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(RESEARCH ARTICLE)

Potential weeds host-reservoirs of *Rice Yellow Mottle Virus* in irrigated rice fields of Niger

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Abstract

Rice Yellow Mottle Virus (RYMV) is spread in the field by insect vectors and weeds. The study contributes to the fight against the spread of the virus. It consisted in collecting and characterizing the weeds present in and around the rice fields and then carrying out a survey followed by field visits to 81 farmers in the target areas. In total, seventy-five (75) species of rice weeds were inventoried. They belong to *Grass* family (28 species), *Cyperaceae family* (10 species) and 21 other broad-leaved herbaceous families (37 species). Among these weeds, *Rotala indica, Cyperus spp* and *Echinochloa spp* are the most widely recognized, frequent and damaging to rice, while most growers are less familiar with *Oryza barthii, Leersia Oryzoides* and *Nymphea tetragonal*. In terms of biological characteristics, twelve (12) weed species were found to be potential host-reservoirs for RYMV. They mainly belong to *Grass* (7 species) and *Cyperaceae* (5 species) families. The *Grass* species are *Echinochloa colona, Cynodon dactylon, Echinochloa crus-galii, Leersia hexandra, Leersia oryzoides, Panicum repens* and *Paspalum scorbilatum* when *Cyperaceae's* especies include *Cyperus difformis, Cyperus esculentus, Cyperus iria, Cyperus distans* and *Pycrerus lanceolatus.* The frequency of RYMV weed hosts-reservoirs varies from family and plot. *Grasses* (58.33%) predominate over *Cyperaceae* (41.67%). *Cynedon dactylon* is the most widespread species, followed by *Echinochloa colona* which is more difficult to control. Among the *Cyperaceae*, *Cyperus difformis* is the most common weed and the most difficult to control. Based on farmers weed control, weeding and/or the application of synthetic herbicides have proved more effective. These results would help to control RYMV and it's spread in African rice-growing ecologies.

Key words: Rice Yellow Mottle; RYMV; Host-Reservoir Weeds; Grasses; Cyperaceae; Rice-Growing Sites in Niger.

1. Introduction

Rice (*Oryza sp.*) is used for human and animal consumption, as well as in industry and handicrafts [1, 2]. It is the most widely grown cereal in the world after wheat [3] and is the staple food of more than half the world's population, particularly the poor [4]. World production of milled rice reached 509.2 million tons in 2020 [3], with Asia accounting for over 90% of global rice stocks. West Africa, meanwhile, with its ever-increasing annual demand for rice, relies on imports to satisfy regional demand for rice [5].

In Niger, rice is the third most important cereal, after millet and sorghum, in terms of both area and production [6, 2]. Annual national rice production was estimated at 75,140 tons of white rice in 2018. However, this production only covers one-sixth (1/6) of the country's annual requirements, estimated at 435,135 tons of white rice [7]. In fact, rice is the foodstuff whose consumption (2.4 to 52.7 kg per person per year, with an average of 20 kg/person/year) is gradually increasing, due to urbanization and ongoing economic and social changes [7].

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In addition, the annual population growth rate is around 3.9%, with an annual increase in rice consumption estimated at 7.6% over the period 2008-2018, which partly explains the relative growth in demand for rice, which remains very strong. Despite the increase in production, the rate of coverage of local rice needs is falling, from 22.33% in 2010 to 17.27% in 2018 [8]. These fluctuations in local rice production are due to abiotic and biotic constraints [5].

Among the biotic constraints responsible for low rice productivity in Niger are yellow mottle disease and rice weeds [9, 10, 2]. Weeds cohabit with rice, compete with it for nutrients and light and transmit viral diseases to it, including rice yellow mottle, a disease that is endemic only in Africa and whose pathogen is the *Rice Yellow Mottle Virus*, RYMV [11, 12, 13].

Rice yellow mottle disease was first described in 1966 in Lake Victoria, Kenya [14]. The disease is not seed-borne [15] and is currently present in almost all rice-growing countries in East, Central and West Africa [16, 17, 18]. It appeared in Niger in 1990 [19], following the intensification of rice growing. Later, an epidemiological characterization of the country's rice-growing area reported the presence of the virus in all irrigated areas, with incidences and crop losses of around 71% and 90% respectively [12]. Production losses of 100% or total abandonment of infested plots have even been reported [11, 5].

Disease incidence and production losses can be considerable, depending on virus isolates, rice cultivars, transplanting date, type of rice cultivation and ecology. This impact is exacerbated by the presence of weed reservoir hosts associated with insect vectors that are omnipresent in and around cultivated rice plots [9, 15, 5]. This is why, given the permanent cohabitation of weeds with cultivated rice, it is necessary to characterize the rice-growing area of Niger in order to: (i) make an inventory of the most frequent rice weeds that are the most difficult to eradicate, (ii) determine farmers' rice weeds perception and their control methods, and (iii) identify the potential RYMV host-reservoir weeds present in the study area.

1.1.1. Study area

The study was conducted at 4 rice-growing sites in the urban commune of Tillabéri, located 115 kilometers (km) from Niamey, the capital, in the western part of the country, between the coordinates 1° and 2° longitude East; 14° and 16° latitude North. The sites concerned are the irrigated perimeters of Daibéri, Toula, Diambala and Namari Goungou, all located along the River Niger (Figure 1).

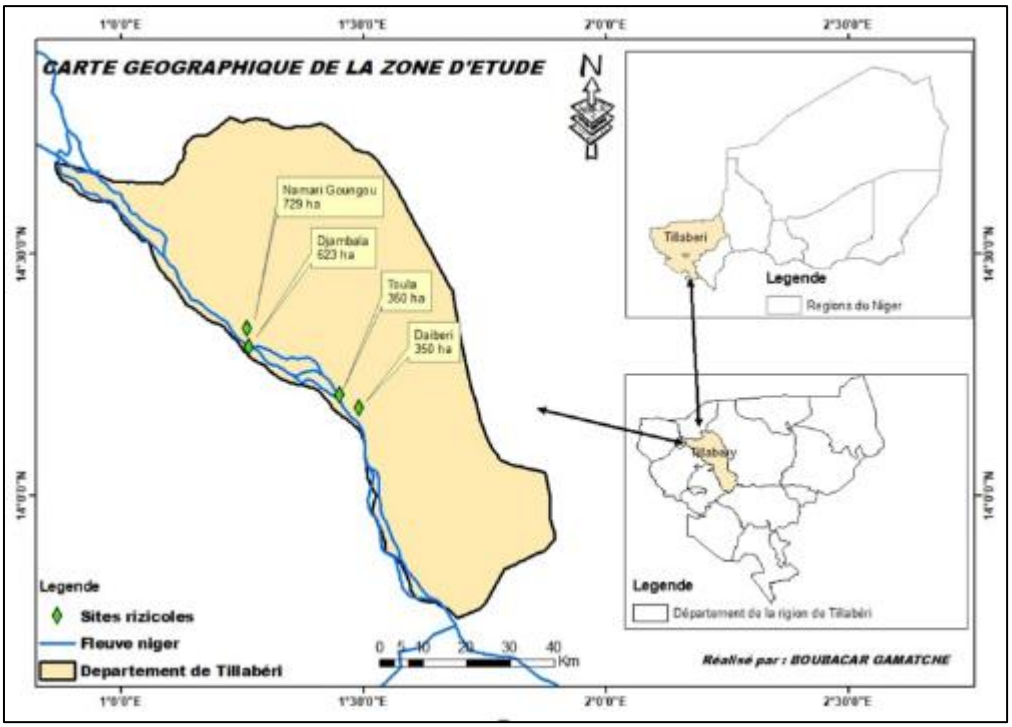

Figure 1 Location map of study sites

2. Materials and methods

2.1. Materials

The plant material consisted of the widely cultivated susceptible rice variety IR1529-680-3-1 and the various rice weed species collected in the study area.

The viral material consisted of four RYMV isolates collected from the 4 rice-growing sites selected. They will be used to characterize the weeds inventoried.

2.2. Study methodology

2.2.1. Rice weed collection, field visits and choice of experimental plots

Rice weed collections were carried out during the 2022-2023 dry season, at random, through surveys of the 4 rice-growing sites in the study area. They consisted of visiting the sites and taking random samples of rice weed leaves and/or stems in or around the grassed plots. Samples of weeds with or without disease symptoms were taken. During sampling, care was taken to wrap the hands in plastic bags and turn them over without touching the leaves with bare hands.

The experiments were conducted at the Toula rice-growing site. The choice of experimental plots consisted, in each section, of following two (2) selected plots, from transplanting to the bolting stage. In all, twelve (12) plots from the six (6) sections of the perimeter were involved.

2.2.2. Surveys of plot holders

The surveys were administered using a questionnaire sent to the farmers, regardless of their responsibility within the cooperative structure. Sampling was based on the list of farmers who were members of the cooperative in the area in question. From this list, 10% of the workforce was considered, i.e. 81 farmers. The purpose of the surveys was to determine farmers' perceptions and methods of controlling weed hosts and reservoirs of RYMV.

2.2.3. Determination of potential RYMV host-weed reservoirs

The weed samples sent to the laboratory were first characterized with a view to their identification; the identification of weed hosts and reservoirs of RYMV was carried out using the “lexicon of Niger plants” [20] and “weeds of Rice in West Africa” [21].

After identification, the weed samples were crushed and used as inoculum in a biological characterization test. The test consisted, firstly, in sowing seeds of the IR1529-680-3-1 variety in pots previously filled with clay soil, then, each weed sample retained with a pinch of fine sand was ground using a mortar.

The crushed material was used to mechanically inoculate young plants of the IR1529-680-3-1 variety in the greenhouse, two to three weeks old, using the method described by Séré et al (2007) [22]. A pool of 4 RYMV isolates originating from 4 rice-growing sites in the study area was prepared in parallel to index the same susceptible variety and serve as a control (Figure 2).

The expression of typical rice yellow mottle symptoms was the only parameter observed at 21 days after sowing (DAS).

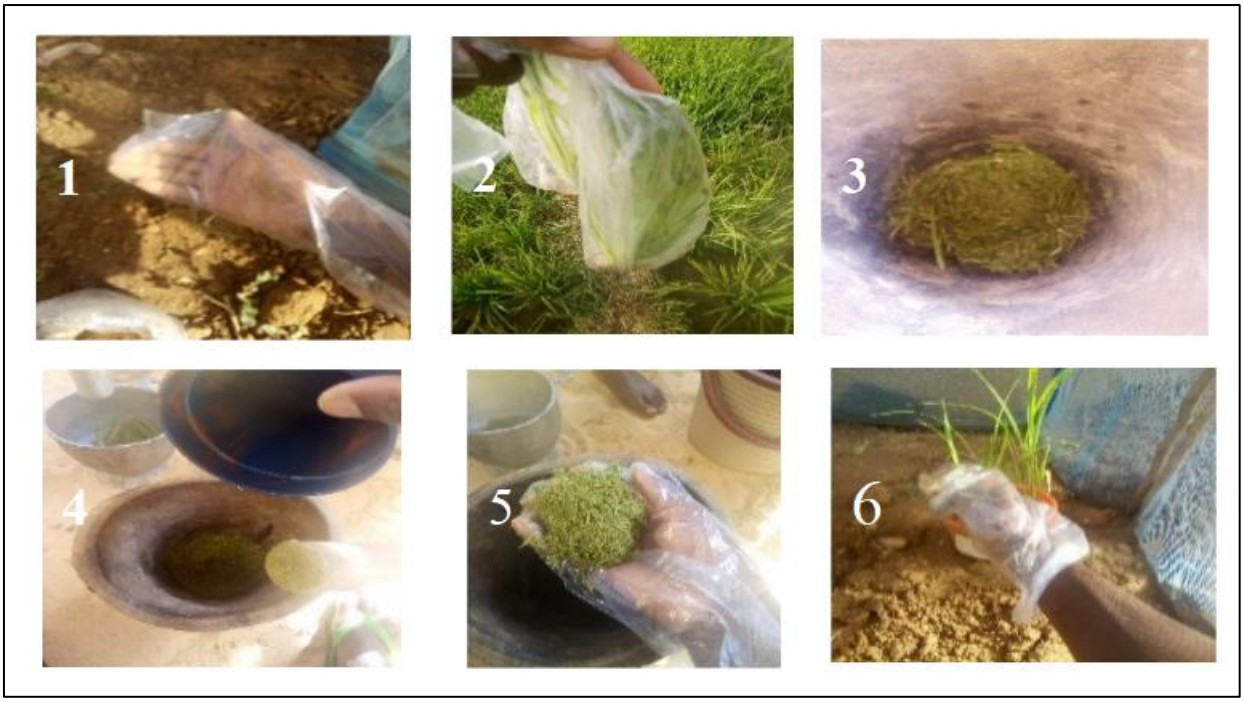

Figure 2 Preparation of weed shred and inoculation of plants at 21 days after sowing (DAS).
1. hand wrapping, 2. leaf collection; 3-4-5. Shredding and 6. Inoculation

3. Results

3.1. Inventory and identification of rice weeds

A total of 126 samples of rice weeds were collected during the survey of the study rice-growing sites. These included 60 samples from Toula, 26 from Namari-Goungou, 20 from Daibéry and 20 from Diambala.

After identification of the 126 weed samples collected in and around the rice fields (Table 1), three (3) major groups were inventoried, including the Grass / *Gramineae* (48.86%), *Cyperaceae* (16.79%) and Broadleaf *Herbaceae* (34.35%) families.

Table 1 Inventory of rice weeds collected

S/N	*Families*	Site (Number of samples)	Ecology	Total of samples	Proportion
1	*Poaceae-Gramineae*	Daibéry (12)	Irrigated rice fields and surrounding area	64	48.86%
		Toula (31)			
		Diambala (7			
		Namari-Goungou (14)			
2	*Cyperaceae*	Daibéry (5)	Irrigated rice fields and surrounding area	22	16.79%
		Toula (10)			
		Diambala (3)			
		Namari-Goungou (4)			
3	Broad-leaved Herbaceous plants	Daibéry (7)	Irrigated rice fields and surrounding area	45	34.35%
		Toula (20)			
		Diambala (10)			

		Namari-Goungou (8)			
Total				131	100%

3.2. Weed families and species identified

Observation, counting and identification of the rice weeds inventoried revealed 23 weed families, including one grass family, one Cyperaceae family and 21 broadleaf herbaceous families (Table 2).

The 21 families of leafy herbaceous plants are: *Amaranthaceae, Apocynaceae, Asteraceae, Caesalpinaceae, Capparidaceae, Convolvulacea, Euphorbiaceae, Juncacceae, Lythraceae, Marsilacea, Molluginaceae, Nymphaeceae, Onograceae, Phyllanthaceae, Plantaginaceae, Pontederiaceae, Portulaceae, Rubiaceae, Salviniaceae, Sphenocleaceae and Sterculiaceae).*

A total of 75 weed species from all families were counted. The Grass family comprised 28 species (37.34%), the *Cyperaceae* family 10 species (13.33%) and the 21 broad-leaved herbaceous families 37 species (49.33%).

Table 2 Number, families and species of rice weeds identified

S/N	Families	*Weed species (number of samples)*	Site/ species number	Number of species
1	*Poaceae-Gramineae*	*Agrostis stolonifera*, *Brachiaria lata (Schum.)*, *Cenchrus clandestinus*, *Cynedon dactylon*, *Dactyloctenium aegyptium (L.)*, *Digitaria cilaris*, *Digitaria sanguinalis*, Diplachne fusca, *Distichis spicata*, *Echinochloa colona* (L), *Echinochloa crus-galli*, *Eleusine indica*, *Glyceria declinata*, *Glyceria Maxima*, *Leersia hexandra*, *Leersia Oryzoides*, *Oryza barthii, Panicum repens L*, *Paspalum digitatum*, *Paspalum distichum*, *Paspalum scorbiculatum var., Phalaris arundinacea*, *Phragmites australis*, *Polygogon monspeliensis*, *Ranunculus flammula*, *Setaria verticillata*, *Setaria viridis*, *Zizania latifolia*	Daibéry (4) Toula(17) Diambala(2) Namari-Goungou(5)	28
2	*Cyperaceae*	*Cyperus difformis*, *Cyperus eragrostis*, *Cyperus esculentus*, *Cyperus fuscu,Cyperus iria*, *Cyperus rotondus*, *Pycreus macrostachyos Lam*, *Pycreus lanceolatus*, *Bolboshoenus planiculmis*, *Schoenoplectiella mucronata*	Daibéry (2) Toula(4) Diambala(2) Namari-Goungou(2)	10
3	*Broad-leaved Herbaceous plants	*Alternanthera ficoidea*, *Alternanthera sessilis*, *Celosia argentea*, *Gomphera serrata*, *Asclepias incarnata, Eclipta prostrata, Secenio inaequidens, Cassia occidentalis,Cleome viscosa L.,Convulvulus arvensis, Ipomoea aquatica,Euphorbia hirta L., Euphorbia hypericifolia,Juncus bufonius,Rotala Indica, Lythrum portula,Marsilea minuta, Marsilea quadrifolia, Mollugo verticillata, Nymphaea tetragona, Ludwigia octovalvis, Ludwigia peploides, Ludwigia palustris, Phyllanthus amarus (Schumach.& Tonn.), Veronica beccabunga, Zoysia japonica, Eichhornie Crassipes, Pontederia cordata, Portulaca oleracea, Ronuncolus flammula, Borreria scabra, Azolla filiculoides, Sphenoclea zeylanica, Melochiacor chorifolia L*	Daibéry (5) Toula (20) Diambala(5) Namari-Goungou(7)	37
Total					75

*The Broad-leaved Herbaceous plants are: Amaranthaceae, Apocynaceae, Asteraceae, Caesalpinaceae, Capparidaceae, Convolvulacea, Euphorbiaceae, Juncacceae, Lythraceae, Marsilacea, Molluginaceae, Nymphaeceae, Onograceae, Phyllanthaceae, Plantaginaceae, Pontederiaceae, Portulaceae, Rubiaceae, Salviniaceae, Sphenocleaceae et Sterculiaceae

3.3. Farmers' perceptions and methods of controlling rice weeds

3.3.1. Farmers' perception of rice weeds

Figure 3 shows the results relating to farmers' perception of rice weeds.

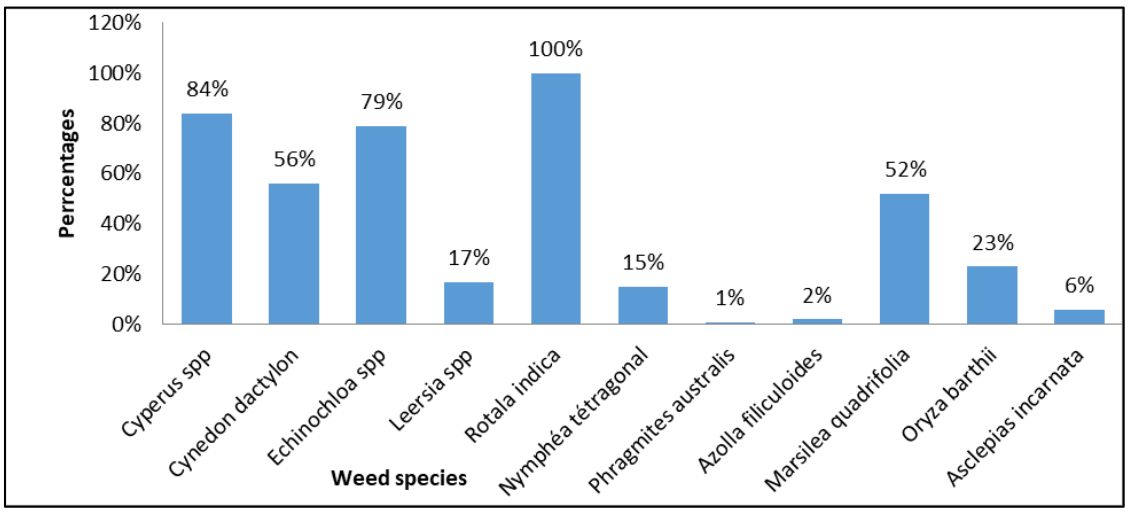

Figure 3 Weeds identified by the surveyed farmers

Analysis of Figure 3 shows that almost all the farmers recognized the species *Rotala indica, Cyperus spp* and *Echinochloa spp* as the most frequent weeds and the most harmful to rice in the experimental area. The species *Cynedon dactylon* and *Marsilia quadrifolia* were recognized by only half the growers surveyed. The other weeds *Oryza barthii, Leersia Oryzoides, Nympha tetragonal, Asclepias incarnata, Azolla filiculoides* and *Phragmites australis* were reported by few farmers.

3.3.2. Farmers' weed control methods

The results of the survey show that the control methods commonly used are manual weeding and the application of synthetic herbicides (LONDAX, GODAX). The results show that all the farmers surveyed use a combination of synthetic herbicides and manual weeding to control the most difficult rice weeds.

Figure 4 shows the proportions of farmers using the herbicide and manual weeding combination to control the most difficult rice weeds.

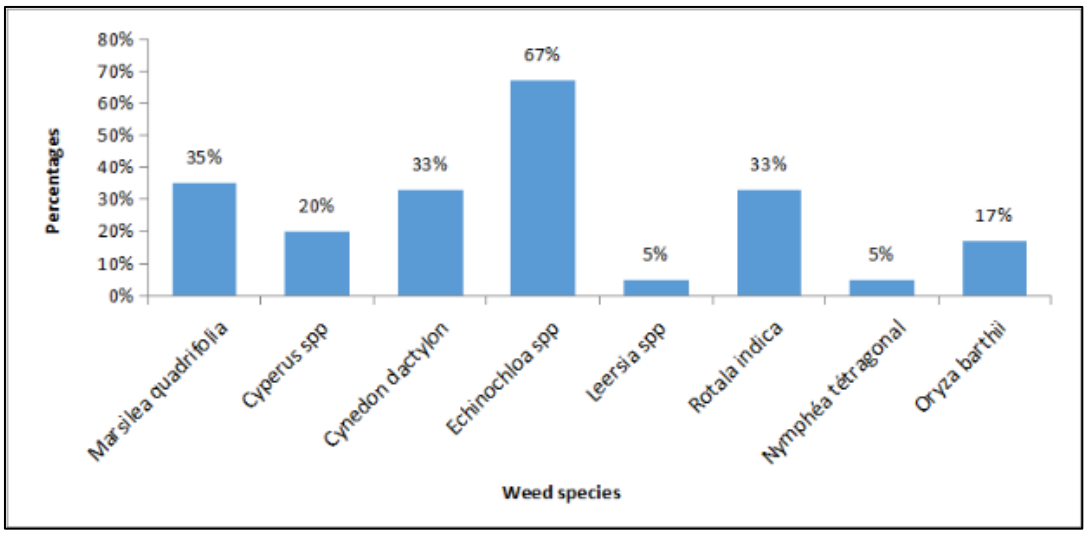

Figure 4 Proportion of farmers using the synthetic herbicide and manual weeding combination against the most difficult rice weeds to control

Analysis of this figure shows that the method based on the combination of synthetic herbicide and manual weeding is used more against *Echinochloa spp* (67%), *Marsilea quadrifolia* (35%), *Cynedon dactylon* (33%) and *Rotala indica* (33%) than against *Nymphea tetragonal* and *Leersia spp* (5%).

3.4. Inventory and frequency of potential RYMV host-weeds

At 21 days after mechanical inoculations (DAI) of shredded material from the most frequent weeds in the experimental perimeter on the susceptible variety IR1529-680-3-1, Twelve (12) weed species were declared to be RYMV host-weeds (Table 3). The species reported belong mainly to *Cyperaceae* (5 species) and *Grass* (7 species) families.

The frequency of virus host-reservoir species varies from one family to another and from one plot to another. *Grasses* (58.33%) predominate over *Cyperaceae* (41.67%). In the Grass family, the weed *Cynedon dactylon* is the most widespread species, followed by *Echinochloa colona*. In the *Cyperaceae family*, *Cyperus difformis* is the most common species in the area.

Table 3 Pathological profiles of rice weed species obtained after mechanical inoculation

Specie	Family	Typology of symptoms	Specie profil
Cyperus difformis	Cyperaceae	Leaf chlorosis	Reservoir host
Cyperus esculentus	Cyperaceae	Leaf chlorosis	Reservoir host
Cyperus Iria	Cyperaceae	Dwarfing and chlorosis of plants	Reservoir host
Cyperus distans	Cyperaceae	Dwarfing and chlorosis of plants	Reservoir host
Pycreus lancéoltus	Cyperaceae	Dwarfing and chlorosis of plants	Reservoir host
Echinochloa colona	Gramineae	Leaf chlorosis	Reservoir host
Echinochloa crus-galli	Gramineae	Dwarfing and chlorosis of plants	Reservoir host
Cynedon dactylon	Gramineae	Leaf chlorosis	Reservoir host
Leersia hexandra	Gramineae	Leaf chlorosis	Reservoir host
Leersia oryzoides	Gramineae	Dwarfing and chlorosis of plants	Reservoir host
Panicum repens	Gramineae	Leaf chlorosis	Reservoir host
Paspalum scorbiculatum	Gramineae	Leaf chlorosis	Reservoir host

Figure 5 below shows the five (5) potential host- reservoir weed species belonging to the *Cyperaceae* family. These are *Cyperus difformis, Cyperus esculentus, Cyperus iria, Cyperus distans* and *Pycrerus lanceolatus*.

The 7 host-reservoir species in the grass family are also shown in Figure 6 below. They are *Echinochloa colona, Cynodon dactylon, Echinochloa crus-galii, Leersia hexandra, Leersia oryzoides, Panicum repens* and *Paspalum scorbilatum.Echinochloa crus-galii, Leersia hexandra, Leersia oryzoïdes, Panicum repens* et *Paspalum scorbilatum*.

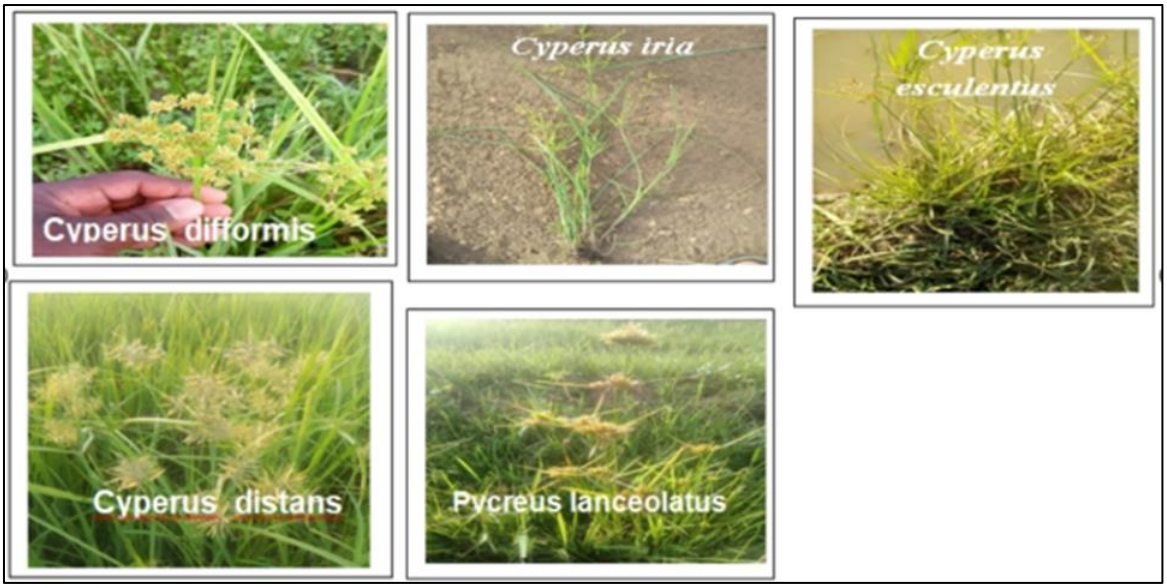

Figure 5 Cyperaceae species identified

Figure 6 RYMV host-reservoir grass species**.**

4. Discussion

The collection of weeds enabled an inventory to be made of those that cohabit with cultivated rice, thus proving that these weeds are indeed present on the study site. At the end of the study, twenty-three families of rice weeds, structured into 75 species, were inventoried in the rice-growing sites. These were the *Cyperaceae* family, comprising 10 species (13. 33%), the *Grass* family with 28 species (37.34%) and 21 families of broad-leaved herbaceous plants

(*Amaranthaceae, Apocynaceae, Asteraceae, Caesalpinaceae, Capparidaceae, Convolvulacea, Euphorbiaceae, Juncacceae, Lythraceae, Marsilacea, Molluginaceae, Nymphaeceae, Onograceae, Phyllanthaceae, Plantaginaceae, Pontederiaceae, Portulaceae, Rubiaceae, Salviniaceae, Sphenocleaceae and Sterculiaceae)* sudvived in 37 species (49. 33%). These results are similar to those of Ouattara, (2017) [23] who showed a predominance of broadleaf weeds on rice fields in western Burkina Faso.

The results of mechanical inoculation of shredded material from the most common weeds to the susceptible rice variety IR1529-680-3-1 (biological characterization) indicate that shredded material from twelve weed species produced typical symptoms of rice yellow mottle on the susceptible variety at 21 days after inoculation; suggesting that the said weeds are capable of harboring RYMV to transmit it later to cultivated rice. These results are similar to those of Issaka et al (2012) [12], who reported on the host-reservoir profile of 10 *Poaceae* and *Cyperaceae* species in around twenty rice-growing areas along the river in the Republic of Niger.

The 12 rice weed species that tested positive following inoculation were classified as RYMV host-reservoir species. They all fall within the host range of the virus. These species belong solely to the Grass (48.33%) and *Cyperaceae* (41.67%) families. Similar results were obtained by Allarangay (2008) [9] in Chad, Amancho et al, (2009) [10] in Côte d'Ivoire, Issaka et al, (2012) [12] in Niger, Ouattara, (2017) [23] in Burkina Faso and Mballo et al, (2018) [24] in Senegal.

All these authors have shown that the host range of RYMV is limited to the two families mentioned above (Grasses and Cyperaceae). The weed host-reservoirs identified include: (i) seven (7) grass species including *Echinochloa colona, Echinochloa crus-galli, Cynedon dactylon, Panicum repens, Leersia oryzoides, Leersia hexandra* and *Paspalum scorbilatum* and (ii) five (5) Cyperaceae species including *Cyperus difformis, Cyperus esculentus, Cyperus iria, Cyperus distans* and *Cuperus pycreus.* These results are similar to those of Issaka *et al,* (2012) [12] and Bouet *et al, (*2013) [25] who reported the presence of most of these weed species as RYMV reservoir hosts in Niger and Côte d'Ivoire respectively. The status of RYMV host-reservoir was earlier conferred to the species *Cyperus difformis* by Banwo and al., (2004) [26].

The evaluation of farmers' perception of weeds showed, on the one hand, that most farmers recognized certain rice weeds such as *Rotalia indica*, *Cyperus sp* and *Cynodon dactylon*, whereas these same farmers were unable to identify a good number of weeds such as *Oryza barthii, Leersia oryzoides* and *Nymphea quadrifolia. Echinochloa colona* is present in almost all the plots surveyed and is the predominant species (67%) within the Gramineae. These results are similar to those obtained in Senegal by Diagne, (1992) [27] and Mbalo and al., (2018) [24] who reported that *Echinochloa colona* was present in more than 98% of the plots visited; *E. colona* is the weed most difficult to control by farmers in the experimental area. The same observation was made in rice fields in Burkina Faso by Ouattara, (2017) [23]. However, our results differ from those of Issaka et al. (2012) [12], who reported that Eleusine indica is the most common weed in the rice ecology of Niger.

Among the Cyperaceae, the weed *Cyperus difformis* is present in most plots and predominates over the other *Cyperaceae*; this result is in agreement with that of Ouattara, (2017) on the characterization of rice weeds in Banfora (Burkina faso) and suggesting the said predominance. Furthermore, the predominance of *Echinochloa colona* and *Cyperus difformis* over other weeds was confirmed by other studies (Banwo et al., 2004; Issaka et al., 2012; Diagne, 1992) [26, 12, 27] which reported that the first weed species encountered in all rice management systems include *Echinochloa colona (L.), Cyperus difformis (L.)* and *Oryza longistaminata.* However, in Senegal, *Oryza longistaminata* appears to be the most difficult host-reservoir to control (Mbalo and al., 2028) [27]. Efficient management of rice weeds, in addition to being a crop maintenance technique, would help to reduce the source of RYMV inoculum in rice-growing sites (Allarangay *et al.,* 2008; Amancho *et al*., 2009; Issaka *et al*., 2013 and Bouet *et al*., 2013) [9, 10, 2, 25]. Indeed, rice weeds are thought to maintain the virus during the inter-season and transmit it to the rice crop during the rice season. Weeds therefore play a major role in the survival and transmission of RYMV in the field (Issaka et al., 2012) [26, 12].

5. Conclusion

Study reported that rice weeds were present in all the areas surveyed. A total of 75 species of rice weeds in 23 taxonomic families were inventoried. These were *Gramineae* family, *Cyperaceae* family and 21 other families of broad-leaved herbaceous plants. The latter constitute the largest group of weeds with 37 species, followed by *Gramineae* (28 species) and *Cyperaceae* (10 species). The biological results confer RYMV host-reservoirs profiles to 12 rice weed species. Among these species, *Echichnloa colona* (grasses) and *Cyperus difformis* (Cyperaceae) are the most frequent host-reservoirs and the most difficult to control by farmers. These weeds are thought to play a major role in the maintenance and spread of RYMV in rice-growing areas, because of their cohabitation with cultivated rice and insect vectors of the virus. These results will help to model the dynamics of the spread of RYMV populations in rice-growing sites in Niger and elsewhere in Africa.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare that they have no conflict of interest.

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