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Full Length Research Paper

Crossability of selected progeny from interspecific crosses between *Oryza sativa* and *Oryza glaberrima* (NERICAs)

S. O. Abebrese[1]*, R. Akromah[1] and P. K. A. Dartey[2]

[1]Department of Crop and Soil Sciences, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
[2]Crops Research Institute, P. O. Box 3785, Fumesua, Kumasi, Ghana.

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Some selected first and second generation upland NERICAs (New Rice for Africa, genetic interspecific materials derived from successful crossing of the two cultivated rice species Oryza sativa and Oryza glaberrima) were intercrossed to find out their level of cross-compatibility and the extent of sterility in the NERICAs. All hybrids were partially sterile and showed significant differences in their seed set (0.6 to 33.09%). The direction of crossing partly affected crossability in the NERICAs. Hybrids from crosses that maintained O. glaberrima cytoplasm, showed significantly higher ($p \leq 0.05$) seed fertility than those that maintained O. sativa cytoplasm. Pollen fertility tests indicated reduced pollen viability in the hybrids. Seed set was improved (up to 65%) when hybrids were backcrossed to either parent. Cytoplasmic factors probably affected the degree of sterility in the NERICAs. Within the selected NERICAs improvement could therefore be possible through conventional crosses and best through backcross breeding.

Key words: Crossability, interspecific crosses, NERICA, hybrid sterility.

INTRODUCTION

Rice has become the most rapidly growing food source in Sub Saharan Africa (Sohl, 2005). This is due to population growth (4% per annum), rising incomes and a shift in consumer preferences in favor of rice, especially in the urban centers (Balasubramanain et al., 2007). The relative growth in demand for rice is faster in this region than anywhere in the world (WARDA, 2005). Africa's annual rice production represents only 3% of the global production (IRRI, 2009) and accounts for 26.1% of global imports (WARDA, 2005). Low yield is one of the main challenges of rice production in Sub Saharan Africa because its locally adapted species *Oryza glaberrima* is inherently low yielding whilst its substitute, *Oryza sativa*, cannot tolerate the biotic and abiotic stresses (Jones et al., 1997). Conventional attempts to combine the desirable characteristics of the two species elsewhere proved futile because F1 plants exhibit complete sterility (WARDA, 2008). WARDA scientists used biotechnological interventions (that is, embryo rescue, anther culture and double haploidization) to develop the first self fertile interspecific rice varieties popularly known as NERICAs (WARDA, 2008). Many such self fertile lines, which differ in several characteristics are available, giving a large gene pool from which desirable characteristics can be combined into a desirable plant type.

For instance, NERICA 1 is aromatic (WARDA, 2008). This aroma gene can be introgressed into other NERICA cultivars, to boost their market acceptability. Though, bridging cultivars has been identified for transfer of traits between sativa and glaberrima (Mande, Personal communication, WARDA), combining desirable characteristics from several NERICA lines into a desirable plant type, may be easier than repeating the

*Corresponding author. E-mail: sam555oppa@yahoo.com.

Table 1. Parentage of varieties.

NERICA lines	Female parent	Male parent
NERICA 1- NERICA 11	WAB 56-104	CG 14
NERICA 12- NERICA 14	WAB 56-50	CG 14
NERICA 15- NERICA 18	CG 14	WAB 181-18

original interspecific crosses. Some selected first generation NERICAs (NERICA 1 to 7) and second generation ones (NERICA 8 to 18), were intercrossed to find out their level of cross compatibility, extent of sterility and the possible cause of sterility in the NERICAs.

MATERIALS AND METHODS

Seeds of NERICA 1, 2, 4 and 8 to 18, originally from the Africa Rice Centre were obtained from Crops Research Institute (CRI), Fumesua, Ghana. Parentages of the varieties are listed in Table 1.

Crosses

Hybridization block was set for the above selected first and second generation NERICAs at Nobewam (N 06° 38' 122", W 001° 16' 54.7", 195 m above sea level). Possible reciprocal crosses were carried out at flowering by emasculating and transferring pollen. The F1 hybrid seeds were harvested at maturity.

Pot culture of F1 plants

The F1 seeds were pre-germinated in white tissue paper for four days, nursed in buckets for 21 days and transplanted one per bucket. The hybrids were provided with 8 g of N.P.K. (15-15-15) fertilizer at tillering, 4 g of Sulphate of ammonia at panicle initiation and watered whenever necessary till maturity. Individual plants were allowed to self. The degree of self sterility/fertility was examined by counting the number of filled and unfilled spikelets per panicle and per plant. Fertile spikelets were identified by pressing the spikelets with fingers to note those that were filled and sometimes by dehusking to be sure. Fertile and sterile spikelets were counted manually.

Data collected

Data were taken on total number of spikelets per plant, number of filled and unfilled spikelets per plant, percentage fertility/sterility per plant and percentage fertility/sterility per cross. Percent fertility per plant was obtained by calculating the number of filled spikelets, as a percentage of the total number of spikelets (filled and unfilled) counted from that plant. To enable statistical comparison of F1 hybrid seed fertility for the various crosses, six panicles per plant for four plants were used, in calculating the percentage seed fertility.

Statistical analysis of F1 seed fertility

Analysis of variance (ANOVA) for seed fertility was first carried out using the arc sine transformed values, for each harvested group. When the results of the analysis of variance revealed significant differences, multiple mean comparisons were carried out using LSD at 0.05.

Backcrossing

Some F1 hybrids were backcrossed to either parent, to find out the possibility of setting seeds and the extent to which sterility can be restored by conventional backcrossing.

Pollen fertility test

Twenty five spikelets were collected at random from the available hybrid plants for each cross at flowering but before anthesis. Ten anthers were sampled at random from the collected spikelets. Pollen grains were stained in 1% iodine-potassium iodide (IKI) solution and observed under a microscope at ×100 magnification. A total of 200 to 300 pollen grains were counted on each slide and classified as sterile or fertile based on their staining behavior (Chaudhary et al., 1981). All dark and brown stained pollen were scored as fertile and irregularly-shaped, yellow or unstained pollen grains were scored as sterile.

RESULTS

Seed and pollen fertility

Sixteen NERICA cross combinations were obtained. The crosses with their mean percent F1 spikelet fertility, as well as percent pollen fertility are summarized in Table 2. Hybrids from two crosses were not included in the analysis of variance because they were not replicated. Pollen fertility was tested for eleven hybrids out of the sixteen crosses.

Backcrosses

Backcrosses were done to find out the possibility of increasing seed set. The following in Table 3 were obtained from backcrosses.

DISCUSSION

Seed fertility

The International Network for Genetic Evaluation of Rice (INGER, 1996) has suggested that hybrid plants with percent seed fertility of < 50% to trace, be classified as highly sterile and that of 0%, as completely sterile. All the hybrids were therefore highly sterile because the highest percent spikelet fertility recorded was 33.09 (NERICA16/NERICA1). None of the hybrids was completely sterile because none recorded percent spikelet fertility of 0.

Semagn et al. (2007) reported that the average proportion of *O. sativa* genome in NERICA1 to NERICA7 is 87.4% while an average of 6.3% was covered by *O. glaberrima* genome based on 130 microsatellite markers.

Table 2. Mean percent spikelet fertility, mean arc sine transformed of percent spikelet fertility and percent pollen fertility of F1 hybrids.

Cross	Mean percent F1 spikelet fertility	Mean, arc sine transformed	Percent pollen fertility
NERICA 16/NERICA 1	33.09	34.98 a	52.00
NERICA 18/NERICA 1	31.26	33.92 a	66.00
NERICA 15/NERICA 1	28.87	32.75 a	59.00
NERICA 1/NERICA 15	19.43	25.02 b	60.20
NERICA 4/NERICA 10	9.26	12.43 c	35.00
NERICA 14/NERICA 2	5.91	14.02 c	**
NERICA 9/NERICA 1	5.74	13.42 c	37.50
NERICA 14/NERICA 11	4.10	11.42 c	47.40
NERICA 1/NERICA 9	3.97	11.43 c	31.40
NERICA 16/NERICA 10	3.31	9.41 c	**
NERICA 14/NERICA 1	3.26	7.96 c	**
NERICA 18/NERICA 10	2.79	*	**
NERICA 17/NERICA 2	2.54	9.03 c	**
NERICA 10/NERICA 4	2.18	11.96 c	50.40
NERICA 14/NERICA 10	1.87	8.85 c	51.30
NERICA 8/NERICA 9	0.6	*	42.00
CV		28.8	
LSD(0.05)		6.822	

* Were not included in the Anova analysis because of non replication. ** Pollen fertility could not be determined for hybrids of that cross.

Table 3. Number of emasculated and pollinated spikelets and their percent seed set.

Backcross	Number of spikelets emasculated and pollinated	Number of spikelets filled	Percent fertility
N15/N1/N15	548	360	65.70
N16/N1/N16	708	388	54.80
N14/N2/N14	76	48	63.12
N14/N1/N14	438	132	30.14
N14/N10/N14	180	96	53.33
N14/N1/N1	168	102	60.71

These findings suggest that each NERICA cultivar, had a genetic background that was mainly *O. sativa*, so the gamete eliminator or pollen killer genes derived from both *O. glaberrima* and *O. sativa* (Sano, 1990), might be operating in the F1 hybrids of this current study. Molecular profiling of the F1 hybrids may be helpful to confirm the true cause.

Significant differences in mean percent F1 spikelet fertility, indicated that the F1 hybrids varied in their inherent fertility. This could be that the gene(s) implicated in hybrid sterility in the NERICAs, was expressing variably or were distributed differently within each NERICA cultivar, during the initial breeding process. Ikeda et al. (2009) crossed upland NERICA cultivars with two accessions of *O. glaberrima* and two cultivars of *O. sativa* and reported percent F1 seed fertility of 0.4 to 91.7%, depending on the cross combination suggesting that the NERICA cultivars varied in their inherent fertility.

Crossability of the NERICAs was irrespective of parentage. Compatibility would have been expected to be higher for cultivars from the same parentage and less for those from different parentages. However, the first three hybrids that ranked high, (NERICA16/NERICA1, NERICA18/NERICA1 and NERICA15/NERICA1) had female parents (that is NERICA16, NERICA18 and NERICA15) developed from CG 14 /3*WAB 181 to 18 parentage while NERICA1 (the male parent) also developed from WAB 56 to 104/CG 14//2*WAB 56 to 104

parentage. NERICA8/NERICA9 which recorded the least mean F1 percent seed fertility, had their parental origin from WAB 56 to 104/CG 14//2*WAB 56 to 104.

NERICA16, NERICA18 and NERICA15, the female parents of the first three hybrids that ranked high, had *O. glaberrima* cytoplasm. These were among the five NERICA cultivars, classified compatible with both japonica and indica cultivars (Ikeda et al., 2009). Ikeda et al. (2009) reported that though it does not deny the possibility that certain nuclear genes were involved in their compatibility, the cytoplasm of *O. glaberrima*, may have a suppressant effect on hybrid sterility.

Cytoplasmic effect could also be implicated from this direction; percent spikelet fertility of NERICA15/NERICA1 was significantly different from its reciprocal NERICA1/NERICA15. NERICA15 was derived from CG 14/3* WAB 181 to 18 and NERICA1 also from WAB 56 to 104/CG 14//2*WAB 56 to 104 (WARDA, 2008). NERICA 15/ NERICA1, had the cytoplasm of *O. glaberrima* CG 14 while NERICA1/ NERICA15 maintained the cytoplasm of *O. sativa*

WAB 56 104, there was no significant difference between NERICA1/NERICA9, NERICA4/NERICA10 and their reciprocals NERICA9/NERICA1 and NERICA10/NERICA4, respectively. All four hybrids maintained the cytoplasm of *O. sativa* WAB 56 104.

Aside from cytoplasmic effect accounting for part of the variability in the NERICA-NERICA crosses, cytoplasmic nucleus interactions could also play a role. There were significant differences among crosses that maintained *O. glaberrima* cytoplasm. The difference could be accounted for by a possible interaction between the cytoplasm and the nucleus composition of the paternal parent. A similar difference was also observed in the crosses that maintained *O. sativa* cytoplasm

Backcrossing

Transfer of pollen grains from either parent to some emasculated spikelets of some F1 hybrids, increased percent spikelet fertility. Hybrids of NERICA14/NERICA1 increased percent seed set by about sixteen times (3.26 increased to 60.71) in the backcross (N14/N1/N1); that of NERICA15/NERICA1 more than doubled the percent spikelet fertility (27.87 to 65.70) in the backcross (N15/N1/N15). This suggested reduced pollen fertility in the hybrids. Sano (1990) reported that the hybrids between *O. glaberrima* and *O. sativa* were male sterile but partially female fertile. The fact that backcrossing increased seed set in hybrids, suggested some pistil/ gynaecium remained functional. Though F2 seeds could be produced indefinitely by ratooning the F1 hybrid plants, the hybrid that improved seed set 16 times in the backcross will require 16 cycles of ratooning, to produce that same seed lot. Hence, genetic improvement within the NERICAs may best be done through backcross breeding.

Pollen fertility

Report by WARDA (2008) indicated that NERICA cultivars are fully fertile resulting in normal seed set. Results of the pollen fertility test indicated reduced pollen viability in the hybrids from the study, indicating the inherent infertility problem in the intraspecific crosses. The differences could be due to variations in cytoplasmic effects as reported by Sano (1985).

Conclusion

Based on the observations and results of the study, it can be concluded that crossability of the first and second generation NERICAs were variable, depending on the type of cytoplasm and cytoplasm nucleus interactions. Reduced pollen viability might be the cause of F1 hybrid sterility. Hybrid sterility within the selected upland NERICAs was not complete. Within the selected NERICAs, improvement can therefore be possible through conventional crosses and best through backcross breeding. Raising a large F1 population through emasculation, as well as ratooning the F1 plants, could help increase F2 populations.

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REFERENCES

Balasubramania V, Sei M, Hijmans RJ, Otuka K (2007). Increasing rice production in Sub- Saharan Africa: Challenges and Opportunities. Adv. Agron., 94: 55-133.

Chaudhary RC, Virmani SS, Khush GS (1981). Patterns of pollen abortion in some cytoplasmic-genetic male sterile of rice. Oryza, 18: 140-142.

Ikeda R, Sokoi Y, Akintayo I (2009). Seed fertility of F1 hybrids between upland rice NERICA cultivars and Oryza sativa L. or O. glaberrima Stued. Breeding Sci., 59: 27-35

INGER (International Network for Genetic Evaluation of Rice) (2006). Standard Evaluation System for RICE. 4th Edn. International Rice Research Institute, Manila, Philipines, pp. 13-46.

IRRI (International Rice Research Institute) (2009). IRRI World Rice Statistics. (WRS). IRRI, Los Banos, Phillipines.

Jones M, Dingkuhn M, Aluko/snm G, Semon M (1997). Interspecific Oryza sativa L. X O. glaberriima stued. Progenies in upland rice improvement. Euphytica, 94: 273-246

Sano Y (1985). Interspecific cytoplasmic substitutions of an indica strain of Oryza sativa.L and O.glaberrima.Steud. Euphytica 34: 587-592.

Sano Y (1990). The genetic nature of gamete elimination in rice. Genetics, 125: 183-191.

Semagn K, Njiondjop MN, Lorieux M, Cissoko M, Jones M, McCouch S

(2007). Molecular profiling of an interspecific rice population derived from a cross between WAB 56-104(Oryza sativa) and CG 14(Oryza glaberrima). Afr. J. Biotech., 6: 2014-2022.
Solh M (2005). Rice is life in 2004 and beyond. Int. Rice commission Newsl., 54: 1-10
WARDA (2005). Rice Trends in Sub-Saharan Africa, WARDA, Bouaké, Côte d'Ivoire, p. 34.
WARDA/FAO/SAA (2008). NERICA®: the New Rice for Africa – a Compendium. Somado EA, Guei RG, and Keya SO (eds.). WARDA, Cotonou, Benin; FAO, Rome, Italy; Sasakawa Africa Association, Tokyo, Japan, p. 210