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Begomovirus prevalence and diversity in the TYLCV resistant tomato cultivar *Shanty* in Nicaragua

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Sammanfattning

I Nicaragua orsakar begomovirus varje år kraftiga reduceringar i landets tomatproduktion. Stora skördeförluster till följd av virusinfektionen leder i sin tur till stora ekonomiska förluster för så väl hela landet som den enskilde bonden. Under de senaste åren har tomatgrödor som är resistenta eller toleranta mot begomovirus börjat användas i större utsträckning i Nicaragua. Men fortfarande är begomovirus infektion ett stort problem för tomatproducenterna. Pågående arbete i Nicaragua syftar därför till att hitta nya och bättre resistenta eller toleranta tomatsorter. Den här studien är ämnad att vara en del av det arbetet, genom att undersöka förekomst och mångfald av begomovirus i den resistenta tomatsorten Shanty. Tomatprover av sorten Shanty samlades in från fyra geografiska områden i Nicaragua (Sebaco, CNIAB (nära Managua), Las Playitas, Tecolostote). Begomovirus infektion bekräftades med hjälp av polymeraskedjereaktion (PCR) och universala begomovirusprimers. Infektion hittades på alla platser där tomatplantor med tydliga symptom på begomovirusinfektion hade samlats in. Infektion kunde även bekräftas i prover som samlats in från tillsynes friska tomatplantor. Rolling circle amplification (RCA) producerade en lämplig produkt för vidare restriktionsanalys i sex prover med positivt resultat från PCR. Restriktionsanalys med de tre restriktionsenzymerna *Eco*RI, *Sal*I and *Hpa*II, utfördes i samtliga sex prov för att undersöka eventuell förekomst av blandad begomovirusinfektion. Blandad infektion kunde bekräftas i ~80% av de analyserade proverna. I korthet visade resultaten att de vanligaste tomatproduktionsområdena i Nicaragua (Sebaco, CNIAB, Las Playitas, Tecolostote) fortfarande är kraftigt påverkade av begomovirusinfektion och att blandade infektioner är relativt vanligt i dessa områden.

Nyckelord: *begomovirus, blandade virusinfektioner, resistens, restriktionsanalys, tomatsort*

Abstract

Every year begomovirus infections cause severe constraints to the tomato production in Nicaragua. Extensive yield losses are followed by large economical losses, both for the country and the single farmer. During recent years the use of begomovirus resistant or tolerant tomato cultivars has increased in Nicaragua as an effort to improve yields. Still, begomovirus infection is a large threat to the tomato production. Therefore, ongoing work in Nicaragua is focusing on finding new, better, begomovirus tolerant or resistant tomato cultivars. This study aims to be a part of this work, by investigating the current prevalence and diversity of begomoviruses in the resistant tomato variety Shanty. Nicaraguan tomato plants of the variety Shanty were collected from four geographical locations around Nicaragua (Sebaco, CNIAB (close to Managua), Las Playitas, Tecolostote). The collected samples were investigated for begomovirus infection using polymerase chain reaction (PCR) and universal begomovirus primers. Infection was confirmed at all locations where plants with clear symptoms of infection had been collected. Infection was also detected in plants, which had been collected without any visible symptoms of begomovirus infections. Rolling circle amplification (RCA) produced a suitable product for restriction analysis in six of the begomovirus positive samples. Restriction analysis with the three restriction enzymes *Eco*RI, *Sal*I and *Hpa*II, showed mixed infection (more than one begomovirus species present in the tomato plant) in ~80% of the analysed samples. The results showed that the most common tomato producing areas in Nicaragua (Sebaco, CNIAB, Las Playitas, Tecolostote) still are heavily exposed to begomovirus infections and that mixed viral infections are relatively common in these regions.

Keywords: *begomovirus, mixed viral infections, resistance, restriction analysis, tomato cultivar*

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1. Introduction

In Latin America, tomato (*Solanum lycopersicum*) is a widely cultivated crop, which has great importance as an export as well as a standard cash crop for the single farmer. However, every year the tomato production is heavily reduced due to begomovirus infection (Rojas 2004; Morales 2006). Begomoviruses belongs to the plant virus family *Geminiviridae.* The majority of begomoviruses holds a bipartite genome of two single-stranded (ss) DNA molecules of approximately 2.6-2.8 kb, referred to as DNA-A and DNA-B (Ala-Poikela et al. 2005; Andrade et al. 2006; Seal et al. 2006). Begomoviruses are transmitted by the whitefly vector *Bemisia tabaci*, and besides tomatoes, they are capable of infecting other important crops including peppers, beans and cucurbits. The virus infection causes symptoms such as mosaic patterns, yellowing and downward curling of leaves, as well as stunting and constraints in photosynthesis of the plant. Early infection of plants often results in complete yield loss (Rojas 2004).

Since the 1980s the diversity and prevalence of begomoviruses infecting tomatoes in Latin America have increased. Agricultural intensification, usage of insecticides and fertilizers in attempts to improve yields, have resulted in increase and resistance in *B. tabaci* populations (Morales 2006; Seal et al. 2006). In addition, the introduction of the more aggressive B biotype of *B. tabaci,* which has a wider host range than the original biotype, has caused further emergence of begomovirus infections in important crops (Ala-Poikela et al. 2005; Morales 2006). Since the presence of *B. tabaci* is essential for causing begomovirus outbreaks, the expansion of *B. tabaci* populations has caused continuous attempts to reduce begomovirus infections by limiting the whitefly populations. Control measures such as chemicals or physical barriers have been used in affected regions. However, in regions where the whitefly attack has been severe the control measures have had little impact on the virus spread (Lapidot and Friedmann 2002). Furthermore, physical barriers, such as greenhouses, cause higher production costs and maintenance, and the use of chemicals may have deleterious effects on the environment. Hence, the research today is more focused on the identification and selection of begomovirus resistant and tolerant tomato cultivars, either through traditional breeding or genetic engineering (Lapidot and Friedmann 2002).

During recent years, several tomato varieties with resistance or tolerance against different begomoviruses, have been introduced to Nicaragua as an effort to improve yields. At present the majority of these cultivars have been genetically improved abroad, e.g. in Israel (Hazera Genetics) and Taiwan (The World Vegetable Centre, AVRDC) (Morales 2006; Personal contact Thomas Laguna 2010-05-27; Personal contact Anders Kvarnheden 2010-04-13). In these regions one of the most widespread and economically important begomoviruses is *Tomato yellow leaf curl virus* (TYLCV). Yield losses due to this virus often reach 100%. Because of the severity of TYLCV infection and ongoing spread of the virus, breeding towards TYLCV resistance has been of high priority (Lapidot and Friedmann 2002; Duffy and Holmes 2007). Therefore, TYLCV resistant tomato cultivars can be found in regions where not yet the virus has been identified (Lapidot and Friedmann 2002). An example of this is the tomato variety Shanty, developed by Dr. Ron Ecker from Hazera Genetics in Florida 2004 (Giras 2009). This variety has recently been introduced to Nicaragua even though it is resistant to TYLCV, which not yet has been identified in Nicaragua (Personal contact Anders Kvarnheden 2010-05-1).

The aim of this project was threefold. The first aim was to investigate the degree of begomovirus infection in the tomato variety Shanty. The variety has been successfully

introduced to several countries in Central America, South America and the Middle East (Giras 2009). In Nicaragua the Shanty variety has become very popular among the farmers and can be found in a wide geographical range, which enabled the second aim of this project: to investigate the present begomovirus prevalence in Nicaragua. The third aim of the project was to investigate the degree of mixed begomovirus infections in Shanty tomato plants. Mixed virus infections are common, but have not been much studied in Central America. However, a recent study by Ala-Poikela et al. (2005) found that mixed begomovirus infection in tomatoes were relatively common. Because mixed infections increase the chance of recombination and consequently the emergence of new, more severe begomovirus strains or species (Padidam et al. 1999; Rojas 2004), the degree of mixed infection is an interesting subject for further investigation.

The three aims of the project presented in short:

1. *Investigation of the degree of begomovirus infection in the tomato variety Shanty*
2. *Investigation of the present begomovirus prevalence in Nicaragua*
3. *Investigation of the degree of mixed begomovirus infections in the tomato variety Shanty*

2. Methods & Materials

2.1 Collection of samples

During the period April to May, 2010, tomato leaves of different age of the Shanty variety were collected from four different locations and in total eight different fields around Nicaragua (Sebaco, CNIAB (close to Managua), Las Playitas and Tecolostote, see Fig. 1). At each field, young leaves from five different plants with typical symptoms of begomovirus infection (stunting, yellowing, mosaic patterns and curling of the leaves) were collected. At two fields, five samples were collected from plants with no visible symptoms, but where *B. tabaci* had been observed. The samples were collected in plastic bags and stored at -20ºC until analysis.

Fig 1. Locations for the collection sites of the variety Shanty. C refers to CINAB (Managua), S Sebaco, T Tecolostote and L Las Playitas.

2.2 DNA extraction and Polymerase chain reaction (PCR)

In total 39 tomato samples were analysed through polymerase chain reaction (PCR) for begomovirus detection (five samples from each field except at field 3, see table 1). Detection of begomovirus DNA was carried out in microcentrifuge tubes. The tubes were pre-incubated with tomato plant extract according to the direct PCR detection method, previously described by Wyatt and Brown (1996). Tomato tissue (0.2 g) was placed in a plastic bag and grinded in 2 ml of extraction buffer (50 mM TRIS and 10 mM EDTA, pH 8). The extract was transferred to an eppendorf tube and centrifuged for 10 min at 13000 rpm. Fifty µl of the supernatant was then transferred to a sterile PCR tube and incubated on ice for 30 min. The extract was removed after incubation and the tube was washed twice with 150 µl TRIS (10 mM), and left to dry at room temperature (22ºC). In order to

detect begomovirus infection PCR was run using the degenerate primers AC1048 and AV494 (Wyatt and Brown 1996). The primers amplify the core region of the coat protein gene *AV1,* which gives a product of 576 bp, including primers (Wyatt and Brown 1996; Rojas 2004). As negative controls, pre-incubated PCR tubes containing only PCR reagents were used. Each PCR contained 0.5 ml DreamTaq polymerase (5 u/µl, Fermentas), 0.5 µl dNTP mix (10 mM), 0.5 µl of each primer (10 mM), 2.5 µl 10x DreamTaq Buffer (Fermentas) and 18.5 µl MilliQ water to a total reaction volume of 23 µl.

The PCR was run under the following conditions: heating of the samples at 94ºC for 2 min, followed by 34 cycles of amplification. Each cycle consisted of 30 s at 94ºC, 1 min at 55ºC and 2 min at 72ºC. The final cycle was followed by 10 min of elongation at 72ºC. Analyses of the amplification products were conducted by running gel electrophoresis with a 1% agarose gel (100 V, 45 min).

2.3 Rolling circle amplification (RCA) and restriction analysis

RCA was carried out in order to produce a suitable product for restriction analysis. In case of positive begomovirus infections detected through PCR analysis, two positive samples from each field were chosen for further analysis with RCA. DNA from positive tomato samples was extracted for RCA analysis using Extract-n-Amp™ Plant PCR kit (Sigma) as previously described by Shepherd et al. (2008). Two deviations were made from the method described by Shepherd et al. (2008): fresh tomato tissue was used and the extracts were stored at -20ºC before RCA reaction (instead of 4ºC). The circular begomovirus genome was amplified using a bacteriophage Φ29 DNA polymerase, included in the commercial kit TempliPhi™ (GE Healthcare) according to the manufacturer's instructions. A sample containing only RCA reagents was used as negative control. Analysis of the amplification products was conducted by gel electrophoresis with a 1% agarose gel (100 V, 60 min).

Restriction analysis was performed on positive RCA products in order to detect mixed begomovirus infections. Each sample was cleaved with three different restriction enzymes (Fermentas): *Eco*RI, *Hpa*II and *Sal*I essentially according to the manufacturer's instructions. One µl of each RCA product was digested with *Eco*RI (10 u), *Hpa*II (10 u) and *Sal*I (1FDU) respectively. Each reaction was incubated at 37ºC for 2 h in a heating block. The reactions were stopped by incubating at 65ºC for 10 min for *Sal*I and for 20 min for *Hpa*II and *Eco*RI. The restriction patterns were analysed through gel electrophoresis with a 1% agarose gel (100 V, 60 min).

Table 1. Detection of begomovirus infection by polymerase chain reaction. The table shows in which area the samples were collected (field 1-8), the age of the plants when collected, the number of plants sampled with symptoms and the number of plants positive for begomovirus infection according to PCR analysis.

Field location	Age of plants (months)	No. samples with symptoms/ No. of plants sampled	No. begomovirus-positive plants/ No. plants analysed	Percentage(%)
1. CNIAB(Managua)	2	5/5	5/5	100
2. CNIAB(Managua)	5	5/5	5/5	100
3. Sebaco (close to experimental centre, cooperative Padre Fabreto)	3	4/4	2/4	50
4. Sebaco	~ 3*	5/5	5/5	100
5. Las Playitas (Dos Montes, Las Tunas)	0,5	0/5	0/5	0
6. Las Playitas (Dos Montes, Las Tunas)	1	0/5	4/5	80
7. Tecolostote	3,5	5/5	4/5	80
8. Tecolostote (Bélen)	3	5/5	5/5	100

* No confirmation of the age of these plants was established, thus the age was estimated to 3 months.

Fig 2. A, typical symptoms of begomovirus infection in a field-grown tomato plant of the cultivar Shanty sampled in Nicaragua. B, a field-grown tomato plant of the cultivar Shanty in Nicaragua without visible begomovirus symptoms.

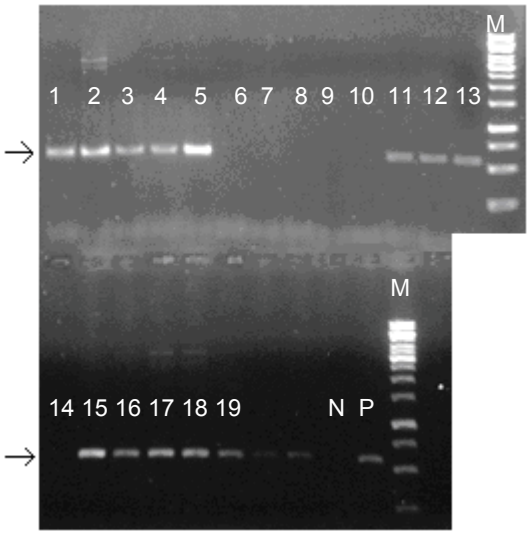

Fig 3. Polymerase chain reaction (PCR) detection of begomovirus infection using the degenerate primers AV494 and AC1048. PCR products (arrow) of approximately 580 bp (including primers) are shown on a 1% agarose gel (100 V, 45 min). Lane 1-5, samples analysed from field 1 (see table 1 for location of fields). Lane 6-10, samples analysed from field 5. Lane 11-13, samples analysed from field 6. Lane 14-15 samples analysed from field 6. Lane 16-20, samples analysed from field 4. Lane N, PCR run without tomato extract (negative control). Lane P, sample containing infected tomato (positive control). Lane M, 1kb Marker (GeneRuler DNA Ladder, Fermentas).

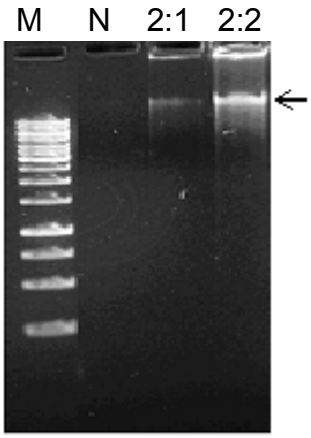

Fig 4. Rolling circle amplification (RCA) products from field 2 shown on a 1% agarose gel (100 V, 60 min). From the left: 1kb Marker (GeneRuler DNA Ladder, Fermentas), sample N containing only RCA reagents (negative control), sample 1 from field 2 (2:1), sample 2 from field 2 (2:2). Both samples showed a clear RCA product (arrow).

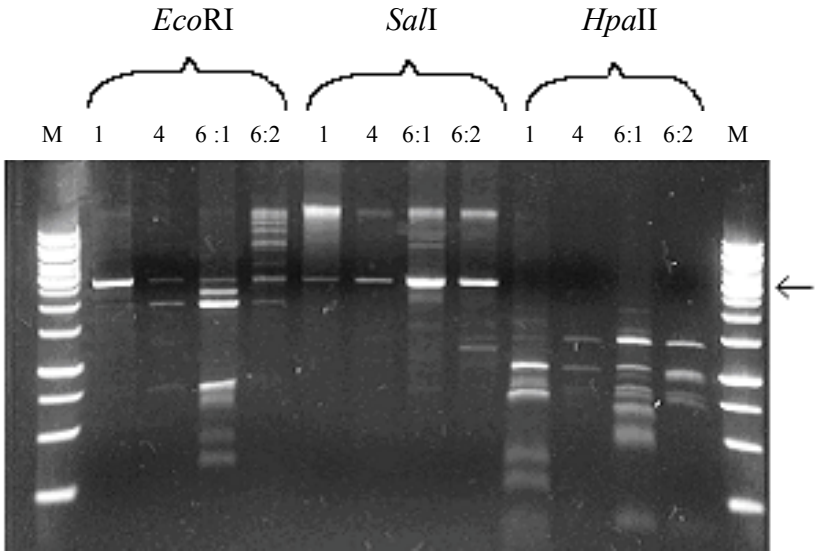

Fig 5. Restriction analysis carried out by the use of the restriction enzymes *Eco*RI, *Sal*I and *Hpa*II. The restriction patterns obtained are shown on a 1% agarose gel (100 V, 60 min). The number written above each lane corresponds to the field where the sample was collected (6:1 and 6:2 correspond to two different samples from field 6). Lane M is a 1 kb Marker (GeneRuler DNA Ladder, Fermentas) The figure shows the four samples (one each from field 1 and 4, and two samples from field 6) after digestion with *Eco*RI, *Sal*I and *Hpa*II, respectively. For all samples digested with *Eco*RI and *Sal*I full-length fragments are visible at approximately 2.7 kb (arrow).

3. Results

3.1 Begomovirus detection by PCR

In the majority of the fields (six of the total eight) visited for collection, almost all tomato plants showed more or less symptoms of begomovirus infection. The sampled plants showed severe symptoms of begomovirus infection such as: yellowing, mosaic patterns and curling of leaves (see Fig. 2). Through analysis by PCR and degenerate primers, PCR products of the expected size (~580 bp) were obtained for seven of the eight fields, thus indicating begomovirus infection. The degree of infection in the tested samples was between 50-100% at the six fields where plants with symptoms had been collected. At the two fields (field 5 and 6, table 1), where plants without symptoms were sampled, field 6 showed begomovirus infection in four of the five tested samples, whereas none of the five tested plants from field 5 were positive (see Fig. 3).

3.2 Detection of mixed infections

Rolling circle amplification was run on PCR-positive tomato samples in order to produce suitable products for restriction analysis. In total, RCA was run on 13 PCR-positive samples and products were obtained from six of the 13 samples: field 1 (one sample), 2 (two samples), 4 (one sample) and 6 (two samples). Fig. 4 shows the two RCA positive samples for field 2. The amplified DNA forms a single high molecular weight band on the agarose gel, which corresponds to the amplified begomovirus genome. One can also observe some RCA products that still are remaining in the wells. This is probably due to the fact that the gel was run in too short time for all of the product to migrate out from the wells.

The restriction analysis suggests that mixed infection is present in five of the six positive RCA products (~80%) (samples are considered positive for mixed infection when two out of three digestions show results of more than three viral DNA components present) (table 2). The restriction enzymes *Eco*RI, *Sal*I and *Hpa*II were used to digest the RCA products to differentiate between distinct viral DNA components. For all samples digested with *Eco*RI and *Sal*I a full-length fragment at approximately 2.7 kb could be found (Fig. 5), which corresponds to one viral component. No such band could be found when the samples were digested with *Hpa*II. In all samples digested with *Sal*I, a high molecular band is visible, corresponding to undigested RCA product. The majority of the *Eco*RI digestions also resulted in a undigested RCA product, while *Hpa*II digested the whole product. The remaining RCA product indicates that there is, at least one, begomovirus genome component that has not been cleaved by the enzyme. For all samples, additional bands could be found that are presented below.

3.2.1 EcoRI digestion
As already mentioned, the restriction pattern obtained after *Eco*RI digestion showed a full-length fragment at ~2.7 kb for all six samples (1, 4, 6:1, 6:2 [Fig. 5]; 2:1, 2:2 [not shown]). Except for sample 4, all samples also yielded an undigested RCA product. The undigested RCA product, together with the full-length fragment, indicates that the samples contain at least two viral DNA components. However, additional bands could be found in the samples, whose sizes could be added up to those of additional viral components (table 2).

In the two samples from field 2, an additional band at 1.4 kb was visible. This band probably corresponds to one viral component (~2.7 kb), cleaved into two fragments of a similar size (~1.4 kb). Hence, the band at 1.4 kb is suggested to be a double band, and it is estimated to correspond to one viral component.

By summarizing the results after *Eco*RI digestion, the results indicate that sample 1, 2:2 and 6:2 contain at least three viral components. Four viral components are estimated in sample 6:1 and 2:1, whereas the result for sample 4 indicates the presence of only two viral components. Thus, the results of the *Eco*RI digestions indicate mixed infections in all samples, except for sample 4 (table 2).

3.2.2 SalI digestion
Similar to the results obtained after *Eco*RI digestion, the *Sal*I digestion resulted in a full-length fragment and an undigested RCA product for all six samples (table 2). For sample 1 and 4, these are the only two fragments present, thus indicating the presence of only two viral components in these samples. The two samples from field 6 (6:1 and 6:2) and the two samples from field 2 (2:1 and 2:2), all showed additional bands that indicate the presence of at least one more viral component (table 2). Hence, the results after *Sal*I digestion indicate mixed infections in all samples, except for sample 1 and 4.

3.2.3 HpaII digestion
In all samples digested with *Hpa*II, neither undigested RCA products were visible, nor a full-length fragment. However, all samples shown in Fig. 5 (1, 4, 6:1, 6:2) showed a band at approximately 1.4 kb as well as one at 1.1 kb. For sample 4, these were the only fragments present, thus indicating that only one viral component was present in this sample. Additional bands could be found in the rest of the samples (1, 6:1, 6:2, 2:1 and 2:2), that could be summarized into further viral components. In sample 1 and 6:1, the bands are estimated to together correspond to four viral components, while for sample 6:2 and 2:1 the sizes of the bands were estimated to correspond to two viral components (table 2). For sample 2:2 only two bands at the approximate sizes 1.0 kb and 0.75 kb were present, thus indicating that no full-length viral component was present in this sample (table 2). The summary of the results after *Hpa*II digestion only shows mixed infections in sample 1 and 6:1.

Table 2. Summary of results from the restriction analysis (see Fig. 5). The table shows the sizes of the fragments obtained for each sample after the three different digestions (*Eco*RI, *Sal*I, *Hpa*II) and if undigested RCA product was present. It also shows the estimated number of begomovirus components (DNA-A and DNA-B), and if the sample is suggested to contain a mixed infection or not.

Sample	Restriction enzyme	Size of fragments (kb)	Nr. of begomovirus components (one component ~2.6-2.8 kb)	Mixed infection
1	*Eco*RI	RCA prod, 2.7, 2.0	≥3	Yes
1	*Sal*I	RCA prod, 2.7	≥2	No
1	*Hpa*II	2.1, 1.8, 1.4, 1.1, 1.0, 0.9, 0.85, 0.8, 0.4, 0.3	4	Yes
4	*Eco*RI	2.7, 2.0, 0.7	2	No
4	*Sal*I	RCA prod, 2.7	≥2	No
4	*Hpa*II	1.4, 1.0	1	No
6:1	*Eco*RI	RCA prod, 2.7, 2.5, 2.0, 0.7	≥4	Yes
6:1	*Sal*I	RCA prod, 2.7, 2.5	≥3	Yes
6:1	*Hpa*II	2.1, 1.6, 1.4, 1.1, 1.0, 0.85, 0.7, 0.6	4	Yes
6:2	*Eco*RI	RCA prod, 2.7, 2.0, 0.75	≥3	Yes
6:2	*Sal*I	RCA prod, 2.7, 2.5	≥3	Yes
6:2	*Hpa*II	1.4, 1.1, 1.0, 0.8, 0.75	2	No
2:1	*Eco*RI	RCA prod, 2.7, 2.0, 1.4, 0.7	≥4	Yes
2:1	*Sal*I	RCA prod, 2.7, 1.4	≥3	Yes
2:1	*Hpa*II	1.4, 1.3, 1.1, 1.0, 0.75	2	No
2:2	*Eco*RI	RCA prod, 2.7, 1.4	≥3	Yes
2:2	*Sal*I	RCA prod, 2.7, 1.4	≥3	Yes
2:2	*Hpa*II	1.0, 0.75	-	No

4. Discussion

The results of this study indicate that the most common tomato productions areas in Nicaragua still are heavily exposed to begomovirus infections. At almost every sampling location all plants in the field showed typical symptoms of begomovirus infection, and in the majority of the sampled plants infection was confirmed by PCR analysis. Even plants that were sampled without any visible symptoms, tested begomovirus positive. These results agree with previous studies regarding begomovirus infections in Nicaragua. For example, Ala-Poikela et al. (2005) performed a study in a very similar manner as in this study. The tomato samples were collected from the same regions (Sebaco, CNIAB (Managua), Las Playitas, Tecolostote) and were analysed for begomovirus infection by PCR, using the same degenerate primers. Their results showed begomovirus infection in all the collected tomato samples. Similar results have also been found in another study (Rojas et al. 2000), hence, the level of begomovirus infections in tomatoes seems not to have changed during the last 10 years. However, it is important to consider that this study was focusing on the relatively new tomato variety Shanty. The Shanty variety is probably less sensitive to begomovirus infection than the tomato cultivars used a decade ago. Thus, the symptoms of infection found in this study are probably milder. It is also important to mention that the result found in this study only refers to the Shanty variety and may not represent the situation for other tomato varieties in the same regions.

The degree of mixed infections present in the samples was also shown to be significant. The results indicate that mixed infections of begomoviruses were present in approximately 80% of the samples analysed. However, there is a possibility that RNA viruses were present in the samples, which then would give a misleading result. But since this was not tested, all viral components in this study are considered to be of begomovirus species. Another possible error to the estimated degree of mixed infection is the undigested RCA product. Because the

undigested RCA product can contain more than one viral component the estimation of viral components in the samples containing undigested RCA product may be incorrect.

In the restriction analysis, the *Hpa*II digestion resulted in a more frequently cut product (i.e. more bands on the gel) for almost all samples compared to when digested with *Eco*RI or *Sal*I. Since *Hpa*II is a "four-cutter" enzyme (recognizing a sequence of four nucleotides), and *Eco*RI and *Sal*I are "six-cutter" enzymes (recognizing a sequence of six nucleotides), *Hpa*II should be able to find more recognition sites in the same genome component, compared to the two other enzymes. Regarding begomoviruses, *Hpa*II frequently cuts all known begomovirus components (DNA-A and DNA-B), whereas *Eco*RI and *Sal*I are known as "single-cutters" (Jeske et al. 2010). Thus, *Sal*I and *Eco*RI are useful when distinguishing different begomovirus species, whereas *Hpa*II is more useful for the identification of particular viral components. Because some samples contained undigested RCA product the properties of the *Hpa*II enzyme was important in order to try to identify the actual number of viral components. In addition, the use of several restriction enzymes gives a more reliable analysis.

From the restriction analysis it is possible to get some indication regarding the diversity of the begomovirus species in the samples, i.e., if the same or different begomoviruses have infected the different samples. In the four samples shown in Fig. 5, two bands can be found in all samples after digestion with *Hpa*II, one at 1.4 kb and one at 1.0 kb. This result indicates that the same begomovirus, or closely related begomoviruses, may be present in these samples. The full-length fragment found in all samples after digestion with the other two restriction enzymes, also indicates that the same or closely related virus species infected the samples. In addition, some of the smaller bands for the different samples after *Eco*RI digestion, have the same size (table 2). Undigested RCA product is also present in the majority of the samples after digestion with *Eco*RI and *Sal*I. However, because the RCA product is likely to contain undigested genome components, it is not possible to draw the conclusion that the same virus is present in the samples, based on remaining RCA product. Nevertheless, by comparing the fragment sizes between the samples, it is possible to conclude that some of the samples probably are containing the same or closely related begomovirus species (table 2).

In the analysis of the restriction patterns a band at 1.4 kb was obtained for all samples (except one) when digested with *Hpa*II. The band was also obtained for the two samples from field 2, after digestion with *Eco*RI and *Sal*I (table 2). What this band corresponds to is not totally clear. In a recent study by Paprotka et al. (2010), it is suggested that such a band represents defective viral DNA. Sometimes half viral genomes can be packed into viral particles, which will have a different shape and size. A half viral genome from such a viral particle may appear at the 1.4 kb position on the gel. Another possibility is that the band represents a DNA-satellite. The role of DNA satellites is a relatively unstudied area, but they are known to occur together with begomoviruses, which may act as helper viruses for the replication and movement of the satellites. DNA satellites known to associate with begomoviruses have the genome size of approximately half a begomovirus component (~1.4 kb) (Seal et al. 2006; Ha et al. 2008). Thus, a DNA satellite genome could create a band at the 1.4 kb position on the gel. There is also a possibility that the RCA product contains amplified tomato DNA that could create a band of that size. However, in this study the 1.4 kb band is explained to be two fragments of approximately the same size. The restriction enzyme is suggested to have cleaved a viral component in such a way that two fragments of the same size (~1.4 kb) were created, and therefore appear at the same position on the gel.

This assumption is, however, unlikely when digesting with *Hpa*II. As already mentioned, *Hpa*II frequently cuts all begomovirus components. Hence, the probability that *Hpa*II only will cut at one position and creating a double band of the genome component is low. In addition, when the 1.4 kb fragment is created from the *Hpa*II digestion it can be summarized with the other created fragments, into a viral component of the approximate size of 2.7 kb. When it appears after digestion with *Eco*RI and *Sal*I it appears as a "free-standing" fragment, thus it is not possible to summarize it together with any of the other fragments to obtain a viral component. Therefore, the conclusion of the 1.4 kb fragment in this study is that it corresponds to a double band when digested with *Eco*RI and *Sal*I, while when digested with *Hpa*II it corresponds to a " normal" single fragment of a begomovirus genome component.

As the results in this study show, the Shanty variety is commonly infected by begomoviruses. Still, the variety is very popular among the farmers (Personal contact Thomas Laguna 2010-05-27). This is probably due to the fact that Shanty, despite begomovirus infection, gives a relatively high yield (Personal contact local farmers 2010-05-13; Hampton et al. 2008). However, the fact that the variety becomes infected makes it a potential reservoir for begomoviruses (Seal et al. 2006). In Nicaragua, it is not uncommon that the farmers grow different crops in the same field or that fields holding different crops are located next to each other. Therefore, a tomato cultivar that acts as a reservoir can cause begomovirus epidemics in other important crops (Ala-Poikela et al. 2005; Seal et al. 2006). The results also indicate that the Shanty variety occasionally becomes infected with more than one begomovirus. Mixed infections are one of the most important factors contributing to begomovirus evolution by providing conditions for recombination (Ala-Poikela et al. 2005; Padidam et al. 1999; Morales 2006). A tomato cultivar, such as Shanty that holds mixed infections provides the precondition for recombination, which may result in more severe begomovirus strains or species (Ala-Poikela et al. 2005). Since the spread of begomoviruses from the Old World into the New World has increased during the last years, there is a higher risk of recombination between begomovirus species (Padidam et al. 1999). Furthermore, the newly introduced B biotype of *B. tabaci* is suggested to have increased the frequency of recombination. The B biotype of *B. tabaci* has a wider host range, which opens the opportunity for recombinant viruses to emerge by infecting new hosts (Padidam et al. 1999). New recombinant viruses are easily maintained in the Nicaraguan cropping system because crops are grown all year round. The constant access to new hosts enables virus epidemics to sustain for a longer time and thereby causing extensive damage in the fields (Seal et al. 2006).

The question is how high the price will be of growing a tolerant tomato cultivar, which gives a relatively high tomato yield, even though it can create new severe begomovirus strains? In addition, Shanty is resistant to TYLCV, a begomovirus not yet identified in Nicaragua, thus, its resistance properties are not optimal for cultivation in Nicaragua. The variety has also been developed in Florida, USA (Giras 2009), which means that when it was introduced into Nicaragua it met different conditions, regarding species of begomoviruses and environment. Hence, the properties of the plant may not be the same when grown in Nicaragua compared to USA.

5. Conclusions

The arguments above indicate that Shanty may not be a suitable tomato cultivar to grow in Nicaragua. However, it is important to remember that the occurring begomovirus situation in Nicaragua does not offer a choice. The tomato producers are dependent on tomato varieties that, despite begomovirus infection, produce relatively high yields of tomatoes. According to

the local farmers, Shanty is one of these varieties. However, a subject for further research would be a more thorough investigation of the Shanty variety status in Nicaragua, i.e., fruit quality and yield. A comparison to other resistant tomato crops grown in Nicaragua would also show its potential value for the tomato production in the country.

In addition, further research should focus on identifying the species of begomoviruses infecting the tomatoes. Identification through sequencing is necessary in order to map the diversity and evolution of begomoviruses and to understand the driving forces behind them (Seal et al. 2006). Since ongoing research in Nicaragua is focusing on creating a local gene bank that will be the foundation for creating local resistant tomato cultivars (Fontagro), research towards understanding the driving forces behind begomovirus evolution is extra important.

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