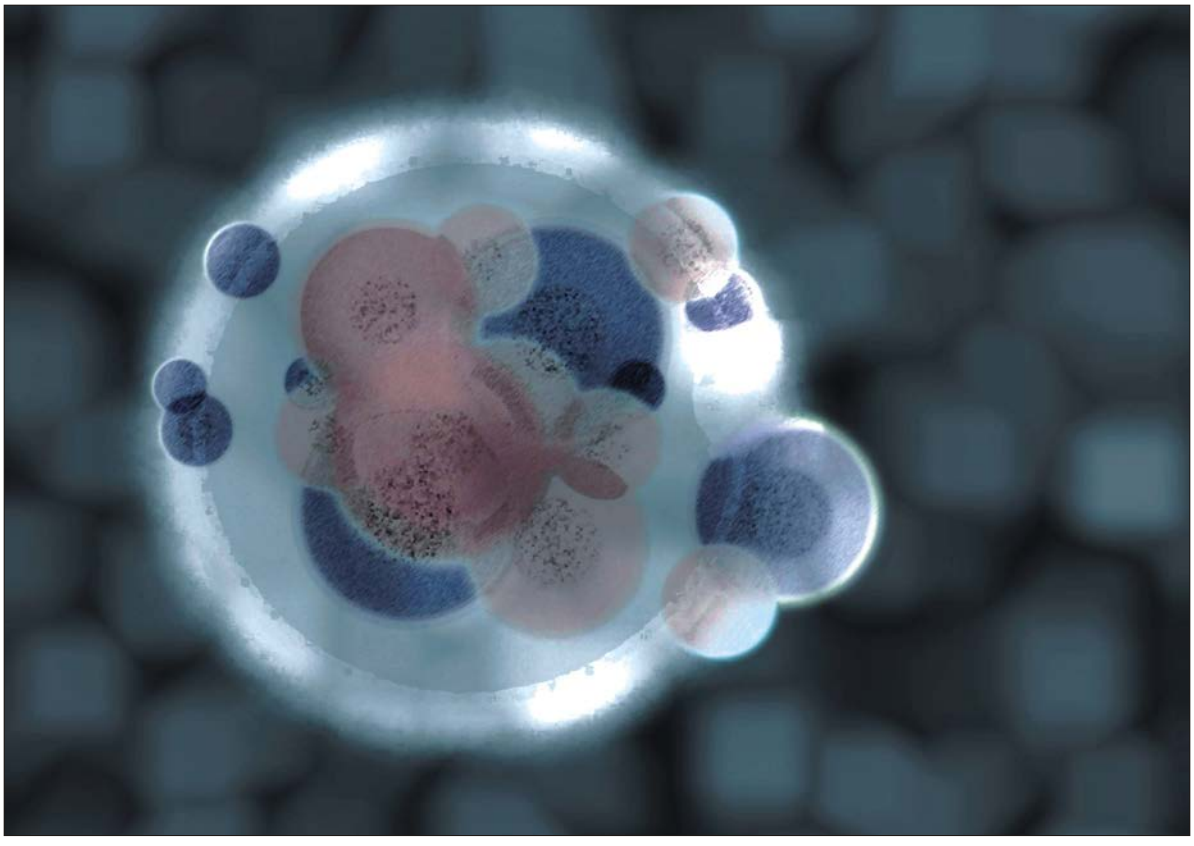

Journal of Biological Sciences

ISSN 1727-3048

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Journal of Biological Sciences 7 (7): 1147-1154, 2007
ISSN 1727-3048

Serological Differentiation Indices and Phylogenetic Analysis of *Rice yellow mottle virus* Isolates in Cote d'Ivoire

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Abstract: Serological diversity of 178 RYMV isolates was determined by phylogenetic analysis of Serological Differentiation Indices (SDI) data generated from antigen coated-plate enzyme-linked immunosorbent assay (ACP-ELISA) using 26 RYMV Polyclonal antisera. These RYMV isolates were obtained from northern, southern, eastern and western Cote d'Ivoire. All the RYMV isolates was classified into three main serogroups (*Sg1*, *Sg2* and *Sg3*) and six subgroups (*Sg1a*, *Sg1b*, *Sg2a*, *Sg2b*, *Sg3a* and *Sg3a*). This indicates the existence and levels of serodiversity among RYMV isolates in Cote d'Ivoire. These results provide evidence of a possible relationship between serological property, host plant and ecological origin of RYMV isolates. Phylogenetic classification of each RYMV isolate defined by SDI data in ACP-ELISA is potentially useful in epidemiological studies to assess isolate identity and interaction as well as to assist breeding programs aiming at the development of cultivars with durable resistant to RYMV in Cote d'Ivoire.

Key words: RYMV, serological differentiation indices, antigen coated-plate enzyme-linked immunosorbent assay (ACP-ELISA), polyclonal antisera, phylogenetic analysis, serological diversity, Cote d'Ivoire

INTRODUCTION

Rice yellow mottle virus (RYMV), genus Sobemovirus (Hull, 1988) is the most rapidly spreading disease of rice (*Oryza sativa* L.) in Africa (Abo *et al*., 1998). First reported in Kenya, East Africa in 1966. It was reported in West Africa in 1975. The disease is now found virtually in all the countries of Africa where rice is grown (Abo *et al*., 1998). The virus causes yellowing, mottling, necrosis and stunting of rice plants leading to incomplete emergence of panicles with sterile grains (John and Thottappilly, 1987). In severity of infected plants death do occur. The means of transmission of the virus is by mechanical contacts and insects (Abo *et al*., 1998). Initially, RYMV was not of great threat to rice production, but with the intensification of rice production under rainfed and continuous irrigation, together with the introduction of many exotic varieties, epidemic out-break occurred. Several yield losses (96-97%) caused by RYMV infection has been reported (Fomba, 1986).

The existence of different RYMV strains in the field (Konate *et al*., 1997) that cause different kinds of disease is often a matter of considerable practical importance. For this reason reliable criteria are needed for distinguishing and identifying these strains. Knowledge of the serological relationships between RYMV isolates is valuable in diagnostic work and may prove to be important in epidemiological studies and disease control.

In previous studies, African RYMV isolates were serotyped using double immunodiffusion gel assay (Pinner *et al*., 1988; Mansour and Baillis, 1994; Sere *et al*., 2005), which were shown to be less sensitive than enzyme linked immunosorbent assay (ELISA). Phylogenetic analysis of some RYMV isolates based on sequences of the coat protein gene has been reported (Ngon *et al*., 1994; N'Guessan *et al*., 2000; Pinel *et al*., 2000; Fargette *et al*., 2001). However, because of relative expensiveness of sequencing many isolates of RYMV, few sequence data are available for phylogenetic analysis. Besides, few data are available on RYMV serodiversity, disease ecology and interactions between different RYMV strains in rice. However, Serological Differentiation Index (SDI) data has been reportedly used for the phylogenetic analysis of plant viruses serological classification (Pinner and Markharm, 1990; Rybicki, 1991; Pinner *et al*., 1992), but never been used for RYMV serodiversity. Construction of phylogeny for plant viruses using SDI data generated from antigen coated-plate enzyme linked

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immunosorbent assay (ACP-ELISA) allows rapid evaluation of serological diversity of many isolates and strains (Pinner and Markharm, 1990). The technique, which can be carried out in any moderately equipped research laboratory, is simple, rapid, cheap and very accurate.

The aim of this study was to investigate the use of serological differentiation indices for phylogenetic analysis of RYMV isolates serological relationships in Cote d'Ivoire. Such information is useful in epidemiological study and for developing rice varieties with durable resistant to RYMV in Cote d'Ivoire.

MATERIALS AND METHODS

Sample collection: In 2001 an intensive RYMV survey and sampling was performed in northern, southern, eastern and western parts of Cote d'Ivoire where rice was produced in upland, lowland and irrigated conditions. Leaf samples were collected based on (i) different host plant species, (ii) typical RYMV symptoms of yellow, mottle and stunting and (iii) different ecosystems. During the surveys, leaf samples were stored in an icebox and there after transferred to the laboratory and were stored in a freezer.

Isolates propagation: Sixty four, 68, 28 and 18 isolates respectively from northern, southern, eastern and western parts of Cote d'Ivoire (Table 1-4) were propagated in the

Table 1: Identity of isolates of *Rice yellow mottle virus* collected from Southern Cote d'Ivoire

Isolate code	Locality	Region	Ecology
SB-U-1	Bakayo-1	Soubre	Upland
SB-U-2	Bakayo-1	Soubre	Upland
SB-U-3	Bakayo-1	Soubre	Upland
SB-U-4	Bakayo-1	Soubre	Upland
SB-L-2	Grand-Zatry-1	Soubre	Lowland
SB-I-1	Sokoyo	Soubre	Irrigated
SB-I-6	Mayo	Soubre	Irrigated
SB-I-7	Mayo	Soubre	Irrigated
SB-I-9	Mayo	Soubre	Irrigated
SB-I-11	Grand-Zatry-1	Soubre	Irrigated
SB-I-12	Grand-Zatry-1	Soubre	Irrigated
AS-L-3	Panapana	Aboisso	Lowland
AS-L-4	Panapana	Aboisso	Lowland
AS-L-23	Akressi-2	Aboisso	Lowland
DB-L-1	Bimbressou	Dabou	Lowland
DB-L-18	Gbougbo	Dabou	Lowland
AZ-I-1	Prison-civile	Adzope	Irrigated
AZ-I-5	Prison-civile	Adzope	Irrigated
AZ-I-8	Assikoi	Adzope	Irrigated
AZ-I-11	Miadzin	Adzope	Irrigated
AZ-I-12	Miadzin	Adzope	Irrigated
AZ-I-13	Miadzin	Adzope	Irrigated
AZ-I-14	Miadzin	Adzope	Irrigated
AZ-I-15	Miadzin	Adzope	Irrigated
GB-L-1	Carrefour-1	Grand-Bassam	Lowland
GB-L-2	Carrefour-1	Grand-Bassam	Lowland
AP-L-1	Konia-1	Alepe	Lowland
AP-L-6	Konia-2	Alepe	Lowland

Table 2: Identity of isolates of *Rice yellow mottle virus* collected from Northern Cote d'Ivoire

Isolate code	Locality	Region	Ecology
KG-1I	Tine	Korhogo	Irrigated
KG-2I	Tine	Korhogo	Irrigated
KG-7I	Tine	Korhogo	Irrigated
KG-20I	Tine	Korhogo	Irrigated
KG-12I	Tine	Korhogo	Irrigated
KG-13I	Tine	Korhogo	Irrigated
KG-23I	Tine	Korhogo	Irrigated
KG-17I	Sologo	Korhogo	Irrigated
KG-18I	Sologo	Korhogo	Irrigated
KG-24I	Sologo	Korhogo	Irrigated
KG-14I	Sologo	Korhogo	Irrigated
KG-6I	Sologo	Korhogo	Irrigated
KG-15I	Sologo	Korhogo	Irrigated
KG-8bI	Sologo	Korhogo	Irrigated
KG-5I	Sologo	Korhogo	Irrigated
KG-16I	Sologo	Korhogo	Irrigated
KG-3I	Sologo	Korhogo	Irrigated
KG-4I	Sologo	Korhogo	Irrigated
KG-32I	Sologo	Korhogo	Irrigated
KG-29I	Sologo	Korhogo	Irrigated
KG-9I	Nombolo	Korhogo	Irrigated
KG-25I	Nombolo	Korhogo	Irrigated
KG-10I	Nombolo	Korhogo	Irrigated
KG-8aI	Nombolo	Korhogo	Irrigated
KG-22I	Nombolo	Korhogo	Irrigated
KG-11I	Nombolo	Korhogo	Irrigated
KG-34I	Nombolo	Korhogo	Irrigated
KG-27I	Nombolo	Korhogo	Irrigated
KG-28I	Nombolo	Korhogo	Irrigated
KG-21I	Nombolo	Korhogo	Irrigated
KG-33I	Nombolo	Korhogo	Irrigated
KG-30I	Nombolo	Korhogo	Irrigated
KG-19I	Nombolo	Korhogo	Irrigated
KG-26I	Nombolo	Korhogo	Irrigated
KG-31I	Nombolo	Korhogo	Irrigated
TG-1U	Kanakono	Tengrela	Upland
TG-2U	Kanakono	Tengrela	Upland
TG-4U	Kanakono	Tengrela	Upland
TG-5U	Kanakono	Tengrela	Upland
TG-3U	Kanakono	Tengrela	Upland
TG-1L	Maniasso-2	Tengrela	Lowland
TG-6L	Maniasso-2	Tengrela	Lowland
TG-2L	Maniasso-2	Tengrela	Lowland
TG-7L	Maniasso-2	Tengrela	Lowland
TG-3L	Maniasso-2	Tengrela	Lowland
TG-8L	Maniasso-2	Tengrela	Lowland
TG-5L	Maniasso-2	Tengrela	Lowland
BD-1U	Ponondougou-1	Boundiali	Upland
BD-2U	Ponondougou-1	Boundiali	Upland
BD-3U	Ponondougou-1	Boundiali	Upland
BD-4U	Ponondougou-1	Boundiali	Upland
BD-5U	Ponondougou-1	Boundiali	Upland
BD-8U	Ponondougou-1	Boundiali	Upland
BD-6U	Ponondougou-2	Boundiali	Upland
BD-7U	Ponondougou-2	Boundiali	Upland
BD-9U	Ponondougou-2	Boundiali	Upland
BD-10U	Ponondougou-2	Boundiali	Upland
BD-11U	Dara	Boundiali	Upland
BD-12U	Dara	Boundiali	Upland
BD-13L	Kpafa	Boundiali	Lowland
BD-14L	Kpafa	Boundiali	Lowland
BD-8L	Fondio-L	Boundiali	Lowland
BD-8I	Gbambiasso	Boundiali	Irrigated
BD-5I	Gbambiasso	Boundiali	Irrigated

Table 3: Identity of isolates of *Rice yellow mottle virus* collected from Eastern Cote d'Ivoire

Isolate code	Locality	Region	Ecology
DK-3U	Akringoua-1	Daoukro	Upland
DK-11U	N'Gattakro-1	Daoukro	Upland
DK-12U	N'Gattakro-1	Daoukro	Upland
DK-14U	N'Gattakro-1	Daoukro	Upland
DK-1L	Akringoua-2	Daoukro	Lowland
DK-2L	Akringoua-2	Daoukro	Lowland
DK-3L	Akringoua-2	Daoukro	Lowland
DK-6L	Kouassi-Dietekro	Daoukro	Lowland
DK-9L	Kouassi-Dietekro	Daoukro	Lowland
DK-11L	Attoungbrekro-2	Daoukro	Lowland
DK-12L	Attoungbrekro-2	Daoukro	Lowland
DK-14L	Attoungbrekro-2	Daoukro	Lowland
DK-15L	Attoungbrekro-2	Daoukro	Lowland
DK-18L	N'Gattakro-2	Daoukro	Lowland
DK-20L	N'Gattakro-2	Daoukro	Lowland
DK-24L	Ouele-1	Daoukro	Lowland
DK-25L	Ouele-1	Daoukro	Lowland
DK-26L	Baya-2	Daoukro	Lowland
DK-27L	Baya-2	Daoukro	Lowland
DK-29L	Baya-2	Daoukro	Lowland
AB-1U	Beki-1	Abengourou	Upland
AB-3U	Beki-1	Abengourou	Upland
AB-4U	Beki-1	Abengourou	Upland
AB-1L	Affalikro	Abengourou	Lowland
AB-4L	Affalikro	Abengourou	Lowland
AB-6L	Niable	Abengourou	Lowland
AB-7L	Niable	Abengourou	Lowland
AB-9L	Niable	Abengourou	Lowland
AB-10L	Niable	Abengourou	Lowland
AB-13L	Beki-2	Abengourou	Lowland
AB-16L	Abloet-Adaou	Abengourou	Lowland
AB-18L	Abloet-Adaou	Abengourou	Lowland
AB-19L	Abloet-Adaou	Abengourou	Lowland
AB-20L	Abloet-Adaou	Abengourou	Lowland
AB-1I	BCEAO	Abengourou	Irrigated
AB-2I	BCEAO	Abengourou	Irrigated
AB-5I	BCEAO	Abengourou	Irrigated
AB-6I	Niable-Station	Abengourou	Irrigated
AB-7I	Niable-Station	Abengourou	Irrigated
AB-8I	Niable-Station	Abengourou	Irrigated
AB-10I	Niable-Station	Abengourou	Irrigated
BK-1U	Korobo-1	Bondoukou	Upland
BK- 6U	Kanasse	Bondoukou	Upland
BK-4L	Korobo-2	Bondoukou	Lowland
BK-10L	Goumere	Bondoukou	Lowland
BK-3I	Karangba	Bondoukou	Irrigated
BO-1U	Niadegue	Bouna	Upland
BO-2U	Niadegue	Bouna	Upland
BO-3U	Niadegue	Bouna	Upland
BO-4U	Niadegue	Bouna	Upland
BO-5U	Niadegue	Bouna	Upland
BO-6U	Koumatan-2	Bouna	Upland
BO-8U	Koumatan-2	Bouna	Upland
BO-9U	Koumatan-2	Bouna	Upland
BO-1L	Baba	Bouna	Lowland
BO-2L	Baba	Bouna	Lowland
BO-3L	Baba	Bouna	Lowland
BO-4L	Baba	Bouna	Lowland
BO-5L	Baba	Bouna	Lowland
BO-7L	Abattoir	Bouna	Lowland
BO-10L	Abattoir	Bouna	Lowland
BO-11L	Nakele	Bouna	Lowland
BO-13L	Nakele	Bouna	Lowland
BO-14L	Nakele	Bouna	Lowland
BO-15L	Nakele	Bouna	Lowland
BO-16L	TchaTchare	Bouna	Lowland
BO-19L	TchaTchare	Bouna	Lowland
BO-22L	Koumatan-2	Bouna	Lowland

method of mechanical transmission was according to Fauquet and Thouvenel (1977). Infected leaf samples were ground with 0.01 M phosphate buffer pH 7.0 at the ratio of 1:10 (w/v) and the resulting homogenate filtered through cheesecloth. Carborundum powder (600 mesh) was added to the inoculum to aid the penetration of the virus into leaf tissues. Four weeks after inoculation, leaves from each RYMV isolate bearing typical yellow mottle symptoms were harvested and used for RYMV serodiversity study.

Polyclonal antiserum production: Twenty-six polyclonal antibodies used in this study were obtained from Plant Pathology Unit, WARDA, raised against different RYMV isolates previously collected from different parts of West Africa (Table 5). These antisera were produced as follows:

Table 4: Identity of isolates of *Rice yellow mottle virus* collected from Western Cote d'Ivoire

Isolate code	Location	Region	Ecosystem
BF-I-1	Garango	Bouafle	Irrigated
BF-I-2	Garango	Bouafle	Irrigated
BF-I-3	Garango	Bouafle	Irrigated
BF-I-4	Garango	Bouafle	Irrigated
BF-I-5	Garango	Bouafle	Irrigated
TL-I-15	Gbahi-2	Tolulepleu	Irrigated
TL-I-17	BeauSoleil	Tolulepleu	Irrigated
TL-I-18	BeauSoleil	Tolulepleu	Irrigated
TL-I-23	Village	Tolulepleu	Irrigated
TL-I-24	Village	Tolulepleu	Irrigated
SG-U-6	CIDT	Seguela	Upland
SG-U-7	CIDT	Seguela	Upland
SG-U-8	CIDT	Seguela	Upland
SG-U-9	CIDT	Seguela	Upland
SG-U-10	CIDT	Seguela	Upland
SG-L-2	BereniDialla	Seguela	Lowland
SG-L-3	BereniDialla	Seguela	Lowland
SG-L-4	BereniDialla	Seguela	Lowland

Table 5: List of antisera code and country of origin

Antiserum code	Region	Country
M-1	Niono4	Mali
M-2	Niono8	Mali
M-3	M'Peniesso	Mali
M-4	Molodo	Mali
M-5	Longorola	Mali
M-6	Kayo macina	Mali
M-7	Selingue	Mali
M-8	Kogoni K7	Mali
BF-1	Banzon	Burkina Faso
BF-2	Kafirguela	Burkina Faso
IITA	IITA	Nigeria
Ng-1	Saga	Niger
Ng-2	Kollo	Niger
Ng-3	Kirkissaye	Niger
Ng-4	Bonfeba	Niger
Ng-5	SayI	Niger
Ng-6	Diomana	Niger
CI-1	M'be	Cote d'Ivoire
CI-2	Danane	Cote d'Ivoire
CI-3	Gagnoa -L	Cote d'Ivoire
CI-4	Gagnoa-U	Cote d'Ivoire
CI-5	Guehiebli	Cote d'Ivoire
CI-6	Tapeguia	Cote d'Ivoire
CI-7	Odienne	Cote d'Ivoire
CI-8	Sakassou	Cote d'Ivoire
CI-9	Sassandra	Cote d'Ivoire

Different RYMV isolates, after propagation on susceptible rice variety Bouake189, were purified using the method of Thottappilly and Rossel (1993) and a modified version of Hull (1988). Two weeks after inoculation, rice seedlings showing typical symptoms of RYMV were harvested for purification. Rabbits were immunized with purified RYMV isolates by intramuscular injections at one-week intervals (Pinner and Markham, 1990). A purified RYMV suspension (0.5 mL, 1 mg mL^{-1}) was emulsified with 0.5 mL of Freund's complete adjuvant and used to immunize a rabbit followed by three more injections prepared with Freund incomplete adjuvant. One week after the fourth injection, the rabbit was bled and serum collected. All the twenty-six antisera produced were preabsorped with healthy plant materials (to remove any possible antibody produced against the host rice plant), as described by Pinner and Markham (1990).

ACP-ELISA: Indirect-antigen coated-plate ACP-ELISA was performed as described by Jaegle and Van Regenmortel (1985). Briefly, the procedure was as follows. Virus saps extracts were directly adsorbed to microtitre plates, followed by blocking with 1% bovine serum albumin. Twofold serial dilutions of antisera were made and bound antibody was detected with goat anti-rabbit serum conjugated to alkaline phosphatase (Sigma). The bound conjugate was detected using p-nitrophenyl phosphate solution and the plates were read at 405 nm.

Serological Differentiation Index (SDI) values between isolates: These values (Jaegle and van Regenmortel, 1985) were determined as described by Pinner and Markham (1990). Each virus was tested against a two fold serial dilution of each antiserum and the process was repeated three times. The SDI represents the average number of twofold dilution steps between homologous and heterologous viruses at a standard absorbance value of 0.5. The SDI values were read directly from the graph and the final value was expressed as a mean of the replicates.

Phylogenetic analysis: Four composite relationships dendrograms were generated, each for 64, 68, 28 and 18 RYMV isolates from northern, southern, eastern and western parts of Cote d'Ivoire from SDI data obtained from ACP ELISA results only (Pinner *et al*., 1992; Rybicki, 1991; Dekker, 1988) using numerical taxonomy and multivariate analysis system (NTSYS-PC), version 2.1 (Rohlf, 2000). SDI data were first converted to pairwise distance matrices, using the Jaccard coefficient of similarity (Jaccard, 1908) present in NTSYS-PC 2.1 and dendrogram was created by UPGMA cluster analysis (Sneath and Sokal, 1973).

RESULTS

SDI validity: All reactions that reached or exceeded the standard value of 0.5 and three times the background level was considered to be positive (Pinner *et al*., 1992). For each antiserum and its homologous, the SDI was defined as 0. The validity of high SDI values was demonstrated by obtaining similar results with virus purified further using CsCl gradient as described by Thottappilly and Rossel (1993). SDI values of the two were considered to be significant (Jaegle and van Regenmortel, 1985).

Diversity among southern isolates: Phylogenetic analysis revealed serological differences among 28 southern isolates (Fig. 1). The similarity ranges from 20 to 70% Jaccard similarity coefficient. At 25% Jaccard similarity level, all the isolates were separated into two main serogroups (*Sg1* and *Sg2*), while at 40% Jaccard similarity level *Sg1* and *Sg2* were further separated into two subgroups (*Sg1a* and *Sg1b)* and (*Sg2a* and *Sg2b*) respectively. However, according to the pairwise genetic distances among the isolates analysed at 100% similarity level all isolates were separated, except in *Sg1a* subgroup in which isolates, SB-U-2 and SB-U-3, AS-L-3 and AS-L-4 were, respectively identical.

Diversity among western isolates: There were considerable diverse serological differences among 18 western isolates (Fig. 1) as indicated by phylogenetic analysis, giving rise to 25 to 75% similarity ranges. All the isolates were separated into two main serogroups (*Sg1* and *Sg2*) at 30% similarity level. *Sg1* and *Sg2* were further separated into two subgroups (*Sg1a* and *Sg1b*) and (*Sg2a* and *Sg2b*), respectively at 40% similarity level. However, *Sg2a* was further divided into $Sg2a^1$ and $Sg2a^2$ higher subgroups at 45% similarity level. At 100% similarity level all isolates were separated, except in $Sg2a^2$ subgroup in which TL-I-18 and SG-U-8 isolates were identical.

Diversity among eastern isolates: Broad serodiversity was obtained among 68 eastern isolates analysed (Fig. 2). The similarity ranges from 20-75% Jaccard similarity coefficient. At 30% Jaccard similarity level, all the isolates were separated into three main serogroups (*Sg1*, *Sg2* and *Sg3*), while at 40% Jaccard similarity level *Sg2* and *Sg3* were further separated into two subgroups (*Sg2a* and *Sg2b*) and (*Sg3a* and *Sg3b*), respectively. However, according to the pairwise genetic distances among the isolates analysed at 100% similarity level, all isolates of subgroups *Sg3a* and *Sg3b* were distinct, while identical isolates were found among *Sg1*, *Sg2a* and *Sg2b* serogroups.

Diversity among northern isolates: Phylogenetic analysis revealed wide serological differences among 64 northern isolates analysed (Fig. 2). The similarity ranges from 25% to 75% Jaccard similarity coefficient. At 25% Jaccard similarity level, all the isolates were separated into three main serogroups (*Sg1*, *Sg2* and *Sg3*), while at 30% Jaccard similarity level *Sg1* and *Sg2* were further separated into two subgroups (*Sg1a* and *Sg1b*) and (*Sg2a* and *Sg2b*), respectively. However, according to the pairwise genetic distances among the isolates analysed at 100% dissimilarity level, all isolates of serogroups *Sg2a* and *Sg3* were distinct, while identical isolates were found among *Sg1a*, *Sg1b* and *Sg2b* subgroups.

DISCUSSION

RYMV has been described as a variable virus with many pathological variants (Thottappilly and Rossel, 1993; Sasaya *et al*., 1997; Konate *et al*., 1997; N'Guessan *et al*., 2000) and the unlimited number of pathological and virulence characters of RYMV and lack of standardisation of pathological conditions and virulence tests among different researchers have led to confusion and uncertainty in the characterization of this pathogen from rice (Taylor *et al*., 1990; Mansour and Baillis, 1994). The classification of all virus isolates into three main serogroups (*Sg1*, *Sg2* and *Sg3*) and six subgroups (*Sg1a*, *Sg1b*, *Sg2a*, *Sg2b*, *Sg3a* and *Sg3a*) indicates the existence and levels of serodiversity among RYMV isolates in Cote d'Ivoire. This conformed to the earlier study of existence of several serotypes of RYMV isolates in Cote d'Ivoire (N'Guessan *et al*., 2000). In this study, many isolates emanating from same locality, field and host were observed to be serologically different (Fig. 1 and 2). For example, northern isolates (KG-34-I and KG-24-I), eastern isolates (DK-2-L and DK-3-L), western isolates (BF-I-1 and BF-I-2) and southern isolates (SB-U-1 and SB-U-2) were in each locality obtained from same host plant but were different in serodiversity. This explains the fact that within a set of isolates of related strains in the same host plant, many possibilities of interaction exist (Matthews, 1991). This possible isolate and host plant interaction varies between one locality to another thus account for diverse serological variability that exist among different RYMV isolates in Cote d'Ivoire. The serological similarities observed between isolates within the same and

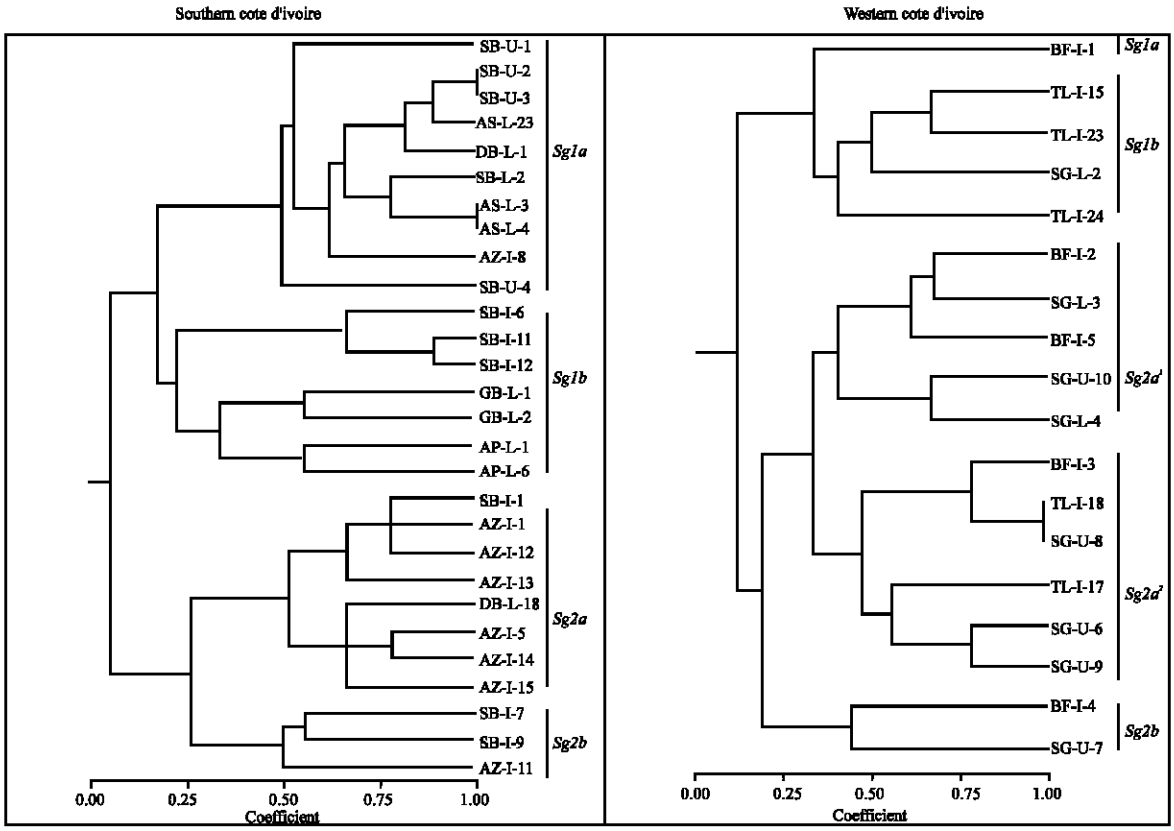

Fig. 1: Composite relationship dendrogram of RYMV isolates from Southern and Western Cote d'Ivoire derived from SDI data using 26 polyclonal antisera in ACP ELISA

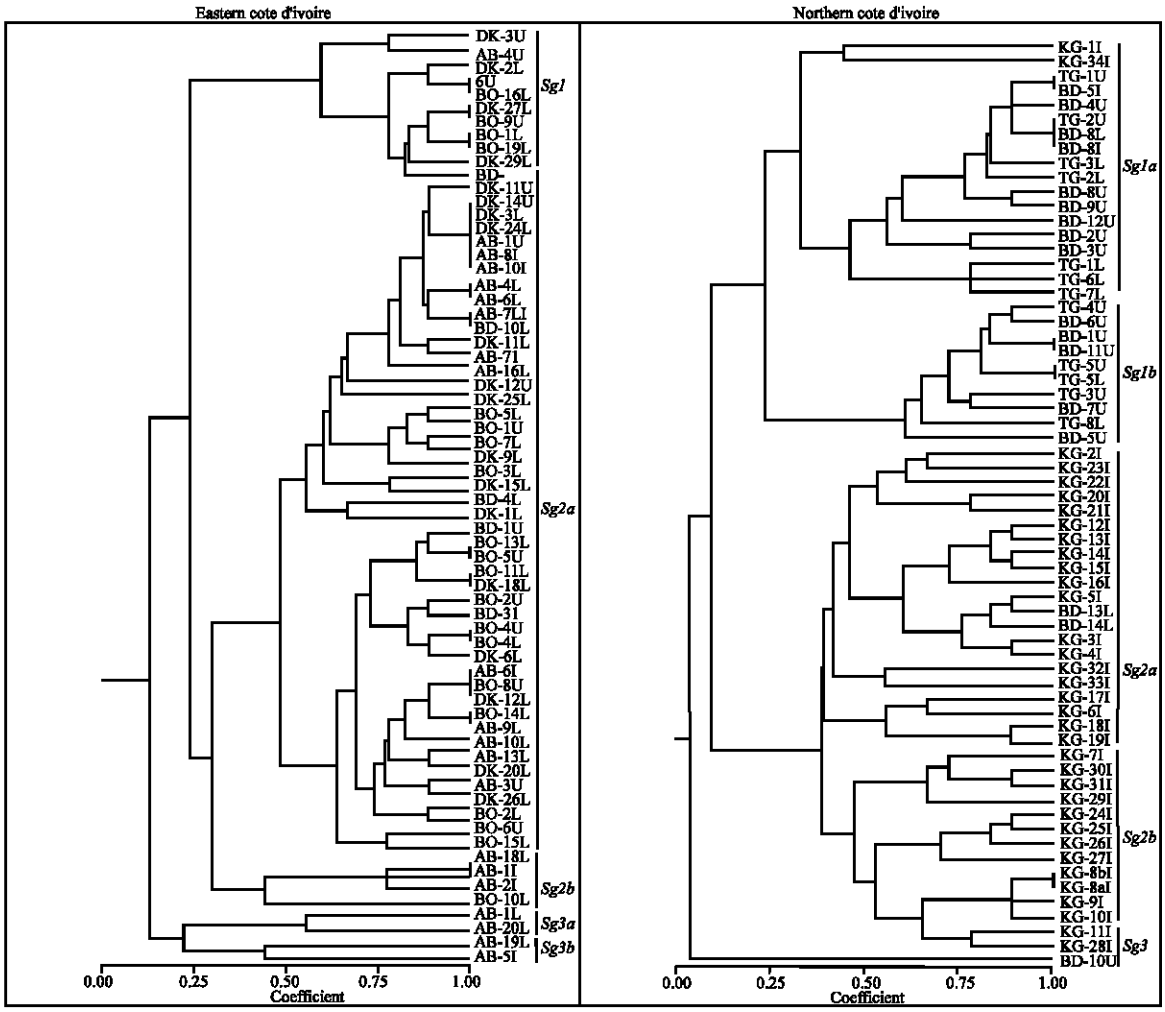

Fig. 2: Composite relationship dendrogram of RYMV isolates from Eastern and Northern Cote d'Ivoire derived from SDI data using 26 polyclonal antisera in ACP ELISA

different localities confirm the great cross-infection potential of RYMV transmitted under natural conditions by different insect vectors (Bakker, 1971; Hammond *et al*., 1999). In this study there were indications of localised micro variation among northern and eastern isolates with the emergence of *Sg3* serogroup which was not found among the southern and western isolates. This is practically important for various RYMV identifications and further strengthens the deployment of durable rice germplasms in the region.

Besides, in the current study, all the isolates were completely separated and distinct. Twenty-two isolates gave homologous reactions with five antisera out of twenty-six antisera used, showing relatively low level of homologous reaction between isolates and the antisera. This might account for complete distinction of all isolates at 80% similarity level (Fig. 1 and 2). These serological distinctions among isolates suggest that they all differed in a specific combination of epitopes (N'Guessan *et al*., 2000). However, some isolates were serologically identical at 100% similarity (Fig. 1 and 2), this might be due to the similarity in the ecological origin of these isolates (Konate *et al*., 1997).

However, isolates from the same leaf and same host plant (KG-34-I and KG-24-I) were observed to be serologically different (Fig. 1). This could probably explain the fact that within a set of isolates of related strains, many possibilities of interaction exist (Matthews, 1991). As a result of possible interaction between different strains of the same isolates in the same host plant, diverse serological variability tends to exist between different isolates of RYMV across different ecologies in Cote d'Ivoire. The serological diversity observed between isolates within the same and from different ecologies in West Africa confirms the great cross-infection potential of RYMV transmitted under natural conditions by

different insect vectors (Bakker, 1971; Hammond *et al*., 1999). Such possibilities of interaction within a set of isolates of related strains might lead to frequent occurrence of mutants which might be responsible for the high level of serological variation among the isolates (Boccard and Baulcombe, 1993).

Present results revealed that the use of SDI data generated from ACP-ELISA has great potential for serological identification and classification of RYMV isolates in Cote d'Ivoire. The specific distinction pattern of each isolate, revealed by phylogenetic analysis of SDI data generated from ACP-ELISA, is consistent, repeatable and reliable. The definition of specific distinct pattern for each isolate or strain should be a simple and straightforward task. Obviously, for these distinctions to have a practical meaning for the rice breeder, specific distinct pattern for each isolate must be related to the degree of virulence present. This could be achieved by a systematic comparison of distinct serotyped isolates or strains contrasting to their degree of virulence to rice. A similar approach has been used to determine the serological relationships of geminivirus isolates from gramineae in Australia (Pinner *et al*., 1992) and to identify maize streak geminivirus strains (Pinner and Markharm, 1990). Phylogenetic classification of each isolate of RYMV defined by SDI data in ACP-ELISA should be useful for the surveillance of RYMV in rice growing regions, in epidemiological studies to assess isolate identity and interaction as well as assist breeding programs aiming at the effective development of cultivars with durable resistant to RYMV in Cote d'Ivoire.

ACKNOWLEDGMENTS

This research was funded by the Department for International Development (DFID), UK and the Government of Japan. The authors would like to acknowledge Mr. Mensah Yao and Mr. Zai Kamelan, for their technical support.

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