$	A	24	12II	,,
$PUT2B_1F_2.12$	A	24	12II	,,
$PUT2B_1F_2.13$	A	24	12II	,,
$PUT2B_1F_2.14$	A	24	12II	,,
$PUT2B_1F_2.15$	A	24	12II	,,
$PUT2B_1F_2.16$	A	24	12II	,,
$PUT2B_1F_2.18$	A	24	12II	,,
PUT2B1F2.19	A	24	12II	,,
PUT2B1F2.20	A	24	12II	,,
PUT2B1F2.21	A	24	12II	,,
PUT2B1F2.22	A	24	12II	Paucity of PMC

Table 3
Continued

Accessions (Backcrosses)	**Genome class**	**Chromosome No.**	**Chromosome configuration (modal)**	**Comment**
PUT2B1F2.23	A	24	12II	,,
PUT2B1F2.24	A	24	12II	,,
PUT2B1F2.25	A	24	12II	,,

PUT2B1F2.26	A	24	12II	,,
PUT2B1F2.27	A	24	12II	,,
PUT2B1F2.28	A	24	12II	,,
PUT2B1F2.29	A	24	12II	,,
PUT2B1F2.30	A	24	12II	,,
PUT2B1F2.31	A	24	12II	,,
PUT2B1F2.32	A	24	12II	,,
PUT7B1F2.1	A	24	12II	2 laggards at anaphase II and non-congression
PUT7B1F2.2	A	24	12II	Paucity of PMC
PUT7B1F2.3	A	24	12II	,,
PUT7B1F2.4	A	24	12II	,,
PUT7B1F2.5	A	24	12II	,,
PUT9B1F2.1	A	24	12II	Showed a laggard at anaphase II

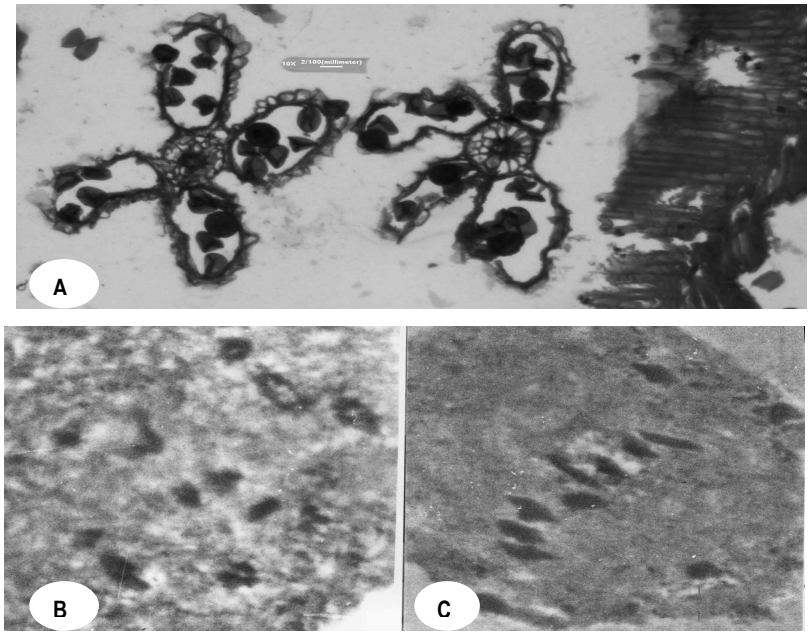

Figure 1: Anther section and chromosome of some representative hybrids. **A**: Anther section of PUT2BC1F_2.30 showing poor formation of pollen grains. **B**: Late diakinesis with 12II chromosomes in PUT7B_1F_2.1 **C**: Laggards and non-congression at metaphase I in PUT7BC_1F_2.1

DISCUSSION

The pollen fertility and seed set was very high in the pure strains, lower in the backcrosses, but much lower in the F_1 hybrids than in the backcrosses (Table 2) implying that the pure strains were fertile while the hybrids were sterile though the backcrosses were less sterile than the F_1 hybrids. This is an indication that though reproductive barriers existing among the parent populations can result in sterility among the hybrid populations, fertility can be gradually restored in the hybrids by backcrossing them to the parent populations. Similar observations were made by Sano (1986) who was able to demonstrate this fact by recovering nearly isogenic F_1 sterile lines from their parental crosses by backcrossing them to the parents.

The chromosomal abnormalities such as non-congression and laggards observed in the meiotic cells of the putative hybrids suggest segregational distortions, as a result of which there might have been some imbalance in the distribution of genes or chromosomes to the poles during meiosis. This is an indication of segregational hybrid sterility.

The paucity of pollen mother cells observed in the cells of the putative hybrids might have resulted from breakdown of meiosis before the formation of pollen mother cells was completed which indicates developmental hybrid sterility. The anatomical evidence for this is the poorly-formed tapetal cells observed in the TS of the anthers of some of the hybrids. Both form of hybrid sterility could have resulted in pollen sterility indicated by the low percentage stainability and seed set of the hybrid population studied. Bhojwani and Bhatnagar (2009) also noted that aberrant meiosis can result in pollen sterility in angiosperms.

The smaller pollen grain sizes of the putative hybrids could also be due to poor formation of the tapetal cells which are responsible for nourishing the sporogeneous tissues during pollen formation. Bhojwani and Bhatnagar (2009) and Pandey (2007) noted that male sterility in angiosperm could arise from aberrant meiosis, aberrant gametophytic development or sporophytic factor.

The failure of the anther to dehisce to release the few normal pollen grains could have also affected the seed set resulting in low seed set percentage. Environmental factors such as photoperiodicity might also have affected the viability of the pollens and the anther dehiscence. These agree with the reports of Bhojwani and Bhatnagar (2009), Matsui et al. (1997a) and Matsui et al. (1997b).

Conclusion

This study reveals that the reproductive isolating mechanism involved in the hybrid swarm studied is post-zygotic, involving both segregational and developmental hybrid sterility but the situation is gradually being ameliorated in the backcrosses through the process of introgression, paving way for gradual restoration of fertility of the hybrid population in the swarm.

REFERENCES

Aladejana FO, Faluyi JO, Nwokeocha CC. 2007. Meiotic and population dynamics in the indigenous representatives of the A-genome complex of the genus *Oryza* Linn. *International Journal of Botany*, **4**(1): 33-40.

Bhatnagar SP, Bhojwani SS. 2009. *The Embryology of Angiosperms* (3rd edn). Vikas Publishing House Pvt Limited: New Delhi.

Bolaji AO, Nwokeocha CC, Faluyi JO. 2012. The occurrence of a hybrid swarm involving *Oryza longistaminata* A. Chev et Roehr., *Oryza glaberrima* Steud. and *Oryza Sativa* Linn. in Jebba, Nigeria. *Ife Journal of Science*, **14**(1): 37-44.

Delouche JC, Burgos NR, Gealy DR, Martin GZS, Labrada R, Larinde M, Rosell C. 2007. *Weedy Rices - Origin, Biology, Ecology and Control*. Food and Agriculture Organization of the United Nations: Rome.

Eleanor L. 2000. *Henderson's Dictionary of Biological Terms* (12th edn). Pearson Education Limited: England.

Faluyi JO. 1985. Cytogenetic Study in the Genus Oryza Linn. I. Investigation of two interspecific hybrids and intervarietal cross. *Nigerian Journal of Genetics*, **6**: 1-16.

Ghesquiere A. 1986. Evolution of *Oryza longistaminata*. Proceedings of the International Rice Genetics Symposium, pp. 15-25.

IBPGR–IRRI RICE ADVISORY COMMITTEE. 1980. *Descriptors for Rice* (*Oryza sativa* Linn.)

Kubo T, Yoshimura A, Kurata N. 2011. Hybrid male sterility in rice is due to epistatic interactions with pollen killer locus. *Genetics*, **189**(3): 1083-1092.

Long Y, Zhao L, Niu B, Su J, Wu H, Chen Y, Zhang Q, Guo J, Zhuang C, Mei M, Xia J, Wang L,Wu H, Liu YG. 2008. Hybrid male sterility in rice controlled by interaction between divergent alleles of two adjacent genes. *Proc. Natl. Acad. Sci.*, **105**(48): 18871-18876.

Majumder ND, Ram T, Sharma AC. 1997. Cytological and morphological variation in hybrid swarms and introgressed population of interspecific hybrids (*Oryza rufipogon* Griff.× *Oryza sativa* L.) and its impact on evolution of intermediate types. *Euphytica*, **94**(3): 295-302.

Matsui T, Namuco OS, Ziska LH, Horie T. 1997a. Effect of high temperature and CO_2 concentration on spikelet sterility in indica rice. *Field Crops Research*, **51**: 213-219.

Matsui T, Omasa K and Horie T. 1997b. High temperature-induced spikelet sterility of japonica rice at flowering in relation to air temperature, humidity and wind velocity condition. *Japanese Journal of Crop Science*, **66**: 449-455.

Oka HI and YE Chu. 1970. Introgression across isolating barriers in wild and cultivated *Oryza* species. *Evolution*, **24**: 61-72.

Olorode O, Hassan SO, Labinjo O, Raimi I. 2011. Tithonia (Asteraceae) in Nigeria. *Ife Journal of Science*, **13**(1): 1-10.

Pandey BP. 2007. *Embryology of Angiosperm* (3rd edn). S. Chand and Company: New Delhi.

Sano Y. 1986. Sterility barriers between *Oryza sativa* and *O. glaberrima*. Proceedings of the International Rice Genetics Symposium, pp. 109-118.

Sano Y, Sano R, Morishima H. 1984. Neighbour effects between co- occurring rice species, *Oryza sativa* and *O. glaberrima*. *Journal of Applied Ecology*, **21**: 245-254.

Vijendra Das LD. 2005. *Genetics and Plant Breeding* (2nd edn). New Age International (P) Ltd., Publishers: New Delhi.

Yang J, Zhao X, Cheng K,Du H,Ouyang Y, Chen J,Qiu S, Huang J, Jiang Y. Jiang L. Ding J, Wang J,Xu C, Li X, Zhang Q. 2012. A killer-protector system regulates both hybrid sterility and segregation distortion in rice. *Science*, **337**: 1336-1340.