## الملخص

**مختار، سناء خليفة، أحمد هاشم أحمد، ميشيل بيتر اسميث ومحمد الخير عبد الرحمن. 2009. دراسات حقلية والتعريف الجزئيي لفيروس تجعد أوراق الطماطم في السودان. مجلة وقاية النبات العربية، 27: 95-98.**

أجريت دراسات حقلية في موسمين زراعيين (2003/2002 و2004/2003) بهدف التعرف على مقاومة ستة أصناف (استرين ب، بيتو 86، امدرمان، عبد الله ، الله كريم و CLN21126B) من البندورة/الطماطم لمرض تجعد أوراق الطماطم الفيروسى. أظهرت النتائج أن هنالك فروقات عالية المعنوية في نسبة انتشار وشدة مرض تجعد الأوراق بين الأصناف في كلا الموسمين. أعطى الصنف "استرين ب" أعلى نسبة إصابة وشدة للمرض تلاه الصنف "بيتو 86"، في حين أظهرت الأصناف "عبد الله" و "الله كريم" نسبة إصابة وشدة مرض أقل، تلاهما الصنفين "ام درمان" و "CLN21126B". أعطى الصنف "استرين ب" أقصر نباتات وأقل عدد من الثمار القابلة للتسويق في كلا الموسمين. خلال الموسم الزراعي 2003/2002، أعطى الصنف "بيتو 86" أعلى إنتاجية ثمار (8.5 طن/هكتار) تلاه الصنف "ام درمان "7.7 طن/هكتار" والصنف "عبد الله" (7.3 طن/هكتار). أما في الموسم الزراعي 2004/2003، فأعطى الصنف "عبد الله" أعلى إنتاجية من الثمار القابلة للتسويق (7.7 طن/هكتار) تلاه الصنف "ام درمان" (7.5 طن/هكتار" و "بيتو 86" (7.2 طن/هكتار). أجريت دراسات بمختبر الفيروسات بالمركز الدولي للتعاون في البحوث الزراعية للتنمية (CIRAD) بفرنسا للتعرف على عزلات الفيروس في منطقة الدراسة الحقلية مقارنة بالعزلات الموجودة في مناطق أخرى في السودان وبنك الجينات، فأظهرت النتائج أن العزلات تابعة لفيروس تجعد أوراق الطماطم/البندورة الموجودة في السودان واليمن.



**كلمات مفتاحية:** البندورة/الطماطم، فيروس تجعد الأوراق، الشجره الوراثية.



**عنوان المراسلة: سناء خليفة مختار، قسم علوم وقاية النبات, جامعة كردفان، السودان، البريد الالكتروني: sanamukhtar@hotmail.com**


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which was highly susceptible in both seasons. In 2002/03 the cultivar Strain B gave the lowest yield of marketable fruits (2.9 t/ha) followed by CLN21126B (4.6 t/ha). In 2003/04 the lowest yield of marketable fruits was given by CLN21126B (2.9 t/ha) followed by Strain B (3.3 t/ha). Alla Kareem cultivar gave 4.8 and 3.8 t/ha of marketable fruits in 2002/03 and 2003/04 seasons respectively (Table 2).

**Table 2.** Yield of marketable fruits of six tomato cultivars during 2002/2003 and 2003/2004 growing seasons.

| Cultivars | Tons/hectare (2002/2003) | Ton/hectare (2003/2004) |
|---|---|---|
| Peto 86 | 8.48 a | 7.20 a |
| Abdalla | 7.27 b | 7.70 a |
| Strain B | 2.90 c | 3.30 b |
| Alla kareem | 4.80 bc | 3.80 b |
| CLN21126B | 4.60 bc | 2.90 b |
| Omdorman | 7.70 ab | 7.50 a |
| Mean | 5.96 * | 5.40 * |

* Significant differences at P=0.05 level.

**Nucleotide sequence data**

The PCR products obtained from the leaf samples were cloned and sequenced to identify the suspected begomovirus (Figure 1). The core region of the CP, which was amplified with the pair of primers Ty1/ Ty2, was sequenced. Comparison with sequences available in genebank, showed that the highest nucleotide identities (> 95%) were obtained with members of the species. ToLCV from Sudan and Yemen: ToLCSDV from Gezira, (ToLCSDV-[Gez]) Ay044137 and Af058031), ToLCSDV from Shambat (ToLCSDV [Sha] (Ay044139), and ToLCV from Yemen (Af065821, Af070926).

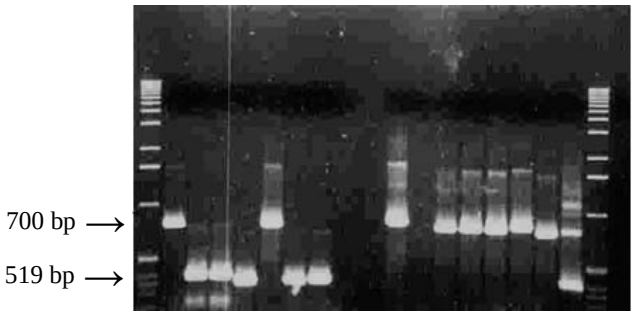


**Figure 1.** PCR amplification of ToLCV cloning using the M13 Reverse and M13 forward:100 bp DNA smart ladder

## Discussion

The effects of cultivars on disease incidence and severity were highly significant in both 2002/03 and 2003/04 seasons. The results showed that the cultivars CLN21126B and Omdorman expressed the lowest incidence and severity, thus they can be classified as resistant or tolerant cultivars (Disease incidence 0.0 %). The cultivars Abdalla and Alla Kareem showed less disease incidence and severity and can be classified as moderately resistant or tolerance Geneif (4) concluded that resistance to ToLCV was probably inhibited multiplication of the virus in the plant at some level after infection.

The highest number of marketable fruits in 2002/03 and 2003/04 was produced by Peto 86 as the yield was not affected by the high incidence. This is in agreement with previous reports (5, 6, 7) which indicated that yield reduction was higher when the tomato plants were infected at early stages leading to drastically reduced production of new fruits. Fruits set before the infection tend to ripen normally. The results obtained in this were in agreement with Geneif (4) who reported that in severe infections none or very few fruits with reduced sizes were produced by the plants. The small fruits and the low fruit weight of the entry CLN21126B is an inherited character.

The results also showed that the disease affected the plant growth of the varieties Strain B and Peto 86 which is in agreed with (11) who emphasized the drastic effects of leaf curl on tomato and poor development of the plants, severe flower shedding that resulted in a low number of small fruits Dafalla (3) also reported that in severe cases of disease there was a marked reduction in plant size, often reaching half or less of normal plant size which is in agreement with earlier report (8).

Sequencing results obtained in this study showed that all samples tested were related to Tomato leaf curl virus. Nucleotide identities (> 95%) were obtained with members of the species *Tomato leaf curl Sudan virus* from Sudan and Yemen. This results was confirmed by (1) who found that tomato samples were collected from different locations from Yemen were infected with isolates that shared around 97% nucleotide (nt) identity with a tobacco isolates from Yemen [AF070926], ToLCSDV- [Gez] [AY044137] and ToLCSDV [Sham] [AY044139].

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plants per plot. In both seasons, the seeds were sown during the last week of September in insect proof cages and transplanted 35 days later. The crop was irrigated once every three days. Harvesting started three months following transplanting. Disease incidence and severity were assessed on the plants 30 days after transplanting and at two- week intervals thenafter until harvest. The virus disease incidence was calculated as follows:

$$\frac{\text{Number of plants exhibiting ToLC symptoms}}{\text{Total number of plants per plot}} \times 100$$

For the determination of disease severity, random samples of 10 plants per plot were used. These plants were then labeled. The disease severity was assessed with a rating scale of 1 (no symptoms) to 4 (severe infection).

**Laboratory testing**

Tomato samples with typical ToLCV symptoms (leaf curl, yellowing and thickening) were tested through the polymerase chain reaction (PCR), to amplify specific DNA fragments of the virus to identify the virus in the samples, the amplified DNA fragments were cloned their sequences determined and compared with sequence of known begomoviruses.

**PCR amplification**

Agarose 1% in 0.5X TBE buffer was prepared. The DNA preparation was mixed with loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol FF, 30% glycerol in water) and loaded and electrophoresed for 4 hrs. at 130 volts. The gel was then stained with a solution of ethidium bromide (1microg/ml) for 10 min and rinsed in a water bath for about 10 min. The gel was photographed under UV light to show the DNA bands.

**Sequencing cloned fragments**

PCR products were sequenced using the PCR primers and then cloned into an *E. coli* plasmid (promega, WI, USA), A culture of *E. coli* strain DH 5a was prepared in 3ml of LB media, 4ml from each cold buffer Tfb 1 and Tfb 2 were added and stored at –80 ºC. The DNA was extracted from *E. coli* using the extraction of the virus DNA from bacteria *E. coli* with Wizard Plus SV MiniPreps DNA purification Systems-centrifugation protocol (Promega, WI, USA)

## Results

**Response of tomato cultivars to ToLCV infection**

Highly significant differences in disease incidence and severity were detected among cultivars in both seasons. Strain B followed by Peto 86 had significantly ($P< 0.01$) higher disease incidence and severity than the other cultivars, in both 2002/03 and 2003/ 04 seasons. The cultivars Abdalla and Alla Kareem showed less disease incidence and severity, whereas the cultivars CLN21126B and Omdorman gave the lowest incidence and severity.

The differences among the cultivars in number of branches, number of unmarketable fruits, weight of unmarketable fruits were not significant in 2002-03 season. On the other hand, variation among cultivars in plant height, number of marketable fruits, shoot and root dry weight and weight of marketable fruits were significant. The cultivar Alla Kareem gave the tallest plants with low number of marketable fruits in both seasons. The cultivar Strain B gave the shortest plants and the lowest number of marketable fruits in both 2002/03 and 2003/04 seasons. The highest number of marketable fruits was produced by Peto 86 which had a high number of branches and short plants in 2002/03 season.

In 2003/04 season the difference among cultivars in number of branches, plant height, number of marketable and unmarketable fruits, weight of marketable fruits and shoot and root dry weights were significant, while the effects of cultivar on weight of unmarketable fruits was not significant (Table1).

**Table 1.** Number of branches, plant height and number of marketable and unmarketable fruits of six tomato varieties during 2002/2003 and 2003/2004 growing seasons

| Variety | No. of branches/ plant | Plant height (cm) | No. of market-able fruits | No. of unmarked-able fruits |
|---|---|---|---|---|
| **2002/2003 growing season** | | | | |
| Peto 86 | 5.00 | 31.30 c | 36.10 a | 2.70 |
| Abdalla | 4.20 | 39.50 bc | 32.40 a | 3.00 |
| Strain B | 4.70 | 32.20 bc | 6.00 c | 4.10 |
| Alla kareem | 5.10 | 69.00 a | 15.30 b | 2.00 |
| CLN21126B | 4.20 | 45.20 b | 33.90 a | 9.30 |
| Omdorman | 3.90 | 37.70 bc | 31.00 a | 2.00 |
| Mean | 4.50 n.s | 36.70 *** | 25.78 * | 3.10 n.s |
| S.E ± | 0.15 | 1.31 | 1.63 | 0.92 |
| C.V (%) | 13 | 12 | 25 | 61 |
| **2003/2004 growing season** | | | | |
| Peto 86 | 5.40 ab | 45.00 bc | 21.00 ab | 5.00 ab |
| Abdalla | 6.20 a | 62.50 ab | 25.00 a | 3.00 abc |
| Strain B | 4.60 b | 43.00 c | 12.30 b | 5.80 ab |
| Alla kareem | 6.30 a | 70.60 a | 15.0 b | 2.80 c |
| CLN21126B | 4.10 b | 54.70 c | 11.90 b | 11.50a |
| Omdorman | 6.20 a | 59.40 ab | 22.90 ab | 2.00 c |
| Mean | 5.50 * | 55.50 ** | 17.90 * | 6.02 * |
| S.E ± | 0.19 | 1.72 | 1.30 | 0.75 |
| C.V (%) | 14 | 13 | 32 | 38 |

*, ** and *** indicates significant differences at 5%, 1% and 0.1% level, respectively.

Values each in column followed by the same letter are not significantly ($P < 0.05$) different according to Duncans multiple range test ( DMRT).

In 2002/03 season, the highest yield of marketable fruits (Table 2) was 8.48 t/ha recorded from Peto 86 followed by Omdorman (7.7 t/ha) and cultivar Abdalla (7.3 t/ha). In 2003/04 season, the highest yield of marketable fruits was obtained from the cultivar Abdalla (7.7 t/ha) followed by Omdorman (7.5 t/ha) and Peto 86 (7.2 t/ha). Whereas the highest number of unmarketable fruits was produced by the cultivar CLN21126B, followed by Srain B

# Field Screening and Molecular Identification of *Tomato leaf curl virus* in Sudan

**Sana K. Mukhtar[1], Ahmed Hashim[2], Michel Peterschmitt[3] and Mohamed K. Abdrahman[4]**
(1) Plant Protection Department, University of Kordofan, Sudan, Email: sanamukhtar@hotmail.com; (2) Plant Protection Department, University of Khartoum, Sudan; (3) Virology Laboratory, CIRAD, Montpellier, France; (4) Department of Agronomy, University of Kordofan, Sudan.

## Abstract

**Mukhtar, S.K., A. Hashim, M. Peterschmitt and M.K. Abdrahman. 2009. Field Screening and Molecular Identification of *Tomato leaf curl virus* in Sudan. Arab Journal of Plant Protection, 27: 95-98.**

Tomato (*Lycopersicon esculentum*. Mill) is one of the most popular vegetable crops in the world. Tomato leaf curl disease is one of the most destructive diseases of tomato crop. *Tomato leaf curl virus* (ToLCV, genus *Begomovirus*, family *Geminiviridae*) is transmitted by the whitefly *Bemisia tabaci* Genn. Two field trials were conducted during 2002/2003 and 2003/2004 winter seasons at Bara area western sudan to identify tomato cultivars resistant to Tomato leaf curl Sudan virus (ToLCSDV, genus *Begomovirus*, family *Geminiviridae*). In both seasons the response of cultivars to disease incidence and severity was highly significant. The cultivar Strain B gave the highest disease incidence and severity followed by the cultivar Peto 86. The cultivars Abdalla and Alla Kareem showed less disease incidence and severity. The cultivars CLN21126B and Omdorman gave the lowest incidence and severity. In 2002/03 season the cultivar Peto86 gave the highest yield of marketable fruits (8.5 t/ha) followed by cultivar Omdorman (7.7 t/ha) and cultivar Abdalla (7.3 t/ha). In 2003/04 season the cultivar Abdalla gave the highest yield of marketable fruits (7.7 t/ha) followed by Omdorman (7.5 t/ha) and Peto 86 (7.2 t/ha). The cultivar Strain B gave the lowest yield of marketable fruits (2.9 and 3.3 t/ha) in 2002/03 and 2003/04 respectively followed by CLN21126B (4.6 t/ha). Laboratory studies were conducted in the virology laboratory at the International Agricultural research center for Development(CIRAD), Montpellier, France, where tomato samples were collected from five different locations in the Sudan were tested. The sequence of the capsid protein gene confirmed that all tomato samples were infected with ToLCV.
**Keywords:** *Tomato leaf curl virus*, Phylogenic tree, Sudan.

## Introduction

Tomato (*Lycopersicon esculentum* Mill.) is a major salad crop in the Sudan and also processed tomato paste, ketchup, sauce and dry tomato slices, and is a main cash vegetable crops production in the Sudan has not reached its full potential, neither in quantity nor in quality, The crop is seriously affected by many pests of which the viral diseases are the most important. Tomato leaf curl virus disease is one of the most destructive diseases of tomato crop (9). *Tomato yellow leaf curl virus* (TYLCV, genus *Begomovirus*, family *Geminiviridae*) causes an extensive damage to tomato crops in many tropical and subtropical regions (2). This virus is particularly important in the Mediterranean region, South America, Africa and South-East Asia. Symptoms caused by geminiviruses on tomato plants include leaf curling, stunting and distortion, interveinal yellowing and necrosis of older leaves.

In the Sudan, tomato leaf curl disease is a serious disease in tomato. It is endemic throughout the country, and often reaches epidemic levels (10).

The most common form of ToLCV is characterized by upward curling, surface reduced and yellow leaflets, without veinal symptoms. Seriously affected plants are markedly reduced in size with serious flower shedding. In severe infections none or very few fruits with reduced sizes are produced (4). This study was conducted to identify tomato cultivars that are resistant to the disease **a**nd to compare partial genomic sequences of field isolates of ToLCV with sequences of isolates collected from elsewhere in the Sudan and from outside the country.

## Materials and Methods

### Experimental site and climate

The research was conducted in the Ministry of Agriculture Nursery, Bara, North Kordofan State (Latitudes 13º 42.78 N and Longitudes 30 21. 24 E), Sudan which falls in the semiarid zone. The soil is sandy soil (superficial deposits) with clay pockets in Khairan area (Bashiri, AlHumara and Abugaidda etc.). The average annual rain fall is around 200 mm.

### Experimental layout

Field experiments were conducted in 2002/03 and 2003/04 winter seasons. This experiment consisted of six treatments including the following six tomato cultivars, Strain B, locally known as Seiko; Peto 86, locally known as Abu Sabah; Omdorman and Abdalla, released by the National Institute for Promotion of Horticultural Exports, Gezira University, Sudan; Alla Kareem a local variety widely cultivated by farmers in Khor Abu Habil area in north Kordofan state and CLN21126B introduced from the Asian Vegetable Research and Development Centre (AVRDC).

The treatments were distributed in a randomized complete block design with three replicates. Plot size was 4.125 m² with three rows and 60 cm between the rows and 50 cm between plants in each row. Plant population was 15