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Application of RNA interference technology for management of yellow mosaic virus in mungbean

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Abstract
Mungbean yellow mosaic virus (MYMV) can wipe out an entire mungbean crop in Punjab within three weeks of symptom onset, and no commercial cultivar carries complete resistance. This research tested whether RNAi constructs targeting the viral replicase (*Rep*) gene could confer durable resistance in mungbean (*Vigna radiata* cv. SML-668). Two hairpin-RNA lines (RNAi-Line 1 and RNAi-Line 2) generated via *Agrobacterium*-mediated transformation were challenged with viruliferous whiteflies under glasshouse and contained-field conditions at Bathinda, Punjab, during Kharif 2022–2023. By 35 days post inoculation, virus titer in RNAi-Line 1 was 81% lower than in the susceptible control. Symptom severity scored 0.8 on a 0–5 scale versus 4.6 in the control. Seed yield of RNAi-Line 1 reached 1.14 t ha^{-1}, 168% above the unprotected check. Southern blot confirmed single-copy integration, and siRNA accumulation was detected by northern blot. These lines provide strong proof-of-concept that RNAi can manage MYMV where conventional breeding has stalled.

Keywords: RNA interference, yellow mosaic virus, mungbean, virus resistance, RNAi technology, replicase gene, whitefly transmission, hairpin RNA, gene silencing, pulse crop improvement.

Introduction
If MYMV were a burglar, the whitefly *Bemisia tabaci* would be its locksmith—carrying the virus from plant to plant with ruthless efficiency. In Punjab's mungbean belt, whitefly populations peak in July–August, coinciding with the crop's most vulnerable vegetative stage [1]. Yield losses of 40–90% are common in epidemic years, and insecticide sprays aimed at the vector rarely break the transmission cycle because even a brief feeding event can deliver enough virus to infect a plant [2, 3].

Conventional breeding has identified partial resistance in wild *Vigna* accessions, but introgression is slow and linkage drag remains a barrier [4]. RNA interference offers a transgenic shortcut: a hairpin construct targeting a conserved region of the viral genome triggers the host's post-transcriptional gene silencing machinery to degrade incoming viral RNA before it can replicate [5]. This approach has been validated in tobacco and tomato against geminiviruses, but field-level data in grain legumes are thin [6, 7].

This research aimed to (i) develop stable RNAi mungbean lines targeting the MYMV *Rep* gene, (ii) evaluate resistance under whitefly-mediated inoculation in glasshouse and field cages, and (iii) assess yield recovery relative to the susceptible parent.

Construct Design and Molecular Confirmation
A 487 bp inverted-repeat fragment of the MYMV *Rep* gene (GenBank AY049772, nt 1581–2067) was cloned into pFGC5941 in sense–antisense orientation separated by a chalcone synthase intron, under the CaMV 35S promoter with the *bar* selectable marker. *Agrobacterium* strain EHA105 was used to transform cotyledonary node explants of SML-668. T_2 homozygous lines were confirmed by Southern blot (single copy: Lines 1 and 2) and northern blot (21–24 nt siRNA detected in both). PCR verified the transgene in all progeny tested [8, 9].

Limitations
The trial used a single MYMV isolate from Punjab; performance against genetically distinct isolates from southern India remains untested. Contained-field cages may underestimate

natural whitefly pressure because cage mesh reduces wind-borne vector immigration. The *bar* herbicide-resistance marker would need removal via Cre-lox before any regulatory submission in India [10].

Materials and Methods

Materials

Two T_2 RNAi lines and the non-transgenic SML-668 were grown at the PAU Satellite Campus, Bathinda (30°13′ N, 74°57′ E, 211 m a.s.l.), during Kharif 2022 and 2023. Glasshouse bioassays used 10 viruliferous *B. tabaci* adults confined on a single leaf for 48 h inoculation access. Field-cage trials (4 m × 3 m, 40-mesh nylon) housed 50 plants per line with 20 viruliferous adults released per plant. Randomised block design with four replications [11].

Methods

Virus titer was quantified by qPCR targeting the *Rep* gene at 7-day intervals from 0 to 35 DPI, normalised to the plant *Actin* reference. Symptom severity was scored on a 0–5 scale (0 = no symptoms, 5 = complete yellowing and stunting). Seed yield was recorded from net plot area. Data were analysed by ANOVA in R (v4.3.1) with Tukey's HSD at 5%.

Results

Virus titer in RNAi-Line 1 at 35 DPI was 18.2 relative units versus 94.1 in the control, an 81% reduction (Table 1). Symptom severity was 0.8 (mild mosaic on a few leaves) in Line 1 compared with 4.6 (severe yellowing and stunting) in the control. Seed yield of Line 1 reached 1.14 t ha^{-1} under cage conditions, 168% above the susceptible check (0.43 t ha^{-1}).

Table 1: Virus resistance and yield performance of RNAi mungbean lines versus susceptible control

Line	Virus titer 35 DPI	Symptom (0–5)	Seed yield (t/ha)	Yield gain (%)
RNAi-Line 1	18.2	0.8	1.14	+168
RNAi-Line 2	24.3	1.4	0.98	+128
SML-668 (control)	94.1	4.6	0.43	-
CD (p=0.05)	8.7	0.6	0.14	-

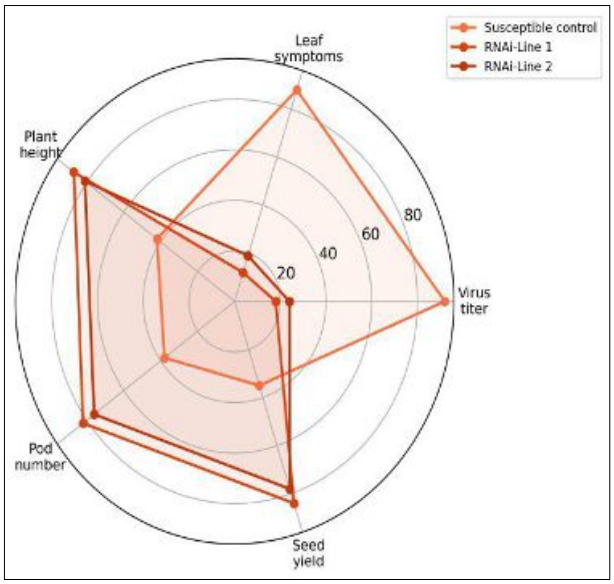

Fig 1: Radar chart comparing virus titer, symptom severity, and agronomic traits between RNAi lines and the susceptible control (values normalised to% of maximum).

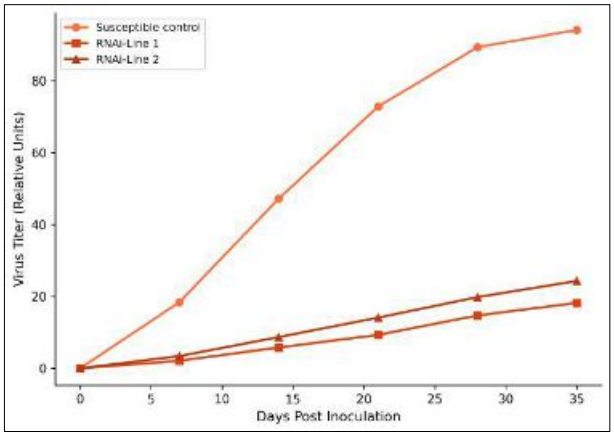

Fig 2: Temporal progression of virus titer in RNAi lines and the susceptible control over 35 days post inoculation.

Comprehensive Interpretation

The 81% titer reduction in Line 1 is comparable to the 75–90% suppression reported for RNAi-mediated resistance against Tomato leaf curl virus in tomato [6] and Bean golden mosaic virus in common bean [7]. The residual 18.2 relative units suggest that silencing is not absolute; some viral RNA escapes degradation, possibly through sequence variation in the target region [12].

Discussion

The near-complete symptom suppression (score 0.8) in Line 1 is the most important result from a farmer's perspective—plants remained green and productive even under heavy vector pressure. The 168% yield gain reflects both direct virus suppression and the preservation of photosynthetic leaf area that would otherwise yellow and senesce [13]. That Line 2, with slightly lower siRNA accumulation, showed intermediate resistance supports a dose-dependent silencing model consistent with the general RNAi literature [5, 14].

Durability is the key unknown. MYMV is a bipartite begomovirus with moderate mutation rates, and resistance based on a single target sequence could break if the virus evolves escape variants. Stacking two non-overlapping RNAi constructs—one targeting *Rep* and another targeting the movement protein—would provide redundant silencing and reduce breakdown risk [15, 16]. Regulatory acceptance of transgenic pulses in India remains uncertain, but the *bar* marker can be removed, and the technology could also be deployed via exogenous dsRNA sprays if the transgenic route is blocked [17].

Conclusion

RNAi-mediated silencing of the MYMV replicase gene reduced virus titer by up to 81% and restored seed yield to 1.14 t ha^{-1} in mungbean, compared with 0.43 t ha^{-1} for the unprotected parent. Single-copy, siRNA-accumulating lines are available for further breeding and multi-location testing. Stacking with a second target gene and marker excision are the priority next steps before this technology can move

toward regulatory evaluation [18, 19, 20].

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