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Screening Rice Accessions Cultivated in Abakaliki Nigeria for Presence of Rice Gall Midge Resistance Genes using Gene Specific SSR Marker

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ABSTRACT

Rice cultivationis the main economic activity inAbakaliki, Ebonyi State, Nigeria, but production level is fastdeclining dueto insect pest attacks. Most allegedpest in the area is gall midge. Amongthe recognized management approaches, host plant resistance is assumedmost effective. Rice gall midgeresistance genes, *gm2* and *gm3*, have been shown to be tightly linked toSSR marker *gm3*del3. In this study, 38 rice accessions from Abakaliki were screened with the *gm3*del3marker for presence of the gall midge resistance genes *gm2* and *gm3*.Resistant allele size for *gm3* is 250 bp while that of *gm2*is 270 bp.The rice seeds were collected from farmers and Agribusiness Company of Ebonyi State University and germinated in pot soil for DNA isolation. DNA was isolatedfrom 14 days old leaves of rice seedlings using CTAB protocoland amplified with SSR primer specific forthe gall midge resistance genes.The PCR amplified clear bandsin 34 of the 38 rice accessions, butall allele sizes were of susceptible type [550 bp] suggestingabsence of the targetgall midge resistance genesin all the rice accessions assessed. This may explain the severity ofrice gall midge attacksin the area.

Keywords: Rice, African rice gall midge, gall midge resistance genes, gm3del3 marker, Abakaliki.

INTRODUCTION

Rice is the most important economic crop in the Abakaliki area of Ebonyi State of Nigeria being the highest source of food, employment and income to the rural populace in the area. Nigeria is recognized as the largest producer of rice in Africa [1], but the country has not been able to produce quantity sufficient to Abakaliki is a major rice producing area in Nigeria but in the area, yield and general production level is severely restricted by the damaging effect of pests and diseases, a situation that has only been recognized but not yet addressed. The most suspected pest in the area is African rice gall midge (*Orseolia oryzivora* Harris and Gagne), but symptoms of others major rice diseases like bacterial leaf light and blast are also observed [5]. African rice gall midge has earlier been identified as a devastating insect pest of rain-fed lowland rice in Africa [6]. Of the management options known for controlling rice pests, host plant resistance is considered the most effective and environmentally-friendly meet the local demand and relies on importation to satisfy the internal demand [2]; [3]. One of the critical production constraints is insect pest infestation [4]. [5] stated that rice plant is vulnerable to numerous insect pest species which attack various parts of the plant and at different stages of growth.

[7]. Host plant resistance refers to the intrinsic capacity of a crop plant to repel attack by an insect pest, and it is a function of specific genetic components (resistant genes) of the plant. Therefore, developing a rice breeding programme for incorporating genes for resistance to the pests in already well-adapted rice cultivars in the area is imperative, but identifying the sources of such candidate genes and finding their locations in the genome is a pre-requisite.

However, molecular marker techniques with capacity to accelerate mapping of specific genes in the genome have been developed. Molecular markers are inheritable and detectable DNA sequences at specific locations of the genome that

can consequently be used to identify specific genes closely situated to it in the genome [8]; [9]; [10]. Several candidate genes have been mapped by this approach [11]; [12]; [13]. The gene (*gm3*) for resistance to rice gall midge has been mapped on chromosome 4 between the sequence delimited by SSR markers RM17480 and *gm3*SSR4 [14] while *Gm2* is mapped on chromosome 4 between the SSR markers RM17473 and RM17503 [15]. The SSR marker *gm3*del3 has also been shown to completely co-segregate with the same gall midge resistance gene *gm3* in a mapping population of three hundred F10 rice inbred lines [16]; [17]. This marker (*gm3*del3) has been shown to exhibit high fidelity in detecting the rice gall midge resistance gene *gm3* and is being used in marker-assisted breeding for introgression of the gene in rice [18].

In this paper, the gene specific marker *gm3*del3 was used to screen a total of 38 rice accessions from Abakaliki area of Ebonyi State in Nigeria for presence of the rice gall midge resistance gene *gm2* and *gm3.*

MATERIALS AND METHODS

Rice samples and collection

Seeds of a total of 38 rice accessions commonly grown in Abakaliki area of Ebonyi State of Nigeria were collected from farmers in different localities of the area and germinated in pot soil in the screen house for DNA extraction. The list and names (as the people call them) of the rice accessions are shown in Table 1.

DNA Extraction

Genomic DNA was extracted from fresh leaves of two weeks old rice seedlings using the CTAB method [19]. A 150 mg fresh leaves was homogenized in small laboratory mortars each containing 500 µl of CTAB buffer. The homogenates were transferred into sterile 1.5 ml microcentrifuge tubes and mixed by brief vortexing. The tubes were incubated at 57°C for 30 min in a water bath. Thereafter, 400 µl of chloroform, phenol and isoamyl in a ratio of 25:24:1 was added and mixed. The tubes were then centrifuged at 14000 x g for 10 minutes after which the supernatants were gently pipetted into clean 1.5 ml tubes. Next, 400 µl of cold isopropanol was added and centrifuged at 10,000 x g for 10 minutes. The supernatant was decanted and the DNA pellet was washed with 70 % ethanol. The obtained DNA pellets were air-dried for about 1 hr. in a laminar flow hood with the tube tilted upside down to remove the ethanol and then re-suspended in DEPC-treated water. The purity and concentration of isolated genomic DNA were estimated by electrophoresis on 1.0% agarose gel and by spetrophotometric measurement respectively. The purified DNA was stored at 4°C for later use for PCR amplification.

Table 1: List of Cultivated Abakaliki Rice Accessions used for the Study

S/N	Accession Name	S/N	Accession Name
1	R8 Izzi	20	Mama Egodi
2	R 54	21	R 27
3	Izzi Mass	22	Awilo Izzi
4	Mirimiri	23	L-34
5	Surugede	24	FARO 47
6	R8	25	Kpuru-kpuru
7	Chinyere	26	Government
8	CP	27	Volume 47
9	Yori yori	28	Atom 47a
10	China	29	R59
11	God Offia	30	FARO 5 months
12	Odumalandi	31	FARO 60
13	Elechi	32	306
14	FARO 15	33	FARO 49
15	Nwangbenya	34	Volume 57
16	Foreign rice Izzi	35	Atom 47b
17	Abiya Izzi	36	FARO 52
18	Iron	37	Upland Izzi
19	Original CP	38	Nwagayi

PCR Analysis and Gel Electrophoresis

The PCR mixture comprised of 2.5 µl of 10x Taq buffer, 2.0 µl of 2.5 mM dNTP mix, 1.0 µl of each of the forward and reverse primers, 1.25 µl of 50 mM of $MgCl_2$, 100 ng of genomic DNA, 0.2 µl of 500 units Taq DNA polymerase and made up to 25.0 µl volume with DEPC-treated water (Invitrogen Corporation, USA). All other reagents were Bioline products. The PCR cycling profile consisted of an initialdenaturation at 94°C for 5 min., followed by 35 cycles of 94°C for 30 s, 72°C for 1min, and a final extension at 72°C for 10 min. Six microliters (6.0 µl) of the PCR products were mixed with 2.0 µl 6x cyber green loading dye and resolved in a 1.5 % agarose gel containing 0.5 mg/ml ethidium bromide and photographed with a gel documentation system (Fotodyne Incorporated, USA).The sequences of the gene specific SSR primer pair (*gm3*del3) used in the study were obtained from [20]: forward (5′-CTGCCAGAGATGGGCCTTCCA-3′) and reverse (5′-CGTACAAATTCCTGTACCACTC-3′).

RESULTS

The marker amplified single and clear bands in the DNA of thirty-four (34) out of the thirty-eight (38) rice accessions screened. The sizes of amplicons are very close among the rice DNAs, ranging between 500 – 600 bp. Eight (8) of the rice accessions (numbers 1, 3, 4, 6, 7, 8, 9 and 10) yielded 550 bpamplicons, six (6) accession (numbers 5, 12, 15, 16, 17 and 18) yielded 600 bp, while twenty (20) accessions (numbers 19 to 38) produced 500 bp DNA fragments(Fig. 1).

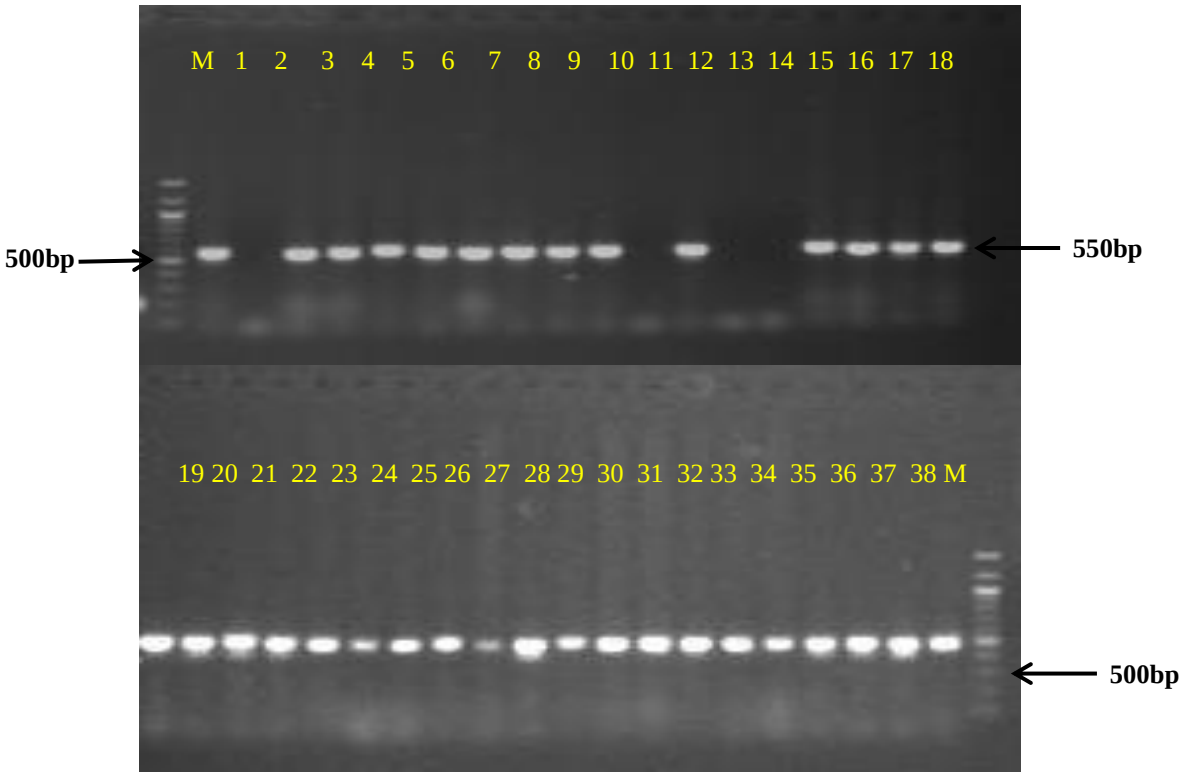

Figure 1: Amplification Profile of 38 Abakaliki Rice Accessions with gm3del3 Marker for presence of Rice Gall Midge Resistance Genes *Gm2* and *gm3* genes. M - 100bp Ladder, 1 – 38 are different rice accessions.

DISCUSSION

Host plant resistance is considered the most effective and environmentally-friendly management approach to insect pest invasion of crop plants [4]. This underscores the importance of techniques for incorporating genes for pest resistance into crop plants. [8] confirmed that gm3del3 marker amplifies

250bp in rice genotypes having the rice gall midge resistant gene *gm3*, 270bp for resistant gene *Gm2* and 550bp in susceptible genotypes. In this study the marker amplified fragment sizes between 500 – 600 bp in thirty-four (34) out of the thirty-eight (38) Abakaliki rice accessions screened. This suggests that none of these rice accessions may have intrinsic capacity to withstand the damaging effect of rice gall midge pest, unless it possesses any of the other gall midge resistance genes such as *Gm1, Gm4, Gm5, Gm6, Gm7, Gm8 and Gm11* [15]; [16].

The rice accessions evaluated in this study are most frequently cultivated by farmers in Abakaliki and the finding here may explain in part the reason for the frequent ravaging rice gall midge pest attack in the area which sometimes leads to total yield failure. In our survey study on "farmers' perception of pest and disease incidence in the area" (Afiukwa et al., unpublished), only a few of the farmers agreed to the knowledge of a rice cultivar (FARO 44) that seem to be able to escape pest invasion. Even the local farmers are aware that this rice cultivar is early maturing and may be able to complete its life-cycles before onset of pest attacks, if planted early. The result of the present study supported by the unpublished survey study revealed the crucial need for breeding efforts to develop gall midge resistance rice cultivars on the background of cultivated accessions in the area.

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