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DEVELOPMENT OF ELISA AND RT-PCR ASSAYS FOR RAPID AND SENSITIVE DETECTION OF IMPERATA YELLOW MOTTLE VIRUS INFECTING MAIZE IN BURKINA FASO

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A B S T R A C T

Imperata yellow mottle virus (IYMV) is an emergent devastating plant viruses infecting maize in Burkina Faso. So, for further epidemiological studies and control of this virus, rapid, sensitive and specific methods are necessary. Two reliable serological assays [Indirect Antigen-Coated Plate enzyme-linked immunosorbent assay (IACP-ELISA) and indirect double antibody sandwich enzyme-linked immunosorbent assay (IDAS-ELISA)] and a reverse transcription polymerase chain reaction (RT-PCR) techniques were developed and compared for IYMV detection. Purified virion from a confirmed IYMV source was used as the immunogen to produce polyclonal antibodies elicited in rabbits and chickens against the virus. Two highly specific antibodies were produced and used for ELISA tests. IACP-ELISA and IDAS-ELISA were highly sensitive and specific for detecting IYMV in local sample collected in field. However, IDAS-ELISA system had a greater absolute sensitivity than the IACP one. Comparing ELISA test, RT-PCR was found more sensitive in detecting local IYMV isolate. The two newly developed serological assays are simple, effective and suitable for large scale indexing. These two assays, particularly the IDAS-ELISA, are useful for high throughput detection of IYMV in host plants and vectors identification. However, the rapid and inexpensive ELISA combined with the highly specific and sensitive RT-PCR are a practical approach for future epidemiological studies of IYMV and acquiring information about the viral genome of samples.

INTRODUCTION

Viruses are a major threat to agricultural crops.The emergence of new viruses is increasingly becoming a common occurrence, possibly as a consequence of agricultural intensification. To control these emerging diseases, developing accurate, quick tests to detect and diagnose the viruses in the host plants and any insect vectors remains very important.Early detection and identification of plant pathogens arean integral part of successful disease management.

In Burkina Faso, *Imperata yellow mottle virus* (IYMV) an emerging virus infecting maize was reported (Kaboré, 2002;Sérémé *et al*., 2008; Sérémé, 2010). IYMV is an RNA virus belonging to the Genus *Sobemovirus*. The complete genome sequence of IYMV consists of 4,547 nucleotides and harbors four open reading frames (ORFs) (Sérémé *et al*., 2008). Infected plants exhibit variable symptoms, ranging from yellow discolouration to mottling of leaves, and stunting (Sérémé *et al*., 2008). The virus has been described in most region of Burkina Faso but it is not presently known if the virus occurs elsewhere in Africa. However, typical symptoms of yellowing and mottling were observed on *Imperata sp*. in Mali (Traoré and Sérémé, 2010). The only known transmission way of this virus is by contact or infected plant material. IYMV disease is a typical example of the need for a quick and sensitive diagnostic procedure to detect the infected material as well as maize is one of the most important food crops of Sub-Saharan Africa. Unfortunately, up to day, only the detection of IYMV by Indirect Antigen-Coated Plate enzyme-linked immunosorbent assay (IACP-ELISA) was reported (Sérémé *et al*., 2008). Add to this, since the description of IYMV (Sérémé, 2005; Sérémé *et al*., 2008), reverse-transcription polymerase chain reaction (RT-PCR) has never been used to detect IYMV in maize. To date, there are no reports comparing ELISA and RT-PCR in detecting IYMV in maize or *Imperata sp*. tissues. The goal of this study was to develop a reliable indirect ELISA and to compare the efficacy of ELISA and RT-PCR in determining IYMV infection and evaluate their use as a diagnostic tool to study IYMV epidemiology in maize and *Imperata cylindrica*. To our knowledge, this is the first study performed worldwide on detection of *Imperata yellow mottle virus* in naturally infected field-grown samples, comparing ELISA and RT-PCR in different source materials. The evidence provided in this

paper showed the usefulness of these two assays (ELISA and RT-PCR) for field-collected samples. We believe that these serological assays, particularly the IDAS-ELISA, can be used for high throughput detection of IYMV infection during field surveys at a low cost. Although, RT-PCR is not appropriate for large scale screening, it showed the best sensitivity than the other two serological methods, may be valuable for acquiring information about the viral genome of samples.

MATERIALS AND METHODS

Sources of virus and field samples

Imperata cylindrica plants showing IYMV characteristic yellow discoloration and mottling symptoms were collected from rice fields in Banzon in Burkina Faso. The virus was inoculated to maize plants *via* mechanical transmission and the inoculated plants were maintained in a greenhouse till virus purification. Field samples showing yellow mottle symptoms were collected from fieldsin the south-western region of Burkina Faso, and stored at -80 °C till use.

Virus purification

Virus was purified from inoculated maize plants after symptoms appearance according to Bakker (1974) with some modifications as described by Sérémé (2005). The concentration of the purified virus particles was estimated by spectrophotometry, assuming an extinction coefficient of E (0.1%, 260 nm) = 6.5, based on the value calculated for RYMV another sobemovirus (Bakker, 1974).

Preparation of viral antigen

Purified Imperata yellow mottle virus was used as viral antigen. Virus was purified from inoculated maize (var. FBC6) plants after symptoms appearance according to Sérémé *et al*. (2008).

Animals and husbandry

Three (2 males and 1 female) rabbits, 2 months old and 1.97 kg body weight, were obtained from the Programme de Development des animaux villageois, based at Ouagadougou, Burkina Faso.Two 40-week-old Bovans Nera hens with 1.65 Kg each were graciously provided by the Animal and livestock Department of the Environment and Agricultural Research Institute (INERA), Burkina Faso. Rabbits and hens were kept singly in 1 m x 0.5 m floor pens. The temperature in the room was 25°C, with a relative humidity ranging from 65 to 70 %. Water was provided *ad libitum.*

Antibodies anti-IYMV production

Immunization of chickens. The two laying hens were immunized weekly by one subcutaneous injection of 500 µl of emulsion containing 0.5 mg of purified IYMV with 500 µl of the incomplete Freund's adjuvant (Calbiochem, Corp., La Jolla CA, USA). The study was conducted in accordance with the Regulations for the Care and Use of Laboratory Animals (Ministry of Animal and livestock, Burkina Faso). Eggs were collected daily, beginning 3 weeks after the first injection, and stored at 4°C until analysis.

Immunization of rabbits. The immunization of rabbits with purified IYMV were performed as described previously by Sérémé *et al*. (2008). Briefly, 0.5 ml of freshly purified IYMV suspension at a concentration of 1 mg/ml was emulsified with equal volume of Freund's incomplete adjuvant and used to immunize a rabbit. Four weekly intramuscular injections of virus preparation were given, alternately using left and right thigh muscles. One week after the fourth injection, the rabbit was bled, and the serum was collected and allowed to clot for about 1 h at room temperature. Then, the clot was loosened with a glass rod to permit separation and left overnight at 4°C. The clot-free liquid was transferred into a tube and centrifuged for 20 min at 1500 g at 4°C. Final serum was stored at 4°C and used for ELISA assays.

IgY production and purification

Two collected eggs were used for IgY extraction and purification. PEG precipitation method described by Polson *et al*. (1980, 1985) was performed. Egg yolk was diluted 1:9 with cold (4°C) distilled water and homogenized for 1 min using a waring blender at high speed. The pH of egg yolk solution was adjusted to pH 5.0 with 1 N HCl. An aliquot of the pooled yolk was diluted 1:2 in PBS containing 0.1 M NaCl and precipitated with PEG 6000 to a final concentration of 3.5% to remove fatty substances. The mixture was centrifuged 10 mn at 10 000 g. IgY were precipitated from the resulting supernatant using 12% PEG 6000, then dissolved in PBS and re-precipitated with 12% PEG 6000. The mixture was centrifuged 50 mn at 14 000 g to remove residual PEG. An emulsification step was incorporated by adding 3% chloroform and re-centrifugation 10 mn at 10 000 g. The supernatant contains purified IgY. Final IgY preparation was stored at 4 °C.

Enzyme-linked immunosorbent assay (ELISA) test

Detection of IYMV particles in purified preparations or in sap extractions of infected leaf tissues was carried out following the standard procedures for IACP-ELISA (Séré *et al*., 2007) and IDAS-ELISA (Clark and Adams, 1977).

For all ELISA tests, leaf extracts used as sources of antigens were obtained by grinding, with a mortar and pestle, leaf samples (1 g) in 10 ml of phosphate-buffered saline, pH 7.4 (PBS) containing 0.05% Tween 20 and 2% polyvinyl pyrolidone (PVP, MW about 24,000). The resulting homogenate was centrifuged for 10 min at 10 000 g. The supernatant was collected and used for virus detection. All buffers used were as described by Clark and Adams (1977). Each sample was put in duplicate wells and four (4) wells filled with healthy maize samples were included in each plate as negative controls. Infected maize plants were used as positive control. Absorbances at 405 nm were recorded using a Metertech Σ960 automatic microplate reader. Three times the mean A405 nm readings from healthy samples was taken as the negative-positive threshold

Determination of the antibodies working dilution/Selection of serum and IgY dilution. The working dilutions of the anti-IYMV serum, IgY, and the goat anti-rabbit IgG conjugated with alkaline phosphatase for IACP and IDAS-ELISA were determined by phalanx tests. Briefly, for IACP-ELISA, the lane wells of ELISA plates coated samples were respectively added four-fold diluted and incubated. The row wells of plates were dispensed the goat anti-rabbit IgG conjugated with alkaline phosphatase at 1: 5,000 dilution and incubated. The alkaline phosphatase conjugate was detected with p-nitrophenyl phosphate. For IDAS-ELISA, the lane wells of ELISA plates were respectively coated two-fold diluted IgY and incubated. After sample incubation, two-fold diluted anti-

IYMV serum were respectively dispensed in row wells of the ELISA plates and incubated. Goat anti-rabbit IgG conjugated with alkaline phosphatase at 1:5,000 dilution was subsequently applied into the wells and incubated. Negative and positive controls were wells incubated with maize extracts from healthy leaf and IYMV-infected leaf tissues, respectively. The bound conjugate was detected using p-nitrophenyl phosphate solution and the plates were read at 405 nm.

IACP-ELISA and IDAS-ELISA for IYMV detection. Indirect-antigen coated-plate IACP-ELISA was performed as follows. A dilution series of virus saps extracts ranging from 1:10 to 1:163,840 dilutions were directly adsorbed toImmunoplates (Nunc) and incubate at 37°C for 2 h. After washing the plates three times with PBS-T, the remaining free-binding sites were blocked with 200 µl of blocking solution (3% dried skimmed milk in PBS-T) each well at 37°C for 1 h. Afterwards, the plates were washed three times with PBS-T and 100 µl diluted anti-IYMV serum was added to each well and incubated at 37°C for 2 h. The contents were removed, washed as above, and at 1:5,000 dilution of goat anti-rabbit alkaline phosphatase conjugate (Sigma-Aldrich) in PBS-T was added to each well and incubated for 2 h at 37°C. The conjugate was removed, the plates washed as above, and 100 µl aliquots of Para-nitro phenol phosphate (PNP) solution (Sigma-Aldrich) at 1.0 mg/ml in 9.7% diethanolamine, pH 9.8, was added to each well,and plates were further incubated in the dark for 2 h at room temperature. The absorbance values were recorded at 405 nm as described above.

IDAS-ELISA was performed by the following procedure. Polystyrene microtiter plates were coated with 100 µl of IgY (at 12.5 µg/ml from immunized chickens with purified IYMV) and incubated at 37°C for 2 h. The plates were washed three times with PBS-T. All subsequent ELISA steps (blocking buffer, crude extracts, anti-IYMV serum, anti-rabbit conjugate and substrate incubations) were as described above for IACP-ELISA.

Total RNA extraction and PCR amplification

RNA extraction. Total RNA was extracted from 100 mg infected plant samples using the Qiagen Plant RNeasy Mini kit following the manufacturer's instructions. The resultant total RNA was eluted in 50 µl nuclease-free H_2O. RNA concentrations were measured using a spectrophotometer and the quality of RNA was determined by RNA gel electrophoresis. Total extracted RNA was stored in aliquots of 10 µl at -20 °C until required.

A two-step reverse transcription-polymerase chain reaction (RT-PCR) protocol was performed with total nucleic acid extracted from infected plant samples.

PCR amplification of cDNA. For RT-PCR analysis, specific IYMV forward primer SDiymvF6 5′-GGCCCTTCTCGGAGTCTTGG-3′, corresponding to the IYMV nucleotide position (3722-3741) and reverse primer SDiymvR5b 5′-GCCTCTCATGGCAACTCTCC-3′, corresponding to the IYMV nucleotide position (4423-4404) were designed according to the IYMV sequences available at the GenBank(AM990928) and used to detect the virus in leaf samples through RT-PCR. This primer pair generated *ca.* 702 bp products, including the 660 pb (3722-4381), out of the 822 (3560-4381) which form IYMV CP ORF.

First-strand cDNA was synthesized with the reverse transcriptase polymerase chain reaction (First-strand RT-PCR kit, Stratagene Ltd) following the manufacturer's instructions, with 7.5 µl of RNA in a 20 µl volumeand using the reverse primer SDiymvR5b. Briefly, 7.5 µl of total RNA was denatured at 65°C for 5 min in the presence of 1 µl of 100 µM reverse primer and 2 µl of 5mM dNTPs in a total volume of 12 ml. An aliquot of 7 µl of reaction mixture containing 2 µl 100 mM DTT, 1 µl of RNAsin (Invitrogen) 20 U/µl, and 4 µl 5X M-MLV RT buffer was added and incubate at 37°C for 2 min. To this mixture was added 1 µl M-MLV-RT (Invitrogen) 200 U/ µl and the RT reactions were performed at 37°C for 50 min, followed by 15 min at 70°Cin an automatic thermal cycle, Perkin-Elmer 9600. RT product was used as a template for the PCR reaction.

The PCR amplification reaction contained 5 µl of 10X Dynazyme reaction buffer, 2 µl of a 10 mM solution of the four deoxynucleotide triphosphates (Promega), 1 µl of 10 µM reverse primer SDiymvR5b, 3 µl of 10 µM reverse primer SDiymvF6, 2 units of Taq DNA Polymerase DyNazyme (Finnzymes OY, Espoo, Finland) and sterile H_2O to 50 µl total volume. The reactions were amplified in a Perkin Thermal Cycler with a cycle profile of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and elongation at 72°C for 1 min for 35 cycles giving the last cycle 10 min elongation step at 72 °C and a final hold step at 4 C.

Amplification products were analysed by agarose (Gibco BRL, electrophoresis grade) 1 % gel in 0.5 X TBE buffer, stained with 0.5% ethidium bromide (Sambrook *et al*., 1989), and visualised, on UV transilluminator, for the presence or absence of PCR bands.

RESULTS

Virus purification. Virion of IYMV was purified from infected maize leaf tissues harvested at 21 days post inoculation (dpi) by differential centrifugation using Optima L-70 Ultracentrifuge (Beckman).

Preparation of polyclonal antibodies against IYMV. Purified IYMV virion was used to immunize rabbits and chickens. After the forth immunization, rabbit was bled, and the serum collected. For chickens, three weeks after the first injection, eggs were collected daily and used for IgY extraction and purification. The IgY yield of the ascitic fluids containing these antibodies was 12.5 µg/ml. The titer of the polyclonal antibodies in ascitic fluids was 1:128,000,000 by an indirect-ELISA. These antibodies were used in IACP and IDAS-ELISA for IYMV detection.

IACP-ELISA and IDAS-ELISA for IYMV detection. IACP-ELISA and IDAS-ELISA, were used for detection of IYMV samples. The working dilutions of the polyclonal antibodies serum (PAb), the goat anti-rabbit IgG conjugated with alkaline phosphatase in ACP- and IDAS-ELISA were determined by phalanx tests. The results of three independent ELISA assays indicated that the dilution of PAb at 1:102,400, goat anti-rabbit IgG conjugated with alkaline phosphatase at 1:1,000 were suitable for IACP-ELISA, and the dilution of PAb at 1:400,000, IgY at 12.5 µg/ml, and goat anti-rabbit IgG conjugated with alkaline phosphatase at 1:1,000 were suitable for IDAS-ELISA.

Sensitivities of these antibodies for IYMV detection were determined through IACP-ELISA and IDAS-ELISA using 1:10 to 1:5,120 diluted crude extracts from the IYMV maize infected plant tissues. Results showed that the highest leaf extract dilutions for the two methods was 1:640 (Fig. 1a) indicating that both IACP-ELISA and IDAS-ELISA were highly sensitive and specific for detecting IYMV. These results indicated that these methods are sensitive and specific for detecting IYMV in field samples. Nevertheless, IDAS-ELISA gave higher optical density and seemed to be a more reliable and sensitive serological method (Fig. 1a). Consequently, IDAS-ELISA was selected for the further assays.

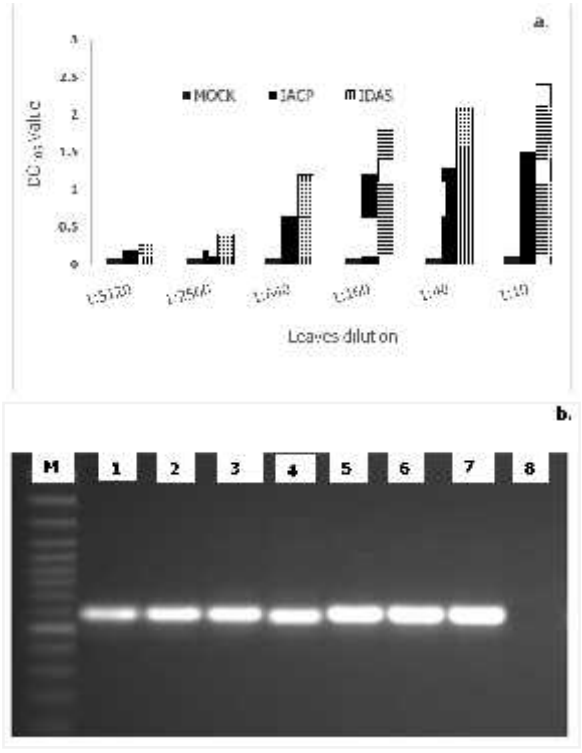

Fig. 1 Sensitivity analysis of the IACP-, IDAS-ELISA and RT-PCR with IYMV-infected tissue extracts. **a.** Sensitivity analyses of IACP- and IDAS-ELISA for IYMV detection. IYMV-infected leaf extracts and healthy leaf extracts (MOCK) were two-fold diluted in PBS buffer from 1:10 to 1:5120 (w/v, g/ml). **b.** Sensitivity analyses of RT-PCR for IYMV detection. Lane M, 100 bp marker. IYMV-infected leaf tissue saps with different dilution from 1:10 to 1:5120 (w/v, g mL^{-1}) corresponding to lane 6 to lane 1. Lane 7 was IYMV-infected maize used as positive control and lane 8 was healthy maizesapused as negative control.

Detection of IYMV in uncharacterized field samples. To determine the usefulness of these methods for field samples, crude extracts from 53 field samples, showing mosaic, mottling, or necrosis symptoms and collected from various crops and wild grasses in different localities of Burkina Faso, were tested by both IACP- and IDAS-ELISA for the presence of IYMV. Of the 53 samples, 11 were tested positive for IYMV infection by the two procedures IDAS-ELISA (Table 1). IDAS-ELISA failed to detect RYMV, another Sobemovirus, in rice RYMV infected sample indicating that there is no cross reactivity. The samples with positive reaction in IDAS-ELISA were then inoculated to *Rottboellia exaltata* and maize (var. FBC6). In all instances, they caused yellow mottle symptoms on the leaves of the above mentioned indicator plants. Interestingly, this result was later validated through RT-PCR. Results showed that the newly developed IDAS-ELISA could be used to detect the virus in field samples.

Table 1 Detection of IYMVin various field sources from different geographic origin

N°	Hosts	Sample source	Field symptoms	ELISA	
				IDAS	IACP
1	Andropogongayanus	Orodora	yellow	0/3[(a)]	0/3[(a)]
2	Brachiaralata	Banfora	necrotic	0/1	0/1
3	Echinochloacolona	Banfora	necrotic	0/1	0/1
4	Eleusineindica	Banzon	yellow	0/2	0/2
5	Imperatacylindrica	Banfora	Mottle, streak	14/14	14/14
6	Oryzalongistaminata	Banzon	mottle	0/3	0/3
7	Panicum infestum	Banzon	mosaic	0/2	0/2
8	Paspallumscrobiculatum	Banfora	mosaic	0/2	0/2
9	Pennisetumpedicellatum	Orodara	yellow	0/1	0/1
10	Rottboelliaexaltata	Banfora	necrotic	0/13	0/13
11	Setariabarbata	Orodara	yellow	0/3	0/3
12	Sorghum bicolor(sorgho)	Banfora	mottle	0/2	0/2
13	Zea mays (maïs)	Vallee du Kou	mottle	2/4	2/4
14	Rice infected by RYMV	Karfiguéla	Mottle, streak	0/2	0/2

[a] Number of samples detected positive/number of samples collectedduring the survey

RT-PCR detection of IYMV isolates.

An RT-PCR method was successfully developed to detect IYMV (Fig. 1b). The primers described above were designed for the amplification of a 702bp fragment of the IYMV genomic RNA (Fig. 1b, 2). Visible bands were observed for the purified virus as well as for the diluted sample (Fig. 2).

A single band (~702bp) including the partial IYMV CP gene was amplified by RT-PCR from both purified virus and diluted IYMV-infected maize samples,while no signals were observed from the healthy *I. cylindrica*, maize or RYMV-infected rice plants (Fig. 2). No differences between the different IYMV infected samples were observed in the mobility of the amplified product after RT-PCR (Fig. 2).

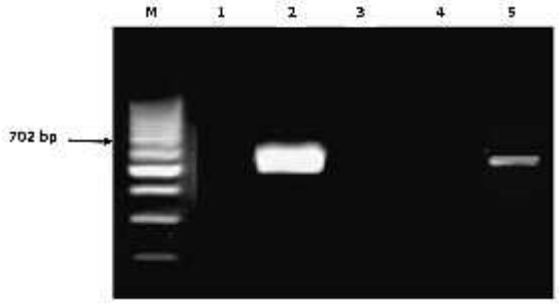

Fig. 2 Analysis of RT-PCR amplification of IYMV -RNA from maize infected samples. M, 100 bp marker, 1, healthy maize sap used as negative control; 2, purified IYMV; 3, healthy *I. cylindrica* sap; 4, RYMV-infected rice plants; 5, maize infected with IYMV. Electrophoresis was performed on a 1% agarose gel stained with ethidium bromide.

Comparison of ELISA and RT-PCR

Since IDAS-ELISA was identified as more reliable and sensitive serological method than IACP one for IYMV detection, it was then used for further assays. Detection of IYMV by ELISA and RT-PCR were compared by analysing a group of biologically characterized two IYMV isolates collected on *I. cylindrica* at Banzon and Banfora, two IYMV isolates collected on maize at Vallee du Kou, and one*Rice yellow mottle virus* (RYMV) isolates from Karfiguela (Table 1). IYMV was detected by the two procedures in all isolates of IYMV independently to origin, but not in the other two RYMV isolates. Interestingly, IYMV was not detected in these two isolates by any of the two procedures tested.

Samples positive by IDAS-ELISA, were also positive by RT-PCR, and those reacting negatively by IDAS-ELISA also failed to react by RT-PCR (Fig. 3).RT-PCR was able to detect IYMV in maize infected plant tissues diluted up to 1:5,120, while IDAS-ELISA detection limit was just 1:640 (Fig.1) These results indicated that RT-PCR is more sensitive and specific than the serological technique used in this study.

Fig. 3 Detection of IYMV in field-samples by RT-PCR. Lane M, 100bp marker; 1, negative control (water used as sample), 2, maize infected with IYMV and used as positive control; 3, IYMV-infected maize collected at Vallee du Kou; 4, IYMV-infected maize collected at Vallee du Kou 5, IYMV-infected *I. cylindrica* collected at Banzon; 6, IYMV-infected *I. cylindrica* collected at Banfora; 7, rice infected with RYMV collected at Karfiguela; 8, healthy maize sap.

DISCUSSION

Imperata yellow mottle virus (IYMV) is an emerging pathogen infecting Imperatacylindrica and maize in Burkina Faso (West Africa). To study the epidemiology and characterize IYMV isolates, it is necessary to develop quick and accurate diagnostic methods, applicable to large numbers of samples. Unfortunately, although Imperata mottle disease have been studied in Burkina Faso since 2005, there are only a few reports on these diseases based on symptomatology, biological indexing and electron microscopy (Sérémé, 2005; Sérémé *et al*., 2008). In this study, we report the development and evaluation of antibody-based ELISA (IACP- and IDAS-) and RT-PCR that are potentially practical, useful tool in IYMV surveillance and diagnosis in Burkina Faso. These assays were compared to determine their sensitivity, specificity, and efficiency as well as their advantages and limitations for IYMV diagnosis.

We have evaluated and compared IACP-ELISA and IDAS-ELISA for the detection of IYMV in field sample. The two ELISA formats were able to detect IYMV in field sample indicating that both IACP and IDAS formats are specific and efficient in detecting the virus. These results are consistent with those reported by Sérémé *et al*. (2008). However, the IDAS test had a greater absolute sensitivity than the IACP one for the detection of IYMV antigen. The increased sensitivity of IDAS-ELISA over IACP-ELISA using the same rabbit anti-mouse immunoglobulins conjugated to alkaline phosphatase, is probably due to two factors.

Firstly, in IACP format, antigen is bound directly to the polystyrene surface. Therefore, quantitation is directly affected by the binding event. Thus, because most proteins are bound to the polystyrene in the coating buffer, it is expected that there would be a competition for available sites by host proteins and viral antigens in crude plant extracts. This interference phenomenon was demonstrated and precluded the use of indirect ELISA for accurate quantitation of viral antigens in crude extracts (Ahoonmanesh *et al*., 1990). Secondly, the use of chickens IgY in IDAS format to coat the polystyrene, introduced an amplification step. Indeed, chicken IgY can react with many epitopes of mammalian antigens due to phylogenic distance between birds and mammals, resulting in amplification of signals. Similar results were reported by many authors (Leslie and Clem, 1969; Altschuh *et al*., 1984; Larsson *et al*., 1991, 1992; Davalos-Pantoja *et al*., 2000). According to these authors, chicken antibodies do not react with mammalian IgG; they do not induce false positive results in immunoassays because they do not activate mammalian complements.

Since, our results have shown IDAS-ELISA to be more sensitive than IACP-ELISA, sample collected from field grown plants were subjected to both IDAS-ELISA and RT-PCR assays and the results were compared. While only a small number of plants were analyzed both IDAS-ELISA and RT-PCR were able to detect the same infected plants that exhibited no viral symptoms. Our results indicate that IYMV can be readily detected, not only by different ELISA formats using antibodies to IYMV, but also by procedures detecting viral RNA with coincident results. Moreover, the information of viral genome can be obtained from sequencing analyses of amplified products of RT-PCR. It is well established that the RT-PCR for RNA virus detection is more sensitive than serological methods and our results proved that again. Then, the procedure of choice on each occasion will depend on the diagnosis purpose and on the facilities available. Even RT-PCR method is considered the most sensitive method for viral RNA detection, it is complicated, time-consuming, expensive, and not appropriate for large scale indexing. So, because of the relative easy and inexpensive cost of ELISA versus RT-PCR, and the results of the current study showing no difference in detection of IYMV, ELISA remains the best choice for routine evaluation of IYMV infection in maize.

However, as in most diagnosis situations, the safest decision is to use more than one detection procedure. The above methods can contribute to simplify and improve the accuracy of future studies on epidemiological studies and the search for IYMV vectors. They canalso assist further research to determine the inoculum concentration thresholds of IYMV in maize growing areas in predicting the risk of disease development.This will lead to better disease management strategies as appropriate preventive measures can be identified more quickly before the spread of IYMV can take place.

In conclusion, both two developed serological assays (IACP- and IDAS-ELISA) in this study are suitable for sensitive, rapid and routine IYMV detection of large-scale samples in the field survey, while RT-PCR is more sensitive and suitable for acquiring information about the viral genome.

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