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Antixenotic and Antibiotic Mechanisms of Resistance to African Rice Gall Midge in Nigeria

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ABSTRACT

African Rice Gall Midge (AfRGM) *Orseolia oryzivora* Harris and Gagné (Diptera: Cecidomyiidae), is a major insect pest mainly of rainfed and irrigated lowland rice in Africa. Of the management options identified for controlling AfRGM, host plant resistance is the most compatible and farmer-friendly manner. Rice varieties have morphological and/or biochemical traits associated with resistance which induces diverse resistance to pests. Two resistance mechanisms (antixenosis and antibiosis) were evaluated on ten rice genotypes under artificial infestation. Level of infestation was assessed while morphological traits were observed physically; leaf samples were collected for biochemical analysis in the laboratory. The results showed that the three *O. glaberrima* varieties were resistant to AfRGM (little or no pest infestation) and all the interspecific genotypes were susceptible to AfRGM. In *Oryza sativa* varieties, long leaf and leaf sheath have been identified to confer antixenotic resistance to AfRGM. But in *Oryza glaberrima* varieties, secondary metabolites-Phenol, Terpenoids, Salicylic acids and Monoterpene have been identified as the key antibiotic traits associated with resistance to AfRGM. The result of the Principal Component Analysis (PCA) of the traits produced four major clusters accounting for 79% of the total variation of the traits which had negative correlation with percentage tiller infestation thereby conferring resistance to AfRGM. Understanding the mechanisms and traits/factors contributing to resistance of host plant is useful in deciding appropriate breeding methodologies for varietal improvement. This work facilitates the effort of plant breeders and entomologists in developing and deploying insect-resistant cultivars to overcome new insect biotypes.

Key words: Rice genotypes, African rice gall midge, mechanisms of resistance, antixenosis, antibiosis, *Oryza glaberrima*, *Oryza sativa*

INTRODUCTION

Rice (*Oryza* spp.) is one of the most important cereal crops for human and livestock consumption (Oyetunji *et al.*, 2012). It is a staple crop in Africa where it has been cultivated for more than 3000 years (Togola *et al.*, 2012; Nwilene *et al.*, 2011). Although local rice production is on the increase yet, it has not been able to meet the local consumption partly due to some constraints including pests and diseases (Togola *et al.*, 2012). African Rice Gall Midge (AfRGM) *Orseolia oryzivora* Harris and Gagné (Diptera: Cecidomyiidae), is a major insect pest primarily of rainfed and irrigated lowland rice in sub-Saharan Africa (Nwilene *et al.*, 2006). Out of several

management strategies that have been proposed to manage the pest (Nwilene *et al.*, 2002), host-plant resistance is the farmer-friendly pest control option and it is recognized as a long-term control measure against this pest (Nwilene *et al.*, 2009).

Substantial achievement has been made in screening and breeding for resistance varieties to AfRGM but slight consideration has been attributed to the mechanisms of resistance in rice genotypes. Plants naturally acquire potential means of resistance which mostly stimulate the morphological and biochemical features of plant (Goncalves-Alvim *et al.*, 2004; Gogi *et al.*, 2010) that impair the normal feeding or oviposition of various insects pest (Afzal and Bashir, 2007) or induce other mortality factors, that collectively create phonetic resistance (Coley and Barone, 1996). These mechanisms of resistance have proved to be valuable tools against the insect pests in many crops and vegetables (Felkl *et al.*, 2005). The mechanisms of resistance in plants are either constitutive or induced (Traw and Dawson, 2002) and are grouped into three main categories such as non-preference or antixenosis, antibiosis and tolerance (Painter, 1951). Antixenotic mechanism of resistance which is employed by the host plants, deters the insects from oviposition (Afzal *et al.*, 2009), feeding, seeking shelter (Sharma and Nwanze, 1997; Woodhead and Taneja, 1987) and colonization (Dhaliwal and Arora, 2003). This mechanism renders the plants undesirable.

Apart from antixenosis, biochemical characteristics are important constituent of host plant resistance. Rice genotypes acquire different biochemical properties which pose their resistance to insect pests. These means enable the plants to circumvent, tolerate or recuperate from the damage caused by insect pest attacks. Plants secondary metabolites do not contribute to growth or reproduction directly but they often contribute to plant defense. These compounds usually belong to one of three large chemical classes: terpenoids, phenolics and alkaloids (Aarts *et al.*, 1998). The development of AfRGM resistant rice varieties has been prejudiced, because of the lack of ample information on the sources traits associated with mechanism for resistance and their influence on the pest burgeoning. Therefore, it becomes imperative to identify morphological and biochemical feature associated with the mechanism of resistance and get knowledge of their influence on oviposition preference, larval performance (Fitt, 1986) and pest multiplication (Dillon *et al.*, 2005) for devising sustainable pest management strategies for AfRGM. Getting the source of resistance could not be achieved without proper evaluation for identification of resistant feature and thorough investigation and series of laboratory analysis. This is the more reason ten lines (checks inclusive) were evaluated on morphological and biochemical traits for resistance to AfRGM. Identification of morphological traits and secondary metabolites in different genotypes as sources of resistance to AfRGM will help in the breeding programme on the choice of traits to introgress to the breeding lines to confer resistance to AfRGM.

MATERIALS AND METHODS

Test entries: The study was conducted in 2011 and 2012 on ten rice genotypes viz., two susceptible *Oryza sativa* (Cisadane, BW348-1); three interspecific progenies (NERICA L-19, NERICA L-25, NERICA L-49); three resistant *O. glaberrima* (TOG 7106, TOG 7206 and TOG 7442) and two *Oryza sativa* as check varieties, TOS 14519 (resistant check) and ITA 306 (susceptible check), under artificial infestation in a paddy screenhouse at AfricaRice, Ibadan, Nigeria.

Experimental design and infestation: The experiment was laid on Randomized Complete Block Design (RCBD) with three replications. The laboratory infested seedlings were transplanted at

21 Days After Sowing (DAS) as infestor band before adult emergence four days to transplanting of test entries for the infestation of the test entries. Test entries were transplanted from the nursery at 21 Days After Sowing (DAS) with two seedling per hill. A basal dose of NPK at the rate 40:40:40 kg ha⁻¹ was applied before sowing and a top-dressing of urea 40 kg ha⁻¹ at 20-25 Days After Transplanting (DAS). Hand weeding was carried out 3 times before harvesting.

Assessment of percentage tiller infestation: Scoring of the number of tillers with gall and the total number of tillers per hill to estimate the incidence and severity of susceptibility and resistance of the rice varieties to AfRGM was done at 45 and 70 DAT. This was evaluated in terms of percentage tiller infestation (Nwilene *et al.*, 2002) and scored according to the Standard evaluation system for rice (IRRI, 1996).

Morphological assessment and biochemical analysis: The morphological traits that were assessed physically include leaf length, seedling vigour, leaf glossiness, leaf surface wetness, ligular hair and colour, leaf sheath and colour. Also, the number of eggs deposited on each leaf sheath was recorded. Biochemical analysis carried out in the analytical laboratory on leaf samples include Total phenols, Monoterpenoids, Salicylic acid, Coumarin, Terpenoids, Sesquiterpenoids, Jasmonic acid, Cystein protease. Phenolic compounds and tannins were determined with the use of UV-Vis spectrophotometer while Flavonoids was determined gravimetrically using the method of Harborne (1973). Total sugar and reducing sugars were determined on the UV at a wavelength of 485 nm.

Statistical analysis: Combined analyses of variance were conducted for the infestation using the General Linear Model (GLM) Procedure of the Statistical Analysis System (SAS, 1996).

Principal Component (PC) analysis was performed on the mean values for each trait to identify a group of traits that accounted for most of the variance in the set of data and could be used to rank the genotypes for antibiotic and antixenotic mechanism of resistance using the PRINCOMP procedure of the SAS package. The contribution of each feature to the PC axis was determined by performing simple correlation analysis. Features that did not have significant correlation with PC scores were considered as inconsequential.

RESULTS

Out of ten genotypes evaluated, one genotype (TOG 7106) was highly resistant or immune to AfRGM. Three genotypes (TOG 7206, TOG 7442 and TOS 14519) showed resistance to AfRGM too. While one rice genotype (BW 348-1) showed moderate resistance to AfRGM; two rice genotypes (NERICAL-19 and NERICAL-25) were moderately susceptible. The remaining three genotypes (CISADANE, NERICAL-49 and ITA 306) were susceptible to AfRGM (Table 1). All the three *O. glaberrima* are either resistant or highly resistant to AfRGM infestation. Out of four *O. sativa*, TOS 14519 was the only resistant genotype while BW 348-1 was moderately resistant; the remaining two are either susceptible or moderately susceptible to AfRGM infestation. All the three interspecific progenies are either susceptible or moderately susceptible to AfRGM infestation (Table 1).

This study showed that the traits for resistance to AfRGM did not make athwart cause on all the genotypes the same rate. While some genotypes possess a particular trait for resistance; others that are deficient in such could possess other traits to compensate for the deficiency among the resistant genotypes. The variation in the resistance/susceptibility of different rice genotypes was

Table 1: Infestation of AfRGM on ten rice genotypes

Genotypes	Tiller infestation (%)		Mean (%)	Resistant ranking (IRRI, SES)
	45 DAT	70 DAT		
TOG 7106	0.00	0.00	0.00	HR
TOG 7206	0.00	6.51	3.26	R
TOS 14519	5.71	2.56	4.14	R
TOG 7442	11.27	0.00	5.63	R
BW 348-1	10.38	10.26	10.32	MR
NERICAL-25	7.04	19.85	13.44	MS
NERICAL-19	13.08	25.70	19.39	MS
ITA 306	16.87	23.22	20.04	S
NERICAL-49	13.43	33.25	23.34	S
CISADANE	27.04	45.69	36.36	S

HR: Highly resistant, R: Resistant, MR: Moderately resistant, MS: Moderately susceptible, S: Susceptible. Source: Standard evaluation system (SES) for rice (IRRI, 1996)

Table 2: Traits for resistance in rice genotypes

Sources of resistance	Level of resistance	Traits associated with antibiotic resistance	Traits associated with antixenotic resistance
<i>Oryza glaberrima</i>			
Tog 7106	Highly resistant	Phenol, terpenoids, salicylic acids and monoterpenoid	Leaf length, leaf glossiness, seedling vigour and leaf sheath
Tog 7442	Resistant	Phenol	Leaf length and leaf sheath
Tog 7206	Resistant	Low phenol	Leaf length and leaf sheath
<i>Oryza sativa</i>			
TOS 14519	Resistant	Sesquiterpenoids, monoterpenoid and terpenoids	Seedling vigour and leaf sheath
CISADANE	Susceptible	Low terpenoids and flavonoid	Leaf surface wetness and high eggs deposition
BW348-1	Moderately susceptible		
ITA 306	Susceptible	Low phenol	Leaf surface wetness and high eggs deposition
Interspecific progenies			
NERICAL-19	Moderately susceptible	Coumarin and phenol	Leaf surface wetness and ligular hair
NERICAL-25	Moderately susceptible	Coumarin and cystein protease	Leaf length and leaf surface wetness
NERICAL-49	Susceptible	Low phenol content and low monoterpenoids	Short internode elongation

partly attributed to some antixenotic traits associated with mechanism of resistance to AfRGM. The percentage tiller infestation was directly linked with some of the morphological characteristics possessed by various genotypes while some show otherwise. Leaf length was found to be a good antixenotic property for resistance to AfRGM as most of the resistant genotypes with low percentage mean tiller infestation possess long leaves (Fig. 1). The leaf length had a significant negative correlation ($r = 0.629$) with the percentage mean tiller infestation and thus resistance to AfRGM. The leaf length in most genotypes was inversely proportional to the percentage mean tiller infestation. TOG 7106 with 0% infestation has longest leaf while ITA 306 and CISADANE with highest infestation have the short leaf among the evaluated genotypes, although with a little variation in between (Fig. 1). The *O. glaberrima* genotypes are resistant to AfRGM and they all have leaf length as the antixenotic mechanism of resistance to AfRGM (Table 2). Therefore, leaf length is a dynamic feature as antixenotic mechanism of resistance to AfRGM. The leaf breadth

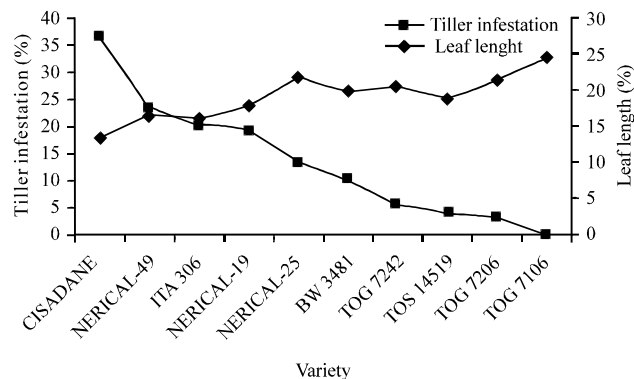


Fig. 1: Effect of leaf length of various rice genotypes on damage by AfRGM

Table 3: Antixenotic traits associated with resistance to AfRGM

Genotype	Seedling vigor	Leaf EGGS	Leaf breadth (cm)	Leaf surface wetness	Leaf sheath	Leaf gloss	Ligular hair	Internode elongation	Trichome
BW348-1	3.00 ^c	11.60 ^d	0.26 ^{cd}	1.10 ^e	1.53 ^{bc}	2.00 ^{abc}	1.57 ^c	2.23 ^d	41.78 ^{ab}
CISADANE	7.00 ^a	15.85 ^a	0.33 ^{abc}	1.57 ^a	1.07 ^d	2.33 ^{abc}	1.43 ^c	2.53 ^{cd}	52.00 ^a
ITA306	5.00 ^b	15.55 ^b	0.35 ^{abc}	1.50 ^c	1.13 ^d	3.00 ^a	1.83 ^{ab}	1.78 ^{de}	19.89 ^{bcd}
NERICAL-19	1.00 ^d	04.05 ^e	0.42 ^a	1.35 ^d	1.10 ^b	2.67 ^{ab}	1.89 ^a	1.97 ^{de}	36.00 ^{abc}
NERICAL-25	5.00 ^b	14.95 ^c	0.23 ^d	1.55 ^b	1.20 ^{cd}	1.33 ^c	1.61 ^{abc}	2.37	8.00 ^c
NERICAL-49	3.00 ^c	04.00 ^f	0.42 ^a	1.10 ^e	1.13 ^d	2.33 ^{abc}	1.82 ^{ab}	1.18 ^e	13.33 ^{bcd}
TOG7106	5.00 ^b	01.75 ^j	0.36 ^{ab}	1.00 ^h	2.80 ^a	2.67 ^{ab}	0.35 ^d	3.68 ^b	0.00 ^d
TOG7206	1.00 ^d	0.70 ^j	0.42 ^a	1.20 ^f	2.73 ^a	2.00 ^{abc}	0.19 ^d	3.80 ^b	29.89 ^{abcd}
TOG7442	3.00 ^c	02.10 ^h	0.38 ^{ab}	1.10 ^e	2.73 ^a	2.33 ^{abc}	0.25 ^d	5.45 ^a	0.00 ^d
TOS14519	5.00 ^b	02.25 ^g	0.31 ^{bcd}	1.25 ^g	2.73 ^a	1.67 ^{bc}	1.51 ^c	3.17 ^{bc}	19.33 ^{cd}

Column means followed by the same letters are not significantly different at $p \leq 0.05$

had a negative correlation with the percentage tiller infestation. The leaf wetness was associated with susceptibility of some susceptible rice genotypes to AfRGM in 2011 and 2012. The rate of leaf wetness tends to hasten and propel the chance eggs had to get to the leaf sheath. It was associated with susceptibility to AfRGM in ITA 306, CISADANE, NERICAL-25 and NERICAL-19. Leaf surface glossiness was associated with the level of resistance to AfRGM in TOG 7106 rice genotypes (Table 3). The percentage mean tiller infestation had significant positive correlation with some morphological traits as well as some secondary metabolites as shown Table 4. The leaf glossiness which in turn was negatively correlated with number of eggs laid though not significant. The leaf sheath was negatively correlated with percentage mean tiller infestation. Leaf sheath was identified as the trait of mechanism for resistance in TOG 7106, TOS 14519, TOG 7442 and TOG 7206 rice genotypes. The internode elongation had significant negative correlation with the percentage tiller infestation. The long internodes tend to impede the larva chance of boring in the plant. The number of eggs on leaf was positively correlated with the percentage mean tiller infestation. The rate of oviposition was associated with the percentage tiller infestation and thus the susceptibility to AfRGM in CISADANE, ITA 306 and NERICAL-25. The oviposition in resistant genotypes (TOG 7206, TOG 7106, TOG 7442 and TOS 14519) was low. Thus, these genotypes possess inherent ability to deter oviposition. Trichome density was positively correlated with the percentage mean tiller infestation and it was not associated with the resistance in this study.

Tables 4: Biochemical traits associated with resistance to AFRGM

Entries	Tiller		Salicylic		Terpenoids		Sesquiterpenoids		Jasmonic	
	infestation (%)	Phenol (%)	Tannin (%)	acid (%)	Coumarin (%)	(%)	Monoterpenoids (%)	(%)	acid (%)	Cystein (%)
ITA306	41.04 ^a	0.23 ^d	44.97 ^{ab}	3.49 ^d	56.08 ^a	1.78 ^d	0.43 ^d	0.57 ^b	19.76 ^a	0.020 ^c
NERICAL-49	28.50 ^b	0.19 ^d	13.51 ^b	2.66 ^f	37.25 ^{bc}	1.28 ^f	0.32 ^f	0.43 ^c	12.42 ^{bc}	0.010 ^e
CISADANE	26.19 ^b	0.40 ^b	34.71 ^{ab}	2.08 ^b	39.19 ^{bc}	1.04 ^b	0.26 ^f	0.15 ^b	12.54 ^{bc}	0.030 ^c
NERICAL-25	15.55 ^f	0.25 ^d	26.28 ^{ab}	3.39 ^d	63.24 ^a	1.69 ^d	0.43 ^d	0.34 ^e	21.08 ^a	0.030 ^d
BW348-1	14.21 ^c	0.29 ^f	52.92 ^a	3.69 ^f	37.70 ^{bc}	1.85 ^f	0.46 ^f	0.21 ^e	12.54 ^{bc}	0.020 ^d
NERICAL-19	12.79 ^f	0.55 ^a	43.95 ^{ab}	4.07 ^b	60.27 ^a	1.97 ^b	0.49 ^b	0.39 ^d	20.09 ^a	0.040 ^a
TOS14519	0.80 ^d	0.28 ^c	22.40 ^{ab}	4.60 ^a	42.03 ^b	2.30 ^a	0.58 ^a	0.77 ^a	14.01 ^b	0.010 ^e
TOG7442	0.80 ^d	0.45 ^b	44.02 ^{ab}	2.93 ^f	37.97 ^{bc}	1.49 ^f	0.38 ^e	0.25 ^f	12.81 ^{bc}	0.020 ^c
TOG7206	0.33 ^d	0.19 ^d	18.86 ^{ab}	3.21 ^e	30.40 ^f	1.59 ^f	0.39 ^e	0.21 ^g	10.14 ^c	0.004 ^e
TOG7106	0.00 ^d	0.44 ^b	33.15 ^{ab}	4.47 ^a	56.35 ^a	2.25 ^a	0.56 ^a	0.75 ^a	18.78 ^a	0.030 ^b

Column means followed by the same letters are not significantly different at p≤0.05

Table 5: Eigen vectors of the first four principal components (PC 1, PC 2, PC 3 and PC 4) axes for 10 rice genotypes evaluated under artificial AfRGM infestation for antixenosis and antibiosis at Africa Rice-Ibadan in 2011 and 2012. Only eigen vectors with values equal to or higher than 0.3 are shown

Character	PC 1	PC 2	PC 3	PC 4
Mean infestation	-0.32676	-	-	-
Tannin	-	-	-	-0.52405
Phenol	-	-	0.572174	-
Salicylic acid	0.373942	-	-	-
Coumarin	-	0.434929	-	-
Terpenoids	0.378331	-	-	-
Monoterpenoids	0.375060	-	-	-
Jasmonic acid	-	0.439858	-	-
Cystein	-	-	0.486339	-
Leaf gloss	-	-	-	0.527853
EGGS	-0.319660	-	-	-

In the result of the multivariate analysis, Eigenvectors equal to or greater than 0.3 were recognized as the logical cut-off points where each selected trait made an imperative contribution to the PC axis. This was based on the genotypes in addition to the knowledge of the characters studied. Applying the restriction that only eigenvectors equal to or greater than 0.3 made a considerable contribution to the PC axis, the variables were grouped into four PCs that together accounted for 79% of the total biochemical and morphological variation among the ten genotypes (Table 5): PC1 (33%), PC 2 (25%), PC 3 (12%) and PC 4 (8%). Mean tiller infestation and the ovipositor i.e., the number of eggs laid had negative loadings on PC 1 whereas Salicylic acid, Terpenoids and Monoterpenoids had a positive loading on the axis. This axis could be termed the Terpenoids component. Coumarin, Jasmonic acid and Ligular hair had positive loadings on PC 2 which may be named the Coumarin component. Phenol and cystein were loaded on PC 3 which could be regarded as the Phenol component. Tannin and leaf glossiness were assigned the heaviest load on PC 4. Tannin had negative weight on PC4. The first two PC axes which together accounted for 58% of the multivariate variation among the ten rice genotypes, were the most important. The traits loaded on the two axes were used to group the ten rice genotypes into closely related clusters. Just as some morphological traits had significant and direct correlation with percentage mean tiller infestation (Table 6); some secondary metabolites have direct correlation with the percentage mean tiller infestation in some rice genotypes while others established a link for direct relationship. Total phenol was negatively correlated with the percentage mean tiller infestation (Table 7). And it was identified as a mechanism of resistance in highly resistant TOG 7106, TOG 7442 and partially susceptible NERICAL-19 genotypes. The *O. glaberrima* genotypes are resistant to AfRGM and they all have phenol as the antibiotic mechanism of resistance to AfRGM. Phenol is always linked with the defense of plants against pests. Therefore, phenol is an integral feature as antibiotic mechanism of resistance to AfRGM. Besides phenol, monoterpenoids and terpenoids had significant negative correlation with the percentage mean tiller infestation. Terpenoids and monoterpenoids were identified as antibiotic mechanism of resistance to AfRGM in TOS 14519 and TOG 7106. Terpenoids always offer a long-term defense against insect pests. Although, the percentage mean tiller infestation was negatively correlated with sesquiterpenoid, it was not significant. However, it was found to be associated with mechanism of resistance in TOS 14519 and TOG 7106. Salicylic acid was negatively correlated with percentage tiller infestation and it was identified as antibiotic

Table 6: Correlation matrix between tiller infestation and some antixenotics of rice genotypes

Parameter	Tiller infestation	Internode elongation	Leaf length	Leaf breadth	Leaf gloss	Leaf wetness	Seedling vigor	EGG No.	Ligule	Trichome density	Leaf sheath
Tiller infestation	1										
Internode elongation	-0.454*	1									
Leaf length	-0.629**	0.402*	1								
Leaf breadth	-0.024 ^{ns}	0.108 ^{ns}	-0.104 ^{ns}	1							
Leaf gloss	0.548*	-0.134 ^{ns}	-0.523**	0.413*	1						
Leaf wetness	0.326 ^{ns}	0.010 ^{ns}	-0.273 ^{ns}	0.271 ^{ns}	0.549**	1					
Seedling vigor	-0.278 ^{ns}	0.005 ^{ns}	0.492**	0.089 ^{ns}	-0.251 ^{ns}	-0.112 ^{ns}	1				
EGG No.	0.486**	-0.063 ^{ns}	-0.448 ^{ns}	0.432*	0.883**	0.876***	-0.197 ^{ns}	1			
Ligule	0.146 ^{ns}	-0.559**	-0.248 ^{ns}	-0.277 ^{ns}	-0.066 ^{ns}	-0.105 ^{ns}	-0.288 ^{ns}	-0.109 ^{ns}	1		
Trichome density	0.162 ^{ns}	-0.282 ^{ns}	-0.360 ^{ns}	-0.142 ^{ns}	0.316 ^{ns}	0.214 ^{ns}	-0.117 ^{ns}	0.287 ^{ns}	0.109 ^{ns}	1	
Leaf sheath	-0.659***	0.614***	0.679***	-0.374*	-0.667***	-0.428 ^{ns}	0.245 ^{ns}	-0.628***	-0.294 ^{ns}	-0.234 ^{ns}	1

Correlation coefficient with ns: Not significant. ****Significant at p = 0.05, 0.01, 0.001, respectively

Table 7: Correlation matrix between tiller infestation and some antibiotics in rice genotypes

Parameter	Tiller infestation	Tannin	Phenol	Flavonoid	Silica	Monoterpenoids	Terpens	Salicylic	Coumarin	Quinone	Total sugar	Reducing sugar
Tiller infestation	1											
Tannin	0.059 ^{ns}	1										
Phenol	-0.112 ^{ns}	-0.171 ^{ns}	1									
Flavonoids	0.171 ^{ns}	0.039 ^{ns}	0.038 ^{ns}	1								
Silica	-0.131 ^{ns}	-0.029 ^{ns}	-0.056 ^{ns}	-0.149 ^{ns}	1							
Monoterpenoids	-0.482*	0.028 ^{ns}	-0.028 ^{ns}	-0.034 ^{ns}	-0.299 ^{ns}	1						
Terpens	-0.493*	0.081 ^{ns}	-0.029 ^{ns}	-0.035 ^{ns}	-0.302 ^{ns}	0.995***	1					
Salicylic	-0.058 ^{ns}	0.183 ^{ns}	-0.284 ^{ns}	0.079 ^{ns}	0.494**	-0.237 ^{ns}	-0.245 ^{ns}	1				
Coumarin	0.031 ^{ns}	0.172 ^{ns}	-0.291 ^{ns}	-0.015 ^{ns}	0.545**	-0.251 ^{ns}	-0.248 ^{ns}	0.924***	1			
Quinone	0.268 ^{ns}	0.455*	-0.243 ^{ns}	0.176 ^{ns}	0.162 ^{ns}	0.109 ^{ns}	0.113 ^{ns}	0.225 ^{ns}	0.3056 ^{ns}	1		
Total sugar	-0.055 ^{ns}	0.206 ^{ns}	-0.009 ^{ns}	0.204 ^{ns}	-0.448*	-0.028 ^{ns}	-0.027 ^{ns}	0.053 ^{ns}	-0.081 ^{ns}	-0.316 ^{ns}	1	
Reducing sugar	-0.187 ^{ns}	-0.062 ^{ns}	-0.016 ^{ns}	0.186 ^{ns}	-0.048 ^{ns}	-0.049 ^{ns}	-0.045 ^{ns}	-0.093 ^{ns}	-0.014 ^{ns}	0.043 ^{ns}	-0.041 ^{ns}	1

Correlation coefficient with ns: Not significant. ****Significant at p = 0.05, 0.01, 0.001, respectively

sources of resistance to AfRGM in TOS 14519 and TOG 7106. Silica content was negatively correlated with the percentage tiller infestation and it was identified as one of the antixenotic mechanisms of resistance in TOG 7206. The silica content deters the pest feeding tendency on plant. Flavonoid was positively correlated with the percentage mean tiller infestation and it was identified as mechanism of resistance in CISADANE. Although Coumarin, Quinone, tannin and Jasmonic acid were positively correlated with the percentage mean tiller infestation, they had little/no upshot on infestation.

DISCUSSION

Among the ten rice genotypes evaluated, the three *O. glaberrima* are either resistant or highly resistant to AfRGM infestation; all the *O. sativa* except TOS 14519 are either susceptible or moderately susceptible to AfRGM infestation; while all the interpecific progenies are either susceptible or moderately susceptible to AfRGM infestation. It therefore depicts that *O. glaberrima* possess inherent ability for resistance to this pest and possibly other biotic stress. This is in conformity with the finding of Nwilene *et al.* (2002) that the glaberrima parent of the NERICAs (CG 14) and many other accessions of *O. glaberrima*, have been found resistant to AfRGM but none of the NERICAL varieties has been identified as resistant (Nwilene *et al.*, 2008). TOS 14519 and the accessions of *O. glaberrima* are resistant because of the inherent ability they possess which could either be morphological or biochemical traits.

The morphological characteristics of the plant are key component of host-plant resistance to insects (Heinrichs, 1992; Nwilene *et al.*, 2009). This attribute aids plant to shun any damage from insect pest. Although trichome density was not associated with the mechanism of resistance in this study, the study however identified long leaf length and leaf surface wetness as antixenotic traits which are significantly associated with the mechanism of resistance to AfRGM. This is in conformity with the findings of Nwilene *et al.* (2009) who reported leaf wetness as mechanism of resistance to gall midge. The rate of survival of hatched eggs and larvae on long leaves is very low. The eggs/larvae could have sway-off the leaf or died before getting to the leaf sheath thus reducing the chance of the insect infestation on the plant. This depicts that the long leaf length tends to reduce the chance eggs/larvae getting to the leaf sheath and boring into the plant tissue. Long leaf length is therefore an important antixenotic property in rice genotypes to AfRGM. This is in agreement with the reports of Nwilene *et al.* (2009) that many morphological features of plants such as leaf hair, surface wax, tissue thickness and allelochemical content are associated with nonpreference of plants for feeding and oviposition by insect herbivores. The result showed that the number of eggs was positively and significantly ($p < 0.05$) correlated with the percentage mean tiller infestation. This depicts high predilection of the adult female gall midge on the susceptible genotypes while the resistant were less preferred for oviposition by the adult female gall midge which was also reported by Nwilene *et al.* (2009) that nonpreference for oviposition by female gall midge was one of the antixenotic components of resistance. Leaf surface wetness was significantly ($p < 0.05$) and positively associated with percentage mean tiller infestation. It was observed that the leaf surface wetness supported the easy movement of the eggs and larvae into the leaf sheath thus causing infestation. It thus implies that it is a vital feature that could aid susceptibility of the rice genotype to AfRGM. Insects are attracted to or repelled by a plant, due to a variety of physio-morphic plant characteristics (Karban *et al.*, 1997; Ernest, 1989; Gogi *et al.* 2010). The physio-morphic characteristics stated by different authors include plant shape, size (Prokopy and Owens, 1983), colour (Prokopy and Owens, 1983; Hirota and Kato, 2001), surface texture (Spencer *et al.*, 1999),

presence of trichomes and wax crystals on the surface, the thickness and toughness of the tissue, tough vascular bundles, length and diameter of the fruits, depth of ribs, flesh-thickness, intensity of ribs and fruit-toughness (Boller and Prokopy, 1976; Dillon *et al.*, 2005). The mechanism of resistance is not limited to the morphological traits alone, the biochemical traits also play vital role. Biochemical traits of varying degree in rice genotypes could be due to genetic make up of the host plant that alters the infestation of insect pest. A broad range of compounds present in plants play significant defensive role against insect pest. In the present study, phenol was negatively correlated with the percentage tiller infestation as it was found as the trait associated with mechanism of resistance to AfRGM. Similar results have been reported by Amudhan *et al.* (1999), who found that phenols or phenolics are attributed to resistance of rice varieties to Asian gall midge *Orseolia oryzae* (Wood-Mason). Phenols have been associated extensively with the chemical defense of plants against microbes, insects and other herbivores (Metraux and Raskin, 1993). Several associations have been reported between phenolics and the resistance of plants to insect damage (Panda and Khush, 1995). Phenols belong to the large class of secondary metabolites produced by plants to defend themselves against pathogens/pest (Panda and Khush, 1995). Phenol contains toxic molecules which interrupt pest/pathogen metabolism. According to Freeman and Beattie (2008), they are produced primarily via the shikimic acid and malonic acid pathways in plants and it includes a wide variety of defense-related compounds. These compounds have the ability to form insoluble complexes with proteins, act as enzyme inhibitors or are oxidized to toxic quinones (Freeman and Beattie, 2008). Terpenoids and Monoterpenoids are observed as the traits of mechanism of resistance to AfRGM in some resistant rice genotypes in the present study. It was equally reported by Jorg (2008) that the range of terpenoid chemicals serves as a multilayered chemical shield in long-lived conifer trees that provides a lasting protection against the much faster evolving insect pests and potential pathogens. Terpenoids, monoterpenoids and Sesquiterpenoids are primary component of essential oil containing alpha- monoterpenoids and beta-pinene which are potent insect toxins and repellents (Freeman and Beattie, 2008). Terpenoid chemicals occur both as constitutive and as massively induced defenses in conifers and it is thought that the successful defense and resistance of conifers against most herbivores and pathogens can be explained partly by the formation of a diverse array of monoterpenoid, sesquiterpenoid and diterpene resin acid defense chemicals (Jorg, 2008). These compounds accumulate in large amounts in form of preformed or induced oleoresin mixtures (Freeman and Beattie, 2008). Salicylic Acid (SA) was found to be associated with mechanism of resistance to AfRGM in some resistant rice genotypes. Although there was no report of Salicylic acid as mechanism of resistance to AfRGM but Ollerstam and Larsson (2003) reported the involvement of Salicylic acid as it mediates resistance in Willow against gall midge *Dasineuras marginemtorquens* which includes some of the key endogenous chemical mediators of plant Defense Signal Transduction (DST).

In conclusion, the antibiotics traits (e.g., Phenol, Terpenoids, Salicylic acids and Monotepernoid) and antixenotic traits (e.g., leaf length and leaf sheath) identified as mechanisms of resistance to AfRGM in some resistant rice genotypes could really assist in breeding for resistant rice varieties. This study suggests that sources of resistance are not stereotyped to cut across board for all the genotypes. Such sources of resistance are yet to be identified. This study also suggests that each antibiotic mechanism identified in each genotype is not the sole source of resistance conferred on such genotypes. There could be other compounds that work in synergy with the sources identified just like the highly resistant TOG 7106 had high Phenol, Terpenoid and Salicylic acid which probably combine with other traits to enhance its resistance to AfRGM. Although the result showed

that some compounds that do not have significant negative correlation with percentage mean tiller infestation, it could be that the synergistic effect of these secondary metabolites had a significant effect in reducing the percentage tiller infestation when combine with or without morphological traits. However, there is need to increase the genotype base to have wider scope of the mechanism for resistance and probably evaluate them under field condition and to determine other antibiotic and morphological traits associated with AfRGM resistance in rice genotypes that could cut across board with all genotypes if any. This present study will help in breeding for resistant rice varieties to AfRGM by introgression of the gene containing the loci of each trait for resistance in rice genotype to greatly improve the resistance of rice genotypes to AfRGM.

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