Research Paper :

Transmission modes of sunflower necrosis virus- determination and confirmation

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SUMMARY

Sunflower (*Helianthus annuus* Linn.), is infected by sunflower necrosis virus (SNV), which causes chlorosis and necrosis of a leaf lamina and distortion of leaf. To know the various modes of transmission, investigations were carried out through sap, thrips, seed and pollen. The disease was successfully transmitted through sap using 0.05 M potassium phosphate buffer with 0.075 per cent thioglycerol as anti-inhibitor. Mean transmission which ranged from 46.80 to 57.40 per cent. The disease could not be transmitted through seeds and pollens. Further, these results were confirmed by subjecting the samples for serological assay through Direct Antigen Coated- Enzyme Linked Immuno Sorbent Assay (DAC-ELISA).

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Sunflower (*Helianthus annuus* Linn.), a member of Asteraceae, is a major edible oilseed crop in importance after soybean and groundnut at the global level. Sunflower is infected by many fungal and bacterial agents. Many virus diseases have also been reported to reduce the yield from several countries (Kolte, 1985). A new virus causing necrotic symptoms and severe yield losses has been reported (Anonymous, 1997 and Singh *et al*., 1997) thus paralyzing the cultivation of this crop. It is considered as one of the threatening diseases because of its fast spreading nature and severity (Nagaraju, *et al.,* 1998). The sunflower necrosis disease is initiated as necrosis of part of the leaf lamina followed by various types of necrosis and mosaic mottling symptoms (Ajith Prasad *et al.,* 2004). It was concluded that the causal virus of Sunflower Necrosis Disease as a strain of tobacco streak Ilarvirus on the basis of serological cross reaction with tobacco streak virus antiserum by Prasada Rao *et al*. (2000). However the knowledge about the source of inoculum and modes of transmission of SNV in the field are still lacking. Therefore the present study was conducted in order to identify the same so that proper control measures can be employed to prevent yield loss.

MATERIALS AND METHODS

The sunflower test plants were raised in 6' x 4' polythene bags, containing mixture of soil, sand and FYM in 2:1:2 ratio (w/w) and were maintained in glasshouse.

Sap transmission:

Standard inoculum of the virus was prepared using 0.05M phosphate buffer. Young tender sunflower leaves showing clear necrosis symptoms were collected from the field. The sample was macerated in pestle and mortar using phosphate buffer (1ml/g of leaf tissue). The resultant extract was used as standard inoculum for sap inoculation. Sunflower seedlings at two leaves stage were used for the experiment. Each set of plants inoculated thus was labeled separately and kept in glasshouse. These plants were maintained for symptom expression up to 30-40 days and per cent transmission of the disease was recorded.

Insect transmission:

Prasada Rao *et al*. (2003) and Sdoodee and Teakle (1987) reported that three thrips species, *Megalurothrips usitatus, Frankliniella schultzei* and *Scirtothrips dorsalis* and *Thrips tabaci*, respectively as the vectors of TSV. Based on these studies, *Thrips palmi* Karny which was very abundantly prevailing on sunflower leaves in Zonal Agricultural Research Station, GKVK, Bangalore were selected for insect transmission studies. The healthy colonies of *T. palmi* were maintained on sunflower, green gram and peanut plants inside a wooden cage kept in glasshouse. Young sunflower leaves showing clear symptoms were kept in the Petriplate. About 20-30 *T. palmi* nymphs were released onto such leaves. The nymphs were allowed for acquisition access period (AAP) of three days under normal room temperature. Nymphs fed on healthy leaves served as check. After three days, 20-25 nymphs were transferred to test plants raised in insect proof wooden cages. Sunflower seedlings at two leaves stage were used for the experiment. The normal movement of thrips was observed to ensure that no injury has occurred during the transfer. The plants were kept undisturbed allowing the nymphs to feed. These test plants were kept under observation upto 50 days for symptom expression.

Seed transmission:

Seeds from naturally infected sunflower plants showing characteristic disease symptoms on different genotypes were collected and stored for 45 days to overcome dormancy period. Twenty five seeds from each genotype were tested for seed transmission of the virus. Per cent seed germination, number of plants showing symptoms till button formation was observed and recorded and the per cent transmission was worked out.

Pollen transmission:

Pollens from naturally infected sunflower plants showing characteristic disease symptoms were collected. Pollen application to test plants was done by two methods.

Dusting method:

Sunflower seedlings at two leaves stage were chosen for this experiment. The leaves were first smeared with an abrasive (celite), to create wounds for the entry of virus. Then the pollens collected from the sunflower field were dusted on to the leaf surface. 50 test plants were used for inoculation. The seedlings were kept undisturbed and observed for the symptom development.

Sap method:

In this method, the pollen collected from infected sunflower plants were macerated in 0.05M phosphate buffer at a dilution of 1:10. The suspension was then filtered using sterilized muslin cloth. This extract was used for inoculating sunflower seedlings. 50 test plants were used for inoculation. These plants were observed for expression of symptoms under glasshouse condition.

Virus detection:

Based on Prasada Rao *et al*. (2000) studies who reported SNV as a strain of TSV, TSV antisera along with alkaline phosphatase (ALP) enzyme and p-nitrophenylphosphate (pNPP) system were employed for Direct Antigen Coated Enzyme Linked Immuno Sorbent Assay (DAC-ELISA). Crude plant extract was prepared in coating buffer of 1:100 dilution (100 mg of leaf sample /1 ml buffer). The filtered extract was dispensed into each well of ELISA plate at the rate of 100 ìl and was incubated at 37^0 C for 1 hour. 2 per cent BSA was used as blocking protien and incubated for 1hr at 37^oC. Antibody and Alkaline phosphatase labeled antigoat IgG were used at 1:1000 and 1:10,000 dilutions and incubated at 37^0 C for 1 hour. p-nitrophenyl phosphate substrate was used for the reaction. Change in the colour of the wells was observed using ELISA reader and OD value was recorded at 405nm.

RESULTS AND DISCUSSION

The virus was successfully transmitted through mechanical sap. A maximum transmission of 57.40 per cent on cultivar Morden, 54.00 per cent on KBSH-44, and 46.80 per cent on KBSH-1 was obtained (Table 1). Symptoms appeared as twisting of freshly emerged leaves 5-6 days after inoculation, which later produced chlorotic and necrotic spots after 8-10 days. Similar results were obtained when TSV was mechanically inoculated onto various plant species like *Vigna unguiculata* cv. C-152 (Fabaceae), *Nicotiana tabaccum* (Solanaceae), *Chenopodium amaranticolor* (Chenopodiaceae) and *Gomphrena globosa* (Amaranthaceae) which produced necrotic lesions on leaves and streaks on stems (Ladhalakshmi *et al*., 2005).

Incase of insect transmission studies, *Thrips palmi* Karny, successfully transmitted SNV to sunflower test plants (KBSH-1, KBSH-44 and Morden), with mean per cent transmission ranging from 16.67 to 26.67 (Table 2).

The seedlings raised from seeds of naturally infected sunflower plants did not show any symptom even after

Table 1: Mechanical transmission of Sunflower necrosis virus (SNV)

Sr. No.	Cultivar	Replication	No. of plants inoculated	No. of plants infected	Transmission (%)	Mean transmission (%)	DAC ELISA reaction
1.	KBSH-1	I	100	15	15	15.80	Positive
		II	100	18	18		
		III	100	16	16		
		IV	100	18	18		
		V	100	17	17		
2.	KBSH-41	I	100	52	52	54.00	Positive
		II	100	53	53		
		III	100	56	56		
		IV	100	55	55		
		V	100	54	54		
3.	Morden	I	100	58	58	57.40	Positive
		II	100	55	55		
		III	100	59	59		
		IV	100	59	59		
		V	100	56	56		
			Mean transmission (%)			52.13	

Table 2: Transmission of SNV through *Thrips palmi*

Sr. No.	Cultivar	Replication	No. of plants inoculated	No. of plants infected	Transmission (%)	Mean transmission (%)	DAC ELISA reaction
1.	KBSH-1	I	10	2	20	16.67	Positive
		II	10	1	10		
		III	10	2	20		
		IV	10	2	20		
		V	10	2	20		
2.	KBSH-41	I	10	2	20	20.00	Positive
		II	10	2	20		
		III	10	2	20		
		IV	10	3	30		
		V	10	2	20	26.67	Positive
3.	Morden	I	10	2	20		
		II	10	3	30		
		III	10	3	30		
		Mean transmission (%)				21.10	

button stage though the seeds recorded good germination percentage, indicating the non-transmissible nature of the virus through seeds (Table 3).

Inoculation of sunflower test plants with pollen grains from SNV infected sunflower plants by both pollen sap and dusting of pollen grains did not produce any

Table 3: Transmission of SNV through seeds

Sr. No.	Genotypes	No. of seeds		Germination (%)	No. of plants infected	DAC ELISA reaction
		Sown	Germinated			
1.	GMU- 402	25	19	76	0	Negative
2.	GMU- 405	25	22	88	0	Negative
3.	GMU- 408	25	20	80	0	Negative
4.	GMU- 410	25	18	72	0	Negative
5.	GMU- 411	25	21	84	0	Negative
6.	GMU- 416	25	20	80	0	Negative
7.	GMU- 424	25	22	88	0	Negative
8.	GMU- 430	25	21	84	0	Negative
9.	GMU- 433	25	21	84	0	Negative
10.	GMU- 432	25	20	80	0	Negative
11.	GMU- 436	25	19	76	0	Negative
12.	GMU- 447	25	22	88	0	Negative
13.	GMU- 454	25	18	72	0	Negative
14.	GMU- 460	25	20	80	0	Negative
15.	GMU- 461	25	17	68	0	Negative
16.	GMU- 476	25	16	64	0	Negative
17.	GMU- 483	25	22	88	0	Negative
18.	GMU- 496	25	18	72	0	Negative
19.	GMU- 498	25	22	88	0	Negative
20.	GMU- 500	25	16	64	0	Negative
21	KBSH-1	25	22	88	0	Negative
22	KBSH-44	25	20	80	0	Negative
23	Morden	25	21	84	0	Negative

Table 4 : Transmission of SNV through pollen dust

Sr. No.	Cultivar	Replication	No. of plants		Transmission (%)	DAC ELISA reaction
			Inoculated	Infected		
1.	KBSH-1	I	50	0	0	
		II	50	0	0	
		III	50	0	0	Negative
		IV	50	0	0	
		V	50	0	0	
2.	KBSH-44	I	50	0	0	
		II	50	0	0	
		III	50	0	0	Negative
		IV	50	0	0	
		V	50	0	0	
3.	Morden	I	50	0	0	
		II	50	0	0	
		III	50	0	0	Negative
		IV	50	0	0	
		V	50	0	0	

Table 5: Transmission of SNV through pollen sap

Sr. No.	Cultivar	Replication	No. of plants		Transmission (%)	DAC ELISA reaction
			Inoculated	Infected		
1.	KBSH-1	I	50	0	0	
		II	50	0	0	
		III	50	0	0	Negative
		IV	50	0	0	
		V	50	0	0	
2.	KBSH-44	I	50	0	0	
		II	50	0	0	
		III	50	0	0	Negative
		IV	50	0	0	
		V	50	0	0	
3.	Morden	I	50	0	0	
		II	50	0	0	
		III	50	0	0	Negative
		IV	50	0	0	
		V	50	0	0	

symptom, indicating that pollen was not involved in SNV transmission (Table 4 and 5). Similar results were reported earlier by Lokesh (2006).

All the above transmission studies were confirmed by subjecting the test samples for serological assay. Positive reactions were obtained for the transmission through sap and thrips while negative for seeds and pollen transmission. This clearly indicates sap and thrips are the two different modes of transmission of SNV and thus management strategies should be focused on them.

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