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PLANT SCIENCES | RESEARCH ARTICLE

Rapid phenotyping for identification of rice resistant varieties to *Diopsis apicalis* (Diptera: Diopsidae) Westwood

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Abstract: Nineteen rice varieties were assessed against *D. apicalis*, under partially controlled environment in a screen-covered cage at AfricaRice-Benin. 20, 30, and 50 couples of insects per square meter per cage were released 20 days after sowing in a complete randomized block design. In a first mock-trial (without shade above the cages), no dead heart was observed. The symptoms appeared in the second trial with the addition of some palm tree branches on top of the cages, showing the importance of shade and humidity in the biology of diopsids. This trial was repeated twice including three repetitions, during the rainy and dry seasons in South Benin. The study showed that the percentage of infested plants increased with the number of released insects. Rice varieties TOG5681, RAM55, NERICA1, NERICA4, NERICA8, and CG14 were the most resistant whereas IR47, IR64, ITA306, and WAB56-104 were the most susceptible. Densities 20 and 30 couples of insects per square meter were the best for an efficient screening. The screen-covered cage made with local materials is a convenient way for national programs in developing countries to screen at a lower cost. This method is easy to implement, fast and can allow simultaneous testing of large inbred rice populations.

ABOUT THE AUTHOR

Bocco Roland is a versatile researcher with laboratory and field experiences. He was previously a Research Assistant in the microbiological control unit against *Striga hermonthica*, cassava green mites and aphids at IITA for 10 years. Then, he joined the biotechnology unit at Africa Rice Center as Research Assistant where for more than 10 years he characterized rice for resistance to drought, the African Rice Gall Midge, the rice yellow mottle virus, the blast, and diopsids. His team improved and fixed introgression lines created through conventional breeding and marker assisted selection to control the aforementioned stresses. He completed a PhD in Plant Genetics and Physiology Applied Entomology in 2017. Dr Bocco is a postdoctoral fellow at the National Institute of Agricultural Sciences in South Korea. He contributes to the isolation, characterization, identification, evaluation of endophytic fungi for biological control, study of interactions, and the writing of scientific articles.

PUBLIC INTEREST STATEMENT

Rice is a strategic crop in sub-Saharan Africa because it is a financial and food resource. The regional production is deficient, hence the need to import and increase production. Climate change is leading to increased diopsid (stalk-eyed) flies creating huge losses. Thus, rice growers use chemical insecticides to minimize losses despite the consequences. This study has shown the genetic heritability of diopsid resistance and aims to exploit the diversity of the species to identify sources of resistance for genetic improvement of rice grown in Africa. The methods used are simple and realistic in developing countries; and revealed rice varieties TOG5681, RAM55, NERICA1, NERICA4, NERICA8, and CG14 as the most resistant to diopsid attacks.

The authors discourage the use of chemicals and support any varietal control initiative for ecofriendly sustainable rice protection.

Subjects: Environment & Agriculture; Agriculture & Environmental Sciences; Plant & Animal Ecology; Entomology

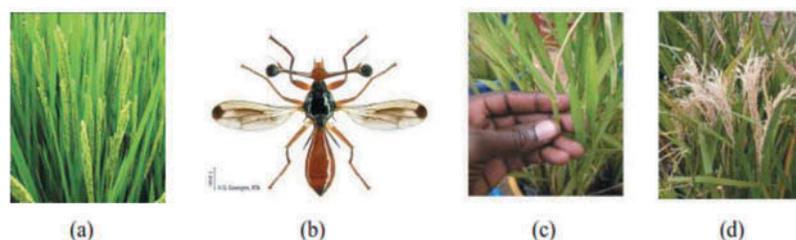
Keywords: *Diopsis apicalis*; resistance; humidity; dead heart; insect density; local materials

1. Introduction

Rice is a key food security commodity in Western Africa (Blein, Soulé, Faivre-Dupaigre, & Yérima, 2008). In this region, rice consumption has increased rapidly as a result of population growth, urbanization, and also the rise of its demand (Africa Rice Center, 2007). Average consumption per capita in the sub-region increased from 32 kg in 1990 to 34 kg in 2000 and reached 49 kg since 2012 (USDA (United States Department of Agriculture), 2014). Unfortunately, West Africa remains a region with regular rice deficit, whereby the local production only satisfies 60% of the total consumption. Countries such as Senegal, Ghana, Benin, and Côte d'Ivoire have a rice self-sufficiency rate lower than 40% (Fofana, Goundan, & Magne Domgho, 2014). The regional imports were estimated in between 7 and 8 million tons in 2011 and 2012, respectively, representing a cost of about \$3.5 to \$4.0 billions (ECOWAS (Economic Community of West African States Commission), WAEMU (West African Economic and Monetary Union Commission), and NPCA (NEPAD Planning and Coordinating Agency), 2014). Nigeria, Senegal, Côte d'Ivoire, and Benin account for more than 50% of the rice importation in West Africa. The main yield-reducing-factors are attributed to abiotic and biotic constraints. Among them, rice stem borers are considered as major insect pests of rice in sub-Sahara Africa (Nwilene, Nwanze, & Youdeowei, 2008; Nwilene et al., 2013). Stalk-eyed fly species (*Diopsis apicalis*) appears among the stem borers that are widely distributed and devastating pests of rice (Heinrichs & Barrion, 2004). The genus *Diopsis* can be found in all rice-producing ecological zones of Tropical Africa (Brenière, 1983) but particularly in humid and shady lowland areas (Appert & Deuse, 1988; Banwo, 2002), including irrigated fields (Heinrichs & Barrion, 2004). A single larva can damage between 3 and 10 rice stems (Heinrichs & Barrion, 2004), by boring into the stem-like lepidopteran stem borers. In cases of high infestation, larvae can attack rice panicles leading to whiteheads (Bijlmakers & Verhoek, 1995). Diopsids (Figure 1(b)) are present on rice throughout the entire growing period but they are particularly abundant in the field at pre-tillering and tillering stages (Joshi, Angela, & Ukwungwu, 1992).

Historically, diopsids control has been relying on the use of insecticides (Autriqueet al., 1989). However, the use of insecticides alone rarely provides long-term solutions. Indeed, the use of chemicals has adverse effects not only on human but also on natural enemies and environment (Pimentel, 2005). Also, inappropriate chemical use can create outbreaks of secondary pests and resistant pest strains. Nowadays, a more sustainable pest management approach includes all compatible control options, such as biological control and the use of resistant/tolerant varieties. Togola et al. (2011) observed diopsids resistance among upland NERICA (New Rice for Africa) rice varieties, which was inherited from their donor CG14. The objective of this study is to identify new resistant rice varieties to diopsids to be used as donors in advanced breeding programs. The development of resistant varieties to diopsids will help to reduce the dependency on pesticides use, will minimize yield loss from pests' infestation and will enable stable rice production across diverse environments.

Figure 1. (a) clean plants; (b) female diopsid; (c) dead heart on seedling; (d) whitehead.



2. Methods

2.1. Experimental site

The study was carried out on the experimental farm of AfricaRice hosted on the IITA-Benin research station (06°25.415N, 02°19.684E and 21 m altitude, 12 km North-West from Cotonou) at Togoudo in Southern Benin. The station is located in the African coastal Savannah zone, with a subequatorial climate (Adam & Boko, 1993).

2.2. Plant material

Nineteen rice varieties (Table 1) were evaluated for resistance to diopsids under screen-covered cages infestation. Among them there were four elite varieties, namely, NERICA1, NERICA4, NERICA6, and NERICA8, which were derived from double-backcrossing of WAB56-104 (*Oryza sativa* L.) and CG14 (*Oryza glaberrima* Steud.) followed by successive selfing (BC2F6) as female and male parents, respectively (Jones, Dingkuhn, Aluko, & Semon, 1997). This successive selfing reduced the heterozygosity from 100% to 3.125% (Amoussou, 2006). The resistant check was CG14 and the susceptible check was WAB56-104, as reported by Togola et al. (2011). Other test entries were TOG7106, TOG5681, and RAM55 belonging to *O. glaberrima* group species that showed a good level of resistance to Rice Yellow Mottle Virus and drought tolerance (Bocco et al., 2012; Thiémélé et al., 2010). The remaining test entries are elite varieties as shown in Table 1.

2.3. Insect collection and infestation

Diopsid adults (male and female) were captured in experimental rice fields and immediately transferred into screen-covered cages (2 m x 2 m size and of 1.6 m height) that were initially developed for the screening of the brown plant hoppers and thereafter adapted to diopsid screening by Togola et al. (2011).

Infestation was carried out at 20 days after sowing (DAS) to meet the susceptible growth stage of rice plants to diopsids including seedling, tillering, and stem elongation stages (Heinrichs, 2000; IRRI, 2002). Three pest densities were used in order to identify the most suitable one: 20, 30, and

Table 1. List of the 19 varieties evaluated for resistance to diopsids at AfricaRice

Entry	Cultivar	Group	Origin	Ecology
1	WAB56-104	<i>O. sativa</i>	AfricaRice	Upland
2	CG14	<i>O. glaberrima</i>	Sénégal	Upland
3	NERICA4	<i>O. sativa</i>	AfricaRice	Upland
4	NERICA6	<i>O. sativa</i>	AfricaRice	Upland
5	ITA306	<i>O. sativa</i>	IITA	Lowland
6	IR64	<i>O. sativa</i>	IRRI	Lowland
7	IDSA 6	<i>O. sativa</i>	Côte d'Ivoire	Lowland
8	NERICA1	<i>O. sativa</i>	AfricaRice	Upland
9	RAM55	<i>O. glaberrima</i>	Mali	Lowland
10	SUAKOKO8	<i>O. sativa</i>	Nigeria	Lowland
11	MOROBEREKAN	<i>O. sativa</i>	Guinea	Upland
12	B6 144-F-MR-6-0-0	<i>O. sativa</i>	IRRI	Lowland
13	NERICA8	<i>O. sativa</i>	AfricaRice	Upland
14	IR47	<i>O. sativa</i>	IRRI	Upland
15	DJOUKEME	<i>O. sativa</i>	Côte d'Ivoire	Lowland
16	TOG5681	<i>O. glaberrima</i>	Nigeria	Lowland
17	IAC165	<i>O. sativa</i>	CIAT	Upland
18	TOG7106	<i>O. glaberrima</i>	Mali	Lowland
19	TOS14519	<i>O. sativa</i>	The Gambia	Lowland

50 couples of diopsids/m². The top of the screening cages was covered with palm leaves to avoid excessive sunlight and heat.

2.4. Experimental set-up

The first experiment was conducted during the main rainy season between April and July 2014, the second one during the main dry season lasting from November 2014 to March 2015. The 19 entries were arranged in a complete randomized block design (CRBD) replicated three times. Each plot consisted of a single row of 50 cm long. To eliminate ants and other pests in the soil, Dursban insecticide (chlorpyrifos-ethyl 50 g.kg⁻¹) was applied at a concentration of 2 ml per liter of water a day before sowing (Monim, Rahman, Monim, Begum, & Costa, 2010). Three seeds were directly sown per hill. The distance between two consecutive hills within a row was 5 cm and the distance between two consecutive rows was 7 cm. A distance of 10 cm was observed between two replications. Thinning was done at 10 DAS in order to keep one plant per seed-hole. In both experiments, the cultural practices (weeding, watering, etc.) were performed on recommended dates for upland rice (Ekeleme et al., 2008).

2.5. Data collection

The relative humidity and temperature were recorded twice a day from the date of infestation to 20 days after infestation (DAI).

At 20 DAI, the number of infested tillers and hills were counted on all 10 plants of each line. Each plant with dead heart was uprooted, the stems were dissected and the diopsid larvae were counted. Percentage of infested tillers (severity) and the percentage of infested hills (incidence) in Figure 1(c,d) were calculated using the following formula (Togola et al., 2011).

At the end, the resistance of each variety was established using the Standard Evaluation System for Rice (IRRI, 2002) in Table 2.

$$\text{Incidence} = \frac{\text{Number of infested hills} \times 100}{\text{Total number of hills}}$$

$$\text{Severity} = \frac{\text{Number of infested tillers} \times 100}{\text{Total number of tillers}}$$

2.6. Statistical analyses

Data were analyzed using a Linear Mixed Model (McCulloch & Searle, 2001) to check for any significant difference between Genotype. For significant effects, a mixed model was fitted for each Season separately, i.e. Genotype × Density, Genotype × Season and Density × Season. Least squares means were estimated for each trait and Analysis of Variance (ANOVA) was followed by multiple comparisons of means using the Dunnett's test (Dunnett, 1955) to compare genotypes

Table 2. Standard Evaluation System (SES) for evaluating rice for resistance to *Diopsis apicalis* (IRRI, 2002)

Scale	Percent of infested tillers (severity)	SES rating
0	No damage	Highly Resistant (HR)
1	1–10%	Resistant (R)
3	11–20%	Moderately Resistant (MR)
5	21–30%	Moderately Susceptible (MS)
7	31–60%	Susceptible (S)
9	61% and above	Highly Susceptible (HS)

with each of the controls (CG14 and WAB56-104). Pearson’s correlation test was used to assess the relationship between Density and Season for each trait. Each variety was classified using Standard evaluation System (SES) for rice against diopsids as described by Table 2 (IRRI, 2002). All analyses were performed using R software version 3.1.2 (R Core Team, 2015).

3. Conclusion

3.1. Results

ANOVA showed a highly significant difference between genotypes in term of their pest severity and their incidence. A similar significant difference was observed for pest densities across seasons. Regarding the interactions Genotype x Density, Genotype x Season, Density x Season and Genotype x Density x Season, the ANOVA did not show any significant difference (Table 3). Therefore, the data corresponding to the two seasons were pooled and analyzed together. However, incidence and severity showed a high heritability (Table 3).

Based on the SES rating (IRRI, 2002), rice varieties CG14, NERICA1, NERICA4, NERICA8, RAM55, and TOG5681 were moderately resistant to diopsids. In contrast, B6144-F-MR-6-0-0, DJOUKEME, IAC165, IDSA6, IR47, IR64, ITA306, MOROBEREKAN, NERICA6, SUAKOKO 8, TOG7106, TOS14519, and WAB56-104 were susceptible to diopsids.

The severity of pest damage on B6144-F-MR-6-0-0 (26.89%), ITA306 (35.56%), MOROBEREKAN (27.61%) and NERICA1 (15.50%) was significantly different ($P < 0.05$ and 0.01) compared to the resistant check CG14 (16.89%). The mean comparison test for severity between WAB56-104 (susceptible check) and B6144-F-MR-6-0-0, CG14, NERICA4, RAM55, and TOG5681 showed a significant difference ($P < 0.05$ and 0.01) with 26.89%, 16.89%, 20%, 15.11%, and 10.56% of infested tillers, respectively, versus 31.94% for WAB56-104 (Table 4).

Regarding the damage incidence, a significant difference ($P < 0.05$ and 0.01) was identified between IR47, IR64, and ITA306 and the resistant check CG14 with 32.94%, 35.56%, and 35.89% of infested hills versus 17.33% for CG14.

The *O. glaberrima* rice varieties TOG5681 and RAM55 showed a significant difference ($P < 0.05$ and 0.01) compared to the susceptible check WAB56-104. Incidence of these two rice varieties was 11.50% and 17.33%, respectively, compared to 31.94% of that of WAB56-104 (Table 4).

Incidence and severity increased gradually with insect densities. There was more dead heart damage during the Season 2 than during the Season 1. Mean values of incidence and severity for each density and season are summarized in Table 5.

Table 3. Evaluation of the different characters across genotypes, season, and pest densities

Source of variation	Incidence	Severity
Genotype	<0.001	<0.001
Density	<0.001	<0.001
Season	<0.001	<0.001
Genotype x Density	0.2347	0.2045
Genotype x Season	0.1077	0.1818
Density x Season	0.5203	0.5084
Genotype: Density x Season	0.4112	0.2405
Heritability	0.58	0.66

<0.001 = significant at 0.001.

Table 4. Varietal performances for severity and incidence values vs CG14 and WAB56-104 during two Seasons

Genotype	Severity				Incidence			
	Severity	SES Rating	vs CG14	vs WAB	Incidence	vs CG14	vs WAB	
B6144-F-MR-6-0-0	26.89	MS	0.04843*	0.0167*	28.94	0.268	0.0738	
CG14	16.89	MR	-	0.0486*	17.33	-	0.0737	
DJOUKEME	22.28	MS	1	0.9937	22.56	0.988	1	
IAC165	30.44	MS	0.3908	0.4927	30.33	0.155	0.5463	
IDSA6	26	MS	0.9749	1	28	0.373	1	
IR4-7	31.83	S	0.0871	0.9673	32.94	0.0447*	0.9995	
IR64	32.33	S	0.5178	1	35.56	0.0095**	1	
ITA306	35.56	S	0.04143*	1	35.89	0.0077**	0.9999	
MOROBEREKAN	27.61	MS	0.03097*	0.9989	28.06	0.366	0.9995	
NERICA1	15.5	MR	0.00356**	0.9991	16.72	1	0.9996	
NERICA4	20	MR	0.3022	0.0215*	20.72	1	0.0541	
NERICA6	23.94	MS	1	0.2177	25.11	0.783	0.3089	
NERICA8	15.44	MR	1	0.7509	15.56	1	0.896	
RAM55	15.11	MR	0.8298	0.0206*	17.33	1	0.0287*	
SUAKOKO 8	29.44	MS	1	1	32.22	0.064	1	
TOG5681	10.56	MR	0.1409	0.001***	11.5	0.968	0.0021**	
TOG7106	22.11	MS	0.9118	0.4679	30	0.178	1	
TOS14519	25	MS	0.9811	0.8894	25.83	0.679	0.9531	
WAB56-104	31.56	S	0.6742	-	31.94	0.074	-	

-: Not available; *Significant at 5%; **Significant at 1%; ***Significant at 0.001

WAB: WAB56-10; MR: Moderately Resistant; MS: Moderately Susceptible; S: Susceptible

Table 5. Multiple comparisons of means between incidence and severity per density and season

Densities/seasons/differences	Incidence	Severity
Density 20	16.11	15.25
Density 30	25.46	24.16
Density 50	35.25	32.99
Season 1	19.37	17.14
Season 2	31.85	31.12
Density 30—Density 20	0.0186*	0.0191*
Density 50—Density 20	<0.001***	<0.001***
Density 50—Density 30	0.0126*	0.0204*
Season2—Season1	<0.001***	<0.001***

*Significant at 5%; ***Significant at 1%

Lastly, the mean values of incidence and severity per density and season exhibited significant differences ($P < 0.05$ and 0.001), except for severity values for Density 50 and Density 30 (Table 5).

4. Discussion

O. glaberrima Steud. (African origin) varieties are known to have great ability to adapt to abiotic and biotic stresses encountered in Africa (Wang et al., 2014). However, these varieties have a very low yield and high grain shattering rates compared to *O. sativa* varieties from Asia. The six rice varieties TOG5681, NERICA1, NERICA4, NERICA8, RAM55, and CG14 identified in this study as the most resistant belong to interspecific and *O. glaberrima*. Rice variety TOG5681 is known for its high drought tolerance capability (Bocco et al., 2012). This same variety (TOG5681) was reported as carrying RYMV-1 gene conferring resistance to rice yellow mottle virus (Thiémélé et al., 2010). Resistance of the interspecific lines NERICA1, NERICA4, and NERICA8 varieties has been inherited from the parent CG14 donor (Togola et al., 2011). According to the same authors, CG14 resistance was similar to the interspecific lines (NERICA4 and NERICA8) resistance. The population density of 50 couples of diopsids/m² was previously reported as capable of breaking plant resistance (Togola et al., 2011). However, the percentage of dead hearts obtained in our study is higher than that of Togola et al. (2011) for the same high diopsid density (50 couples/m²). It is important to point out that the insects used by Togola et al. (2011) were collected from the village of Koussin (about 200 km North-East of Cotonou), whereas the diopsids for our study were captured on the AfricaRice research station. Hence, the dissimilar results could be explained by the possible difference of biotypes (or ecotypes) between diopsids strains in Koussin and the one in Cotonou. High heritability estimated for severity confirms results of Singh, Sharma, and Rao (2011). Traits investigated could therefore be improved using pedigree breeding schemes, along with breeding for diopsid resistance.

NERICA1, NERICA4, and NERICA8 are interspecific upland rice lines, which suffer relatively less damage of diopsids compared to *O. sativa* rice varieties. The resistance in these varieties may confirm their status of potential donors for breeding programs despite NERICA8 being known as moderately susceptible to insect pests like grain borer *Rhyzopertha dominica* (Chougourou et al., 2013). In a recent study, RAM55 was found to be resistant to African rice gall midge (AfrGM caused by *Orseolia oryzivora*) (AfricaRice unpublished data in 2013). Therefore, this rice variety could be used as donor parent to elite varieties in order to confer double resistance to AfrGM and diopsids via double or triple backcrossing followed by selfing through six or eight generations (Jones et al., 1997). A similar strategy used for the generation of the NERICAs could be used here to generate resistant lines (to both diopsids and AfrGM), which could be exploited to produce mapping populations. Therefore, the genes involved in those resistances could be located using molecular markers.

Unfortunately, that same assessment made in 2013 (unpublished data) at AfricaRice had revealed the susceptibility of CG14 to AfRGM, whereas this variety is resistant to diopsids. Based on these results, we can deduce that different genes may confer resistance to CG14 and RAM55.

During our experiments, some varieties of *O. sativa* namely, DJOUKEME, IAC165, IR47, SUAKOKO 8, WAB56-104, and B6144-F-MR-6-0-0 suffered heavy pest attacks and tended to produce new tillers to compensate for lost ones. These new tillers were also likely to be attacked and flowered with a delay compared to healthy tillers (Osisanya, 1984). This ability of varieties to initiate new tillers following an attack could be induced by cytokinins (Liu et al., 2011). We know that these hormones play an important role in the germination process and promote cell division (Dante, Larkins, & Sabelli, 2014). They activate the initiation of leaves, tillers, stems and stolons, and promote the expansion of leaves and cotyledons (Champault, 1973). This ability of the rice plant to withstand pest damage by producing new tillers can be considered as a tolerance mechanism.

The difference between incidences, as well as the difference between severities, could be interpreted in the light of climate dissimilarities between the two seasons. Environmental factors such as temperature, relative humidity, and sunlight are known to have a big influence on pest development and activity (Heinrichs & Barrion, 2004). It is known since that climatic phenomena such as temperature and relative humidity can affect the behavior of insects (Astuti, Mudjiono, Rasminah, & Rahardjo, 2013). The harmattan (dry) season characterized by poor hygrometry may be unfavorable to diopsids as the case in the studies on the dipteran *Aedes aegypti* by negatively affecting egg production, oviposition time and changing oviposition patterns (de Almeida Costa, de Mendonça Santos, Correia, & de Albuquerque, 2010). As the case for AfRGM, research against the diopsids may also be directed towards the production of rice during periods, which do not permit the development of the diopsids by providing a minimum of water, although enough to fulfill the needs of the rice seeds (Nwilene, Nwanze, & Okhidievbie, 2006). Those previous authors mentioned that AfRGM populations are lower in upland rice while higher in lowland rice ecology. Moreover, Ogah, Umeh, and Oselebe (2006) had noted a substantial reduction in the population of AfRGM by modifying rice sowing dates. Because most of the current elite irrigated rice varieties used in the sub-region are susceptible to diopsids, the modification of the sowing dates could possibly be a valuable control option while resistant varieties are being developed.

Different reactions are observed on rice varieties when they are exposed to diopsids. Some plants can stimulate the emission of several tillers often less productive than in the absence of attacks (Osisanya, 1984). Other rice varieties are less damaged and that could be explained by the presence of volatiles attracting or repelling insects (Omoloye & Vidal, 2007). During a phytochemical screening of three rice cultivars, those previous researchers identified four major phytosterols (secondary metabolites): campesterol, 24-methylenecholesterol, stigmaterol, and β -sitosterol in susceptible and resistant rice cultivar. However, 24-methylenecholesterol was the most abundant sterol in the resistant *O. glaberrima* cultivar TOG7442, whereas β -sitosterol was most abundant in the two susceptible *O. sativa* cultivars (Cisadane and ITA 306). They concluded that the high concentration of 24-methylenecholesterol in this cultivar of *O. glaberrima* could be an indicator of resistance to AfRGM. Moreover, it was found that a plant low in saponins has a repellent or toxic effect on insects (Morrissey & Osbourn, 1999, quoted by Francis, Kerem, Makkar, & Becker, 2002). In general, females choose their hosts for laying eggs and the development of their progenies (Courtney & Kibota, 1990); they are moving towards plants with good nutrient content to reproduction (Awmack & Leather, 2002; Colsey, Bateman, & Kursar, 2006; Janz & Nylin, 1997; Morewood, Simmonds, Gries, Allison, & Borden, 2003). The low severity (percentage of infested tillers) compared to incidence (percentage of infested hills) may reflect a resistance mechanism known as antibiosis displayed by the plant after being attacked (Omoloye, Odebiyi, Williams, & Singh, 2002). In general, antibiosis reactions are related to the presence and activity of endophytic microorganisms, enzymes, R gene-mediated that emit repulsive or toxic molecules (da Silva Ribeiro et al., 2018; Suhandono, Kusumawardhani, & Aditiawati, 2016). In rice, elicitors derived from herbivore insect oral secretions or oviposition fluids activate phytohormone signaling, and transcriptomic changes mediated mainly by transcription factors lead to accumulation of defense-related secondary metabolites (Qi et al.,

2018). According to those same authors, direct defenses, such as trypsin protein inhibitors in rice, have anti-digestive or toxic effects on insect herbivores. Herbivory-induced plant volatiles, such as terpenes, are indirect defenses, which attract the natural enemies of herbivores. The resistance noted in the varieties belonging to the African rice species *O. glaberrima* could result from the coevolution between that species and the microorganisms in an environment marked by the presence of several biotic and abiotic stresses (Holtz et al., 2010). Research directed towards the isolation of endophytes in the plant tissues of the rice varieties studied would reveal a difference in diversity between susceptible and resistant individuals (Zakaria, Yaakop, Salleh, & Zakaria, 2010). As a result of this difference, bioassays would focus on the behavior of the insect against each microorganism through an olfactometer. Chromatography will identify the chemical compounds present and finally determine their influence on the insect behavior (Daisy et al., 2002). This inexpensive approach would contribute to the sustainable and ecological protection of rice and therefore provide a healthy diet for consumers.

Our study did not reveal neither the genes involved in conferring resistance nor their inheritance, in spite of this resistance found in NERICAs 1, 4, and 8. However, extending this screening to other rice accessions might open the possibility of gene pyramiding in order to broaden this resistance; and by doing so preventing pests to overcome it easily (Fukuoka et al., 2015).

The occurrence of resistance in the rice cultivars CG14, RAM55, and TCG5681, will enable the generation of new resistant lines (like the ones resistant to the Brown Planthopper *Nilaparvata lugens* (Stål) from Wild Rice Species) for the greater benefit of small-holder farmers (Sarao et al., 2016). This program should be beneficial to African rice farmers in areas where *D. apicalis* is a problem and should reduce the need of chemical use and therefore their environmental impact (Damalas & Eleftherohorinos, 2011). This resistance could be introduced into susceptible elite varieties in the sub-region similarly to the NERICAs.

Overall this work has enabled the identification of resistant rice varieties to *D. apicalis* but did not reveal any metabolic pathway or sensory basis for this resistance. Regarding the characterization of the physiological basis of the rice resistance towards *D. apicalis*, it would be advisable to conduct olfactometric and electroantennogram studies to identify each volatile molecule to study them individually for their involvement in the insect orientation process. It would also be important to quantify reducing sugars contained in the rice varieties in order to better understand their roles in the resistance against *D. apicalis*.

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Competing Interests

The authors declare no competing interest.

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