



Research article

Assessment of Korean rice lines for their reaction to rice yellow mottle virus in Ghana



Maxwell Darko Asante^{a,*}, Braima Amadu^a, Valentin Stanislas Edgar Traore^b, Allen Oppong^a, Moses Adeolu Adebayo^c, Phyllis Aculey^a, Esther Agyemang Marfo^a, Kyung-Ho Kang^{d,e}

^a Council for Scientific and Industrial Research–Crops Research Institute (CSRI-CRI), P.O. Box 3785, Fumesua-Kumasi, Ghana

^b INERA Institut de l'Environnement et de Recherches Agricoles, 04 BP 7192 Ouagadougou 04, Burkina Faso

^c Seed Co Nigeria, No. 3, Ribadu Road, Unguwan Rimi, Kaduna, Kaduna State, Nigeria

^d National Institute of Crop Science, RDA, South Korea

^e AfricaRice, St Louis, Senegal

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ABSTRACT

Rice yellow mottle virus (RYMV) is the most damaging viral disease of rice in Africa and can cause yield losses of up to 100%. The objective of this study was to characterize newly introduced rice lines from Korea into Ghana for their reaction to RYMV infection. One hundred and seventy-two rice lines from Korea were screened for their level of resistance RYMV in a screen house at Fumesua, Ghana. Four checks consisting of two highly resistant lines (Tog7291 and Gigante with *rymv1-2* (resistant gene1-allele2) and *rymv2* (resistant gene2) respectively), a moderately resistant line (CRI-Amankwatia) and a susceptible cultivar Jasmine 85 were used. The experiment was carried out in a 4 x 44 lattice design with four replicates. Screening for RYMV resistance was conducted by visual symptom scoring and virus-assessment through serology using enzyme linked immunosorbent assay (ELISA) test. Disease incidence and severity were assessed from 2 to 42 dpi. Data for disease severity and incidence were transformed (Log x+1) for ANOVA.

Five lines (8261112, 8261119, 8261133, 8261588, and 8261634) were identified to be highly resistant to the disease just like Tog7291 and Gigante. The study also revealed 24 lines that were resistant but not grouping with Tog7291 and Gigante, whereas 100 moderately resistant lines clustered with the moderately resistance check CRI-Amankwatia in a distinct group. Forty-three (43) susceptible lines were identified with the susceptible check Jasmine 85 falling in this group. No highly susceptible line was identified. The newly identified resistant genotypes can be used by breeders to develop RYMV resistant varieties.

1. Introduction

Rice is one of the most important food crops in the world. It is mostly consumed directly as cooked meal but it is also processed into various industrial products (rice cakes, rice bran oil, and wine) and the straw for livestock feed and mushroom production. Rice is increasingly becoming a regular staple for the populations in sub-Saharan Africa (Séré et al., 2013). It is the second largest cereal consumed in Ghana coming after maize, and the fastest growing food source (USDA 2018). This has been attributed to increasing urbanization and the ease with which rice is prepared compared to other traditional meals.

The 2007–2008 crisis on rice availability and price prompted African countries to develop initiatives to increase their domestic production by

increasing the area under rice cultivation and/or the productivity per unit area through the use of high-yielding varieties and fertilizers. However, the rice industry faces major challenges, with diseases featuring prominently (Kouassi et al., 2005; Séré et al., 2013). Of all the rice diseases, Rice yellow mottle virus (RYMV), discovered in Kenya by Bakka in 1966, is the most damaging in Africa (Okioma and Sarkarung 1983; Kouassi et al., 2005; Traore et al. 2006, 2015; Traoré et al., 2009). RYMV disease has been reported in all the major rice producing zones of sub-Saharan Africa (East, West, Central and Southern Africa, and Madagascar) and was first reported in Ghana in 1980 (Kouassi et al., 2005).

The disease incidence and accompanying yield losses due to RYMV could be high depending on the rice genotype grown, the date of

* Corresponding author.

E-mail address: mdasante@gmail.com (M.D. Asante).

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Table 1. Pedigree of rice lines tested against RYMV.

S/No	Genotype	Cross	Source
1	8210002	JH15-1-1-1/SR34598	Korea
2	8210004	JH15-1-1-1/SR34598	Korea
3	8210007	Milyang23*4/O.glaberrima	Korea
4	8210009	M23//(Milyang23*4/O.glaberrima)-133/Japonica 1/HB4052	Korea
5	8210011	SR34040/SAGC-06,china	Korea
6	8210012	SR34040/SAGC-06,china	Korea
7	8210013	JH15-1-1-1/SR34598	Korea
8	8210016	JH15-1-1-1/SR34598	Korea
9	8210021	JH15-1-1-1/SR34598	Korea
10	8210023	Yang/V046	Korea
11	8210024	(M23*4/O.glaberrima)-128/KhaoHaLon	Korea
12	8210026	M23//(Milyang23*4/O.glaberrima)-133/Japonica 1/HB4052	Korea
13	8210027	Hwaseong/IR84421-4-47-B-1-3	Korea
14	8210039	JH15-1-1-1/SR34598	Korea
15	8210044	Yang/V046	Korea
16	8210045	JH15-1-1-1/SR34598	Korea
17	8210050	JH15-1-1-1/SR34598	Korea
18	8210052	M23//(Milyang23*4/O.glaberrima)-133/Japonica 1/HB4052	Korea
19	8210055	Yang/V046	Korea
20	8210065	Milyang23*4/O.glaberrima	Korea
21	8210066	Yang/V046	Korea
22	8210058	JH15-1-1-1/SR34598	Korea
23	8210079	Sahel 134	Senegal
24	8210084	JH15-1-1-1/SR34598	Korea
25	8210087	Sahel 210	Senegal
26	8220001	Milyang23*4/O.glaberrima	Korea
27	8220004	M23//(Milyang23*4/O.glaberrima)-133/Japonica 1/HB4052	Korea
28	8220005	Japonica1/Mogyang	Korea
29	8220006	JH15-1-1-1/SR34598	Korea
30	8220007	JH15-1-1-1/SR34598	Korea
31	8220009	JH15-1-1-1/SR34598	Korea
32	8220012	JH15-1-1-1/SR34598	Korea
33	8220013	Yang/V046	Korea
34	8220014	(Hwaseong/049556)	Korea
35	8220021	(Hwaseong/049556)	Korea
36	8220022	Samgwang/SR31986	Korea
37	8220023	Ungwang/Dongnong429	Korea
38	8220033	(Milyang23*4/O.glaberrima)-128/KhaoHaLon//Mogyang	Korea
39	8220042	TY 14	Korea
40	8220043	TY 34	Korea
41	8220044	HB4052/Ungwang	Korea
42	8220045	PBR1000922	Korea
43	8220055	SR34042-HB3368-224//Suweon559/SR34589	Korea
44	8220056	SR34042-HB3368-224//Suweon559/SR34589	Korea
45	8220057	SR34042-HB3368-224//Suweon559/SR34589	Korea
46	8220058	SR34042-HB3368-224//Suweon559/SR34589	Korea
47	8220060	SR34042-HB3368-224//Suweon559/SR34589	Korea
48	8220061	Japonica 1/HB4052	Korea
49	8220062	Japonica 1/HB4052	Korea
50	8220063	Japonica 1/HB4052	Korea
51	8220066	(Milyang23*4/O.glaberrima)-128/KhaoHaLon//Mogyang	Korea
52	8220073	(Hwaseong/049556)F2	Korea
53	8220086	SR33705/Milyang23	Korea
54	8220095	HB4057/Japonica 1	Korea
55	8230006	SR34796-1-32-8/SR32037-1-106	Korea
56	8230008	SR34796-1-40-8/SR34053(#3-47)-8-28-1	Korea
57	8230009	Nunkeunheugchal/SR32037-1-114	Korea
58	8230012	Dasan1//MS11/Hanmaeum	Korea

(continued on next page)

Table 1 (continued)

S/No	Genotype	Cross	Source
59	8230015	Dasan1//MS11/Hanmaeum	Korea
60	8230016	Dasan1//MS11/Hanmaeum	Korea
61	8230023	HB4055/Japonica1//MS11//M23/(Milyang23*4/O.glaberrima)-133//HB4055/MS11	Korea
62	8230024	HB4055/Japonica1//MS11//Suweon559//V046/Milyang23	Korea
63	8230028	HB4055/Japonica1//MS11//Suweon559//V046/Milyang23	Korea
64	8230029	HB4055/Japonica1//MS11//Suweon559//V046/Milyang23	Korea
65	8230030	HB4055/Japonica1//MS11//Suweon559//V046/Milyang23	Korea
66	8230033	HB4055/Japonica1//MS11//Suweon559//V046/Milyang23	Korea
67	8230034	HB4055/Japonica1//MS11//Suweon559//V046/Milyang23	Korea
68	8230035	HB4055/Japonica1//MS11//Suweon559//V046/Milyang23	Korea
69	8230037	HB4055/Japonica1//MS11//Suweon559//V046/Milyang23	Korea
70	8230045	HB4055/Japonica1//MS11//M23*4/O.glaberrima)-128/KhaoHaLon	Korea
71	8230061	Jinmi/V046/unknown	Korea
72	8230080	Sahel 134	Senegal
73	8230100	Sahel 210	Senegal
74	8230102	Nerica8_AC-23	Korea
75	8230103	Nerica8_AC-32	Korea
76	8230104	Nerica8_AC-39	Korea
77	8260012	SR34590/SR34071(#5-16)-5-1	Korea
78	8260014	SR34590/SR34071(#5-16)-5-1	Korea
79	8260019	SR34590/SR34071(#5-16)-5-1	Korea
80	8260029	SR34590/SR34071(#5-16)-5-1	Korea
81	8260031	SR34590/SR34071(#5-16)-5-1	Korea
82	8260034	SR34590/SR34071(#5-16)-5-1	Korea
83	8260050	SR34590/SR34071(#5-16)-5-1	Korea
84	8260086	SR34590/SR34071(#5-16)-5-1	Korea
85	8260781	Milyang295/SR32037-1-139	Korea
86	8260782	Milyang295/SR32037-1-139	Korea
87	8260817	Milyang296/SR32037-1-114	Korea
88	8261024	SR34071(#5-16)-5-1-2/Milyang23	Korea
89	8261203	SR32037-1-110/SR34590	Korea
90	8261401	Milyang288/SR32037-1-147	Korea
91	8260159	SR32037-1-110/SR34590	Korea
92	8261487	Milyang296/SR32037-1-114	Korea
93	8260179	SR32037-1-110/SR34590	Korea
94	8260195	SR32037-1-110/SR34590	Korea
95	8260198	SR32037-1-110/SR34590	Korea
96	8260230	SR32037-1-110/SR34590	Korea
97	8260325	SR34590/Nunkeunheugchal	Korea
98	8260326	SR34590/Nunkeunheugchal	Korea
99	8260332	SR34590/Nunkeunheugchal	Korea
100	8260347	SR34590/Nunkeunheugchal	Korea
101	8260355	SR34590/Nunkeunheugchal	Korea
102	8260385	Milyang285/SR32037-1-106	Korea
103	8260392	Milyang285/SR32037-1-106	Korea
104	8260418	Milyang295/SR32037-1-106	Korea
105	8260461	Milyang296/SR32037-1-106	Korea
106	8260505	Milyang286/SR32037-1-147	Korea
107	8260543	Milyang286/SR32037-1-147	Korea
108	8260570	Milyang286/SR32037-1-147	Korea
109	8260581	Milyang286/SR32037-1-147	Korea
110	8260585	Milyang286/SR32037-1-147	Korea
111	8260586	Milyang286/SR32037-1-147	Korea
112	8260588	Milyang286/SR32037-1-147	Korea
113	8260592	Milyang286/SR32037-1-147	Korea
114	8260599	Milyang286/SR32037-1-147	Korea
115	8260607	Milyang286/SR32037-1-147	Korea
116	8260612	Milyang288/SR32037-1-147	Korea
117	8260641	Milyang288/SR32037-1-147	Korea

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Table 1 (continued)

S/No	Genotype	Cross	Source
118	8260681	Milyang288/SR32037-1-147	Korea
119	8260701	Milyang288/SR32037-1-147	Korea
120	8260715	Milyang288/SR32037-1-147	Korea
121	8260753	Milyang288/SR32037-1-147	Korea
122	8260754	Milyang288/SR32037-1-147	Korea
123	8260783	Milyang295/SR32037-1-139	Korea
124	8260811	Milyang296/SR32037-1-114	Korea
125	8260844	Milyang296/SR32037-1-114	Korea
126	8260770	Milyang295/SR32037-1-139	Korea
127	8260903	Milyang296/SR32037-1-114	Korea
128	8260906	Milyang296/SR32037-1-114	Korea
129	8260956	Milyang296/SR32037-1-114	Korea
130	8260979	Milyang296/SR32037-1-114	Korea
131	8260987	SR34071(#5-16)-5-1-2/Milyang23	Korea
132	8261007	SR34071(#5-16)-5-1-2/Milyang23	Korea
133	8261023	SR34071(#5-16)-5-1-2/super	Korea
134	8261042	SR34071(#5-16)-5-1-2/super	Korea
135	8261061	SR34071(#5-16)-5-1-2/super	Korea
136	8261046	SR34071(#5-16)-5-1-2/super	Korea
137	8261048	SR34071(#5-16)-5-1-2/super	Korea
138	8261069	SR34071(#5-16)-5-1-2/super	Korea
139	8261099	SR34071(#5-16)-5-1-2/super	Korea
140	8261078	SR34071(#5-16)-5-1-2/super	Korea
141	8261081	SR34071(#5-16)-5-1-2/super	Korea
142	8261085	SR34071(#5-16)-5-1-2/super	Korea
143	8261087	SR34071(#5-16)-5-1-2/super	Korea
144	8261096	SR34071(#5-16)-5-1-2/super	Korea
145	8261107	SR34071(#5-16)-5-1-2/NERICA 8	Korea
146	8261109	SR34071(#5-16)-5-1-2/NERICA 8	Korea
147	8261112	SR34071(#5-16)-5-1-2/NERICA 8	Korea
148	8261119	SR34071(#5-16)-5-1-2/NERICA 8	Korea
149	8261126	SR34071(#5-16)-5-1-2/NERICA 8	Korea
150	8261133	SR34071(#5-16)-5-1-2/NERICA 8	Korea
151	8261135	SR34071(#5-16)-5-1-2/NERICA 8	Korea
152	8261161	SR34071(#5-16)-5-1-2/NERICA 8	Korea
153	8261186	SR34796-1-5-4/SR32037-1-147	Korea
154	8261211	SR32037-1-110/SR34590	Korea
155	8261251	SR32037-1-110/SR34590	Korea
156	8261255	SR32037-1-110/SR34590	Korea
157	8261288	Milyang286/SR32037-1-147	Korea
158	8261293	Milyang286/SR32037-1-147	Korea
159	8261310	Milyang286/SR32037-1-147	Korea
160	8261341	Milyang286/SR32037-1-147	Korea
161	8261372	Milyang286/SR32037-1-147	Korea
162	8261390	Milyang286/SR32037-1-147	Korea
163	8261398	Milyang286/SR32037-1-147	Korea
164	8261475	Milyang295/SR32037-1-139	Korea
165	8261486	Milyang296/SR32037-1-114	Korea
166	8261492	Milyang296/SR32037-1-114	Korea
167	8261496	Milyang296/SR32037-1-114	Korea
168	8261405	Milyang286/SR32037-1-147	Korea
169	8261551	Milyang296/SR32037-1-114	Korea
170	8261588	SR34071(#5-16)-5-1-2/PBR1000653	Korea
171	8261634	SR34071(#5-16)-5-1-2/PBR1000653	Korea
172	8261620	Japonica 1	Korea
173	Jasmine 85	Susceptible check	Ghana
174	CRI-Amankwatia	Moderately resistant check	Ghana
175	Gigante	Resistant check	Mozambique
176	Tog7291	Resistant check	Ghana



Figure 1. Scoring scale for RYMV disease severity. where 1 = no symptoms, 3 = leaves green but with sparse dots or streaks and less than 5% of height reduction, 5 = leaves green or pale green with mottling and 6–25% of height reduction, flowering slightly delayed, 7 = leaves pale yellow or yellow and 26–75% of height reduction, flowering delayed, and 9 = leaves turning yellow or yellow orange, more than 75% of height reduction, no flowering or some dead plants.

infection and the viral strain (Bakker 1970). A survey conducted by Hubert et al. (2016) in Tanzania demonstrated that the farmers experienced 100% loss due to attack by RYMV in spite of heavy investment on control measures. It was also observed that yield losses to RYMV are strongly influenced by host cultivars as well as time of virus infection (Onwughalu et al., 2010).

Rice plants infected by RYMV show pale yellow mottling on their leaves, stunted growth, fewer tillers, asynchronous flower formation, poor panicle exertion, spikelet discoloration, and brown to dark-brown discoloration of grains (Séré et al., 2013; Sereme et al., 2016). RYMV reduces grain yield by 10–100% and severe attacks can lead to plant death (Kouassi et al., 2005). RYMV disease is transmitted when the sap of infected leaves comes into contact with the cells of healthy leaves (Séré et al., 2013). It is transmitted by livestock as they graze from field to field (Ochola and Tusiime 2011), by insects (Koudamilo et al. 2015, 2019) and by wind (Sarra et al., 2004). Contaminated scissors with small blades can transmit the virus up to 12 seedlings during continuous cutting (Uke et al., 2014). The intensive usage of irrigation in rice cultivation is implicated to have enhanced growth and continuity of a wide range of weeds that act as alternative hosts and reservoirs from which the viral inoculum spreads to healthy rice crops (Okioma and Sarkarung 1983; Fargette et al., 2008; Traoré et al., 2009; Ochola and Tusiime 2011). Among the cropping practices that have facilitated the build-up of the virus inoculum and the perpetuation of RYMV is the incorporation of rice

stubble back into soil during land preparation (Fargette et al., 2008; Ochola and Tusiime 2011).

Several management strategies have been recommended to control the disease but the use of resistant cultivars is the most cost-effective, environment-friendly, and sustainable (Salaudeen 2014). Cultural practices and prophylactic methods are more effective if combined with the use of resistant varieties (Kouassi et al., 2005). Partial and highly resistant genes, used alone or in pyramiding approaches, are of particular interest in breeding new varieties (Kouassi et al., 2005). Most rice accessions are susceptible to RYMV (Sereme et al., 2016), including *O. glaberrima* species in which most sources of resistance to RYMV have been found earlier (Traore et al., 2015). Partially and highly resistant varieties have been identified, but currently the disease is not controlled adequately, and its incidence continues to increase in Africa (Kouassi et al., 2005; Salaudeen 2014). In Nigeria, an increase in the incidence of the disease was observed in addition to increase in severity from 30 to 90 % after one year period (Odedara et al., 2016). No variety is yet commercially available that has both high resistance and other desirable agronomic traits (Kouassi et al., 2005; Suvi et al., 2018). In Ghana, the most popular rice varieties are the Jasmine types but they very susceptible to RYMV disease (Asante et al., 2013; Traore et al., 2015). In view of this, there is a need to identify or develop rice varieties with desirable agronomic traits and with a high level of resistance to the disease. The scarcity of resistance in rice genotypes against RYMV disease calls for

introduction of resistant lines/cultivars from foreign sources and transferring of their resistant genes/alleles into the existing commercial cultivars in Africa. The objective of this study was to characterize the response to RYMV infection among newly introduced rice lines from Korea into Ghana and to identify new sources of resistance for breeding.

2. Materials and methods

2.1. Source of seed, sowing and agronomic practices

One hundred and seventy-two rice lines (Table 1) introduced into Ghana in 2018 by Korea-Africa Food and Agricultural Cooperative Initiative (KAFACI) were screened for their level of resistance to RYMV infection. Before their introduction into Ghana, the lines had been previously multiplied at the AfricaRice station at St Louis, Senegal, thus the germplasm included two Senegalese varieties Sahel 134 and Sahel 210. The experiment was carried at the Council for Scientific and Industrial Research-Crops Research Institute of Ghana (CSIR-CRI), Fumesua, Ghana in 2019. It was done in a screen house to prevent insects, birds and rodents attack. The experimental design was a 4 x 44 lattice with four replicates. Four checks consisting of two highly resistant lines (Gigante and Tog7291 lines with *rymv1-2* (*rymv* resistant gene1-allele2) and *rymv2* (*rymv* resistant gene2) respectively), a moderately resistant line (CRI-Amankwatia) and a susceptible cultivar Jasmine 85 were used (Table 1). Two seeds were sown per hill and later thinned to one seedling/hill. Each entry consisted of one row with three plants at a spacing of 20 cm within row. The field was kept weed free and supplementary irrigation was applied as and when required. The recommended fertilizer rates in Ghana (90 kg N: 60 kg P: 60 kg K per hectare) were applied.

2.2. Virus source and inoculation

The virus inoculum for this study was sourced from a field with high incidence and severity of RYMV infection. The samples were preserved in pots while in transit to ensure the viability and the infectivity of the virus. The virus inoculum was tested on Tog7291 and Gigante for confirmation of the isolate. The virus inoculum was applied to the susceptible check, Jasmine 85. Infected Jasmine 85 plants were subsequently used as the source of inoculum for the test lines. The inoculum was prepared by grinding infected leaf tissue with cold distilled water at the ratio of 1:10 (w/v) using sterile mortars and pestles following the procedure of Thiemele et al. (2010). Carborundum (600 mesh) was added to the extracts to ease penetration of the virus in the plant tissue. At 14 days after planting, when most plants have achieved a 3-leaf stage, inoculation was done by rubbing the leaves of the test plants with fingers moistened with the prepared inoculum. Inoculation was repeated one week later to avoid any escapes.

2.3. Serological assay

Double Antibody Sandwich Enzyme-linked Immunosorbent Assays (DAS-ELISA) was conducted to ensure the reliability of the visual evaluation of symptoms. A polyclonal antibody that reacts strongly and similarly with all the RYMV isolates of West and Central Africa was sourced from DSMZ, Germany, and used as the coating antibody. The same antibody was coupled to alkaline phosphatase and used as conjugate. All buffer used and incubation times were done as previously described by Konate et al. (1997) at the Virology Laboratory of the CSIR-CRI. For detection of RYMV in the samples, analysis was done directly on extracts obtained after grinding the leaves (1 g in 10 ml of buffer) and centrifugation at $8,000 \times g$ for 10 min. Reading of the microplate reader was done at 405nm but a sample is declared infected if microplate reader reads a value that is at least twice the negative control.

2.4. Data collection

Days to symptoms appearance, disease incidence and severity were recorded. Appearance of symptoms and disease progress were assessed daily after the initial inoculation for the first 21 days and subsequently at a week-interval for the next 21 days. A Standard evaluation system (SES) for RYMV symptoms based on the one described by John and Thottapilly (1987) was employed for severity scoring, where 1 = no symptoms, 3 = leaves green but with sparse dots or streaks and less than 5% of height reduction, 5 = leaves green or pale green with mottling and 6–25% of height reduction, flowering slightly delayed, 7 = leaves pale yellow or yellow and 26–75% of height reduction, flowering delayed, and 9 = leaves turning yellow or yellow orange, more than 75% of height reduction, no flowering or some dead plants as shown in Figure 1.

Incidence was calculated using the following formula:

$$I\% = \frac{PA \times 100}{PT} \quad (\text{Eq. 1})$$

where I: disease incidence; PA: number of infected or dead plants (a plant was considered as infected as soon as a visible symptom was observed); PT: total number of plants inoculated.

2.5. Data analysis

The data recorded for each of the varieties were used to detect the resistant, tolerant, or susceptible lines, based on the 1–9 disease rating scale and ELISA results. Disease severity was evaluated using the means of the scores recorded. Average severity ranges of 1–1.5; 1.6–3.5; 3.6–5.5; 5.6–7.5; and 7.6–9 were recorded as 1, 3, 5, 7 and 9, respectively. Hence highly resistant (HR) for score 1, resistant (R) for score 3, moderately resistant (MR) for score 5, susceptible (S) for score 7, and highly susceptible (HS) for score 9 (Sereme et al., 2016). Analysis were performed using PBIB test of R statistical software, version 3.6.1 (R Core Team, 2019). The disease incidence data (calculated using Equation 1) were log transformed to conform to the assumptions of ANOVA. Mean disease incidence on rice genotypes were detected using ANOVA and separated by Tukey test at $P = 0.05$. Genotypes were compared for disease incidence and severity.

3. Results and discussion

This study aimed at identifying sources of resistance to RYMV in 172 Korean lines in breeding programs for resistance to the disease in Africa. Because RYMV epidemic occurs randomly, screening for resistance under field conditions is difficult. Thus, the genotypes were evaluated for reaction to RYMV infection by mechanical inoculation under screen house conditions. The plants were scored for symptoms severity based on visual observations from 2 to 42 dpi.

3.1. Serological assay

DAS-ELISA test was conducted to check the viral concentration and identify asymptomatic plant. The result of the ELISA test was highly correlated to that of the visual scoring ($r = 0.99$) and no asymptomatic plant was identified. About 170 lines representing 98.8% showed symptoms of RYMV disease and only 2 lines (genotypes 8261112 and 8261119) constituting 1.2% did not show any symptom of the disease at all. This confirms the scarcity of resistance to the disease as earlier reported (Longue et al., 2018; Sereme et al., 2016; Traore et al., 2015).

3.2. Days to symptoms appearance

Plants inoculated with RYMV expressed symptoms of the virus at different days after inoculation (Table 2). Typical symptoms of RYMV observed in this study included sparse elongated yellow spots, mottled

Table 2. Results of RYMV disease symptom appearance, disease incidence, disease severity score and ELISA test of Korean rice lines inoculated with RYMV isolate in Ghana.

S/No	Genotype	Days to Symptoms Appearance	No. of Infected plants/Total plants	Mean Incidence	Mean Severity	ELISA	Class
147	8261112	NS	0	0.00d	1.00	-	HR
148	8261119	NS	0	0.00d	1.00	-	HR
175	Gigante(RC)	NS	0	0.00d	1.00	-	HR
176	Tog7291(RC)	NS	0	0.00d	1.00	-	HR
150	8261133	13	5/11	45.46c	1.47	+	HR
170	8261588	19	3/9	33.33bc	1.49	+	HR
171	8261634	18	2/9	22.22c	1.52	+	HR
152	8261161	20	5/11	45.46bc	2.09	+	R
139	8261099	14	5/10	50.00abc	2.16	+	R
149	8261126	14	8/11	72.73a	2.16	+	R
146	8261109	21	7/11	63.64abc	2.17	+	R
60	8230016	14	7/10	70.00a	2.40	+	R
59	8230015	19	7/8	87.50a	2.50	+	R
144	8261096	14	8/11	72.73a	2.54	+	R
14	8210039	9	9/12	75.00a	2.70	+	R
42	8220045	13	7/9	77.78ab	2.96	+	R
58	8230012	13	8/11	72.73a	3.04	+	R
151	8261135	14	10/11	90.91a	3.19	+	R
57	8230009	13	8/8	100.00a	3.25	+	R
172	8261620	13	10/10	100.00a	3.26	+	R
67	8230034	12	11/12	91.67a	3.27	+	R
101	8260355	20	8/9	88.89a	3.32	+	R
71	8230061	11	11/11	100.00a	3.34	+	R
55	8230006	10	9/9	100.00a	3.38	+	R
80	8260029	13	9/9	100.00a	3.38	+	R
25	8210087	12	12/12	100.00a	3.40	+	R
8	8210016	14	11/11	100.00a	3.42	+	R
5	8210011	20	9/10	90.00a	3.48	+	R
4	8210009	14	7/10	70.00a	3.51	+	R
10	8210023	13	11/11	100.00a	3.52	+	R
2	8210004	13	8/9	88.89a	3.54	+	R
168	8261405	14	10/10	100.00a	3.56	++	MR
69	8230037	21	7/8	87.50a	3.59	++	MR
63	8230028	12	11/11	100.00a	3.62	++	MR
27	8220004	11	10/12	83.33a	3.63	++	MR
29	8220006	9	9/11	81.82a	3.66	++	MR
6	8210012	11	10/10	100.00a	3.70	++	MR
62	8230024	19	7/8	87.50a	3.70	++	MR
120	8260715	13	9/10	90.00a	3.82	++	MR
21	8210066	14	10/11	90.91a	3.84	++	MR
90	8261401	13	12/12	100.00a	3.87	++	MR
66	8230033	13	11/11	61.11a	3.90	++	MR
73	8230100	13	10/11	90.91a	3.91	++	MR
163	8261398	13	12/12	100.00a	3.97	++	MR
65	8230030	14	11/11	100.00a	4.01	++	MR
46	8220058	21	9/9	100.00a	4.01	++	MR
31	8220009	12	11/11	100.00a	4.04	++	MR
45	8220057	20	8/9	88.89a	4.06	++	MR
141	8261081	13	11/12	100.00a	4.10	++	MR
44	8220056	14	11/11	100.00a	4.13	++	MR
140	8261078	9	12/12	100.00a	4.23	++	MR
39	8220042	14	11/11	100.00a	4.26	++	MR
43	8220055	9	10/10	100.00a	4.27	++	MR
131	8260987	11	12/12	100.00a	4.27	++	MR
68	8230035	12	12/12	70.83a	4.30	++	MR
122	8260754	9	10/10	100.00a	4.30	++	MR
26	8220001	13	11/11	100.00a	4.31	++	MR
137	8261048	12	12/12	100.00a	4.33	++	MR
49	8220062	10	10/10	100.00a	4.34	++	MR

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Table 2 (continued)

S/No	Genotype	Days to Symptoms Appearance	No. of Infected plants/Total plants	Mean Incidence	Mean Severity	ELISA	Class
169	8261551	12	10/10.	100.00a	4.37	++	MR
92	8261487	11	10/10.	100.00a	4.40	++	MR
117	8260641	11	9/10.	90.00a	4.41	++	MR
35	8220021	9	10/10.	100.00a	4.47	++	MR
121	8260753	11	12/12.	100.00a	4.47	++	MR
16	8210045	11	11/11.	100.00a	4.47	++	MR
136	8261046	8	9/10.	90.00a	4.49	++	MR
15	8210044	9	12/12.	100.00a	4.53	++	MR
32	8220012	13	12/12.	100.00a	4.53	++	MR
23	8210079	12	10/10.	100.00a	4.57	++	MR
162	8261390	13	11/11.	100.00a	4.58	++	MR
81	8260031	14	10/11.	90.91a	4.60	++	MR
30	8220007	11	10/11.	90.91a	4.62	++	MR
12	8210026	12	9/10.	90.00a	4.66	++	MR
18	8210052	9	11/12.	91.67a	4.67	++	MR
114	8260599	9	11/11.	100.00a	4.68	++	MR
100	8260347	12	11/11.	100.00a	4.70	++	MR
165	8261486	11	11/11.	100.00a	4.70	++	MR
84	8260086	8	10/10.	100.00a	4.70	++	MR
135	8261061	11	12/12.	100.00a	4.70	++	MR
134	8261042	9	11/11.	100.00a	4.73	++	MR
110	8260585	11	9/9.	100.00a	4.74	++	MR
74	8230102	9	11/11.	100.00a	4.76	++	MR
153	8261186	12	12/12.	100.00a	4.77	++	MR
167	8261496	12	12/12.	100.00a	4.77	++	MR
41	8220044	12	11/11.	100.00a	4.79	++	MR
20	8210065	11	12/12.	100.00a	4.87	++	MR
61	8230023	12	11/11.	100.00a	4.87	++	MR
115	8260607	10	11/11.	100.00a	4.88	++	MR
75	8230103	12	11/11.	100.00a	4.94	++	MR
53	820086	14	11/11.	100.00a	4.96	++	MR
38	8220033	13	11/11.	100.00a	4.97	++	MR
72	8230080	9	12/12.	100.00a	4.97	++	MR
70	8230045	13	8/8.	100.00a	5.00	++	MR
102	8260385	13	12/12.	100.00a	5.00	++	MR
17	8210050	10	11/11.	100.00a	5.04	++	MR
64	8230029	11	8/8.	100.00a	5.06	++	MR
160	8261341	10	10/10.	100.00a	5.09	++	MR
118	8260681	11	11/11.	100.00a	5.10	++	MR
156	8261255	8	12/12.	100.00a	5.10	++	MR
34	8220014	11	10/10.	100.00a	5.14	++	MR
174	Amankwatia (MRC)	10	9/9.	100.00a	5.15	++	MR
9	8210021	12	12/12.	100.00a	5.17	++	MR
93	8260179	8	11/11.	100.00a	5.17	++	MR
113	8260592	11	10/10.	100.00a	5.17	++	MR
124	8260811	8	11/11.	100.00a	5.18	++	MR
54	820095	8	10/11.	100.00a	5.19	++	MR
98	8260326	11	9/9.	100.00a	5.19	++	MR
47	8220060	13	12/12.	100.00a	5.20	++	MR
111	8260586	12	12/12.	100.00a	5.20	++	MR
130	8260979	11	12/12.	100.00a	5.20	++	MR
104	8260418	9	11/11.	100.00a	5.21	++	MR
36	8220022	10	11/11.	100.00a	5.23	++	MR
82	8260034	10	10/10.	100.00a	5.27	++	MR
142	8261085	14	10/10.	100.00a	5.30	++	MR
7	8210013	8	10/10.	100.00a	5.31	++	MR
145	8261107	13	11/11.	100.00a	5.32	++	MR
40	8220043	9	12/12.	100.00a	5.33	++	MR
103	8260392	11	11/11.	100.00a	5.36	++	MR
143	8261087	10	12/12.	100.00a	5.37	++	MR

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Table 2 (continued)

S/No	Genotype	Days to Symptoms Appearance	No. of Infected plants/Total plants	Mean Incidence	Mean Severity	ELISA	Class
91	8260159	9	11/11.	100.00a	5.40	++	MR
96	8260230	10	11/11.	100.00a	5.40	++	MR
109	8260581	8	10/10.	100.00a	5.40	++	MR
127	8260903	10	12/12.	100.00a	5.40	++	MR
19	8210055	9	12/12.	100.00a	5.43	++	MR
132	8261007	11	10/10.	100.00a	5.46	++	MR
123	8260783	9	12/12.	100.00a	5.47	++	MR
3	8210007	8	11/11.	100.00a	5.49	++	MR
48	8220061	9	11/11.	100.00a	5.53	++	MR
28	8220005	12	11/11.	100.00a	5.53	++	MR
128	8260906	12	12/12.	100.00a	5.53	++	MR
37	8220023	11	11/11.	100.00a	5.54	++	MR
83	8260050	11	9/9.	100.00a	5.54	++	MR
99	8260332	9	10/10.	100.00a	5.57	+++	S
116	8260612	9	11/11.	100.00a	5.58	+++	S
52	820073	12	11/11.	100.00a	5.60	+++	S
50	8220063	13	8/8.	100.00a	5.66	+++	S
85	8260781	9	10/10.	100.00a	5.68	+++	S
105	8260461	11	9/9.	100.00a	5.68	+++	S
87	8260817	11	11/11.	100.00a	5.68	+++	S
51	820066	9	12/12.	100.00a	5.70	+++	S
159	8261310	9	12/12.	100.00a	5.70	+++	S
79	8260019	9	10/10.	100.00a	5.73	+++	S
106	8260505	11	12/12.	100.00a	5.73	+++	S
119	8260701	9	9/9.	100.00a	5.74	+++	S
133	8261023	10	10/10.	100.00a	5.76	+++	S
78	8260014	11	11/11.	100.00a	5.83	+++	S
129	8260956	9	12/12.	100.00a	5.83	+++	S
125	8260844	11	12/12.	100.00a	5.87	+++	S
126	8260770	9	11/11.	100.00a	5.87	+++	S
158	8261293	9	10/11.	90.91a	5.87	+++	S
89	8261203	11	12/12.	100.00a	5.93	+++	S
77	8260012	8	11/11.	100.00a	5.95	+++	S
76	8230104	9	11/11.	100.00a	5.97	+++	S
1	8210002	12	10/10.	100.00a	6.07	+++	S
33	8220013	7	9/10.	90.00a	6.10	+++	S
94	8260195	8	12/12.	100.00a	6.10	+++	S
161	8261372	9	9/9.	100.00a	6.12	+++	S
112	8260588	10	12/12.	100.00a	6.13	+++	S
107	8260543	12	11/11.	100.00a	6.14	+++	S
166	8261492	9	11/11.	100.00a	6.16	+++	S
157	8261288	8	11/11.	100.00a	6.17	+++	S
22	8210058	8	10/11.	90.91a	6.19	+++	S
155	8261251	9	12/12.	100.00a	6.23	+++	S
154	8261211	9	10/10.	100.00a	6.23	+++	S
108	8260570	9	11/11.	100.00a	6.26	+++	S
24	8210084	8	11/11.	100.00a	6.28	+++	S
13	8210027	11	12/12.	100.00a	6.30	+++	S
86	8260782	9	11/11.	100.00a	6.39	+++	S
164	8261475	10	12/12.	100.00a	6.47	+++	S
95	8260198	12	11/11.	100.00a	6.60	+++	S
11	8210024	5	11/11.	100.00a	6.87	+++	S
88	8261024	8	12/12.	100.00a	6.87	+++	S
97	8260325	8	11/11.	100.00a	7.04	+++	S
138	8261069	10	10/10.	100.00a	7.09	+++	S
56	8230008	9	9/9.	100.00a	7.24	+++	S
173	Jasmine 85 (SC)	8	8/8.	100.00a	7.24	+++	S

HR = highly resistant; R = resistant; MR = moderately resistant; S = susceptible; HS = highly susceptible; RC = resistant check; MRC = moderately resistance check; SC = susceptible check; NS = no symptom; (-) = negative reaction; (+) = weak reaction; (++) = weak, but clear reaction; (+++) = strong reaction.

The bold values represents the various checks.

Table 3. Mean squares from the analysis of variance table for disease severity and incidence of RYMV.

Source of variance	DF	SEVERITY	INCIDENCE
Replicate	3	30.828**	0.641*
Block/Rep	172	0.0196**	0.226 ^{ns}
Genotype	175	5.5899**	2.44**
Residual	353	0.0121	0.2079
CV (%)		2.6	10.3

^{ns} Non-significant at $P \leq 0.05$; *Significant at $P \leq 0.05$; **Significant at $P \leq 0.01$.

green or pale leaves, pale yellow, yellow or orange leaves and death of plants. Genotype 8210024 showed symptoms as early as 5 dpi and symptoms appeared on 8220013 at 7 dpi as shown in (Table 2). Sixteen genotypes as well as the susceptible check, Jasmine 85 showed symptoms at 8 dpi whilst 36 genotypes exhibited symptoms of RYMV at 9 dpi. Symptoms appeared in 14 tested genotypes as well as the moderately resistant check (CRI-Amankwatia) at 10 dpi whereas it appeared on 30, 23, 23, 15, 1, 3, 4 and 3 genotypes at 11, 12, 13, 14, 18, 19, 20, and 21 dpi, respectively (Table 2).

The results of this study corroborate the finding of Kouassi et al. (2005) who reported that infected rice plants exhibit most of the typical RYMV symptoms if infected within 20 days after planting, may stop growing, and eventually die. Our results also supported the observation made by Bakker (1970) that typical symptoms of RYMV disease appeared seven days after inoculation. The time taken for symptoms development is a function of the level of susceptibility or resistance of each genotype to RYMV infection. Susceptible genotypes develop symptoms faster while resistant genotypes delay symptoms development. The delay in symptoms expression till about five to six weeks after inoculation observed for five genotypes among the 172 lines evaluated indicate that these genotypes were good sources of resistance to RYMV. Mogga et al. (2012) reported significant differences in the number of days to symptoms expression on rice accessions subjected to RYMV infection in Uganda.

3.3. RYMV disease severity and incidence

There were highly significant differences ($p \leq 0.01$) among the genotypes for disease severity (Table 3). Five genotypes (8261112, 8261119, 8261133, 8261588 and 8261634) were similar to the resistant checks in disease severity. These resistant genotypes had significantly

lower symptoms than the remaining 167 genotypes including the moderately resistant and susceptible checks. A total of 100 genotypes had symptoms scores that were not significantly different from score of CRI-Amankwatia, which is the moderately resistant check while 43 genotypes were similar to the susceptible Jasmine 85 check in symptoms severity (Table 2).

Highly significant differences were detected among the means of the genotypes ($P < 0.01$) for disease incidence (Table 3). Clear variability for disease incidence was shown by the 172 tested lines (Table 2). The disease incidence ranged between 0 and 100%. One hundred and thirty-two lines representing 76.7% exhibited high susceptibility in comparison with the susceptible check (Jasmine 85), recording 100% incidence. However, two lines namely, 8261588 and 8261634 recorded low disease incidence of 33.3 and 22.2%, respectively. Two lines, 8261112 and 8261119 did not show any symptom of the disease together with the highly resistant checks (Gigante and Tog7291), confirming their resistance to the disease. Tukey test revealed that six lines were significantly different from the susceptible check (Jasmine 85) (Table 2).

Among the lines, only genotypes 8261588 and 8261634 were not significantly different from the highly resistant checks (Gigante and Tog7291) as shown in Table 2.

3.4. Classification of the genotypes

The genotypes were classified into five reaction classes based on the incidence, severity and ELISA test (Table 2). These were: highly resistant, resistant, moderately resistant, susceptible and highly susceptible and the proportion of the lines in percentages were 2.9, 14.0, 58.1, 25.0 and 0, respectively, (Figure 2). It is was observed that majority of the tested

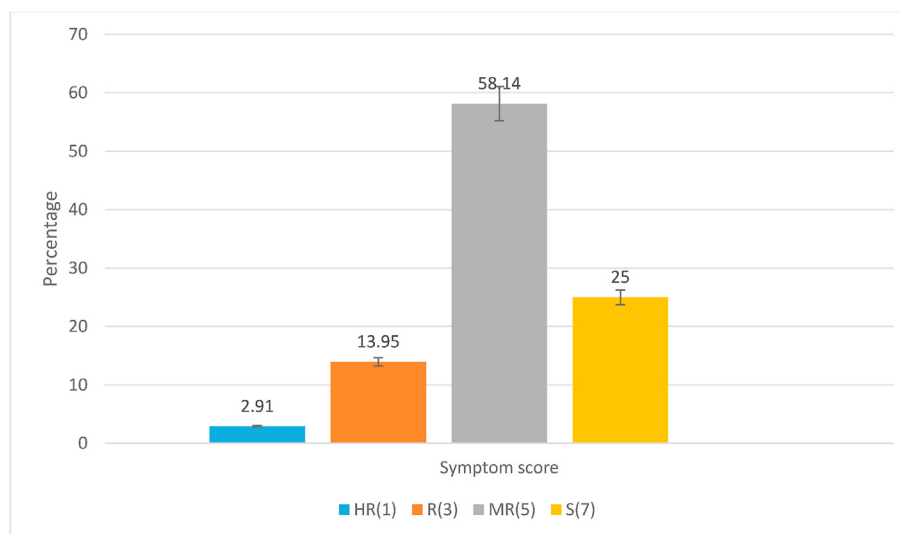


Figure 2. Population distribution according to their response to virus inoculation of 172 rice genotypes assessed by their symptom intensity using the 1–9 standard evaluation system: HR (1) = Highly Resistant, R (3) = Resistant, MR (5) = Moderately Resistant, S (7) = Susceptible, HS (9) = Highly Susceptible (there were no highly susceptible lines).

genotypes had some level of resistance to RYMV, which disagrees with the overall susceptibility of rice genotypes to RYMV as reported by Sereme et al. (2016) and Longue et al. (2018). Among the five highly resistant lines discovered in this study, two lines namely, 8261112 and 8261119, that did not show any symptoms have Nerica 8 as one of their parents. This suggests that these two identified highly resistant lines had the resistance gene from Nerica 8. Nerica 8 has already been identified as a potential source of resistant to RYMV (Mogga et al., 2012; Oludare et al., 2016). This strengthens the hypothesis that *O. glaberrima* is a potential source for resistance to the biotic stresses including RYMV disease (Agnoun et al. 2012, 2019; Mogga et al., 2012; Orjuela et al., 2013; Pidón et al., 2017; Sereme et al., 2016; Sié et al., 2012). Our results also supported Mogga et al. (2012) and Oludare et al. (2016) who showed that upland genotypes are mostly resistant to RYMV while those of lowland and irrigated ecologies are mostly susceptible. Altogether, the concept of a susceptible, tolerant or resistant variety, therefore, has to be considered with considerable caution because of different environmental conditions and ecology.

4. Conclusion

This study confirms the host resistance of the checks Tog7291 and Gigante to RYMV. The field and ELISA test data were highly correlated ($r = 0.99$). Five highly resistant lines (genotypes 8261112, 8261119, 8261133, 8261588, and 8261634) and 24 resistant lines were identified from the Korean germplasm. These lines can be incorporated into breeding programs for RYMV disease management in Ghana and beyond.

Declarations

Author contribution statement

M. D. Asante: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

B. Amadu: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

V. S. E. Traore: Conceived and designed the experiments; Wrote the paper.

A. Oppong: Contributed reagents, materials, analysis tools or data; Wrote the paper.

M. A. Adebayo: Analyzed and interpreted the data; Wrote the paper.

P. Aculey: Performed the experiments; Wrote the paper.

E. Agyemang Marfo: Performed the experiments; Contributed reagents, materials, analysis tools or data.

K.-H. Kang: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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