LETTER TO THE EDITOR

MOLECULAR VARIABILITY AND DISTRIBUTION OF RICE YELLOW MOTTLE VIRUS IN TANZANIA

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Rice yellow mottle virus (RYMV, the species *Rice yellow mottle virus*, the genus *Sobemovirus*) is restricted to the continent of Africa. It has been known to occur in East and West Africa and in Madagascar (*1*). Very recently the disease has been reported also in Central Africa (*2*). RYMV is transmitted mechanically and by vectors. Transmission by rice seeds is not known to occur (*3*). Infected plants show yellow discoloration and mottling of leaves, stunting, poor tillering, reduced panicle exertion and sterility. Early infection of susceptible varieties can result in high yield losses to plant death. A yield loss of 92% has been reported and the search for resistant variants that would be acceptable to the farmers in Tanzania is in progress (*4*). RYMV is rapidly spreading between and within rice growing countries in Africa. Serological differences have been reported between and within isolates from various countries (*5, 7, 8*). Though the diversity of RYMV isolates is believed to be the highest in East Africa (*5*), such an information is limited to Tanzania, the largest producer and consumer of rice in East Africa where the disease continues to be a major constraint to rice growers. An earlier study (*5*) has been performed only on three isolates from Tanzania.

Therefore the goal of this work was to determine the molecular diversity among 26 isolates of RYMV from different regions of Tanzania using coat protein (CP) gene sequences. This study will provide useful information on the diversity of RYMV in Tanzania, and will permit the identification of RYMV isolates to be selected as source of inoculum in breeding programs for ensuring resistance against RYMV.

The virus isolates used in this study were collected from rice plants showing characteristic mottling symptoms in eight rice growing regions of Tanzania in 1999–2001. RYMV-infected rice plants were multiplied in the susceptible rice cultivar Kusabue, which reacts with characteristic mottling and yellowing symptoms. Infection with RYMV was confirmed by double-antibody sandwich ELISA (DAS-ELISA) according to Clark and Adams (*6*). The DSMZ D-0074 DAS-ELISA kit, which detects all hitherto known RYMV isolates was utilized for serological indexing. The CP genes of the RYMV isolates collected and those of two reference RYMV isolates (PV 0478 from Nigeria and PV 0479 from Kenya) were amplified by RT-PCR after extraction of total RNA from RYMV-infected leaves. For RT-PCR, the primer pair described by Pinel *et al*. (*5*) was employed to amplify the 872 bp dsDNA fragment comprising the complete CP gene (720–723 bp) and a portion of the 3'-non-translated region of the genome. All dsDNA fragments obtained were sequenced and the amino acid sequences were deduced. The obtained sequences were aligned and a phylogenetic tree was constructed. The known sequences were obtained from the Genbank (their accession numbers are shown on the figure).

The comparison of CP amino acid sequences of the RYMV isolates from Tanzania revealed a close relationship

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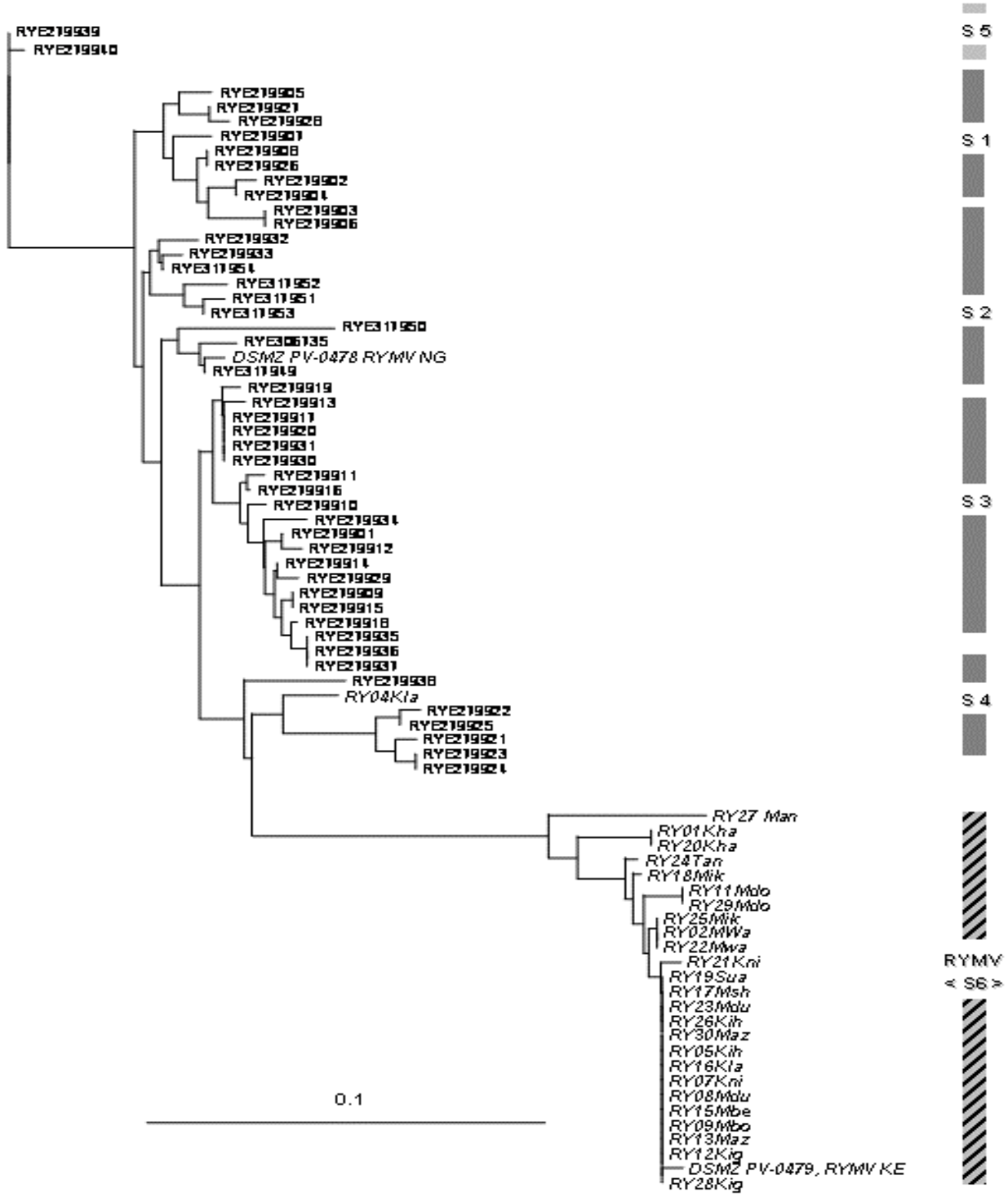
Abbreviations: CP = coat protein; DAS-ELISA = double-antibody sandwich ELISA; RYMV = Rice yellow mottle virus


RYE219939
RYE219940
RYE219905
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RYE219904
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RYE219932
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RYE311954
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RYE311953
RYE311950
RYE306135
DSMZ PV-0478 RYMV NG
RYE311949
RYE219919
RYE219913
RYE219911
RYE219920
RYE219931
RYE219930
RYE219911
RYE219916
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RYE219935
RYE219936
RYE219937
RYE219938
RY04Kia
RYE219922
RYE219925
RYE219921
RYE219923
RYE219924
RY27 Man
RY01Kha
RY20Kha
RY24Tan
RY18Mik
RY11Mdo
RY29Mdo
RY25Mik
RY02MWa
RY22Mwa
RY21Kni
RY19Sua
RY17Msh
RY23Mdu
RY26Kih
RY30Maz
RY05Kih
RY16Kia
RY07Kni
RY08Mdu
RY15Mbe
RY09Mbo
RY13Maz
RY12Kig
DSMZ PV-0479, RYMV KE
RY28Kig
0.1
S 5
S 1
S 2
S 3
S 4
RYMV
< S6 >

with 95–99% identity. In general, the divergence amongst the isolates from Tanzania ranged from 0.3% to 6%, with the most diverging isolate RY27MAN from Mangula, the Morogoro region, reached a divergence of 8%. This isolate originated from a region known to have a very high incidence of RYMV and rice plants showing severe disease symptoms. Phylogenetic relationships were inferred and the resulting phylogenetic tree identified 3 groups (S4, S5 and S6) in Tanzania (see the figure; the scale bar represents a 0.1 nucleotide replacement per site).

Five strains (S1-S5) of RYMV occur in Africa (*5,* 9), while the isolates from Central and West Africa fall in the S1-S3 groups and the East African isolates in the S4 and S5 groups. RY4KLA, one of the two isolates from the Kyela District, the Mbeya Region, fall in the S4 group consisting of the isolates from Madagascar and Tanzania (*5*), while PV-0478, the reference isolate from Kenya, and all the 26 isolates from 8 regions in Tanzania (Dar-es-Salaam, Kilimanjaro, Shinyanga, Mbeya, Morogoro, Dodoma, Tanga and Pwani) falls within a new group/strain of RYMV which was observed in this study for the first time and which is referred to as S6.

It has been reported earlier (*5, 9*) about an isolate from Kenya belonging to the S4 group; at the same time it has been stated (*5*) that some isolates collected in Kenya from the places located only a few kilometers apart belong to different strains.

A similar finding was observed also in this study since the isolates RY4KLA and RY16KLA from Kyela, the Mbeya Region, fell into the S4 and S6 groups, respectively. In contrary, this has not been reported earlier to occur in Tanzania (*9*).

This study showed that the RY4KLA isolate belonging to the S4 group was found only in the Mbeya Region, while the S6 group proved to have a wider distribution since it was observed in all the following 8 regions: Pwani, Morogoro, Dar-es-Salaam and Tanga in eastern Tanzania; Dodoma in central Tanzania; Kilimanjaro in northern Tanzania; Shinyanga in the Lake Zone, and Mbeya in the southern highlands. The S4 strain, which is less severe is believed to be present in the Lake Zone (Mwanza and Shinyanga Regions), while the S5 strain is thought to occur in Kilimanjaro, Morogoro and Mbeya Regions.

This study revealed that 3 strains, S4, S5 and S6 occur in Ivory Coast.

Breeders in Tanzania have been therefore advised to use three different strains of RYMV as sources of inoculum in studies of resistance of rice against RYMV, since the use of resistant varieties currently remains the most economical and effective means in the management of the disease.

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