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Screening Rice (*Oryza sativa* L.) Varieties for Resistance to *Rice stripe necrosis virus* (RSNV)

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Abstract

Rice stripe necrosis virus (RSNV) is one of the main viruses infecting rice in Burkina Faso, causing significant damage. The objective of this work was to identify varietal resistance to *Rice stripe necrosis virus* (RSNV). The tests were carried out in a real environment, in the lowland of the Banfora experimental site (Tannounyan region) located at the INERA research station. Seeds of 30 improved rice varieties have been planted. For each variety, direct seeding was carried out at the rate of one (01) grain per pocket in elementary plots of 6.24 m^2 (5.2 m × 1.2 m) on five (5) rows. The spacing between the lines and between the pockets is identical and corresponds to 20 cm. NPK-based base fertilizer (14-23-14) was applied at a rate of 300 kg per ha. Three weedings were carried out and during the ripening-harvesting phase water was maintained until 14 days before harvest. Thus, through a three-replicate alpha lattice device, varietal field screening followed by impact assessment, identified 28 RSNV resistant/tolerant rice genotypes while two (02) were susceptible to the disease with significant production losses reaching 31.7% and 32.8% respectively for the cultivar WAB2066-WAT21-1-B-1-TGR2 and ARC1-20-B-1. Genotypes that have developed partial resistance and acquired total Resistance 110 days after sowing (DAS) with a production loss of between 0 and 30% can be used by producers for production and by breeders for their use in varietal improvement programs.

Keywords

RSNV, Rice, Resistance/Tolerance, Burkina Faso

1. Introduction

Rice is the staple food of more than half of the world's population. It is one of the world's most important cereals and ranked third after wheat and corn. It is a strategic crop, given its importance in food security. In West Africa, demand for rice is growing at a rate of 6% per year, outpacing population growth and faster than anywhere else in the world [1]. Its consumption is increasing by 7% annually due to population growth, urbanization and changes in eating habits [2]. In Burkina Faso, among cereals, rice occupies 4th place, both in terms of cultivated areas, quantity produced and annual per capita consumption [3]. In addition, rice demand is estimated at 840,000 tons per year compared to an average annual production of 450,000 tonnes [4].

The state often resorts to imports, at around 400,000 tonnes, to make up this deficit [5]. Thus, to succeed in resolving this situation, particular emphasis must be placed on increasing local production characterized by low yields, of the order of 2 tons/ha on average [4]. Indeed, rice cultivation is linked to abiotic and biotic constraints that plague rice fields and considerably reduce production by impacting the yield of the varieties developed. Among the biotic constraints are insect pests, weeds, nematodes and pathogen attacks, the most important of which are viruses, bacteria, and fungi [6]. Other constraints are a lack of knowledge of new improved varieties, climate change, which also contribute to the low rice production yield. As far as viruses are concerned, the rice striped necrosis virus (RSNV), recently discovered by [7] in Burkina Faso, causes a lot of damage. It is capable of causing yield losses of the order of 80% ± 5% of crops [7]. It is a virus that is re-emerging in West Africa and emerging in South America, where it is causing an increase in yield losses. Symptoms of the disease appear as bright yellow stripes on the leaves with excessive tillering, but the tillers remain stunted. The young shoots often have a tendril appearance. On rice, the *Rice stripe necrosis virus* (RSNV) is transmitted by *Polymyxa graminis* (Pog). *Polymyxa graminis* is an endoparasitic protist of the roots of soil-dwelling plants [8]. It is classified in the genus *Benyvirus* of viruses and little information exists about this pathogen. The disease is not spread through seeds from diseased plants. Disease control is mainly based on the use of fungicides against the fungus vector to treat soil and seeds, and the use of resistant varieties to limit the spread of the disease in the field.

Previous work suggests that sources of resistance exist in the African rice Oryza glaberrima [9]. These sources of resistance are being used for the genetic improvement of Asian rice varieties (*O. sativa*) for resistance to RSNV. In addition, some varieties used so far in Burkina Faso could show a certain vulnerability to this virus. A screening of varieties from research centers such as INERA is necessary to identify varieties resistant to this virus. Indeed, these varieties are used by local producers in the slums of the country. Knowledge of their behaviour in the face of RSNV becomes an imperative in order to plan an effective control.

This study was devoted to screening in a real environment where RSNV is present on 30 rice varieties originating from Banfora (Burkina Faso), while at the same time determining the effect of the incidence of the virus on the agro-morphological parameters of these 30 varieties.

2. Material

2.1. Study Site

The study was carried out in the lowland of the INERA/Banfora experimental site (Tannounyan region), which represents an RSNV hot spot. This site, located between the 1000 mm and 1200 mm isohyets, consists of a simple layout with compacted earth bunds. Figure 1 shows the location of the experimental site with the following geographical coordinates: longitude 4˚46'38.56", latitude 10˚37'47.89". The site is located in the sudanian climatic zone of Burkina Faso, characterized by an average annual rainfall ranging from 900 to 1200 mm, a rainy season lasting approximately six months (May to October), and a mean annual temperature of about 27˚C [10].

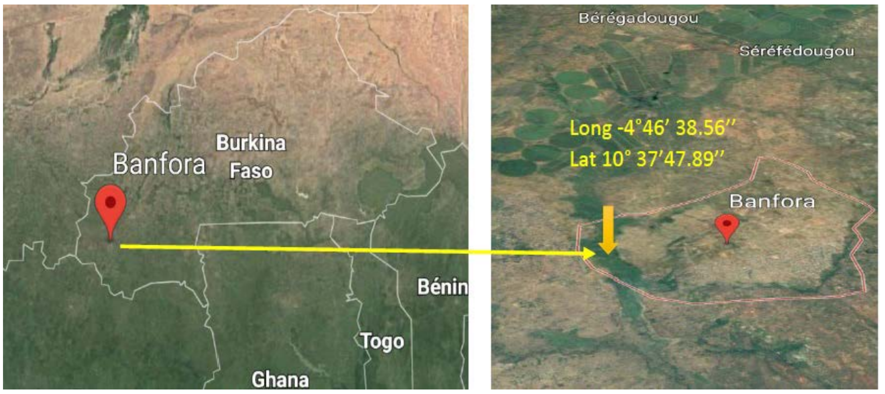

Figure 1. Varietal screening site in the presence of the RSNV.

2.2. Plant Material

The plant material consisted of 30 improved high-yielding rice varieties from the INERA rice and rice cultivation programme (Table 1).

Table 1. List of varieties used.

Code	Variety	Pedigree	Origin	Species	Ecology
V1	ART346-10-2-1		Benin (Africarice)	*Oryza sativa*	Lowland
V2	ART349-1-1-1		Benin (Africarice)	*Oryza sativa*	Lowland
V3	WAB2066-WAT21-1-B-1-TGR2	WAB2066-WAT21-1-B-1-TGR2	Benin (Africarice)	*Oryza sativa*	Lowland
V4	ARC1-432-B-1		Benin (Africarice)	*Oryza sativa*	Lowland
V5	ART348-4-1-1		Benin (Africarice)	*Oryza sativa*	Lowland
V6	WAB2135-WACB-2-TGR3-WAT8-1		Benin (Africarice)	*Oryza sativa*	Lowland

Continued

V7	WAB2138-WACB-2 -TGR2-WAT5-1	WAB 2138-WACB-2 TGR2-WAT5-1	Benin (Africarice)	*Oryza sativa*	Lowland
V8	FKR19*TSF6-36		Benin (Africarice)	*Oryza sativa*	Lowland/Irrigated
V9	ART346-10-1-1		Benin (Africarice)	*Oryza sativa*	Lowland
V10	ART347-9-1-1		Benin (Africarice)	*Oryza sativa*	Lowland
V11	ARC1-235-B-1		Benin (Africarice)	*Oryza sativa*	Lowland
V12	ART346-10-4-1		Benin (Africarice)	*Oryza sativa*	Lowland
V13	WAB2066-WAT21-1 -B-1-TGR3	WAB 2066-WAT21-1 -B-1-TGR3	Benin (Africarice)	*Oryza sativa*	Lowland
V14	ARC1-448-B-1		Benin (Africarice)	*Oryza sativa*	Lowland
V15	WAB2152-TGR2-WAT1-2		Benin (Africarice)	*Oryza sativa*	Lowland
V16	WAB2081-WAC2-2-TGR2WAT1-8-TGR3		Benin (Africarice)	*Oryza sativa*	Lowland
V17	WAB2081-WAC2-2 -TGR2-WAT1-9-TGR3		Benin (Africarice)	*Oryza sativa*	Lowland
V18	ART29-6-62-1-4-1		Benin (Africarice)	*Oryza sativa*	Lowland
V19	FKR 19	TOX 728-1 (Local-Nigeria)	Burkina Faso (INERA)	*Oryza sativa*	Lowland/Irrigated
V20	WAB2104-WAC5-2 -TGR1-BAT8-1		Benin (Africarice)	*Oryza sativa*	Lowland
V21	ART347-8-2-1		Benin (Africarice)	*Oryza sativa*	Lowland
V22	ARC1-20-B-1		Benin (Africarice)	*Oryza sativa*	Lowland
V23	IR88638-62-1-1-1-1-1		Burkina Faso (INERA)	*Oryza sativa*	Lowland
V24	ART315-BC1-6		Benin (Africarice)	*Oryza sativa*	Lowland
V25	ART347-7-1-1		Benin (Africarice)	*Oryza sativa*	Lowland
V26	FKR 56N	WAB450-I-BL-1-736-HB	Burkina Faso (INERA)	Interspecific	Lowland/Irrigated
V27	ART347-1-1-1		Benin (Africarice)	Interspecific	Lowland
V28	ARICA 2 (CHECK)	WAB-2056-2-FKR 2-5-TGR 1-B	Benin (Africarice)	Interspecific	Lowland
V29	FKR 62N	WAS 122-IDSA-1-WAS-6-1	Burkina Faso (INERA)	Interspecific	Lowland/Irrigated
V30	NERICA-L19 (Check)		Benin (Africarice)	Interspecific	Lowland/Irrigated

3. Methods

3.1. Soil Preparation and Experimental Design

In the second ten-day period of June, 1050.5 m^2 (38.2 m × 27.5 m) of surface was ploughed with a disc plough and pulverized with a hoe before the basic plots (5.2 m × 1.2 m) were set up. The preparation of the seedbed was done with a hoe during the first ten days of July. The experimental device used was a three-replicate alpha lattice (**Figure 2**). Each repetition was composed of six blocks and each block was subdivided into five plots. The size of each plot was 5.2 m long and 1.2 m wide. The distance between the lines and between the pockets was 20 cm × 20 cm.

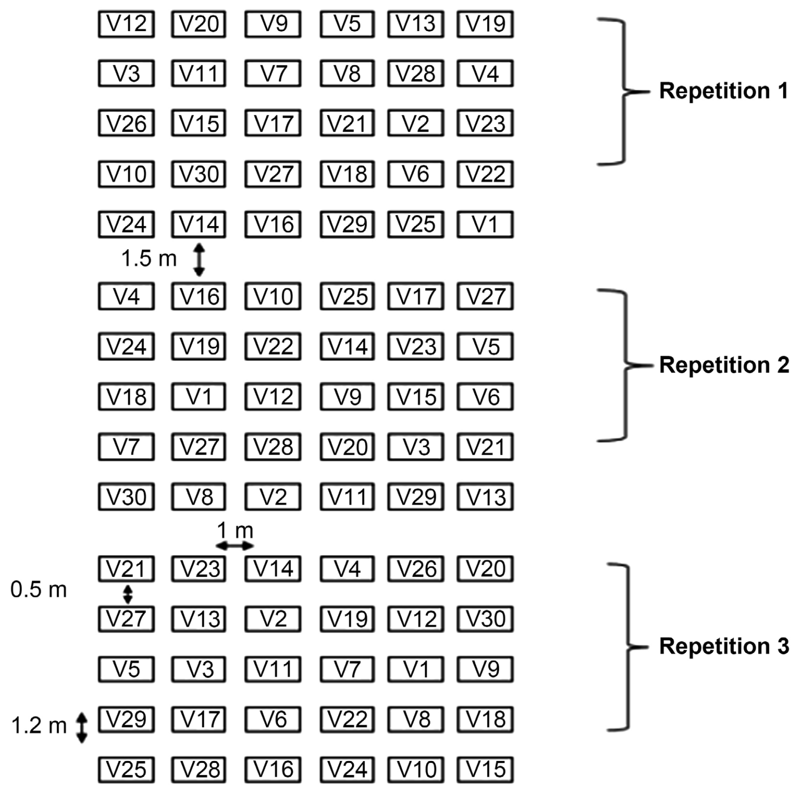

Figure 2. Experimental design of the RSNV trial.

3.2. Conduct of the Test

For each variety, direct sowing is carried out at the rate of one (01) grain per pocket in elementary plots of 6.24 m^2 (5.2 m × 1.2 m) on 5 rows. The spacing between lines and between pockets is identical and corresponds to 20 cm. NPK-based base fertilizer (14-23-14) was applied at a rate of 300 kg per ha. Then, urea-based top-dressing at a dose of 100 kg/ha is divided into two applications. The first application is made at a dose of 35 kg per ha at 15 days after sowing (DAS) and the second at a dose of 65 kg per ha at the initiation of panic at about 63 DAS in accordance with research recommendations to producers. Three weddings were carried out manually by hand given the grass cover of the trial. During tillering, a 5 cm water gap (drain when the fertilisers are spread) was maintained in the plots. This water was reduced to 8 cm during the bolt-flowering phase. During the ripening-harvesting phase, water was maintained for up to 14 days before harvest.

3.3. Data Collected

The observations made were related to the agro-morphological characteristics of the different varieties of seven (07) traits:

- The number of tillers per m^2 at 60 DAS to assess the tillering ability of each variety, which is one of the parameters directly influencing yield.
- The sowing heading cycle (SHC) 50% corresponding to the difference between the date on which half of the tillers have sprung and the sowing date on each plot.
- The sowing maturity cycle (SMC) 85%, corresponding to the difference between the date from which 85% of the panicles on each plot have a straw colour and the sowing date.
- The height of the plants measured on five (5) plants randomly chosen per elementary plot at 60 DAS during the maturity phase. The height measurement is taken from the collar of the plants to the top of the panicle leaf or at the end of the highest panicle when the latter exceeds the size of the panicle leaf.
- The number of panicles per m^2 was obtained by counting five (5) plants randomly chosen per elementary plot at 110 DAS.
- Yield was determined by measuring the weight of the grains in each plot. Indeed, winnowing and weighing using a scale were carried out for each variety in a given plot.

In addition to these seven (7) traits observed, the number of infected plants per variety was determined.

3.4. Impact of the RSNV

The incidence of the disease was calculated according to the formula described by [11]:

$$I(\%) = \frac{PA \times 100}{PT}$$

where I: incidence of the disease; PA: number of infected and/or dead plants (a plant was considered infected when symptoms were immediately observed); PT: total number of plants.

A slightly modified standard of the evaluation system described by Sereme *et al.* (2016) was used to determine the status of each variety with respect to the RSNV: highly resistant (HR) for 0% - 10% infection, resistant (R) for 11% - 20% infection, moderately resistant (MR) for 21% - 30% infection, susceptible (S) for 31% - 85% infection, and highly susceptible (HS) for infection greater than 85%.

3.5. Statistical Analysis of the Data

The data were processed with Microsoft Office's Excel 2016 software and analysed with XLSTAT 2014 and STATISTICA version 7.1. Excel 2016 was used for data entry, data arrangement, frequency calculation, sector realization and curves. XLSTAT, on the other hand, was used to analyse the performance of varieties in the presence of the virus through analysis of variance (ANOVA). Finally, STATISTICA was used for correlation analysis.

4. Results

4.1. Evaluation of the Agronomic Performance of Varieties in the Presence of the RSNV

The diagram in Figure 3 shows the variation in the average number of tillers per m^2 at 60 days after sowing (DAS), the semi-heading cycle and the sowing-maturity cycle 85% of the different varieties. The number of tillers varied according to the variety but also from one replicate to another. The maximum average number of tillers was obtained with variety ARC1-20-B-1 (383 tillers) and the minimum average number was obtained with variety ARC1-235-B-1 (235 tillers). That is a difference of 148 tillers. The shortest semi-heading cycle (50%) was obtained at 84.66 DAS with the ART315-BC1-6 varieties, and the longest cycle was obtained at 99 DAS with the variety IR88638-62-1-1-1-1-1-1-1, a difference of 14.34 days. The shortest seeding-maturity cycle (85%) was obtained at 110.33 days with the varieties ART347-9-1-1 and the longest cycle was obtained at 129.66 days with the variety FKR 56N, a difference of 19.33 days.

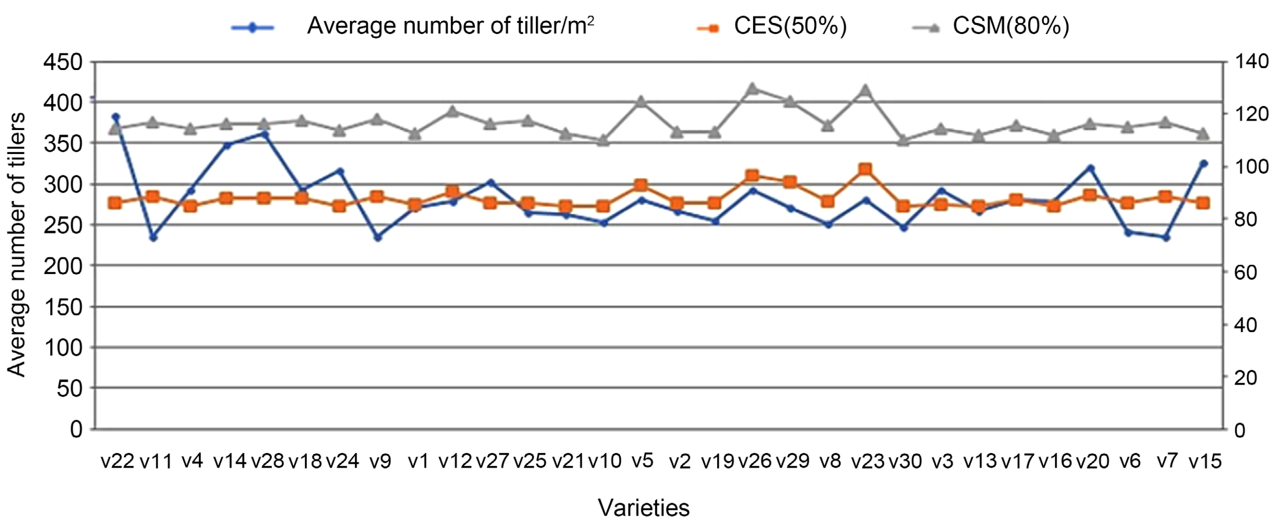

Figure 3. Variation in the average number of tillers (60 DAS), the CSE (50%) and the CSM (85%) of the different varieties.

Figure 4 shows the average heights at 60 DAS and at maturity for the different rice varieties. The greatest mean height at 60 DAS was 95.06 cm observed in the FKR 56N variety. The smallest mean height at 60 DAS of 56.86 cm was noted in variety ART349-1-1-1.

The greatest mean height at maturity was 129.66 cm observed in FKR 56N. The smallest mean height at maturity of 110.33 cm was noted in variety ART347-9-1-1.

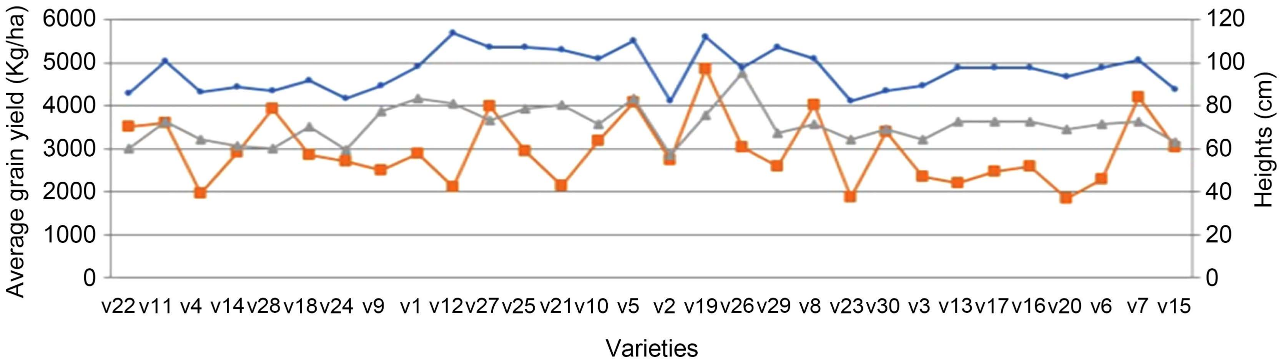

Figure 4. Average heights (60 DAS and at maturity) and the average yield of the varieties.

The best average grain yield in the presence of RSNV was 4852.87 kg/ha for FKR 19 and the lowest average yield of 1837.39 kg/ha was observed for WAB2104-WAC5-2-TGR1-BAT8-1.

The analysis of the different agronomic performances revealed the existence of variability within the varieties studied in the presence of RSNV in the environment. The analysis of variance showed the existence of a significant difference ($P < 0.05$) within varieties for the mean number of tillers at 60 days after sowing: the average plant heights at 60 DAS and the average grain yield. On the other hand, the analysis of variance showed the absence of a significant difference within the varieties sown for the number of panicles ($F = 0.8006$; $P = 0.7404$). At the level of traits such as the 50% seed-heading cycle; the sowing cycle at 85% maturity; mean plant height at maturity, a highly significant difference ($P < 0.001$) existed within varieties for these variables.

4.2. Correlation

The statistical analysis also revealed the existence of a very strong correlation ($P < 0.01$) between incidence and certain agro-morphological parameters. The incidence is positively correlated with the number of tillers per m^2 ($P = 0.001$; $R = 0.3405$) and the number of panicles per m^2 ($P = 0.01$; $R = 0.3477$). It is negatively correlated with height at 60 DAS ($P = 0.033$; $R = 0.2255$) and height at maturity ($P = 0.001$; $R = -0.3737$). There is also a positive correlation between the height at maturity and the grain yield ($p = 0.003$; $F = 0.3140$).

4.3. Incidence of the Disease

Analysis of variance revealed a highly significant difference ($P < 0.001$) in the mean incidence of RSNV that varied by variety (Table 2). Thus, the highest average incidences were observed on the varieties ARC1-20-B-1; WAB2066-WAT21-1-B-1-TGR2 and WAB2081-WAC2-2-TGR2-WAT1-9-TGR3 with average incidences of around 32.80%; 31.73% and 29.86%, respectively.

The ART346-10-2-1 varieties; ART347-9-1-1; ART346-10-4-1 and WAB2135-WACB-2-TGR3-WAT8-1 had the lowest mean incidence of 0.80%. In contrast, ART346-10-1-1 and NERICA-L19 did not show any symptoms of RSNV.

Table 2. Impact of RSNV and average grain yield of varieties.

Varieties	Incidence (%)	Yield (kg/ha)	Status
NERICA-L19 (Check)	0a	3388.1bcdefg	HR
ART346-10-1-1	0a	2500.0abcd	HR
ART346-10-2-1	0.8a	2892.3abcdef	HR
ART347-9-1-1	0.8a	3184.3abcdef	HR
WAB2135-WACB-2-TGR3-WAT8-1	0.8a	2274.0abc	HR
ART346-10-4-1	0.8a	2113.5abc	HR
WAB2081-WAC2-2-TGR2WAT1-8-TGR3	0.8a	2574.0abcde	HR
FKR 62N	1.1a	2596.1abcde	HR
WAB2104-WAC5-2-TGR1-BAT8-1	1.1a	1837.4a	HR
ART348-4-1-1	1.1a	4071.8efg	HR
ART347-8-2-1	1.6a	2137.3abc	HR
ART347-7-1-1	1.9a	2928.7abcdef	HR
FKR19*TSF6-36	2.4a	4006.6defg	HR
WAB2066-WAT21-1-B-1-TGR3	2.4a	2274.0abc	HR
WAB2152-TGR2-WAT1-2	2.7a	3025.6abcdef	HR
ART347-1-1-1	2.9a	3976.4defg	HR
FKR 56N	3.5a	3043.3abcdef	HR
ARC1-448-B-1	4.3a	2923.1abcdef	HR
WAB2138-WACB-2-TGR2-WAT5-1	4.8a	4185.9fg	HR
FKR 19	6.4a	4852.9g	HR
ARC1-235-B-1	6.9a	3601.1cdefg	HR
IR88638-62-1-1-1-1-1	8a	1867.5ab	HR
ARICA 2 (CHECK)	9.9a	3917.9defg	HR
ART315-BC1-6	12.8ab	2713.3abcdef	R
ART349-1-1-1	14.7abc	2746.2abcdef	R
ARC1-432-B-1	15.5abc	1956.0ab	R
ART29-6-62-1-4-1	17.1abc	2860.7abcdef	R
WAB2081-WAC2-2-TGR2-WAT1-9-TGR3	29.9bc	2465.8abcd	MR
WAB2066-WAT21-1-B-1-TGR2	31.7c	2359.6abc	S
ARC1-20-B-1	32.8c	3523.1cdefg	S
F	2.1335	2.0065	
Probability	<0.0067	<0.0116	
Signification	Significant	Significant	

The analysis also revealed that the varieties ART348-4-1-1 (V5); FKR19*TSF6-36 (V8); WAB2138-WACB-2-TGR2-WAT5-1 (V7); FKR 19 (V19); NERICA-L19 (Check) (V30); ART347-9-1-1 (V10); WAB2152-TGR2-WAT1-2 (V15); ART347-1-1-1 (V27); FKR 56N; ARC1-235-B-1 (V26); ARICA 2 (CHECK) (V28) showed a very low incidence against RSNV and gave both good yields. However, the first 4 varieties mentioned above gave the best yields with a very low incidence of RSNV.

Components assigned to the same letter in the same column are not significantly different at the 5% probability threshold. Highly resistant (HR) for 0% - 10% infection, resistant (R) for 11% - 20% infection, moderately resistant (MR) for 21% - 30% infection, susceptible (S) for 31% - 85% infection, and highly susceptible (HS) for infection greater than 85%.

4.4. Frequency of Impacts

Figure 5 shows the frequency of RSNV impacts observed on the 30 varieties. Indeed, the majority of varieties, 77%, have a very low incidence compared to the RSNV, between 0 and 9.9%. Low incidences of 12.8% for one group and between 14.17% and 17.1% for another were obtained for 13% of the varieties. Finally, the highest incidences concern 10% of varieties, including an incidence of 29.9% for one group and incidences between 31.7% and 32.8% for another group.

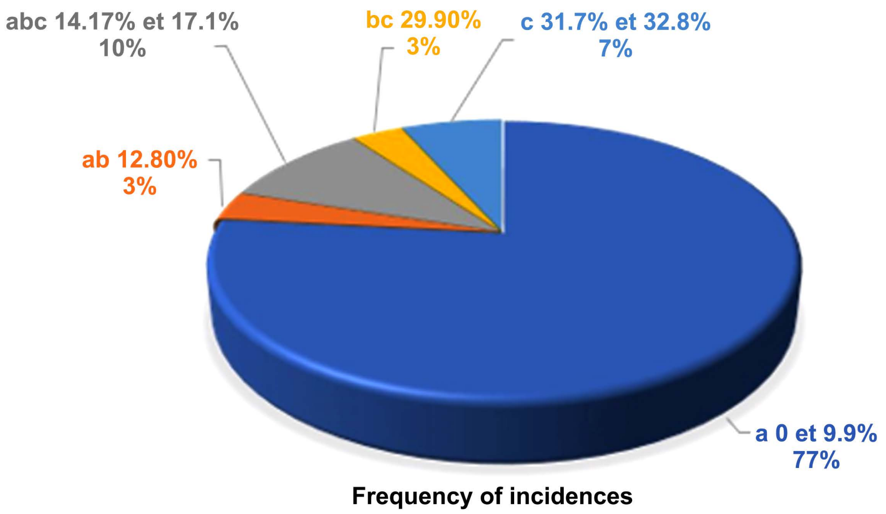

Figure 5. Frequencies of varieties in the groups for the incidence of RSNV.

5. Discussion

In order to identify rice genotypes that are tolerant or even resistant to RSNV, a varietal screening was carried out in the field. The results obtained showed that the incidence of RSNV was a function of the varieties. Some varieties have shown a very low incidence against RSNV with fairly high yields. This low incidence (0% to 17.1%) could be explained by a low level of inoculum at the level of the study plot this year. In addition, the general low incidence observed could also be due to the fact that this disease recently declared by [7] has not yet developed sufficient mechanisms to negatively impact the development of varieties used in Burkina Faso. Indeed, the impact of RSNV on rice development depends not only on the age of the plants during infection but also on the number of tillers affected and the variety present [12]. Our work is in line with the screening carried out by [13] in Benin when they observed some accessions of dead plants due to serious attacks. After screening, they found incidences of 0 to 37% in the highlands and 0 to 26.6% in the lowlands.

In addition, these low incidences could also be explained by the good resistance of the improved varieties used, suggesting the presence of resistance genes in the different genotypes with low incidence. The statistical analysis also revealed the existence of a very strong correlation ($P < 0.01$) between incidence and certain agro-morphological parameters. The incidence positively correlated with the number of tillers per m^2 and the number of panicles per m^2 means that the greater the incidence, the greater the number of tillers and the number of panicles. It is negatively correlated with height at 60 DAS and height at maturity. This means that the higher the incidence, the lower the heights at 60 DAS and at maturity. Since height at maturity is positively correlated with yield, this could mean that the incidence indirectly decreases variety yields.

However, one outstanding variety (ARC1-20-B-1) exhibited a high average tillering count and average good yield despite the high incidence (32.8%) of RSNV.

In the presence of the disease, descriptive analyses of agro-morphological parameters showed significant discrepancies between the minimum and maximum values for the traits studied. This demonstrates that inter-accession variability is significant.

6. Conclusion

RSNV is a virus that has emerged recently and is mainly present in the Saudi area of the country where rice is widely grown. Our work has made it possible to identify in field conditions, 27 resistant varieties, one (01) tolerant variety and two (02) susceptible varieties. The first two will be made available to producers. In addition, they could also be used in breeders' varietal improvement programs. However, it should be noted that we had only carried out this experiment once in the agricultural season. For greater reproducibility, this experiment should be repeated for at least two to three crop years. As the presence of inoculum required varies from year to year, a varietal screening in a greenhouse should be considered to allow a better choice of resistant/tolerant varieties.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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