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SERODIAGNOSIS OF *TOBACCO STREAK VIRUS* INFECTING SUNFLOWER IN VILLAGES OF KADAPA DISTRICT, ANDHRAPRADESH

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ABSTRACT: *Tobacco streak virus* (TSV) is an economically important plant virus infects both dicots and monocots. It was distributed all over the world and account for huge crop loss. In India, its epidemic occurs in peanut and sunflower. In the present study, we have collected leaf samples from sunflower plants showing chlorotic and necrotic lesions from the fields of Sreenivasapuram and Machupalli villages of Kadapa district and screened for TSV by direct antigen coating (DAC)-ELISA. Out of 61 field samples, we found 2 samples were positive for TSV. The ELISA positive samples were also confirmed as TSV by westernblot-immunoassay. The remaining 59 samples negative for TSV and may be infected by other plant pathogens.

KEYWORDS: Sunflower, *Tobacco streak virus,* Enzyme linked immunosorbent assay (ELISA), Sunflower necrosis disease (SND).

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1.INTRODUCTION

Sunflower (*Helianthus annuus.* L) is one of the most important oilseed crops grown in temperate countries. In India it is locally known as Suryakanti or Suryaful a major source of vegetable oil [1].The crop was introduced during 1969 in India and now the country is occupied third place in world sunflower oilseed production [2]. The major sunflower cultivating states in India are Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu [1]. In Andhra Pradesh, the major sunflower producing districts are Kadapa, Kurnool, Prakasam and Anantapur. Sunflower is grown in Kharif, Rabi and spring seasons throughout India. Sunflower is susceptible to fungal, bacterial

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and viral diseases. The common viral diseases in sunflower are Sunflower mosaic disease, Sunflower ring spot disease, Yellow blotch disease (YBD) and Leaf crinkle disease (LCD), Leaf curl disease, Sunflower necrosis diseases (SND), Sunflower mosaic disease, Sunflower leaf curl disease by *Cucumber mosaic virus*, *Sunflower ring spot virus*, *Sunflower crinkle virus* (SuCV), *Tobacco leaf curl virus*, *Tobacco streak virus* (TSV), *Sunflower mosaic virus* (SuMV), Sunflower leaf curl virus (SuLCuV), respectively [3]. Among these viruses, TSV poses potential threat to the cultivation of sunflower in India. TSV belongs to the family *Bromoviridae* in the genus *Ilarvirus*. It has a wide host range and reported in all over the world. It infects various crops, mainly *Asteraceae*, *Brassicaceae*, *Cucurbitaceae* and *Solanaceae* families and responsible for huge economic loss [4]. TSV does not generally lead to epidemics, with the exception of sunflowers in India and Australia, and peanuts in India [5, 6]. There were at least 18 different field crops and 16 alternative weed hosts susceptible to TSV infection in India. Severe disease incidences by TSV reported in sunflower [7, 8, 9], peanut [5], okra [10] and cucurbits [11] in India. In a study found that the weed hosts over the side of the field crops responsible for TSV disease incidences. In the epidemic locations, TSV was identified in many weed hosts of which *Parthenium hysterophorus* probably plays a major role in spread of TSV by thrips [6]. The major method of TSV transmission is by infected pollen, which can be spread by wind or carried by various species of Thrips viz., *F. schultzei, S. dorsalis, T. palmi, M. usitatus, H. gowdeyi, B. melonicornis and T. hawaiiensis* [12, 13, 3]. As in other *bromoviridae* viruses, TSV consists positive sense genomic ssRNA of three different ssRNAs including RNA1, RNA2 and RNA3 of 3.5 kb, 2.9 kb, and 2.2 kb in size, respectively. The RNA4 is a subgenomic RNA derived from RNA3. RNA1 and RNA2 encode proteins for viral replication, whereas RNA3 encodes movement protein for cell-to-cell movement and the viral coat protein (CP) is expressed by a subgenomic RNA4 [14]. In the present study, we have detected TSV in naturally infected sunflower plants by DAC-ELISA.

2. MATERIALS AND METHODS

Sample collection

A total of 61 sunflower leaf samples showing chlorotic and necrotic spots were collected (August, 2016 to February, 2017) randomly from villages of Surathkhanpalli (16 samples), Sreenivasapuram(15 samples), Machupalli (15 samples), Kopparthi and Mittameedhapalli (15 samples) of Kadapa District in Andhra Pradesh. All these samples subjected for detection of TSV by DAC-ELISA.

DAC-ELISA

It was performed as described by Clark and Bar-Joseph [15]. The infected leaf samples were washed twice with distilled water and crushed in carbonate buffer (0.2 M Na_2CO_3; 0.2 M $NaHCO_3$; pH 9.6; 1:10 w/v). A healthy leaf extract was also used as a healthy control. The prepared extracts of 200 µl

were loaded into the wells of the microtitre plate and incubated at 37 ^{0}C for 90 mins. Then the wells were washed thrice with PBS-T buffer (0.1 M PBS with 0.1 % Tween 20) and once with PBS buffer (0.1 M, pH 7.4). TSV antiserum made into 1:5000 dilution (v/v) in the antibody buffer (PBS-T; 2% PVP; 0.2% egg albumin) was dispensed 200 µl per well and incubated at 37 ^{0}C for 90 min. Again, the plate was washed thrice with PBS-T and once with PBS. Alkaline phosphatase (ALP) labeled goat anti rabbit antibodies (secondary antibody) diluted in antibody buffer (1: 2000, v/v) and added 200 µl per well and incubated at 37 ^{0}C for 90 min. Then the plate was washed thrice with PBS-T and once with PBS buffer solution. Finally, a freshly prepared substrate solution (Diethanolamine, 9.7 ml; Distilled water, 80 ml; p-Nitrophenyl phosphate 0.5 mg/ml; pH 9.8) was added to the wells and plates were incubated in dark for 30 min. The reaction was terminated by adding 50 µl of the reaction stopping solution (2 N NaOH). The readings were taken at A_{405nm} in an ELISA plate reader (BIORAD).

Western blot Immunoassay

TSV positive and negative sunflower leaf samples were grounded with PBS buffer (1:10 w/v) and centrifuged at 8000 rpm for 10 mins. The supernatant was carefully collected and SDS PAGE was carried out as described by Hill and Shepherd [16]. Further the proteins were transferred onto the nitrocellulose membrane according to the Li *et al.,* [17] method with few modifications. After blotting of proteins, membrane was carefully removed from blotting unit and incubated in blocking solution [TBS (0.2 M Tris, 0.5 M NaCl) with 5% dried milk powder, (w/v)] on a shaker for 1 hr at room temperature. Then the membrane was incubated for 1 hr in antibody solution (TBS-T with 5% dried milk powder) containing primary antibodies (TSV polyclonal antibodies) (1:1000, v/v). After three washings with TBS-T (TBS with 0.05% Tween-20), the membrane was incubated in secondary antibody solution (IgG conjugated with ALP). Finally, the membrane was washed thrice with TBS-T and immersed in substrate solution (BCIP/ NBT, Genei). Color development was observed and reaction was stopped by washing the membrane with distilled water.

3. RESULTS AND DISCUSSION

Sunflower (*Helianthus annuus* L.) is an important edible oilseed crop in the country next to groundnut and soybean [3]. TSV is one of the main constraints to the sunflower crop, causes sunflower necrotic disease (SND) and leads to severe yield loss. The virus has wide host range including weed hosts and spread rapidly with infected pollen by insect vectors and wind, causes epidemics in susceptible crops. In India, TSV was initially identified in sunflower and peanut causing necrosis disease [9, 5]. In Tamil Nadu, Nakkeeran (AICRP report 2010) first reported the association of TSV in cotton. In the early 2000s, TSV has been identified as the cause of wide spread crop losses in many regions in India [9]. Although disease levels fluctuate from season to season, actual losses of 5-70 % are reported from sunflower and a single epidemic of TSV in groundnut

during 2000 reportedly caused crop losses of greater than $ AUD 60 million [18, 10]. TSV appeared as an epidemic form from 1997 to 1999 in Southern India and caused yield loss up to 90 % [3]. In this study we have observed some of the sunflower plants infected by plant pathogens. The infected plants showing symptoms such as chlorotic and necrotic lesions on the leaves, severe necrosis of stem petioles and wilting (fig.1). The early infection of TSV kills entire plants and produces characteristic symptoms, namely, necrosis of leaf, petiole, stem, and bracts and malformation of head with chaffy grains [3]. Initially we have suspected TSV infection and to diagnose the virus, 61 symptomatic leaf samples were collected randomly from the sunflower fields of Sreenivasapuram (15 samples), Surathkhanpalli (16 samples), Machupalli (15 samples), Kopparthi and Mettamedap alli (15 samples) villages of Kadapa district. All these villages were closely located with an average distance of two kilometers. These field samples were tested for TSV by DAC ELISA. ELISA is a simple and sensitive technique use widely to diagnose plant virus diseases. Hence, in this study we have used DAC-ELISA as a diagnostic tool to detect TSV infection in sunflower. The polyclonal antiserum for TSV was positively reacted with 2 sunflower samples of Sreenivasapuram (1 sample) and Machupalli (1 sample). The two positive samples were given more than thrice OD value than the healthy control samples. The OD value of the test sample is more than two folds than the OD values of the healthy control sample considered as test positive [19]. Further these two ELISA positive samples were also confirmed by westernblot-immunoassay (fig.2). The remaining 59 samples were negative with TSV antisera and may be infected by other than TSV. It seems the sunflower necrosis disease was not wide spread during the time (August, 2016 to February, 2017) of sample collection in villages of the Kadapa district. The presence of TSV in necrosis disease affected leaf samples collected from major sunflower growing areas of Andhra Pradesh, Karnataka, Maharashtra and Tamil Nadu was confirmed by DAC-ELISA using TSV specific polyclonal antiserum [20]. In addition to sunflower, TSV also infects many commercial crops including groundnut, cotton, soyabean, mungbean, chickpea. Recently, the rate of incidence of TSV in cotton was surveyed in various states of India by DAC-ELISA, where Telangana (India) had the highest incidence when compared to Tamil Nadu, Andhra Pradesh and Maharashtra [21]. Similarly, TSV was first identified in cluster bean by DAC- ELISA using TSV polyclonal antibodies [22]. However, molecular tools like PCR and real time PCR were highly sensitive but expensive and not suitable for field applications. DAC- ELISA is inexpensive, highly sensitive and reliable technique to detect TSV in sunflower.

Fig.1. The sunflower plants showing chlorotic and necrotic symptoms on the leaves

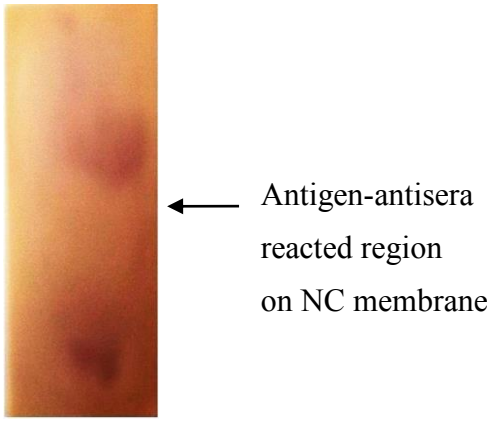

Fig.2. The crude leaf extract of TSV positive sunflower samples reacted with TSV antisera in westernblot-immunoassay.

4. CONCLUSION

Sunflower is one of the most important oilseed crops grown in Andhra Pradesh in both Kharif and Rabi seasons. We have collected 61 field infected sunflower samples showing chlorotic and necrotic lesions from five villages of Kadapa district (AP) during the period of August, 2016 to February, 2017 and tested for TSV infection by DAC-ELISA. The TSV antisera positively reacted with only two samples by DAC-ELISA indicating the sunflower necrosis disease was not severe and wide spread at the time of sample collection in growing sunflower crop areas.

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