8. APPENDIX

8.1 Buffers and reagent for ELISA

8.1.1 Coating buffer, pH 9.6 (0.05 M carbonate buffer)
Na₂CO₃ 1.59 g
NaHCO₃ 2.93 g
Distilled water 1 L

8.1.2 Phosphate buffer saline (PBS), pH 7.4
Na₂HPO₄ 2.38 g
KH₂PO₄ 0.4 g
KCl 0.4 g
NaCl 0.4 g
Distilled water 2 L

8.1.3 PBS-T (washing) buffer
PBS 1 L
Tween-20 500 µl

8.1.4 Conjugate (Antibody) buffer
PVP-40000 2 g
Ovalbumin 0.2 g
PBS-T 100 ml

8.1.5 10% Diethanolamine (substrate buffer), pH 9.8
Diethanolamine 100 ml
Distilled water 1 L
Adjust the pH to 9.8 with concentrated HCl

8.2 Inoculation buffer used sap transmission of virus

8.2.1. 50 mM potassium phosphate buffer, pH 7.0
KH₂PO₄ 2.4 g
K₂HPO₄ 5.4 g
Distilled water 1 L

8.2.2. 100 mM potassium phosphate buffer, pH 7.0
KH₂PO₄ 4.8 g
K₂HPO₄ 11.6 g
Distilled water 1 L
Reducing agent:
Mono-Thioglycerol 750 µl in 1 L of inoculation buffer (or)
2-mercaptoethanol 1 ml in 1 L of inoculation buffer (or)
Sodium sulphite 2 gm in 1 L of inoculation buffer
8.3 Buffers used for virus purification

8.3.1 Homogenization buffer (0.1 M Phosphate buffer, pH 8.0)

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH₂PO₄</td>
<td>0.68 g</td>
</tr>
<tr>
<td>K₂HPO₄. 3H₂O</td>
<td>20.52 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1 L</td>
</tr>
</tbody>
</table>

Note: 0.01 M DEICA and 0.075% mono-thioglycerol were used as a reducing agent and was added just before starting purification procedure to the homogenization buffer.

8.3.2 Re-suspension buffer (0.01 M homogenization buffer, pH 8.0)

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenization buffer</td>
<td>100 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>900 ml</td>
</tr>
</tbody>
</table>

8.3.3 30% sucrose cushion

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>60% sucrose</td>
<td>50 ml</td>
</tr>
<tr>
<td>Re-suspension buffer</td>
<td>50 ml</td>
</tr>
<tr>
<td>Polyethylene glycol (PEG)</td>
<td>6 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.16 g</td>
</tr>
</tbody>
</table>

8.3.3.1 60% sucrose solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>100 g</td>
</tr>
<tr>
<td>Re-suspension buffer</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

8.3.3.2 40% sucrose solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>60% sucrose solution</td>
<td>40 ml</td>
</tr>
<tr>
<td>Re-suspension buffer</td>
<td>20 ml</td>
</tr>
</tbody>
</table>

8.3.3.3 30% sucrose solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>60% sucrose solution</td>
<td>20 ml</td>
</tr>
<tr>
<td>Re-suspension buffer</td>
<td>20 ml</td>
</tr>
</tbody>
</table>

8.3.3.4 20% sucrose solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>40% sucrose solution</td>
<td>20 ml</td>
</tr>
<tr>
<td>Re-suspension buffer</td>
<td>20 ml</td>
</tr>
</tbody>
</table>

8.3.3.5 10% sucrose solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>20% sucrose solution</td>
<td>20 ml</td>
</tr>
<tr>
<td>Re-suspension buffer</td>
<td>20 ml</td>
</tr>
</tbody>
</table>

8.4 Polyacrylamide gel electrophoresis reagents and buffers

8.4.1 Stacking gel buffer (0.5 M Tris-HCl, pH 6.8)

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris base</td>
<td>6.05 g</td>
</tr>
</tbody>
</table>

Dissolve in 80 ml of sterile water. Adjust the pH to 6.8 with 1 M HCl and make up the volume to 100 ml with sterile water.
8.4.2 Resolving gel buffer (1.5 M tris-HCl, pH 8.8)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris base</td>
<td>18.15 g</td>
</tr>
</tbody>
</table>

Dissolve in 80 ml of sterile water. Adjust the pH to 8.8 with 1 M HCl and make up the volume to 100 ml with sterile water.

8.4.3 Acrylamide / Bis mix (30%)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylamide</td>
<td>29.2 g</td>
</tr>
<tr>
<td>Bis-acrylamide</td>
<td>0.8 g</td>
</tr>
</tbody>
</table>

Distilled water to 100 ml

Filter and store the solution at 4°C in an amber colored bottle or wrap the bottle with aluminum foil to avoid exposure to light.

8.4.4 10% Ammonium persulphate (APS)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>APS</td>
<td>100 mg</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

Note: Always prepare fresh solution before use.

8.4.5 Running buffer, pH 8.3

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris base (25 mM)</td>
<td>3 g</td>
</tr>
<tr>
<td>Glycine (250 mM)</td>
<td>14.4 g</td>
</tr>
<tr>
<td>SDS (1%)</td>
<td>1 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1 L</td>
</tr>
</tbody>
</table>

Note: No need to adjust pH. Store at room temperature.

8.4.6 Stacking gel composition (4%)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>30% Acrylamide: Bis mix</td>
<td>800 μl</td>
</tr>
<tr>
<td>Stacking gel buffer</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>3.575 ml</td>
</tr>
<tr>
<td>10% SDS</td>
<td>60 μl</td>
</tr>
<tr>
<td>10% APS</td>
<td>60 μl</td>
</tr>
<tr>
<td>TEMED (HiMedia)</td>
<td>5 μl</td>
</tr>
<tr>
<td>Final volume</td>
<td>6 ml</td>
</tr>
</tbody>
</table>

8.4.7 Resolving gel composition (12%)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>30% Acrylamide: Bis mix</td>
<td>6 ml</td>
</tr>
<tr>
<td>Resolving gel buffer</td>
<td>3.75 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>4.945 ml</td>
</tr>
<tr>
<td>10% SDS</td>
<td>150 μl</td>
</tr>
<tr>
<td>10% APS</td>
<td>150 μl</td>
</tr>
<tr>
<td>TEMED</td>
<td>5 μl</td>
</tr>
<tr>
<td>Final volume to</td>
<td>15 ml</td>
</tr>
</tbody>
</table>

Note: Mix acrylamide:bis mix solution, gel buffer, distilled water, SDS and APS mix well and then add TEMED, mix and immediately pour into the gel mould.
8.4.8 Laemmli buffer (2x SDS sample loading dye)
Stacking gel buffer 2.5 ml
Glycerol 2 ml
10% SDS 2 ml
2-mercaptoethanol 500 μl
Bromophenolblue 1 mg
Distilled water to 10 ml

8.4.9 Staining solution
Coomassie Brilliant Blue-R (R-250) 200 mg
Methanol 40 ml
Glacial Acetic acid 7 ml
MilliQ water 53 ml

8.4.10 Destaining solution
Methanol 20 ml
Glacial Acetic acid 7 ml
MilliQ water 73 ml

8.5 Buffers and reagents in Western analysis

8.5.1 Transfer (Towbin) buffer, pH 8.3
Tris base 9.1 g
Glycine 43.2 g
Methanol 600 ml
Distilled water to make up to 3 L

8.5.2 Tris-buffered saline (TBS)
Tris base 2.42 g
NaCl 29.74 g
Dissolve in 900 ml of distilled water. Adjust the pH to 7.5 with 1 N HCl and make up the volume to 1 L with distilled water.

8.5.3 TBS-Tween (TBS-T)
TBS with 0.05% Tween-20

8.5.4 Blocking solution
Non-fat dried milk powder 5 g
TBS 100 ml

8.5.5 Antibody buffer
Non-fat dried milk powder 5 g
TBS-T 100 ml
8.6 Buffers and reagents used in bacterial culture techniques

8.6.1 LB medium
Tryptone 1 g
Yeast extract 500 mg
NaCl 1 g
Dissolve in 100 ml of distilled water and adjust the pH to 7.0. Sterilize by autoclaving.

8.6.2 Transformation and storage buffer (TSB)
LB medium (pH 6.1) 5 ml
10% PEG 500 µl
DMsO 500 µl
1 M MgCl$_2$ 100 µl
1 M MgSO$_4$ 100 µl
Glycerol 1 ml
Distilled water 2.762 ml

8.6.3 5x KCM
1 M KCl 5 ml
1 M CaCl$_2$ 1.5 ml
1 M MgCl$_2$ 2.5 ml
Distilled water 1 ml

8.6.4 SOC medium
Trypton 2 g
Yeast extract 500 mg
NaCl 58 mg
KCl 18 mg
MgCl$_2$.6H$_2$O 203 mg
MgSO$_4$.7H$_2$O 246
Glucose 360 mg
Dissolve in 100 ml of distilled water and filter sterilize.

8.6.5 GTE
1 M Glucose 5 ml
1 M Tris-Cl, pH 8.0 2.5 ml
0.5 M EDTA, pH 8.0 2 ml
Distilled water 90.5 ml

8.6.6 Lysis solution
10 N NaOH 200 µl
Distilled water 8.8 ml
10% SDS 1 ml
Note: Prepare fresh from the stocks
8.6.7 Neutralizing solution
Potassium acetate 29.2 g
Glacial acetic acid 11.2 ml
Distilled water to 100 ml

8.6.8 3 M potassium acetate, pH 5.2
Potassium acetate 29.4 g
Glacial acetic acid 11.2 ml
Dissolved with distilled water to make up the volume to 100 ml

8.6.9 3 M Sodium acetate, pH 4.8
Sodium acetate 24.6 g
Dissolve in 80 ml of distilled water and adjust the pH to 4.8 by adding glacial acetic acid.

8.6.10 70% Ethanol
Absolute alcohol 70 ml
Distilled water 30 ml

8.7 Buffers used in over-expression and purification of rTSV CP

8.7.1 Bacterial lysis buffer
Tris base 1.21 g
NaH₂PO₄ 13.8 g
Urea 480.5 g
Dissolve in 600 ml of distilled water. Adjust the pH to 8.0 with 1 N NaOH and make up the volume to 1 L.

Note: Just before use add 0.1% β-mercaptoethanol and lysozyme (20 μg/ml).

8.7.2 Equilibration buffer
Bacterial lysis buffer 85 ml
Glycerol 15 ml

8.7.3 Wash buffer 1
Equilibration buffer with 10 mM imidazole

8.7.4 Wash buffer 2
Equilibration buffer with 25 mM imidazole

8.7.5 Wash buffer 3
Equilibration buffer with 40 mM imidazole

8.7.6 Bradford's reagent
Bradford's reagent (BIO-RAD) 1 ml
Distilled water 4 ml
8.8 Buffer and Reagents used in genomic DNA isolation

8.8.1 Dellaporta's DNA extraction buffer

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M Tris-Cl, pH 8.0 (10 mM)</td>
<td>1 ml</td>
</tr>
<tr>
<td>5 M NaCl (50 mm)</td>
<td>1 ml</td>
</tr>
<tr>
<td>0.5 M EDTA, pH 8.0 (5 mM)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Distilled water up to</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

Add 100 µl of 2-mercaptoethanol just before use.

8.8.2 3 M potassium acetate, pH 5.2

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium acetate</td>
<td>29.4 g</td>
</tr>
<tr>
<td>Glacial acetic acid</td>
<td>11.2 ml</td>
</tr>
</tbody>
</table>

Dissolved with distilled water to make up the volume to 100 ml.

8.8.3 CTAB extraction buffer

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M Tris-Cl, pH 8.0</td>
<td>10 ml</td>
</tr>
<tr>
<td>0.5 M EDTA, pH 8.0</td>
<td>10 ml</td>
</tr>
<tr>
<td>1 M Glucose</td>
<td>20 ml</td>
</tr>
<tr>
<td>PVP-40000</td>
<td>2 g</td>
</tr>
<tr>
<td>DIECA</td>
<td>0.13 g</td>
</tr>
</tbody>
</table>

Dissolve in distilled water to make up the volume to 100 ml.

Add 200 µl of β-mercaptoethanol and 100 mg of L-Ascorbic acid just before use.

8.8.4 CTAB Lysis buffer

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M Tris-Cl, pH 8.0</td>
<td>10 ml</td>
</tr>
<tr>
<td>0.5 M EDTA, pH 8.0</td>
<td>4 ml</td>
</tr>
<tr>
<td>5 M NaCl</td>
<td>28 ml</td>
</tr>
<tr>
<td>CTAB</td>
<td>2 g</td>
</tr>
<tr>
<td>PVP-40000</td>
<td>2 g</td>
</tr>
<tr>
<td>DIECA</td>
<td>0.13 g</td>
</tr>
</tbody>
</table>

Dissolve in distilled water to make up the volume to 100 ml.

Add 200 µl of β-mercaptoethanol and 100 mg of L-Ascorbic acid just before use.

8.8.5 10x TBE buffer, pH 8.3

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris base</td>
<td>108 g</td>
</tr>
<tr>
<td>Boric acid</td>
<td>55 g</td>
</tr>
<tr>
<td>0.5 M EDTA, pH 8.0</td>
<td>40 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1 L</td>
</tr>
</tbody>
</table>

Note: No need to adjust pH

8.8.6 10x DNA loading dye

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromophenol blue</td>
<td>2 mg</td>
</tr>
<tr>
<td>Distilled water</td>
<td>650 µl</td>
</tr>
<tr>
<td>Glycerol</td>
<td>350 µl</td>
</tr>
</tbody>
</table>
8.9 Buffers and reagent used for southern analysis

8.9.1 Depurination solution
37% HCl (0.2 N) 4.5 ml
Make up the volume to 250 ml with distilled water.

8.9.2 Denaturation solution
NaCl (1.5 M) 43.8 g
5 M NaOH (0.5 M) 50 ml
Dissolve in 500 ml distilled water and autoclave.

8.9.3 Neutralization solution
NaCl (3 M) 87.7 g
Tris (1.5 M) 90.8 g
Dissolve in 400 ml distilled water and adjust the pH to 7.0 and make up the volume to 500 ml.

8.9.4 20x SSC
Sodium Citrate (0.3 M) 88.2 g
NaCl (3 M) 175.3 g
Dissolve in 800 ml of distilled water. Adjust pH to 7.0 with 1 N HCl and make up to 1 L. Sterilize by autoclaving.

8.9.5 Maleic acid buffer
Maleic acid (0.1 M) 11.6 g
NaCl (0.15 M) 8.76 g
Dissolve in 800 ml of distilled water. Adjust pH to 7.5 with 1 N NaOH and make up to 1 L. Sterilize by autoclaving.

8.9.6 10% Blocking agent
Blocking agent 10 g
Maleic acid 100 ml
Dissolve gently under continuous stirring at heating mode. Sterilize by autoclaving.

8.9.6a 1% Blocking solution
10% Blocking agent 10 ml
0.15 Maleic acid buffer 90 ml

8.9.7 Pre-hybridization buffer (50 ml)
20x SSC 12.5 ml
10% Lauryl sarcosine Na salt 500 µl
10% SDS 100 µl
10% Blocking agent 5 ml
Distilled water 31.9 ml
8.9.8 Washing solution
Maleic acid buffer with 0.3% Tween-20

8.9.9 Detection buffer
Tris base 1.21 g
NaCl 0.584 g
Dissolve in 90 ml of distilled water and adjust the pH to 9.5 with 1 N HCl. Make up the volume to 100 ml.

8.10 Buffers and reagents used in RNA analysis

8.10.1 DEPC treated water
DEPC 1 ml
Distilled water 1 L
Incubate at 37°C for 24 h and autoclave.

8.10.2 Disruption buffer
0.5M EDTA pH 8.0 1 ml
10% SDS 10 ml
DEPC treated water 39 ml

8.10.3 10x MOPS (Formaldehyde gel) buffer
MOPS (200 mM) 83.72 g
Sodium acetate (50 mM) 8.23 g
0.5 M EDTA (10 mM) 20 ml
Adjust the pH to 7.0 with 10 N NaOH and make up the volume to 1 L with DEPC treated water. The solution is sterilized by autoclaving.

8.10.4 RNA extraction buffer
Sodium acetate 1.64 g
0.5 M EDTA 1 ml
10% SDS 20 ml
Dissolve in DEPC treated water to make up to 100 ml.

8.10.5 5x RNA loading dye
10x MOPS buffer 400 μl
37% Formaldehyde 70 μl
Formamide 300 μl
Glycerol 200 μl
DEPC treated water to 1 ml

8.10.6 Formamide hybridization buffer
20x SSC 12.5 ml
10% Lauryl sarcosine Na salt 500 μl
10% SDS 100 μl
10% Blocking agent 5 ml
Formamide 25 ml
DEPC-treated water 6.9 ml
8.11 Miscellaneous reagents

8.11.1 1 M Tris-Cl buffer, pH 8.0

Tris base: 121.1 g
Dissolved in 800 ml of distilled water and pH is adjusted to 8.0 with 1 M HCl. The final volume was made up to 1 L with distilled water.

8.11.1.2 0.5 M EDTA, pH 8.0

EDTA, disodium salt: 186.1 g
Dissolve in 800 ul of distilled water by continuous stirring on a magnetic stirrer. Adjust the pH to 8.0 with 1 N NaOH. Make the volume to 1 L with distilled water.
Note: The disodium salt of EDTA will not go into solution until the pH of the solution is adjusted approximately 8.0 by the addition of NaOH. Sterilize by autoclaving.

8.11.1.3 TE, pH 8.0

1 M Tris Cl, pH 8.0: 1 ml
0.5 M EDTA, pH 8.0: 200 µl
Make up the volume to 100 ml with sterile distilled water.

8.11.1.4 10% SDS

SDS: 10 g
Distilled water: 90 ml
Dissolve and adjust the volume to 100 ml with distilled water.
Note: Warm if necessary to assist dissolution. No need to sterilize this solution.

8.11.1.5 5 M NaCl

NaCl: 292.2 g
Dissolve in distilled water to make up the volume to 1 L.
Natural Occurrence and Distribution of Tobacco Streak Virus in South India

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Abstract

Tobacco streak virus (TSV) is emerging as an economically important virus causing severe reduction in yield of sunflower and groundnut in India by causing sunflower necrosis disease (SND) and groundnut stem necrosis disease (GSND). In order to monitor the natural occurrence and distribution of TSV, surveys were conducted in the predominant sunflower and groundnut growing regions of Andhra Pradesh, Karnataka and Maharashtra during Kharif 2002 to 2004. SND was widely prevalent in the three states with a maximum disease incidence upto 95%, 75% and 80% during Kharif 2002, 2003 and 2004, respectively. GSND was most predominant in Andhra Pradesh and adjoining regions of Karnataka with the incidence ranging from 0 - 15%, 0 - 3% and 1 - 80% during Kharif 2002, 2003 and 2004, respectively. TSV also found to infect other economically important crops viz., cotton, okra and soybean with incidence upto 20%, 25% and 40%, respectively, in different regions of the three states. Virus isolates collected from various crops viz., sunflower, groundnut, cotton, okra, soybean, cowpea, mungbean, sesame, safflower and niger; ornamentals like marigold, lantana and crossandra; and several weed species were found positive to TSV in direct antigen coating - enzyme linked immunosorbent assay (DAC-ELISA). Mechanical sap inoculation of the representative ELISA positive TSV isolates on cowpea (Vigna unguiculata cv. C-152), sunflower (Helianthus annuus cv. PAC36) and tobacco (Nicotiana tabacum cv. Xanthi) exhibited symptoms typical of TSV.

Keywords: Tobacco streak virus, sunflower necrosis disease, groundnut stem necrosis disease, Enzyme linked immunosorbent assay

Introduction

Sunflower (Helianthus annuus L.) and groundnut (Arachis hypogea L.) are the two economically important oilseed crops in India. Both the crops are susceptible to several biotic stresses, among which fungi, bacteria and viruses are of relatively major yield reducers. In recent years, sunflower necrosis disease (SND) and groundnut stem necrosis disease (GSND) are the two economically important diseases affecting sunflower and groundnut crops, respectively, in India. The SND was first noticed in sunflower in Karnataka state during 1997 which later spread to the major sunflower growing regions of Karnataka, Maharashtra, Andhra Pradesh and Tamil Nadu (Anonymous, 1998). Generally the infection starts with chlorosis and or necrosis of leaf followed by vein, petiole, stem and head necrosis. Early infection either kills the plant or causes severe stunting with malformed head or heads filled with chopped seeds (Anonymous, 1998; Anonymous, 2000, Ramiah et al., 2001, Ravi et al., 2001). The epidemics of SND have been reported for three consecutive years (1997-99), with the disease incidence ranging from 10 to 80% (Anonymous, 2000). The increased severity of SND was considered to be the major factor responsible for the decline in the sunflower cultivation during the four years (Prasada Rao et al., 2000). Similarly, an epidemic of stem necrosis disease on groundnut characterised with necrosis of terminal leaflets and stem followed by death of the plant was recorded in Andhra Pradesh during the year 2000 and affected nearly 0.25 m ha with an estimated crop loss of more than 300 crore rupees (Reddy et al., 2002). The causal agent of both SND and GSND was identified as Tobacco streak virus (TSV) belonging to flavivirus genus (Prasada Rao et al., 2000, Ravi et al., 2001, Ramiah et al., 2001, Reddy et al., 2002, Bhat et al., 2002). In our present study, attempts were made to monitor the disease incidence, natural occurrence and distribution of TSV in predominant sunflower and groundnut growing regions of Andhra Pradesh, Maharashtra and Karnataka states during Kharif 2002 to 2004.

Materials and methods

Survey

Survey was conducted in the predominant sunflower and...
groundnut growing regions of Anantapur and Kurnool regions of Andhra Pradesh; Bidar, Bellary, Bijapur, Gularga, Raichur, Haveri, Davangere and Bagalkot regions of Karnataka and Jalna, Osmanabad, Beed, Latur, Solapur and Parbhani (Marathwada) regions of Maharashtra during Kharif 2002 to 2004. The plots were selected in random and the disease incidence in sunflower and groundnut was recorded by counting the number of infected plants to the total number of plants in a row and in 1 m² area, respectively. In each plot at least 4 to 5 observations were taken, leaving the border rows. Suspected leaf samples from the infected sunflower, groundnut, cotton, okra, soybean etc. and weed species in and around the infected plots with virus like symptoms were collected for further diagnosis.

**Virus detection by ELISA**

Direct antigen coating - enzyme linked immunosorbent assay (DAC-ELISA) was performed as described by Hobbs et al., (1987) for the detection of TSV in the suspected samples. The infected leaf samples were tested by ELISA using TSV specific antiserum. The leaf samples were macerated at 1:10 w/v in 0.05 M carbonate buffer, pH 9.6 with 0.01 M DEB CA (diethyldithiocarbamate). TSV antiserum (Mahyco Research Center) diluted to 1:5000 was used as primary antibody. Secondary antibody, goat anti-rabbit IgG conjugated to alkaline phosphatase (ALP) (Sigma-Aldrich, USA) was used at 1:10,000 dilution. Substrate, para-nitrophenyl phosphate, was used at 1 mg/ml in 10% diethanolamine pH 9.8. Absorbance values were taken at 405 nm with a Multiskan ELISA reader (Lab system) one hour after adding the substrate. Only the samples showing an absorbance values three times higher to the healthy control were considered positive.

**Mechanical transmission**

The representative ELISA positive TSV isolates collected from different regions and crops were tested for infectivity assay by mechanical sap inoculations. Virus inoculum was prepared by grinding the young infected leaf samples in a pre-chilled mortar and pestle using chilled 0.05 M phosphate buffer, pH 7.0 containing 0.075% (v/v) thioglycoleric. Carborundum powder (600 mesh) was used as an abrasive and was dusted on the test plant leaves before inoculation. The inoculum was applied on upper surface of the leaf and inseed with tap water five minutes after inoculations. The representative TSV isolates were further inoculated on propagating hosts like sunflower (*H. annuus* cv. PAC 36) or tobacco (*N. tabacum* cv. Xanthi). The inoculated plants were kept under greenhouse and observed for symptom expression.

**Results and discussion**

**Survey**

TSV was widely prevalent in the sunflower growing regions of Andhra Pradesh, Karnataka and Maharashtra (Table 1). GSND was more prevalent in Anantapur and Kurnool regions of Andhra Pradesh and adjoining regions Raichur, Bellary and Bijapur of Karnataka state. In addition to sunflower and groundnut, the virus was found to infect number of other economically important crops, ornamentals and weed species. Among the crop species, high incidence of TSV was commonly observed in cotton, okra and soybean. The prominent disease symptoms of TSV in various crops were necrosis of infected plant parts, whereas in okra the symptoms were chlorotic ring spot or mosaic mottling without any necrosis (Table 2).

**Sunflower necrosis disease.** SND incidence ranged from 0 to 95% during Kharif 2002 in the three sunflower growing regions. High disease incidence was observed in Jalna region of Maharashtra; Davangere, Gularga and Raichur regions of Karnataka state. During Kharif 2003, the disease incidence ranged from 0 to 75% with the maximum incidence in Latur region of Maharashtra, followed by Osmanabad, Solapur and Beed regions of Maharashtra; and Bidar, Gularga and Raichur regions of Karnataka. During Kharif 2004, the incidence ranged from 1 to 80% with the maximum incidence in Anantapur regions of Andhra Pradesh followed by Solapur and Beed regions of Maharashtra. Similar widespread of SND was reported in Andhra Pradesh, Karnataka, Maharashtra and Tamil Nadu states with an incidence of 10 to 80% (Anonymous, 2000). The infection in sunflower resulted in severe yield loss since the early infection resulted in death of the plants whereas late infection resulted in malformed head without any seed set.

**Groundnut stem necrosis disease.** During Kharif 2002, GSND incidence was up to 15% in Raichur and Kurnool regions of Karnataka and Andhra Pradesh, respectively. The incidence in Anantapur region of Andhra Pradesh ranged from 1 to 10% with an average incidence of 3%. There was a low incidence (up to 3%) during Kharif 2003 in Andhra Pradesh, but there was an epidemic (up to 80%) in Anantapur and Kurnool regions of Andhra Pradesh during Kharif 2004 and resulted in a serious crop failure. Similarly, an epidemic of GSND was reported in groundnut during the year 2000 in Andhra Pradesh (Reddy et al., 2002). Anantapur and Kurnool regions of Andhra Pradesh were considered as hot spots for TSV in groundnut as major epidemics occurred during 2000 and 2004 resulting in huge crop loss. The reason for the frequent epidemics in groundnut is yet to be established.
The virus inoculum, collateral weed hosts, cropping pattern (like sunflower and groundnut mixed cropping), thrips population and environmental conditions may contribute for the inoculum build-up and frequent epidemics. On the contrary, the necrosis disease in sunflower was endemic in northern Karnataka (Bidar, Gulbarga, Raichur and Bellary regions) and Maharashtra (Jalna, Solapur, Osmanabad and Beed regions) causing considerable damage to sunflower production every year.

**TSV in cotton, okra and soybean.** During the present survey, occurrence of TSV in cotton, okra and soybean were also monitored which showed up to 20%, 25% and 40% disease incidence, respectively. TSV in cotton is prevalent in the cotton growing regions of Maharashtra (Marathwada and even in Vidarbha zone), Karnataka (Raichur, Gulbarga, Haveri and Bellary regions) and Andhra Pradesh (Kurnool region). The disease incidence was up to 20% during Kharif 2002 to 2004. Similarly, the chlorotic ringspot in okra caused by TSV was observed in Jalna and Beed regions of Maharashtra; Gulbarga and Raichur regions of Karnataka and Kurnool region of Andhra Pradesh with the incidence from 0 to 25%. Soybean necrosis caused by TSV was also widely prevalent in Jalna, Beed and Osmanabad regions of Maharashtra with an incidence ranged from 0 to 40%. The present surveys indicated that apart from sunflower and groundnut, the virus infects number of other economically important crops like cotton, okra and soybean etc., which can emerge as serious threat to these crops.

**Virus detection by ELISA.**

Out of 450 samples tested 342 samples were found to be positive for TSV. Samples tested positive include sunflower (95 out of 100), groundnut (106 out of 136), cotton (58 out of 110), okra (33 out of 40), soybean (25 out of 30), marigold (Tagetes patula L.) (6 out of 9), cowpea (5 out of 7), mungbean (4 out of 5), sunflower (2 out of 3), nilger (Guzmania abyssinica Cass.) (2 out of 3), sesame (Sesamum indicum L.) (2 out of 2), ixora (Ixora coccinea L.) (2 out of 2) and cornsandra (Crossandra infundibuliformis L.) (2 out of 3). Among the 95 suspected weed samples tested, 54 samples comprising Parthenium hysterophorus L., Xanthium strumarium L., Croton bonplandianum Baill., Achyranthes aspera L., Acanthospermum hispidum DC., Commelina benghalensis L. and Tridax procumbens L. were tested positive to TSV. Whereas, none of the samples collected from crops like tomato (Lycoopersicon esculentum MILL.), brinjal (Solanum melongena L.), chilli (Capsicum L.) and watermelon (Citrullus lanatus Thumb.) was positive to TSV. Prasada Rao et al., (2003) reported the occurrence of TSV in

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**Table 1. TSV incidence in different districts of Maharashtra, Karnataka and Andhra Pradesh during Kharif 2002, 2003 and 2004**

<table>
<thead>
<tr>
<th>State</th>
<th>District</th>
<th>Season</th>
<th>No. of plots</th>
<th>Per cent incidence min-max</th>
<th>No. of plots</th>
<th>Per cent incidence min-max</th>
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a wide range of host and weed species based on serological assays.

**Mechanical transmission**

Upon inoculation, ELISA positive samples produced characteristic local necrotic lesions on cowpea leaves within 2 to 3 days post inoculation (dpi). The representative virus isolates exhibited symptoms typical of TSV on sunflower and tobacco. On sunflower cv. PAC36, the virus produced necrotic lesions on inoculated leaves within 5 to 7 dpi followed by systemic mosaic mottling (7 to 9 dpi), leaf deformation, stunted growth and malformed heads. In tobacco cv. Xanthi, the virus produced local necrotic lesions with ringspots within 2 to 3 dpi. The newly emerged leaves exhibited necrotic rings, which spread along the mid-rib living oak leaf like pattern within 7 to 9 dpi. Similar symptom expression by TSV inoculation on these crops was reported by Ravi et al., (2001), Reddy et al., (2002) and Prasada Rao et al., (2003).

**TSV is also considered as an emerging threat to other economically important crops like cotton, okra and soybean.** The disease spread in various economically important crops needs to be monitored and factors responsible for TSV epidemics in groundnut and sunflower have to be established.

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**References**


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