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Screening Upland Varieties of NERICA and its Parents for Resistance to Stalk-eyed Fly, *Diopsis* sp. (Diptera, Diopsidae) in Benin

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Abstract: The stalk-eyed fly or Diopsid, *Diopsis* sp. (Diptera, Diopsidae) is an economically significant insect pest of rice in Tropical Africa. The objective of this study is to develop a fast screening method of rice varieties for resistance to *Diopsis* sp. in a view to advice breeders on resistance sources and to guide producers in the use of resistant varieties to reduce or avoid insecticidal treatments of the fields. The study was conducted in 2008 at the AfricaRice/IITA Station, Cotonou where, 18 upland NERICA varieties and their parents (*Oryza glaberrima* and *O. sativa*) were twice screened under artificial infestation of Diopsid's eggs and adults. The screening method was based on Brown Plant Hopper screening techniques using small cages. As results, only NERICA16 and NERICA18 showed good resistance to Diopsid attack at 20 Days after Infestation (DAI) under egg infestation. The remaining 18 entries were more or less susceptible. Under adult infestation, 16 NERICA varieties showed very good resistance to the stalk-eyed fly attack at 20 DAI. Of these, NERICA18, NERICA11, NERICA6 and NERICA15 were highly resistant (% deadhearts <7%). Only NERICA9 and NERICA17 were moderately susceptible. The African *O. glaberrima* parent CG14 was the most resistant entry. The Asian parent line WAB56-104 also suffered less damage from *Diopsis* sp. than most of the NERICAs. In this experiment, the adult infestation appeared to be more realistic than infestation with eggs because it is a free choice method in which pest pressure is weak and where varieties can demonstrate their real behaviour.

Key words: Diopsid, *O. glaberrima, O. sativa*, damage, rice

INTRODUCTION

The stalk-eyed fly or Diopsid, *Diopsis* sp. (Diptera, Diopsidae) is key pests for rice crop in Tropical Africa. Its larva or asticot is very harmful to rice plants. The two main species commonly found in Benin are *Diopsis thoracica* Westwood and *Diopsis apicalis* Westwood (Diptera, Diopsidae) (Heinrichs and Barrion, 2004). Diopsid can be found in all rice ecological zones of Tropical Africa (Breniere, 1983) but preferentially in humid and shady lowland (Appert and Deuse, 1988; Banwo, 2002) and also in irrigated rice fields (Heinrichs and Barrion, 2004). Damage from Diopsid larvae is similar to the primary damage made by Lepidopteran larvae. The central meristem of the plant is bored, resulting in the deadheart (Descamps, 1957; Breniere, 1983). One larva can destroy between 3 to 10 rice stems (Heinrichs and Barrion, 2004). In cases of high infestation, larvae can attack rice panicles (Bijlmakers and Verhoek, 1995). *Diopsis* sp. are present on rice throughout the growing period but they are more abundant in the field at pre-tillering and tillering stages (Joshi *et al*., 1992). Feeding by the larvae significantly decreases tiller density, effective panicles, grain weight and the total yield (Heinrichs and Barrion, 2004) and increases the number of immature panicles. In endemic areas, 66% of the tillers and 100% of the hills can be infested (Scheibelreiter, 1974). Varieties with improved resistance appear to be a sustainable and effective option for controlling this pest. However, rice varieties with high levels of resistance to *Diopsis* sp. are not yet available. In addition, screening for resistance to *Diopsis* sp. has been very difficult to be achieved until now.

In the early 1990s, the Africa Rice Center (AfricaRice) and its partners developed upland improved interspecific rice varieties known as NERICA (New Rice for Africa) from the crossing of the two cultivated rice species, *Oryza sativa* and *O. glaberrima* (Jones *et al*., 1997; Dzomeku *et al*., 2007). The NERICA varieties combine the

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useful traits of the two parents, but information on their resistance to stalk-eyed fly was previously unavailable. The present study screened upland NERICA rice varieties for resistance/tolerance to *Diopsis* sp. using a new artificial screening method.

MATERIALS AND METHODS

The experiment was conducted during the 2008 season (from March to June) at the sub station of Africa Rice Center (AfricaRice) located on the domain of IITA (International Institute For Tropical Agriculture) at Cotonou, Benin (between 6°25' latitude North and 2°19' longitude East, with 15 m of altitude above sea level).

Screening method: The screening method used in that experiment is an adapted BPH (Brown Plant Hopper) screening technique that uses small cages as experiment area and requires a short duration.

Infestation was carried out at ten days after sowing (10 DAS) because it is reported that the susceptible stage of rice plants to Diopsid flies is from seedling to tillering (Breniere, 1983, 1966; Cochereau, 1978; Pollet, 1978; Alghali, 1983; Alam, 1988; Appert and Deuse, 1988; Chiasson and Hill, 1993; Heinrichs, 2000).

Two screenings were done in two different trials. The first trial used Diopsid eggs at the rate of 5 eggs per row, the eggs being placed singly on the central leaf of the plants at 10 DAS. The second trial involved adults of *Diopsis* sp. whereby 25 individuals were released in each screening cage at 10 DAS to give a critical density of 50 individuals m^{-2} (Breniere, 1986).

Rice varieties: Twenty rice varieties, including 18 upland NERICAs (labeled from 1 to 18), one *Oryza glaberrima* (CG14, African parent) and one *Oryza sativa* (WAB56-104, Asian parent) were sown at random under artificial conditions. The African O. *glaberrima* was considered as the resistant check to *Diopsis* sp. Table 1 shows the list of varieties.

Planting techniques: Three screening cages, each 90 cm long, 50 cm wide and 70 cm high, were built for the experiment. Upland field soil was collected and heated at 100°C for 30 min in an electric oven to sterilize it. A 10 cm soil layer was put in the bottom of each cage. A basal fertilizer of 40 kg N, 40 kg P_2O_5, 40 kg K_2O ha^{-1} was applied. Screening cages were located in a shady environment under ambient conditions. The whole plot was watered daily for one week. The seeds of each variety were soaked in water for two days after which the pre-germinated seed was sown in hills at 1 cm×4 cm spacing. Gaps were filled two days after germination. Test entries were sown in single rows. No weeding was carried out. A Randomized Complete Block Design (RCBD) with three replications was used.

Table 1: List of rice varieties used in the experiment

Variety	Pedigree	Origin
NERICA1	WAB450-I-B-P-38-HB	Africa Rice Center
NERICA2	WAB450-11-1-P31-1-HB	Africa Rice Center
NERICA3	WAB450-I-B-P-28-HB	Africa Rice Center
NERICA4	WAB450-I-B-P-91-HB	Africa Rice Center
NERICA5	WAB450-11-1-1-P24-H3	Africa Rice Center
NERICA6	WAB450-I-B-P-160-HB	Africa Rice Center
NERICA7	WAB450-I-B-P-20-HB	Africa Rice Center
NERICA8	WAB450-I-BL1-136-HB	Africa Rice Center
NERICA9	WAB450-B-136-HB	Africa Rice Center
NERICA10	WAB450-11-1-1-P41-HB	Africa Rice Center
NERICA11	WAB450-16-2-BL2-DV1	Africa Rice Center
NERICA12	WAB880-1-38-20-17-P1-HB	Africa Rice Center
NERICA13	WAB880-1-38-20-28-P1-HB	Africa Rice Center
NERICA14	WAB880-32-1-2-P1-HB	Africa Rice Center
NERICA15	WAB881-10-37-18-3-HB	Africa Rice Center
NERICA16	WAB881-10-37-18-9-P1-HB	Africa Rice Center
NERICA17	WAB881-10-37-18-13-P1-HB	Africa Rice Center
NERICA18	WAB881-10-37-18-18-12-P3-HB	Africa Rice Center
CG14	CG14	Africa
WAB56-104	WAB56-104	Africa Rice Center

Rearing for egg production: About 100 adults of *Diopsis* sp. were introduced into a plastic box (40 cm high and 30 cm diameter). The cover of the box comprised a large hole covered with nylon tissue. Cotton tissue wetted with a sweet solution was placed in the bottom to feed the adults, while fresh rice leaves were put inside the box to bear the eggs. Eggs laid on both rice leaves and the internal compartment of the box were collected and used as screening agents. This rearing was carried out under natural conditions with the box kept in a shady environment.

Data analysis for field screening: At 10 and 20 DAI (days after infestation), the severity of *Diopsis* sp. damage (percentage of tillers with deadhearts) was recorded in each cage. ANOVA analysis was used to determine the significant effects of the treatment (rice varieties). The Student-Newman-Keuls method was used to compare the mean percentages of deadhearts for the 21 entries. Clustering analysis and dendrograms were performed to determine the resistance/tolerance status of NERICA varieties in relation to their parents. The analysis was done using SAS 9.1 (SAS Institute, 2002-2003).

RESULTS

Screening with eggs: Of the 20 upland NERICA rice varieties evaluated under artificial infestation with *Diopsis* sp. eggs, nine varieties showed early high tiller infestation

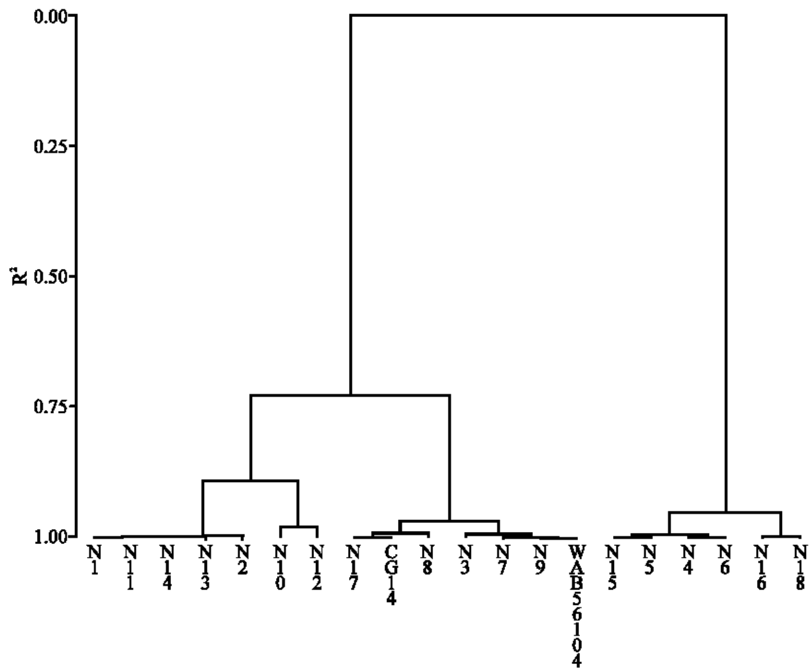

Fig. 1: Egg infestation dendrogram showing the minimum distance between clusters groups. N= NERICA

Table 2: Evaluation of rice lines for resistance/tolerance to *Diopsis* sp. under artificial infestation of eggs in a screen cage at Africa Rice Benin Station, Cotonou, 2008 wet season

	% Deadhearts±SE	
Varieties	10 DAI	20 DAI
NERICA1	27.4±1.0ab	43.5±5.6a
NERICA2	40.9±6.6a	45.6±4.7a
NERICA3	32.6±3.2ab	38.0±4.0a
NERICA4	15.6±4.9ab	21.1±4.1a
NERICA5	19.2±6.0ab	22.3±4.7a
NERICA6	8.0±3.1b	21.9±2.2a
NERICA7	27.7±5.0ab	35.0±4.8a
NERICA8	14.3±3.9ab	29.7±2.3a
NERICA9	24.1±5.5ab	36.0±2.4a
NERICA10	46.4±4.7a	49.3±6.7a
NERICA11	17.1±6.2ab	43.1±0.9a
NERICA12	40.6±3.8ab	56.5±7.9a
NERICA13	23.8±4.9ab	42.3±3.1a
NERICA14	17.2±5.6ab	42.9±2.4a
NERICA15	12.0±5.3ab	20.0±4.1a
NERICA16	12.0±2.2ab	15.9±4.3a
NERICA17	16.7±5.2ab	33.4±8.4a
NERICA18	12.6±2.2ab	14.0±3.5a
CG14	14.7±5.1ab	32.4±3.7a
WAB 56-104	36.2±5.1ab	37.9±5.2a

SE: Standard error. Means with the same letter are not significantly different at p>0.05 according to SNK test

(mean deadhearts >20% at 10 DAI) whereas 11 varieties were moderately resistant. At 20 DAI, only NERICA16 and NERICA18, respectively scoring 15.9 and 14% for tiller infestation-showed good resistance to *Diopsis* sp. attacks under egg infestation. The remaining 18 entries varied in susceptibility between 20.0 and 56.5% tiller infestation (Table 2). The African parent, CG14, recorded a low level of deadhearts at 10 DAI (14.7%) but ranged to a high level of damage at 20 DAI (32.4%). As for the Asian parent (WAB56-104), it was severely damaged at both assessment timings, with 36.2 and 37.9%, respectively, at 10 DAI and 20 DAI.

At 10 DAI, 16 NERICA varieties were more resistant than WAB56-104, but at 20 DAI, 11 NERICAs were more resistant than the Asian parent. On the other hand, the African parent was more resistant than 15 NERICAs at 10 DAI and also more resistant than 13 NERICAs at 20 DAI (Table 2).

The cluster of mean percentage of infested tillers at 20 DAI ranked NERICA16 and NERICA18 as the varieties most resistant to *Diopsis* attack. The group comprising NERICA12, NERICA10 and NERICA2 was the most susceptible (Fig. 1).

Screening with adults: The damage recorded during the adult infestations was far lower than that recorded under egg infestation (Table 3). Of the 20 entries screened under adult infestation, 18 were resistant (% deadhearts <20%) at 10 DAI and two were moderately susceptible (% deadhearts between 20 and 26%). At 20 DAI, the 18 varieties remained resistant while the two others were further damaged (% deadhearts between 26 and 30%). The extent of tiller infestation in the test entries ranged between 0 and 26.6% at 10 DAI and between 3.0% and

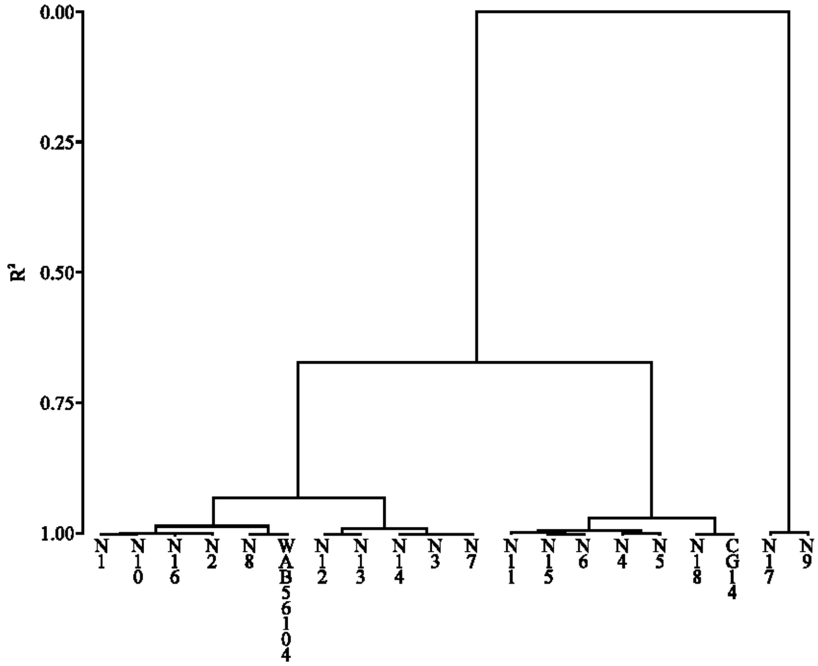

Fig. 2: Adult infestation dendrogram showing the minimum distance between clusters groups. N= NERICA

Table 3: Evaluation of rice lines for resistance/tolerance to *Diopsis* sp. under artificial infestation of adults in a screen cage at AfricaRice Benin Station, Cotonou, 2008 wet season

	% Deadheart±SE	
Varieties	10 DAI	20 DAI
NERICA1	11.6±4.2abc	11.6±4.2ab
NERICA2	7.8±5.9bc	12.3±1.4ab
NERICA3	10.6±4.5bc	16.1±4.2ab
NERICA4	7.1±7.1bc	8.5±5.7b
NERICA5	5.3±0.4c	7.7±6.3b
NERICA6	5.3±3.9c	6.7±3.0b
NERICA7	12.4±4.8abc	16.2±3.9ab
NERICA8	5.4±3.2c	14.4±4.9ab
NERICA9	26.6±3.9a	32.1±3.7a
NERICA10	7.7±2.7bc	11.4±2.6ab
NERICA11	2.9±2.9c	5.6±4.3b
NERICA12	13.3±4.5abc	18.5±5.7ab
NERICA13	13.6±7,9abc	18.0±4.8ab
NERICA14	12.5±2.6abc	16.6±2.4ab
NERICA15	3.4±2.2c	6.7±5.3b
NERICA16	9.4±4.8bc	12.9±3.5ab
NERICA17	23.5±5.6ab	33.6±1.6a
NERICA18	2.4±1.4c	3.8±2.1b
CG14	0.0±0.0c	3.3±0.0b
WAB 56-104	5.4±2.2c	14.2±2.7ab

SE: Standard error. Means with the same letter are not significantly different at p>0.05 according to SNK test

33.6% at 20 DAI. The most resistant variety was CG14, which scored 3.0% for deadhearts, followed by NERICA18 (3.8%), NERICA11 (5.6), NERICA6, NERICA15 (6.7), NERICA5 (7.7%) and NERICA4 (8.5) at 20 DAI (Table 3). Good resistance was also displayed by the varieties NERICA10(11.4%), NERICA1 (11.6%), NERICA2 (12.3%), NERICA16 (12.9%), WAB56-104 (14.2) and NERICA8 (14.4%) showed good resistance.

The cluster of mean percentages of infested tillers at 20 DAI showed CG14 and NERICA18 as the most resistant varieties to *Diopsis* sp. attack under adult infestation. The group comprising NERICA9 and NERICA17 was the most susceptible, followed by the group containing NERICA3, NERICA7, NERICA12, NERICA13, NERICA14, NERICA8 and WAB 56-104 (Fig. 2).

This result showed that 16 NERICAs recorded less than 20% of tiller infestation damage.

DISCUSSION

The results showed the differing behavior of rice varieties according to the type of infestation (eggs or adults) and also according to the number of days after infestation.

Under egg infestation, most of the screened varieties recorded very high percentages of deadhearts after 10 DAI. However, the damage level was low at both 10 DAI and 20 DAI following infestation by adults. In effect, the use of eggs in screening was a non-choice method whereas screening with adults resulted in a free-choice for the pests and the method appeared to be more realistic than egg screening in which the pest pressure was too high. Screening under adults is more appropriate for evaluating the resistance of rice varieties to *Diopsis* sp. because it more closely resembles natural infestation.

In general, the 20 entries and the NERICA varieties in particular showed good resistance to Diopsid attack despite using 50 adults m^{-2}, which is considered to be a critical high Diopsid density (Breniere, 1986). The resistance/tolerance to *Diopsis* sp. shown by most of the NERICA varieties was possibly inherited from the *O. glaberrima* parent, CG14. This *O. glaberrima* variety appeared to have a high level of resistance to *Diopsis* sp. possibly because it has co-evolved with the pest since its cultivation began in Africa over 3000 years ago (Bidaux, 1978; Carpenter, 1978; Jacquot *et al*., 1997; Ukwungwu *et al*., 1998). Without help or interference from man it has developed adaptive or protective mechanisms for resisting major biotic and abiotic stresses. It represents a rich reservoir of useful genes for resistance to diseases and pests (Jones *et al*., 1997).

This result confirms the high adaptability of *O. glaberrima* to environmental conditions in the African continent (Sarla and Swamy, 2005). It also demonstrated the good performance of NERICA varieties against field insects (WARDA, 1992; Akintayo *et al*., 2008).

The Asian variety WAB-56-104 was highly susceptible under egg infestation but it was moderately resistant under adult infestation. The performance of the *O. sativa* is not as good as that of *O. glaberrima* because of its more recent introduction in Africa and its low resistance behavior to many of the stresses that affect upland rice in West Africa (WARDA, 1992).

The screening with adults (free choice) indicated that most of upland NERICAs don't need chemical treatment against Diopsid flies even in conditions of high infestation. The genetic resistance of the varieties, combined with their strong tillering recovery capacity can keep them in a stable yield condition. As indicated by Harris (1962), rice varieties with high tillering ability have good properties to tolerate stem borer damage. The good resistance displayed by NERICA varieties in the present study confirms results obtained by Akintayo *et al*. (2008), who explained the performance of NERICA material in resisting field insect attack. The study confirmed the resistance of NERICA2 and NERICA4 to stem borers as reported by Nwilene *et al*. (2008) and Rodenburg *et al*. (2006).

The good performance of NERICAs and *O. glaberrima* varieties in terms of resistance to Diopsids could be due to genes of possible resistance inherited from the African parent. The performance of upland rice varieties has been improved thanks to conventional breeding over more than three decades in West Africa (Bidaux 1978; Takeoka, 1965). The benefit of *O. glaberrima* as a source for upland rice improvement was confirmed by Jones *et al*. (1997). Apart from genetic resistance, NERICAs and their *O. glaberrima* parent demonstrate high seedling vigor, resulting in rapid initial growth (Jones *et al*., 1997). This agronomic character can play a big role in the early resistance to *Diopsis* sp. According to Breniere (1983); the resistance of rice varieties to pests depends on their genetic or agronomic characteristics.

The high severity of *Diopsis* sp. damage in screening with eggs compared to adult screening can be explained by the no-choice element that characterizes the first method. In addition, most of the eggs were those of *D. thoracica* which attacks only safe tissues (Descamps, 1957) and one larva can damage 3-10 tillers (Feijen cited by Heinrichs and Barrion, 2004).

Under adults infestation 16 NERICAs recorded less than 20% of tiller damage, which is held to be the economic threshold (Nwilene *et al*., 2009).

Egg screening appears to be appropriate only in studies aiming the evaluation of antibiosis effect of varieties.

CONCLUSIONS

The present study has provided evidence that the upland NERICA rice varieties show good tolerance/resistance to Diopsid flies in general. The results showed that majority of upland NERICAs don't need chemical protection against *Diopsis* sp. even in conditions of high infestation. They can therefore be recommended for adoption by farmers in all environments in which *Diopsis* sp. are present. In particular, NERICA16, NERICA18 and CG14 are ranked as highly resistant to stalk-eyed fly and could be used in future hybridization rice programs.

The study showed that on-cage artificial screening under Diopsid adults infestation is a fast and appropriate method to test the resistance of rice varieties.

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