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Screening of Sunflower Genotypes for Resistance to Sunflower Mosaic Virus using Molecular and Serological Assays

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ABSTRACT: Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops but it has been found prone to many fungal, bacterial and viral diseases, particularly sunflower mosaic virus (SuMV). Since no effective chemical control measures are available, host resistance remains the most sustainable management strategy. To evaluate the resistance of selected sunflower genotypes against SuMV using serological assay (DAS-ELISA) and molecular confirmation (RT-PCR), eleven sunflower genotypes were screened under glasshouse conditions using mechanical inoculation. Symptom expressed was recorded and plants were further tested through DAS-ELISA and RT-PCR targeting the coat protein gene of potyvirus. Three genotypes (KBSH-41, KBSH-53, and AHT-5) exhibited complete resistance, showing no visible symptoms, negative ELISA results and absence of amplification in RT-PCR. The remaining eight genotypes displayed varying degrees of mosaic, mottling and chlorotic lesions with disease incidence ranging from 22 % to 42 %. RT-PCR confirmed SuMV presence in symptomatic plants, producing a 500 bp amplicon and DAS- ELISA results showed positive results in symptomatic plants. These findings highlight KBSH-41, KBSH-53 and AHT-5 as promising resistant sources for SuMV management, suitable for resistance breeding programs. The integration of phenotypic, serological and molecular assays provides a reliable framework for screening resistance in sunflower.

Keywords: Sunflower, Sunflower mosaic virus, Resistance, RT-PCR, DAS-ELISA, Genotype Screening.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an economically important oilseed crop cultivated widely in tropical and subtropical regions. This herbaceous annual plant belongs to the Asteraceae family and is native to North American regions (Blackman *et al.*, 2011). This plant is important as its seeds are an accessible source of premium oil dedicated primarily to human consumption. Aside from other factors, sunflower production can be affected by diseases caused by nematodes, bacteria, fungi and viruses (Kolte, 1985 and Bello *et al.*, 2022) but particularly SuMV. Its oil is valued for nutritional quality and industrial applications. In India, sunflower cultivation has expanded steadily to meet the increasing demand for edible oil. However, productivity is often constrained by several diseases, particularly those caused by viruses.

Globally, sunflower viral diseases may result in yield losses of up to 40-60 % during severe epidemics. Sunflower mosaic disease is a significant problem in India, with an incidence of 5-10 % typically occurring during the *Kharif* season (Jindal *et al.*, 2001; Verma *et al.*, 2009). Sunflower mosaic virus (SuMV), a potyvirus, is considered one of the most destructive pathogens, inducing chlorotic rings, systemic mosaic, mottling, leaf curling and stunting symptoms. Its effects are most severe at early growth stages, leading to poor seed set, reduced photosynthesis and low oil content. Because chemical control options are unavailable for viral diseases, resistance breeding is the most practical and environmentally sustainable approach.

Earlier studies revealed that while many sunflower genotypes are highly susceptible, some carry natural resistance that can be utilized in breeding programs. Accurate identification of resistant genotypes is thus critical for integrated disease management and yield stability. Traditionally, resistance screening was based on field observations and symptom assessments. However, advances in biotechnology have enabled the use of serological and molecular assays such as DAS-ELISA and RT-PCR, which improve detection accuracy and identify symptomless carriers.

The present study aimed to evaluate eleven sunflower genotypes under controlled glasshouse conditions for their resistance or susceptibility to SuMV using phenotypic, serological, and molecular approaches.

MATERIAL AND METHODS

Plant material and experimental design: Eleven sunflower genotypes *viz.*, Ganga Kaveri, Sandoz, Kaveri, ITC, KBSH-41, Suvarna, KBSH-53, KBSH-44, KBSH-78, ITC-1068 and AHT-5 were evaluated in a completely randomized design (CRD) with five replications. Each replication consisted of ten plants, totaling 50 plants per genotype.

Virus source and Maintenance: SuMV-infected plants were collected from the Zonal Agricultural Research Station (ZARS), Bengaluru. Typical field symptoms included mottling, mosaic, chlorotic lesions (A) and severe stunting (B) (Fig. 1). The virus was maintained under glasshouse conditions on the susceptible variety Ganga Kaveri, which served as the inoculum source.

A.

B.

Fig. 1. Naturally infected sunflower plant showing symptoms of SuMV under field conditions.

Mechanical inoculation: Leaf samples showing severe SuMV symptoms were ground in chilled 0.1 M phosphate buffer (pH 7.0) using a sterile pestle and mortar. The sap was filtered through muslin cloth and applied to two-leaf stage sunflower seedlings pre-dusted with carborundum powder. Gentle rubbing was followed by rinsing with distilled water to remove excess inoculum (Hull, 2009; Ashfaq *et al.*, 2010). Plants were maintained in insect-proof cages for symptom expression.

Disease recording and incidence calculation: Symptom observations began from 10 days after inoculation (DAI) and continued up to 30 DAI. Symptoms included mild mosaic, mosaic, chlorotic lesions and mottling. Disease incidence was calculated as:

$$\text{Disease Incidence (\%)} = \frac{\text{No. of plants infected}}{\text{Total No. of plants}} \times 100$$

Double antibody sandwich ELISA: DAS-ELISA was performed using potyvirus-specific antibodies, polystyrene plates were coated with capture antibodies, blocked and incubated with leaf sap extracts from inoculated plants. Secondary antibodies conjugated with alkaline phosphatase was added and colour was developed using *p*-nitrophenyl phosphate substrate. Absorbance was measured at 405 nm. Values twice that of buffer controls were considered as positive (Basavaraj, 2014).

RT-PCR assay: Total RNA was extracted from infected and resistant genotypes using the TRIzol method (Kavyashree, 2014). Reverse transcription was carried out at 39 °C for 30 min to synthesize cDNA. PCR amplification targeted the SuMV coat protein (CP-500bp) gene using specific primers (Dujovny *et al.*, 2000). Cycling conditions included initial denaturation at 94 °C, annealing at 52 °C, and extension at 72 °C. Amplicons were resolved on 1% agarose gels, stained with ethidium bromide and visualized under UV light.

RESULTS AND DISCUSSION

Symptom expression: Among the genotypes screened, three (KBSH-41, KBSH-53, and AHT-5) exhibited no visible symptoms, indicating strong resistance. The remaining eight genotypes-Ganga Kaveri, ITC, Kaveri, Sandoz, Suvarna, KBSH-78, KBSH-44 and ITC-1068-displayed varying degrees of mosaic, mottling and chlorotic lesions (Fig. 2) on younger leaves. Disease incidence ranged from 22 % (ITC-1068) to 42 % (Ganga Kaveri) as presented in Table 1.

The results are in line with the findings of Bello *et al.*, (2022), who reported that sunflower chlorotic mottle virus (SuCMoV) infected plants showed chlorosis, mosaic, chlorotic ringspot and necrosis on younger leaves with more disease incidence. Similarly, Clara and Zein (2012) worked on pea seed- borne mosaic potyvirus (PSbMV) in cowpea in Egypt and observed symptoms *viz.*, necrotic local lesions, mottle and systemic mosaic. After biological purification, the identity of the virus isolate was confirmed by indirect ELISA also.

RT PCR detection: RT-PCR analysis confirmed SuMV infection in eight symptomatic genotypes, all producing a 500 bp amplicon. Resistant genotypes (KBSH-41, KBSH-53, and AHT-5) and negative controls showed no amplification (Fig. 3).

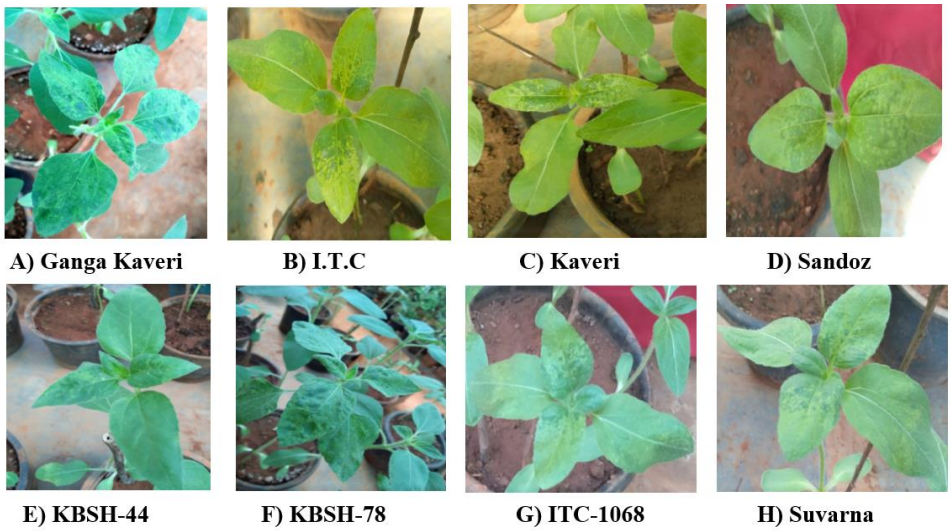

Fig. 2. Genotypes showing symptoms of SuMV under glasshouse condition.

Table 1: Screening of genotypes through mechanical inoculation against SuMV.

Sr. No.	Hybrid	No. of plants infected	Disease incidence	Symptoms exhibited	OD value at 405nm
1.	Ganga Kaveri	21	42.00	MM, M, Mo, Cl	2.10
2.	Sandoz	15	30.00	M, Cl	1.38
3.	I.T.C	30	34.00	M, Y	1.55
4.	Kaveri	34	36.00	M	1.60
5.	KBSH-41	00	00.00	-	0.42
6.	KBSH-53	00	00.00	-	0.46
7.	Suvarna	12	24.00	M, Cl	1.36
8.	KBSH-44	13	26.00	MM, M	1.13
9.	KBSH-78	14	28.00	M, Mo	1.41
10.	ITC-1068	11	22.00	M, Cl	1.32
11.	AHT-5	00	00.00	-	0.70

*No. of plants inoculated: 50, MM-Mild mosaic, M-Mosaic, Cl-Chlorotic lesion, Mo-Mottling

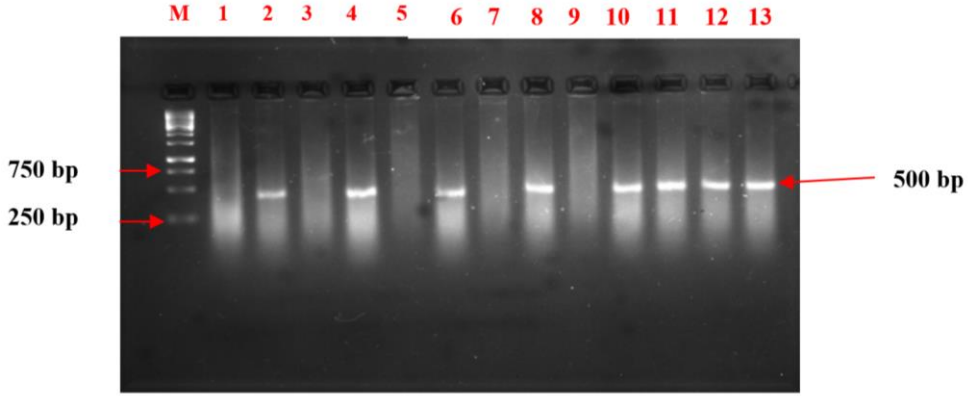

Fig. 3. Screening of different hybrids of sunflower against SuMV by RT-PCR.

Lane M: Marker (1kb), Lane 1: Water control; Lane 2: SuMV infected sample as positive control; Lane 3: Healthy sunflower sample as control; Lane 4, 6 and 8: Ganga Kaveri, I.T.C, Sandoz; Lane5, 7 and 9: KBSH-41, KBSH-53 and AHT-5; Lane 10, 11, 12 and 13: Suvarna, ITC-1068, KBSH-44 and Kaveri

DAS-ELISA detection: DAS-ELISA results indicated positive reactions in eight genotypes, producing bright yellow colour with potyvirus-specific antisera. The highest OD value (2.10) was recorded in Ganga Kaveri, while the lowest (0.42) was recorded in KBSH-41 (Table 1). Positive,

negative and buffer controls had OD values of 2.37, 0.27, and 0.36 respectively thus confirming the reliability of test.

The results of SuMV were consistent with earlier reports of El-Kady *et al.*, (2014), used ELISA and RT-PCR against Egyptian BCMV isolate, yielding a ~500 bp amplicon of the coat protein and positive results in ELISA, respectively. Sharma *et al.,* (2015) similarly employed DAS-ELISA and RT-PCR in bean yellow mosaic virus (BYMV), detecting an ~800 bp coat protein fragment with positive ELISA results.

Overall comparison with earlier findings: The present investigation demonstrated that most sunflower germplasm is susceptible to SuMV, with only a few genotypes showing resistance. These findings are consistent with earlier studies. Shah *et al.,* (2011) reported that most local chilli cultivars were susceptible to chilli veinal mottle virus (ChiVMV), except for a few resistant lines. Similarly, Venugopal Rao *et al.,* (1987) found all six sunflower cultivars (BSH-1, Morden, Surya, C0-1, EC-68414, and EC-68415) tested to be susceptible to SuMV, while Gulya *et al.,* (2002) reported that both inbred and hybrid sunflower lines responded with high virulence of SuMV with variable symptom expression across genotypes. Desai (1998) conducted field trials during 1993-94 in the Northern Dry Zone 3 (Region II) of Karnataka, India, to screen 44 sunflower genotypes for mosaic disease caused by SuMV. The percentage disease incidence ranged from 0.01-11.02 in different trials.

Comparable trends have been observed in other crops infected with potyvirus where, Mohamad and Hassan (2002) reported susceptibility in several soybean genotypes against soybean mosaic virus (SMV) under glasshouse conditions. Wani *et al.,* (2017) found that only 8 out of 32 cowpea genotypes were resistant to BCMV. Likewise, Bachkar *et al.,* (2019) observed resistance in only a few soybean genotypes against SMV with several showing moderate resistance or susceptibility under different conditions. The similarity of our findings to these studies reinforces the understanding that viral pathogens often pose a uniform threat across a wide genetic base, leaving limited options for natural resistance within cultivated germplasm.

The overall concurrence between our results and earlier studies emphasizes the urgent need to strengthen breeding programs aimed at incorporating durable resistance genes from exotic or wild relatives into cultivated varieties. The identification and deployment of resistant sources like AVRDC lines in chilli, could play a pivotal role in developing varieties capable of withstanding ChiVMV pressure. Thus, the present study not only validates earlier reports but also underscores the necessity of continuous screening and resistance breeding for sustainable crop improvement.

This consistency highlights the scarcity of natural resistance in cultivated germplasm and reinforces the need to incorporate resistance genes from exotic or wild relatives into breeding programs.

Disease progression: Symptom progression was gradual, with mild chlorotic patches appearing at 10 DAI, systemic mosaic by 20 DAI, and severe stunting in susceptible genotypes by 30 DAI. Resistant genotypes maintained normal growth, similar to healthy controls.

CONCLUSIONS

Virus-resistant genotypes act as barriers to disease spread and can enhance sunflower productivity. In this study, KBSH-41, KBSH-53 and AHT-5 were identified as resistant, showing no viral symptoms, negative ELISA reactions, and absence of viral amplicons in RT-PCR. These genotypes represent valuable sources for resistance breeding programs aimed at developing cultivars with durable resistance.

The disease incidence patterns revealed significant genetic variability among susceptible genotypes, with Ganga Kaveri being the most vulnerable. Early resistance screening under controlled inoculation conditions is essential, as SuMV is most damaging at early crop stages. The combined use of phenotypic, serological, and molecular assays ensures accurate resistance identification and should be adopted for large-scale germplasm evaluation.

FUTURE SCOPE

The use of resistant genotypes can reduce dependence on chemical management, lower production costs, and enhance yield stability. Incorporating these resistant sources into breeding programs will facilitate the development of high-yielding hybrids with durable resistance, thereby improving sunflower productivity and profitability.

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Conflict of interest: None declared.

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