



Sunflower Necrosis Disease – A Threat to Sunflower Cultivation in India: A Review

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Received for publication: October 26, 2013; Accepted: November 17, 2013.

Abstract: Among different diseases infecting sunflower (*Helianthus annuus* L.), sunflower necrosis disease (SND) which was observed for the first time in 1997 has emerged as a major disease of sunflowers in India. The disease has been noticed in an epidemic form consecutively for the last twelve years and the incidence is ranging from 5-90 per cent both in open pollinated varieties and hybrids. Disease incidence is a function of various aspects of host, vector, weather, time and its transmission. Effect of necrosis disease on yield and yield parameters of sunflower are very adverse and it includes reduction of plant height, leaf area and chlorophyll content of plant at all growth stages of infection. In this review, we describe the incidence, transmission, and recent advances of molecular and serological detection of virus and their preventive measures. Finally concluded that an integrated control measures have evident benefits and should be fostered and promoted as a means of enhancing crop productivity to meet the increasing demands of a rapidly increasing human population.

Keywords: Tobacco streak virus, Necrosis Disease, Sunflower plant, Transmission, Thrips.

Introduction

Sunflower (*Helianthus annuus* L.), a member of compositae family is the second most important edible oilseed crop next to soybean in the world. It was first domesticated in central parts of the United States of America. Oil extracted from sunflower is a light, healthy and nutritious that is easy to digest. Sunflower oil is rich in natural vitamins, it consists mainly of poly-unsaturated fatty acids (PUFA) and is low in saturated fats. This crop was introduced into India during 1969, which accounts for nearly 5 percent of the current oil seed production. In India, Sunflower crop is cultivated in an area of 1.48 million hectares with production of 0.9 million tonnes (DOR Annual Report, 2010) and Karnataka, Andhra Pradesh, Maharashtra and Tamil Nadu are the major sunflower growing states. The crop is highly vulnerable to stress factors such as various diseases incited by viruses, bacteria, phytoplasma and fungi [1] resulting in severe economic losses. Several viruses belonging to Cucumo, Ilar, Poty, Tospo and Umbra virus groups are known to infect sunflower severely [2]. Virus and virus like diseases of sunflower have been reported by various workers, both in India and abroad are enlisted in Table 1. Sunflower mosaic, chlorotic mosaic, yellow ring mosaic, yellow mosaic, yellow spot, chlorotic leaf/mosaic, greening, cucumber mosaic and mycoplasma like organisms (strain of tomato big bud, aster yellows and

phyllody) have been reported from this crop. All these diseases continues to be the main factor for low productivity and for the last few years, there has been a decline in the acreage to less than 2 million ha. Among these diseases, major factors in this reduction are attributed to the occurrence of sunflower necrosis disease. The disease has now spread to sunflower growing areas and up to 80% incidence of the disease was recorded. Following are the brief account of major viral diseases of sunflower in India

Sunflower mosaic disease:

The causal organism of this virus is Sunflower mosaic virus (Strain of *Cucumber mosaic virus*). The affected plants show mosaic patterns accompanied by ring spots or chlorotic spots that show tendencies to coalesce to form mosaic pattern, cupping and malformation of leaves [3, 4]. The virus is restricted to sunflower only. *Nicotiana tabacum*, *N. glutinosa* and *Capsicum annum* are not infected by the virus. Another mosaic disease was reported which shows the presence of small circular chlorotic spots on leaves, which coalesce to form typical mosaic patterns [5]. Reduction in seed yield, cupping and malformation of leaves, poorly developed root system; seed viability and pollen fertility are some of the associated symptoms.

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Table 1: Viral diseases of Sunflower and their transmission

S.No	Disease	Virus	Genus	Transmission	Reference
1	Sunflower mosaic disease	<i>Cucumber mosaic virus</i>	<i>Cucumovirus</i>	Seed and A. gosipi; Myzes <i>persicae</i>	Arnott and Smith, 1967; Orellana and Quacquarelli, 1968; Gupta & Gupta 1977; Gupta, 1981; Gupta and Roy, 1981; Venugopal Rao et al., 1987; Jitendra Mohan, 1992; Nagaraju et al., 1997.
2	Sunflower ring spot disease	<i>Sunflower ring spot virus</i>	<i>Iilarvirus</i>	Thrips sps.	Behncken, 1974.
3	Yellow blotch (YBD) and leaf crinkle disease (LCD)	<i>Sunflower yellow blotch virus</i> ; <i>Sunflower crinkle virus</i> (SuCV)	<i>Umbravirus</i>	<i>A.gossipi</i>	Theuri et al., 1987. Ndunguru & Hanumantha Rao, 1999.
4	Leaf curl disease	<i>Tobacco leaf curl virus</i>	<i>Begomovirus</i>	<i>Bemesia tabaci</i>	Chatterjee and Pandey, 1977.
5	Sunflower stem necrosis diseases (SND)	<i>Tobacco streak virus</i> (TSV)	<i>Iilarvirus</i>	Thrips sps.	Prasada Rao et al., 2000; Ravi et al., 2001 and Bhat et al., 2002b.
6	Sunflower mosaic disease	<i>Sunflower mosaic virus</i> (SuMV)	<i>Potyvirus</i>	<i>Myzus persicae</i> and <i>Capitphorus elaeagni</i> ,	Jindal et al., 2001, Verma et al., 2009.
7	Sunflower leaf curl disease	<i>Sunflower leaf curl virus</i> (SuLCuV)	<i>Begomovirus</i>	<i>Bemesia tabaci</i>	Govindappa et al., 2011.
8	Sunflower chlorotic mottle virus	<i>Sunflower chlorotic mottle virus</i> (SuCMoV)	<i>Potyvirus</i>	<i>Aphids</i>	Dujovny et al., 2000)

Transmission and host range of Sunflower mosaic disease:

The virus is reported to be sap transmitted [6]. It is also transmitted through graft and seeds. The important vectors are aphid species i.e. *Aphis. gossypii*, *A. craccivora* and *Myzus persicae*. The virus has a dilution end point of 1:500- 1: 1000, thermal inactivation point of 55-65°C and longevity in vitro for 66-72 h at room temperature [7]. The host range of this virus is narrow infecting only one weed host (*Galinsoga parviflora*).

Yellow ring mosaic disease:

Yellow ring mosaic virus is the causative organism of this disease and this disease shows severe mosaic accompanied by stunting and malformation of young leaves in the form of yellow rings [8]. Affected plants do not produce flowers or reduction of flower number.

Transmission and host range of Yellow ring mosaic disease:

The virus is transmitted both by mechanical sap and leaf grafting. The virus has limited host range producing chlorotic

local lesions only on *Chenopodium amaranticolor*.

Yellow blotch and leaf crinkle diseases:

The causative organism of this disease is virus particles which are isometric, 26 nm in diameter and serologically related to *Beet western yellows virus*. In yellow blotch disease (YBD), initially short, irregular yellow vein bands appear, later they coalesce to form distinct bright yellow blotches measuring 1-3 cm in diameter, but the plant height and leaf morphology are unaffected. In leaf crinkling disease (LCD), irregular yellow bands appear as in yellow blotch, but also include severe leaf puckering, starting with the youngest leaves, reduced leaf size and stunted plants. This disease was reported from African countries [9] and affected 80 % of the plants in certain fields.

Transmission and host range of Yellow blotch and leaf crinkle diseases:

Both the diseases can be transmitted by mechanical sap and aphid (*A. gossypii*) was able to transmit YBD. Viruliferous aphids which transmit YBD include *Arachis hypogaea*, *Glycine max*, *Lactuca sativa*, *Nicotiana clevelandii* and *Zinnia elegans*. There are no

known sources of resistance against this virus. Clean cultivation by removing the weeds both inside the field and neighboring fields, removal and destruction of infected plants reduce further spread of the disease. Spraying of suitable insecticides to control the insect vectors as a prophylactic spray is recommended [10].

Sunflower mosaic disease:

In India, *Sunflower mosaic virus* (SMV) incidence ranged from 5-10 per cent and disease appears mostly in *kharif* season. It belongs to the genus *Potyvirus*. The disease will not have much impact on yield reduction. However, there are reports that SMV infection reduced the plant height, stem girth, leaf area, head size and seed weight of sunflower hybrids [11, 12]. So far, limited research work has been attempted with regard to sunflower mosaic disease since they do not cause much economic loss.

Sunflower leaf curl disease:

Recently *Begomo* viruses transmitted by whitefly *Bemisia tabaci* causing symptoms like leaf curl (Fig. 1), leaf thickening, leaf enations and stunting symptoms are emerging threat to sunflower cultivation [13]. Sunflower leaf curl disease (SuLCuD) was observed to the extent of 40 per cent on 'Sunbred 275' hybrid during *rabi* 2009 in the fields of UAS, Raichur, Karnataka. Phylogenetic analysis of the core CP gene sequence of the virus with those of other begomoviruses clustered next to *Tomato leaf curl virus* isolate and shared 97.5 % nucleotide identities. However, exact taxonomic status requires sequencing of the complete ssDNA viral genome. Tentatively virus name has given as Sunflower leaf curl virus.

Sunflower necrosis disease (SND):

Sunflower necrosis disease (SND) is a potential threat in all traditional sunflower growing areas in India. SND was reported as an epidemic consecutively for the three years (1997-99), with the incidence ranging from 10 to 80 percent and causing yield losses up to 90 percent in most of the sunflower growing regions of Southern India (DOR Annual Report, 2001). The causal agent of SND was identified as *Tobacco streak virus* (TSV), belonging to the genus *Illavirus* and Family *Bromoviridae* [14].

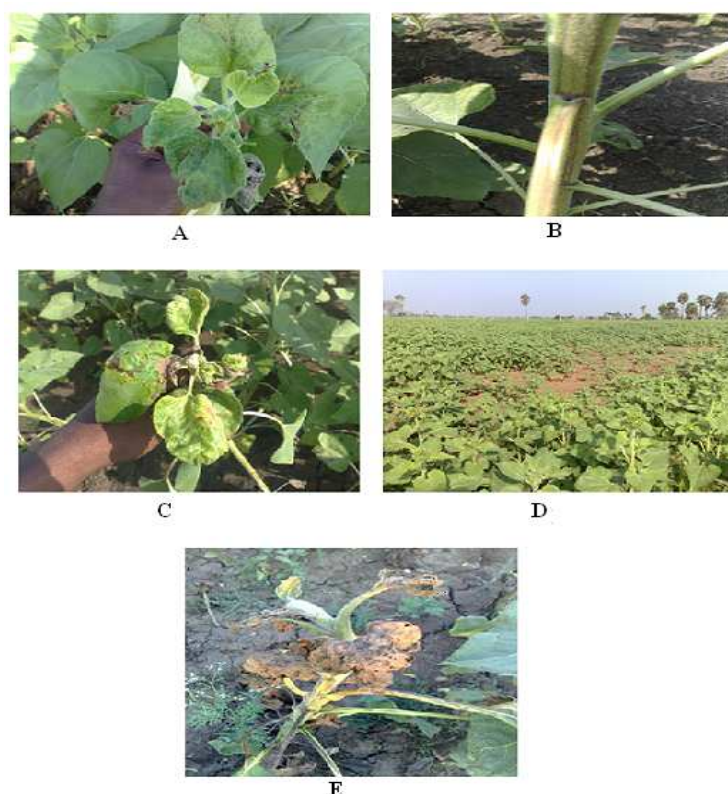


Fig.1: Symptoms of sunflower necrosis disease in sunflower plants.

(A) Sunflower plant with mosaic and leaf necrosis (B) Stem and Petiole necrosis (C) Sunflower flower bract necrosis (D) Virus affected sunflower fields (E) Complete death of plant.

Occurrence of SND & vector population in India:

SND was reported from Netherlands, Australia [24] and India. It was reported for the first time in India at Bagepalli village of Kolar district, Karnataka in 1997 [25] which later spread to AP, TN and Maharashtra states. The disease was noticed in an epidemic form consecutively for the three years (1997-99) in most of the sunflower growing regions of Southern India (DOR Annual Report, 2000). In Andhra Pradesh, maximum incidence of SND (38 percent) was reported during October, 2003. Similarly, highest average thrips population/head (16.3 thrips/head) was recorded during March, 2004 on sunflower cv. *Morden* at Chevella, Narkhoda and Kavvaguda villages of Ranga Reddy district during fortnightly survey [26].

The necrosis disease monitoring in Aland taluk alone in Gulbarga district in Karnataka, India, indicated that out of 23,000 ha area planted, 12,142 ha (52.79%) area suffered severe crop loss due to necrosis virus

at early growth stage (45 days) during 2002-03 [27]. Further, the survey during 2003-05 in three districts of Gulbarga, Bidar and Raichur indicated the mean disease incidence of 19.81% with disease ranging from 0.0-100%, and recently in 2011 [28]. were reported the disease incidence in November 0.67 percent shown then immediately it increased 39.30 percent shown in January.

In Southern Karnataka, highest incidence of 22 percent was reported on cv. KBSH-1 with mean thrips population of 2.42 per five plants at Bagepalli taluk on May, 2006 sown crop [29], whereas in Northern Karnataka highest incidence of SND (36%) and thrips population (9.6 thrips/3leaves) was reported in Raichur district [30]. The intensity of disease ranged from 2 to 100 percent. The seed yield losses are as high as 89 per cent under severe conditions (DOR Annual report, 2001). The disease has significant impact on the crop as early infection causes death of the plants or severe stunting with malformed head or heads filled with chaffy seeds [14, 31]. Early infected plants remain stunted and develop malformed heads with poor or no seed setting, resulting in complete loss of the crop [32]. There has been a continuous threat to sunflower production in India due to TSV epidemics and reduction of over 40% in the yield since 1997, amounting to annual loss of Rs.76 crores [32].

Symptoms and Host range:

The disease is observed at all growth stages starting from seedlings to mature plant. The characteristic field symptoms of the disease include mosaic on leaves that leads to extensive necrosis of leaf lamina, petiole, stem, floral calyx and complete death of seedlings eventually. (Fig.1 A-E). Early infection either kills the plant or causes severe stunting with malformed head filled with chaffy seeds [14, 31] Necrosis at bud formation stage makes the capitulum to bend and twist resulting into complete failure of seed setting and maturation. [33]. Fig.1 Shows, A; Sunflower plant with mosaic and leaf necrosis; B: Stem and Petiole necrosis; C: Sunflower flower bract necrosis; D: Virus affected sunflower fields E: Complete death of plant.

Host range studies clearly indicated that several workers revealed that the virus causing SND could also infect members belonging to families. Amaranthaceae,

Chenopodiaceae and Fabaceae [34]; Asteraceae, Leguminosae and Cucurbitaceae [35] and Fabaceae, Malvaceae, Cucurbitaceae and Solanaceae [36]. Thirty three plant species from six families Asteraceae, Chenopodiaceae, Cucurbitaceae, Leguminosae, Malvaceae and Solanaceae expressed visible symptoms [37].

Lavanya et al., [36] revealed that the weeds such as *Trianthema portulacastrum*, *Priva leptostachya*, *Digeria arvensis*, *Clitoria ternata*, *Solanum nigrum*, *Vernonia cineraria*, *Trichodesma indicum* and some other species were found to serve as hosts for sunflower necrosis virus. Natural occurrence of TSV infection has also been recorded from other crops, shown in Table. 2. The host range studies clearly indicated that several weed species occurring in sunflower fields and in crop plants such as groundnut, green gram, black gram, cowpea, and soybean were most commonly TSV hosts under field conditions cultivated in *Capsicum annuum*, *Gossypium hirsutum*, *Vigna unguiculata*, *Cucumis sativus*, *Cucumis anguria*, *Arachis hypogaea*, *Tagetes erecta*, *Guizotia abyssinica*, *Abelmoschus esculentus*, *Carthamus tinctorius*, *Glycine max*, *Helianthus annuus*, *Cratalaria juncea* and *Vigna mungo*, *Vigna radiat* and also observed in weed species *Abutilon indicum*, *Acalypha indica*, *Achyranthes aspera*, *Acanthospermum hispidum*, *Calotropis gigante*, *Cleoma viscosa*, *Commelina benghalensis*, *Croton bonplandianum*, *C. sparsiflorus*, *Digera arvensis*, *Euphorbia hirta*, *E. geniculata*, *Lagasca mollis*, *Leucas aspera*, *Parthenium hysterophorus*, *Tridax procumbens* and *Xanthium strumarium*.

Table 2: Natural occurrence of TSV recorded from other crops.

Host	Reference
Cotton	Bhat et al., 2002a
sun hemp	Bhat et al., 2002a
Mungbean	Bhat et al., 2002a
Groundnut	Reddy et al., 2002
Cucumber	Krishna Reddy et al., 2003a & b
Gherkin	Krishna Reddy et al., 2003a & b
Okra	Krishna Reddy et al., 2003a & b
Safflower	Chander Rao et al., 2003b
Chilli	Jain et al., 2005
Urdbean	Ladhalaxmi et al., 2006
Niger	Arunkumar et al., 2007
Onion	Sivaprasad et al., 2010
Kenaf	Bhaskara Reddy et al., 2012
Guar	Sivaprasad et al., 2012
Jasmine	Seshadri Goud et al., 2013
Horse gram	Seshadri Goud et al., 2013
Pigeon pea	Vemana et al., 2013

Transmission of Sunflower necrosis disease (SND):

Mechanical / Sap transmission:

The disease was transmitted by mechanical or sap inoculation from sunflower to sunflower cv. Morden under laboratory conditions [19,51]. In general, *Ilarvirus* had wide host range as they were efficiently sap transmissible to many of the host plants belonging to Amaranthaceae, Chenopodiaceae and Fabaceae under glass house conditions [32,34].

Sundaresha *et al.*, [52] developed a rapid and efficient sap inoculation method for *tobacco streak virus* (TSV-SF). Their study has demonstrated that a high throughput methodology for sap inoculation of TSV has been developed and the symptoms confirmed as SND by both expression and transcript accumulation of the viral proteins. Conventionally, sap inoculation of TSV has been through the swabbing of leaf lamina along with an abrasive that makes it a very tedious and cumbersome procedure

Seed transmission: As per the best of our knowledge there are no reports regarding seed transmission of sunflower necrosis virus. The same is proved by Prasada Rao *et al.*, (53); Bharati bhat *et al.*, (37) with their experimental results. Pankaja *et al* [54] also conducted experiments to find different modes of transmission in sunflower necrosis virus and proved that sunflower necrosis was never transmitted through seed. Hence, it is concluded that non seed transmissible strain of TSV might be existing in India. Even in the absence of seed transmission, primary inoculum of the TSV is provided by secondary hosts and weed hosts prevalent in and around the sunflower fields by thrips vector.

Vector transmission: The major mode of transmission of TSV-SF is by infected pollen, which can spread by wind or carried by Thrips which transport infected pollen on their bodies [55]. So far, no virus-vector specificity was established for this virus. Chander Rao & Santha Lakshmi Prasad, [55] reported that pollen and thrips collected from TSV infected *Parthenium* weed released together, recorded 58.3 and 70 per cent disease incidence at vegetative and flowering stages of the sunflower crop. So far, five species of thrips viz., *F. schultzei*, *S. dorsalis*, *T. palmi*, *M. usitatus* and *H. gowdeyi* in A.P. [26] and *T. palmi*, *F. schultzei*, *B.*

melonicornis, *H. gowdeyi* and *T. hawaiiensis* in Northern Karnataka [30] were recorded on sunflower. *T. palmi* successfully transmitted the virus to sunflower test plants on acquisition access period (AAP) of 2-3 days and Inoculation access period (IAP) of 3-5 days. [56, 57, 58]. Pankaja *et al.*, [59] proved that a single thrip was enough to acquire and transmit the virus from an infected to healthy sunflower plant and they experimentally revealed that the vector Thrips palmi (Karny) could acquire the virus with an Acquisition Access Period (AAP) of 3 days from the cotyledonary leaves of an infected sunflower plant, with a resultant 16.67% transmission. Similarly, Inoculation Access Period (IAP) of 6 days was necessary for successful transmission of the virus with 13.33 % transmission of the virus.

Genome of Tobacco streak virus (TSV)

SND Causative *Tobacco streak virus* (TSV) was first described by Johnson *et al.*, [60] is the type species of the genus *Ilarvirus*, of the family *Bromoviridae* that includes viruses having tripartite quasi isometric particles of size 27 to 35 nm. Fig. 2 showed that the virus has three nucleoprotein particles designated as RNA-1 (3.4 kb), RNA-2 (3.1 kb) and RNA-3 (2.2 kb). RNAs 1 to 3 are genomic and encodes proteins 1a (119 kDa), 2a (91 kDa) and 3a (32 kDa), respectively; whereas RNA-4a (0.9 kb) and RNA-4 (1.0 kb) are subgenomic expressed from RNA-2 and RNA-3. RNA 4a encodes 2b (22 kDa) and coat proteins (28 kDa), respectively. TSV genome is infectious only in presence of its coat protein or RNA-4. Till to date, none of the SND causing TSV-SF full genomes were sequenced and reported, but many researchers have sequenced and reported full length RNA3 which hosts the Movement Protein and Coat Protein gene.

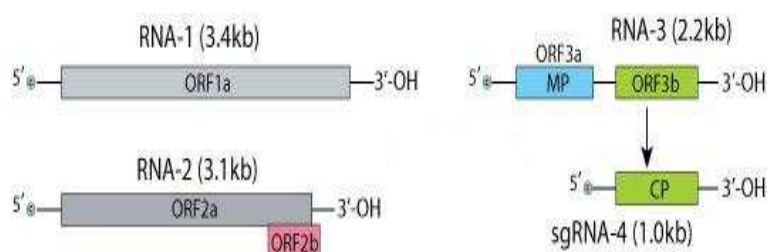


Fig. 2: viral genome of *Tobacco streak virus*

Source:

http://viralzone.expasy.org/all_by_species/136.html

Epidemiology:

Studies on SND revealed the survival of virus throughout the year on several weeds viz., *Parthenium hysterophorus*, *Tridax procumbens*, *Phyllanthus* sp., *Euphorbia geniculata* and *Digeria arvensis* (DOR Annual Report, 2002). Of the 35 common weed species collected from in and around the sunflower fields, 12 weeds showed natural infection of TSV such as *D. arvensis*, *A. aspera*, *Lagasca mollis*, *P. hysterophorus*, *A. hispidum*, *A. conyzoides*, *C. bengalensis*, *E. geniculata*, *Phyllanthus niruri*, *Malvastrum coromandelianum*, *Abutilon indicum* and *Physalis minima* by back inoculation on assay host (Cowpea cv. C-152) and further confirmed by DAC-ELISA [37]. *Parthenium hysterophorus*, *Abutilon indicum*, *Ageratum conyzoides*, *Croton sparsiflorous*, *Commelina bengalensis*, *Cleome viscosa*, *Euphorbia hirta*, *Lagasca mollis* and *Tridax procumbense* were found to be the most common weed hosts for TSV under field condition. Of these, *Parthenium* is the most widely distributed and is a symptomless carrier of TSV and produces several flushes of flowers during its life cycle ensuring continuous supply of TSV infected pollen. It hosts the virus as well as thrips and produces copious pollen throughout season and acts as a primary source of inoculum initiating and sustaining the TSV infection during a crop season. Besides, Thrips colonizing flowers of these plants can become externally contaminated with pollen and movement of these thrips to new hosts results in introduction of the virus into fields. Wind blown pollen of *Parthenium* contaminates the leaves and thrips arriving independently may well contribute to infection.

Epidemiological studies on SND indicated the positive correlation between thrips population and the weather parameters viz., maximum and minimum temperature, sunshine and dry spells whereas, negative correlation was observed with rainfall and relative humidity (DOR Annual Report, 2006). Positive correlation of thrips population with maximum temperature and negative correlation with minimum temperature, RH-I, RH-II and rainfall were established. Disease incidence showed a positive correlation with minimum temperature, RH I, RH-II and rainfall. However, negative correlation with maximum temperature was observed. Besides, positive correlation between thrips

population and disease incidence existed [61, 62, 63].

The disease incidence is higher in *kharif* and summer seasons whereas it is low in *rabi* season. The sunflower cultivars sown during July and August had high necrosis incidence compared to post-rainy season i.e. September onwards [64]. Higher population of thrips during *kharif* sowing was reported in sunflower cv. Morden [62,63,65]. Dry weather (July-Aug) with moderate temperature of 30-32°C and 55-75 per cent relative humidity is conducive for thrips incidence.

Diagnosis:

Ramiah et al., [31, 34] raised Polyclonal antiserum against sunflower necrosis disease and developed a rapid diagnosis method of DAC- ELISA. Bhat et al., [66] developed that serological electroblot immunoassay diagnosis method for coat protein of sunflower necrosis disease. Bhat et al., [38] developed an efficient Reverse Transcription Polymerase Chain Reaction (RT-PCR), Recently Sarovar et al., [67] were developed an high efficient Immunocapture-Reverse Transcriptase-Polymerase Chain Reaction (IC-RT-PCR) for RNA3 of TSV-SF and also they were developed a serological and probe-based blotting technique for the detection of Tobacco streak virus infected sunflower plants[68].

Management of SND:

The most economical and convenient way to manage TSV is to grow resistant varieties. So far, complete resistant varieties/hybrids were not available in sunflower. Even though, effective screening was carried out at Regional Research station, Nandyal, Directorate of oilseeds Research (DOR), Hyderabad, AP and University of Agricultural Science (UAS), Dharwad.

At DOR, sap inoculation technique has been optimized for large scale screening of sunflower genotypes against SND. About 500 genotypes comprising of the released cultivars, diverse inbreds, cytoplasmic male sterile (CMS) lines and restorer lines, germplasm accessions and few derivatives of wild sunflower species were screened against SND and the degree of severity varied among the lines tested [33]. Twenty perennial wild *Helianthus* species were screened against SND and none of them showed symptoms

probably due to the indeterminate and perennating habit of the species studied. Systematic studies have been undertaken for identification of reliable sources of resistance to SND in wild sunflowers [69]. Babu *et al.*, [70] screened thirty hybrids along with their parents against sunflower necrosis disease under natural conditions, using 0-4 scale. Fourteen hybrids (CMS 378A x RHA 265, CMS 378A x DSI 218, CMS 378A x RHA 344, CMS 234A x RHA 265, CMS 234A x RHA 271, CMS 234A x RHA 344, CMS 234A x RHA 345, CMS 234A x RHA 346, CMS 7-1A x RHA 345, DCMS 41 x RHA 274, DCMS 41 x SF 216, DCMS 42 x RHS 273, DCMS 42 x RHA 859 and DCMS 43 x DSI 218) and two parents (CMS 378 A and CMS 234A) recorded resistant reaction, compared to cv. Morden which showed high susceptibility.

Mantur *et al.* [71] took five improved cultivars *viz.*, KBSH-44, KBSH-1, Surya, PAC-1091 and Jwalamukhi to evaluate the incidence of sunflower necrosis. The study revealed that the highest incidence was observed in Surya (16.44%) followed by KBSH-44 (14%), Jwalamukhi (8.2%), PAC-1091 (5.5%) and least incidence was observed in KBSH-1(3.9%). Ranganatha *et al.*, [72] screened sunflower genetic stocks for necrosis disease in three sets during 2001-02. The hybrids PCSH-245, KBSH-1, exotic selection REC-430 and REC-435 recorded low necrosis (6.8 to 7.5%). The populations DRSF-111, GAUSUF-15 and selections REC-436 and Sel-Master recorded high disease (34.3 – 40.8%). Among gene pool in hybrids, GP 9-217-4-4, 9-472-7-4, 9-163-8, 96-33E-4-2, 9-755-2, 9-709-4, 1-1737, 1-1880, 1334-21 and 2150-4 recorded very low disease incidence (3 to 5%). However, GP9-152-5-4, 9-152-7, 1-1341 and 1-2087 inbreds recorded higher necrosis (42.5 to 47.5). In general hybrids indicated better tolerance than the populations and inbreds. Ajit Prasad [73] reported that among the 96 genotypes screened during summer season, only eight (RHA-284, RHA-5D-1, RHA-265, RHA-859, RHA-297, RHA-365, CR-1 and R-214-NBR) were not infected by the disease whereas, the incidence in the field it ranged from 0.0 to 16.66 per cent. In *kharif* 2003, among the 167 genotypes screened, 40 were not infected by the disease and the incidence ranged from 0.0 to 54.54 per cent. Bestar, [74] reported that out of 115 germplasm lines screened against SND, 27 germplasm lines were free from disease.

The germplasm accessions, GMU 3, 6, 9, 15, 17, 21, 88, 33, 34, 38, 41, 47, 53, 58, 59, 66, 73, 74, 79, 83, 84, 90, 91 and 96. The GMU 22 recorded the highest incidence of 24.00 per cent followed by 21 per cent in GMU 8 and 20.

Cultural Practices:

Several cultural practices have been suggested for management of various virus diseases in crop plants that could help to prevent and contain the diseases. Cultural practices such as dates of sowing, border cropping, intercropping, rouging, optimum plant population and removal of weed hosts etc., have been advocated by several workers to reduce disease incidence and intensity [10, 33, 75, 76, 77, 78,]

Removal of virus sources especially weeds germinated with early rains, in fallow lands, road side and on field bunds helps in reducing secondary inoculum can reduce the TSV incidence. Moreover, sunflower and groundnut should not be grown side by side or at least avoid synchronization of flowering period of sunflower with groundnut crop as sunflower crop provides infective pollen inoculum with TSV. Similarly, removal of early infected sunflower will not reduce disease incidence as early infected sunflower does not produce flowers. TSV susceptible crops like marigold and chrysanthemum should not be grown adjacent to sunflower fields.

Natural barriers such as tall grasses in the field protected the adjacent crops from the disease. The tall grasses might obstruct not only wind born infected pollen from outside weeds but also wind-born thrips. Sowing 7-11 rows of fast growing cereals (pearl millet, sorghum or maize) as border crop around fields which obstruct the movement of thrips from landing on crop plants were found to reduce disease incidence in sunflower [10, 33] and groundnut [75]. Mesta *et al.*, [79] reported that use of border crop like sorghum reduced incidence of SND from 18 to 37 per cent.

Bare patches in the field attract thrips landing. Optimum plant population discourages thrips landing on the sunflower crop indicating maintenance of optimum plant population is one of the options for the management of TSV infection.

Date of sowing of crops mainly depends on rainfall pattern and distribution. Shirshikar, [63] opined that SND could be minimized if sunflower is sown in the post rainy season, i.e. from September onwards. Upendhar *et al.*, [61, 62] reported that SND incidence was high during August (35.04 per cent) and September (49.93 per cent) and low during October (20.75 per cent) months.

Intercropping with red gram or castor was found to reduce disease intensity compared to monocropping of sunflower and groundnut (75, 80). Pearl millet, sorghum and maize as intercrops in mungbean maintained their severity in containing thrips population with 71.4, 61.5 and 57 per cent reduction and 68.9, 61.7 and 60.2 per cent reduction over sole crop during *kharif* and *rabi* seasons, respectively. The same intercrops suppressed the disease incidence with 85, 83.5 and 79.2 per cent reduction and 65.8, 60.7 and 59.2 per cent reduction over sole crop during *kharif* and *rabi* seasons, respectively [81].

Chemical methods:

Seed treatment with imidacloprid @ 5 g/kg seed and imidacloprid (0.5 per cent) spray reduced disease incidence with higher yield compared with other treatments [82]. Management trial for SND at AICRP on oilseeds revealed that seed treatment either with imidacloprid at 5g/ kg seed or thiomethoxam at 4g/ kg seed followed by two sprays at 30 and 45 days found to reduce necrosis disease and increase seed yield significantly over untreated control [83].

Antiviral compounds:

Use of various anti viral materials such as *Prosopis*, goatmilk and *Bougainvillea* in combinations were used to induce the resistance in sunflower against TSV-SF [84]. Among them, *Bougainvillea spectabilis* with goat milk, *Prosopis chilensis* with goat milk, *Bougainvillea spectabilis* alone, and *Prosopis chilensis* alone were found highly effective in inducing the resistance in sunflower against SND. The combinations of treatments which involve plant products with goat milk were more effective than the individual ones. Significantly enhanced PR-proteins like β -1, 3 glucanase and oxidative enzymes like Peroxidase, Polyphenol oxidase, and Phenylalanine ammonialyase were observed in sunflower using above anti viral materials.

Biological methods:

Srinivasan & Mathivanan, [85] reported plant growth promoting microbial consortia (PGPMC) mediated biological control of SND under field conditions for the first time. Powder and liquid formulations of two PGPMCs (PGPMC-1: consisting of *Bacillus licheniformis* strain MML2501+*Bacillus* sp. strain MML2551+*Pseudomonas aeruginosa* strain MML2212+*Streptomyces fradiae* strain MML1042; PGPMC-2: consisting of *B. licheniformis* MML2501+*Bacillus* sp. MML2551+*P. aeruginosa* MML2212) were evaluated along with farmers' practice (imidacloprid + mancozeb) in farmers' fields. Significant disease reduction, increase of seed germination, plant height and yield parameters with an additional seed yield of 840 kg ha⁻¹, an additional income of Rs. 10,920/ha and benefit cost ratio of 6.1 were recorded using powder formulation of PGPMC-1.

Transgenic approach:

In a number of crops, transgenic resistant to an infective virus have been developed by introducing a sequence of the viral genome in the target crop by genetic transformation. Virus-resistant transgenics have been developed in many crops by introducing either viral CP or replicase gene encoding sequences. Pradeep *et al.*, [86] amplified, cloned and sequenced the coat protein (CP) gene of *Tobacco streak virus* (TSV) from sunflower (*Helianthus annuus* L.). In their study, a 421 bp fragment of the TSV coat protein gene was amplified and gene constructs encoding the hairpin RNA (hpRNA) of the TSV-CP sequence was sub-cloned into the binary vector pART27. This gene construct was then mobilized into *Agrobacterium tumefaciens* strain LBA4404 via triparental mating using pRK2013 as a helper. Sunflower (cv. Co 4) and tobacco (cv. Petit Havana) plants were transformed with *A. tumefaciens* strain LBA4404 harboring the hpRNA cassette and in vitro selection was performed with kanamycin. The integration of the transgene into the genome of the transgenic lines was confirmed by PCR analysis. Infectivity assays with TSV by mechanical sap inoculation demonstrated that both the sunflower and tobacco transgenic lines exhibited resistance to TSV infection and accumulated lower levels of TSV compared with non-transformed controls.

Final remarks:

The necrosis disease of sunflower is of recent origin. Initially the causal virus of SND was reported as *Tomato spotted wilt virus* (TSWV) of Genus *Tospovirus* and has been later identified as *Tobacco streak virus* (TSV) of Genus *Iilarvirus*. [87, 51,] reported an association of a *Tospovirus* with necrosis disease of affected sunflower samples collected from Bangalore, Dharwad and Hyderabad that has serological affinities to *Peanut bud necrosis virus* (PBNV). Jain et al., [86] studied the serological relationship of sunflower necrosis virus with the antiserum of the members of Genus *Tospovirus* at Indian Agricultural Research Institute (IARI), New Delhi and reported a strong reaction of this virus with the antiserum of *Water melon silver mottle virus* (WSMV) and PBNV and concluded that the virus belonged to '*Tospovirus*' serogroup. Later, based on serological cross-reaction with *Tobacco streak virus* antiserum and similarities in nucleic acid species, molecular weight of virus coat protein and aphid non-transmissible nature, the causal virus of SND was confirmed as *Tobacco streak Iilarvirus* [88]. Serological relationship was demonstrated only with *Tobacco streak virus* (TSV), which was confirmed by western blot analysis and IEM decoration assays using SNV and TSV antisera [14]. Bhat et al., [38] developed RT-PCR amplification of TSV from sunflower using coat protein (CP) primers of *Tobacco streak virus*. On the basis of serological relatedness and sequence identity, it was proposed that the sunflower *Iilarvirus* from India should be considered as a strain of TSV belonging to subgroup I and designated as TSV-SF.

Conclusions

Among different viruses infecting sunflower crop in India, *Tobacco streak virus* causing severe threat to sunflower production. Desired level of resistance to TSV both in cultivated species as well as in the germplasm of sunflower is not available as the screened cultivars/germplasm/CMS lines/R lines were found susceptible against TSV in laboratory screening. Therefore, there is an urgent need to search the resistance sources both in native and exotic germplasm, which can be exploited to augment TSV resistance through breeding programmes. As an alternative strategy, developing transgenic resistance is also desirable to combat this dreaded disease in sunflower. A combined effort of biotechnology and traditional

breeding may further enhance opportunities for development of resistant varieties to SND. Effective screening should be carried under artificial epiphytotics using *Parthenium* infector border. Adoption of appropriate cultural practices and effective management of vectors and alternate hosts are also equally important. Moreover, marker assisted selection (MAS) cannot be exploited in sunflower crop. To develop a marker, choose either DNA route or protein route (reverse genetics) using resistant or susceptible sources. Complete molecular characterization of virus associated with SND in order to identify the strains of TSV in different geographical regions of the country. Further studies are needed to ascertain the prevalence of TSV pathotypes /serotypes originating from different sunflower locations in the world.

Acknowledgements

Authors are thankful to Sri Venkateswara University, Tirupati, India for providing necessary facilities to do research on Sunflower Necrosis Diseases.

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Source of support: Nil

Conflict of interest: None Declared