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Full Length Research Paper

Production of polyclonal antibodies to various strains of rice yellow mottle virus (RYMV) obtained across different agro-ecological zones in West Africa

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High titer polyclonal antibodies for serological diagnostic purposes were locally produced to forty isolates of rice yellow mottle virus (RYMV) collected from different sites across four West African countries (18- from Cote d'Ivoire, 2- from Burkina Faso, 5- from Mali and 15- from Niger Republic). This was achieved by immunizing rabbits with purified viral preparations. The primary goal was to obtain high titer, high affinity antisera for use in experiments and diagnostic tests for all WARDA collaborating partners (NARS). RYMV from each isolate was purified from infected rice leaves collected from each location. For quarantine and bio-safety reasons, none of the foreign isolate was used as inoculum for multiplication of virus in the screen houses in Bouaké, Côte d'Ivoire; instead, sufficient leaf samples were collected from different locations, transported and kept in the freezer until use for virus purification. Polyclonal antibodies were produced using the purified virus preparations with combination of complete and incomplete Freund's adjuvant. These high titer serological reagents, kept at the Africa Rice Center (WARDA), are available for use by all WARDA collaborators (National Agricultural Research laboratories) for identifying, classifying and typing of RYMV isolates.

Key words: Polyclonal antibody, rice yellow mottle virus (RYMV), agro-ecological zones of West Africa.

INTRODUCTION

The production of paddy rice (Oryza spp.), a staple cereal crop in most countries of Africa, was estimated at more than 12 million metric tons for Sub-Saharan Africa in 2003 (Food and Agriculture Organization of the United Nations - FAO, www.fao.org). Despite a continuous increase in rice area under cultivation, yields remained very low (1 to 3 t/ha) and thus, rice production does not fulfill the needs for feeding the growing population. Consequently, substantial quantities of rice are imported yearly - about 2 million metric tons of husked rice in 2002 (www.fao.org).

Rice production on the African continent is further threatened by rice yellow mottle, a disease caused by rice was yellow mottle virus (RYMV), genus Sobemo-virus which first noticed in 1966 in Kenya (Bakker, 1970). This virus is endemic in Africa, south of the Sahara, and occurs mainly in irrigated rice cultivation systems (Abo et al., 1998). RYMV has also been reported from several East, Central and West African countries, including the islands of Zanzibar and Madagascar (Bakker, 1970; Banwo et al., 2001; Reckhaus and Andriamasintseheno, 1997; Rossel et al., 1982). The virus infects most, if not all, wild rice species (*Oryza spp.*), and some other grass species (Awoderu, 1991; Bakker, 1974; Fauquet and Thouvenel, 1977). The wild rice species *Oryza longistaminata* is believed to form a natural virus source in the Soudano-sahelian zone. The virus may also be spread from infected *Oryza barthii* and the grass species *Echinochloa colona*, *Panicum repens*, and *Ischemia rugosum* (Konaté et al., 1997).

The observation that rice variety resistant to RYMV in one location could be susceptible in another location, led to the idea that there could be isolate variability of the virus. Consequently, the objective of this work was to collect different viral isolates from many localities and produce high titer, high affinity antisera to each of them. These antisera would then serve two purposes:

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i.) Serological diagnostic reagents for dissemination to all NARS collaborators,
ii.) Comparative serological analysis of RYMV in different location using agar gel diffusion. Results of the latter objective had been reported elsewhere (Séré et al., 2005, 2007).

MATERIALS AND METHODS

Sample collection

Intensive survey and sample collection in the major agro-ecological zones of Mali, Nigeria, Burkina Faso, Niger and Côte d'Ivoire where rice was produced in upland, lowland and irrigated conditions was carried out between 1997 and 1999. Leaves samples were collected based on typical RYMV symptoms. The samples were labeled with the name of the village/town of collection, stored in an icebox, transferred to the laboratory and stored in a freezer for subsequent processing.

Virus purification

Virus purifications from collected infected leaves were carried out using the method of Thottappilly and Rossel (1993), which is a modified version of Hull (1988) and Ball et al. (1988). Briefly, it entailed the following:

i.) Infected leaves were macerated in phosphate buffer (pH 7.1) into which the reducing agent ascorbic acid (1%) and equal volume of a mixture of Chloroform and n-Butanol (1:1) (as a clarifying agent) was added before emulsifying in a Warring blender.
ii.) The macerated and emulsified material was centrifuged at low speed (2000 rpm) for 20 min to remove plant debris while the aqueous layer was subjected to 10,000 rpm centrifugation to remove the residual plant material.
iii.) The pH of the solution was adjusted to 4.1 with HCL/NaOH and 10% Polyethylene glycol (PEG) and 1% NaCl were added and stired at 4°C for 2 h. This was centrifuged at 12,500 rpm for 15 min to precipitate the virus.
iv.) The precipitate was dissolved in phosphate buffer and centrifuged at 10,000 rpm for 10 min to remove any plant material still present in the preparation.
v.) Supernatant was subjected to centrifugation at 22,500 rpm for 2.5 h to precipitate the virus. The pellet was then dissolved in 100 Mm sodium acetate pH 5.0 and the particles were allowed to dissolve. It was the subjected to low centrifugation of 10,000 rpm for 10 min to remove any plant residue.
vi.) The virus preparation was then laid on 20% sucrose dissolved in sodium acetate buffer pH 5.0 (sucrose cushion) to remove host plant protein that may be present in the preparation.
vii.) The processing of the material through $CsCl_2$ was not performed.

Virus concentration was determined spectrophotometrically by taking 20 µl of the sample in 3 ml of sterile buffer, mixed and optical density (O.D.) read at 260 nm against blank distilled water and concentration calculated using 5.8 as the extinction coefficient.

Rabbit

Forty inbreed rabbits, progenies of two males and three females locally purchased rabbits, were used in the production of polyclonal antibodies. Each rabbit was kept in a locally made wooden cage fed with locally obtained rabbit feed supplemented with weekly ration of weeds. The rabbits were immunized with purified viral isolates intradermally at the back of the neck.

Polyclonal antisera

Purified RYMV suspension (from each isolate) of 0.5 mg (0.5 ml of 1 mg/ml), emulsified with 0.5 ml of Freund's complete adjuvant was used to immunize a rabbit; this was followed by three injections with emulsified suspension with Freund incomplete adjuvant. One week after the fourth injection, each rabbit was bled and serum collected. Briefly, the rabbit's ear was dilated with xylene and the marginal vein slightly lacerated; about 10 ml of blood was collected. The collected blood was allowed to clot in the refrigerator for about 2 h. The serum was removed (using a pipette), spun at 5,000 rpm to precipitate unneeded red blood cells. 0.1 mg/ml of sodium azide was added to the crude antisera to prevent bacterial growth. All the forty antisera produced were pre-adsorbed with healthy plant materials, as described by Pinner and Markham (1990) to remove antibody against host tissue.

RESULTS AND DISCUSSION

Polyclonal antibodies represent a group or mixture of antibodies produced by different B-lymphocytes in response to the same antigen; thus, different antibodies in the group recognize different parts of the antigen. The diversity of antibodies provides an advantage by allowing the detection of multiple epitope sites on the protein of interest.

Forty putatively different isolates of rice yellow mottle virus (RYMV), collected from four West Africa countries at different locations, were purified from infected leaves. For the antibody production, forty rabbits were immunized with these isolates. The titers of the polyclonal antibodies (using isolates that produced them), determined by checkerboard titration in Enzyme-Linked Immunosorbent Assay (ELISA) are shown in Table 1.

The production of antibodies in laboratory animals has become an essential part of many research projects. The production of these antibodies to specific and nonspecific antigens is a tool utilized in nearly all diagnostics fields, -biomedical research (Robson, 1995), Salmonella diagnosis (Ibebuike et al., 2008), plant virology (Thottappilly et al., 1998). Monoclonal antibodies that are discreet in certain epitopes have also been very useful (Agindotan et al., 2003, 2006).

As a necessary step in the diagnosis and management of RYMV, the production of high quality serological reagent is very crucial. Although polyclonal antiserum to any of the isolate could be identify the others but with less sensitivity, it was our desire in this work to produce antibodies to all available isolates for two major reasons: a.). It offers the excellent possibility of identifying, classifying and typing different isolates into their respective serological-group. As an International center with a regional mandate in rice, Africa Rice Center (WARDA) has the responsibility of collecting and storing of all available isolates of RYMV both in purified form and in leaves tissues (RYMV Bank). b) It will confirm or deny if, actually, there exists the phenomenon of "isolate variability" which renders incomparable the result of the same variety of rice artificially inoculated with the RYMV tested at different location. This was achieved by mixing all different antisera produced against each isolate together and testing the isolates with

Table 1. Different isolates of RYMV collected and the titer of produced polyclonal antibody. Titer was determined by check-board ELISA titration.

Isolate	Bottle number	Country of collection	Antibody titer
Mbé - site1	1	Côte d'Ivoire	1: 1000
Mbé - site 2	2	Côte d'Ivoire	1: 2000
Mbé - site 3	3	Côte d'Ivoire	1: 1000
Sakassou - site 1	4	Côte d'Ivoire	1: 1000 (2000)
Sakassou - site 2	5	Côte d'Ivoire	1: 1000
Sakassou - site 3	6	Côte d'Ivoire	1 : 1000
Sakassou - site 4	7	Côte d'Ivoire	1: 1000
Tapeguia - site 1	8	Côte d'Ivoire	1: 1000
Tapeguia - site 2	9	Côte d'Ivoire	1: 2000
Tapeguia - site 3	10	Côte d'Ivoire	1: 1000
Tapeguia - site 4	11	Côte d'Ivoire	1: 2000
Tapeguia - site 5	12	Côte d'Ivoire	1:2000
Tapeguia - site 6	13	Côte d'Ivoire	1:1000
Odienne - site 1	14	Côte d'Ivoire	1: 2000
Odienne - site 2	15	Côte d'Ivoire	1: 2000
Odienne - site 3	16	Côte d'Ivoire	1: 1000
Guehiebli - site 1	17	Côte d'Ivoire	1: 1000
Guehiebli - site 2	18	Côte d'Ivoire	1: 2000
Kollo cuvette-site 1	19	Niger Republic	1: 2000
Kollo cuvette-site 2	20	Niger Republic	1: 2000
Kollo cuvette-site 3	21	Niger Republic	1: 2000
Diomana-site 1	22	Niger Republic	1: 2000
Diomana-site 2	23	Niger Republic	1: 2000
Diomana-site 3	24	Niger Republic	1: 2000
Say-site 1	25	Niger Republic	1: 2000
Say-site 2	26	Niger Republic	1: 2000
Say-site 3	27	Niger Republic	1: 2000
Saga-site 1	28	Niger Republic	1: 1000
Saga-site 2	29	Niger Republic	1: 2000
Saga-site 3	30	Niger Republic	1: 2000
Bonfeba-site 1	31	Niger Republic	1: 2000
Bonfeba-site 2	32	Niger Republic	1: 2000
Kirkissaye	33	Niger Republic	1: 2000
Longorola(Sikasso)	34	Mali	1: 2000
Niono-site 1	35	Mali	1: 2000
Niono-site 2	36	Mali	1: 1000
Molodo-site 1	37	Mali	1: 2000
Molodo-site 2	38	Mali	1: 2000
Banzon (Banfora)	39	Burkina Faso	1: 1000
Banzon (Banfora) SC	40	Burkina Faso	1: 2000

them in agar diffusion test. The presence of "spur" formation confirms the presence of another antigenic determinant unique to such isolate. This is easier and simpler than PCR or other molecular technique in Africa where technology adaptation is still in its infancy, due to budgetary constraints. The results of this part of the work were reported elsewhere (Séré et al., 2005, 2007). Consequently these two objectives, one of which is reported here, were achieved.

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