



Unravelling the Genetic Complexity: Enhancing MYMV Resistance in Blackgram [*Vigna mungo* (L.) Hepper] Through Trait-based Selection

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ABSTRACT

Background: Limited genetic diversity in blackgram, stemming from its common ancestry with greengram, presents challenges in combating Mungbean Yellow Mosaic Virus (MYMV), leading to yield stagnation. To address this, genetic screening and selection of segregating generations in F_2 are essential for refining resistance traits with enhancing productivity.

Methods: A two-season experiment was conducted in Panpoli, Tamil Nadu, during the *Kharif* 2021 and *Kharif* 2022 in a randomized block design using, F_2 seeds from specific crosses (MDU 1 $\times$ Mash 114 and ADT 3 $\times$ PU 31), along with F_1 and parent plants. MYMV disease scores were recorded using the infector row method for all the genotypes studied.

Result: The study unveiled a complex genetic basis of MYMV resistance in blackgram, characterized by di-genic dominant genes displaying duplicative dominance type of epistatic interaction. This complexity underscores the insufficiency of simple selection methods. Estimates of genetic variability for "number of pods per plant" exhibited high genetic advance and moderate heritability, indicating control by additive genes. Positive correlations were observed between plant height, number of clusters per plant and number of pods per plant with yield. Path analysis revealed that traits like number of seeds per pod, 100 seed weight and number of pods per plant directly and positively influenced yield, which exhibit minimal influence from MYMV. These traits offer crucial selection criteria for enhancing MYMV resistance in blackgram.

Key words: Complexity, Digenic, Dominant, Genetic gain, Selection.

INTRODUCTION

Blackgram [*Vigna mungo* (L.) Hepper] is a crucial legume in Asia and the Asian Subcontinent, valued for its protein, carbohydrates, minerals and essential nutrients (Mehra *et al.*, 2016). In India, it covers 5.44 mha, producing over 3.56 mt with an average productivity of 655 kg/ha, mainly in Andhra Pradesh, Karnataka, Maharashtra, Tamil Nadu and Uttar Pradesh (Singh *et al.*, 2021). Despite its significance, blackgram productivity has plateaued due to a limited genetic base. Cultivated in diverse environments, it faces biotic and abiotic challenges, with the mungbean yellow mosaic virus disease (MYMVD) being particularly detrimental (Vadivel *et al.*, 2023). This *Begomovirus*, transmitted by the whitefly *Bemisia tabaci* (Malathi and John, 2009), can reduce yield by up to 85% (Nene, 1973), affecting crops in both *Kharif* and *Summer* seasons. Breeding efforts thus focus on enhancing MYMVD resistance to boost yields. Given the narrow genetic variability shared with greengram, hybridization is a key to introducing new genetic variations for MYMVD resistance. In self-pollinated crops like blackgram, selection relies on heritable variation (Bishnoi *et al.*, 2017). Identifying high-yielding genotypes in segregating generations requires understanding trait associations and cause-and-effect relationships. This study aims to identify recombinants with desirable MYMVD resistance without compromising yield, examining trait associations and variability in the F_2 generation to formulating breeding strategies that enhance both MYMVD resistance and yield in blackgram.

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MATERIALS AND METHODS

Plant materials and development of F_2 segregants

During the *Kharif* season of 2020 at the Vanavarayar Institute of Agriculture (VIA), Pollachi, four blackgram genotypes were cultivated in 3 m $\times$ 3 m plots with 30 cm row to row spacing and 10 cm plant to plant spacing. High-yielding but MYMV-susceptible genotypes ADT 3 and MDU 1 and MYMV-resistant genotypes PU 31 and Mash 114 were used (Table 1). Using hand emasculatation and pollination techniques by Sen and Ghosh (1959), hybridization was carried out, staggering parents for synchronized flowering was followed. The resulting F_1 hybrids, ADT 3 $\times$ PU 31 (Cross I) and MDU 1 $\times$ Mash 114 (Cross II), were confirmed and self-

Table 1: Details of genotypes and their MYMV inheritance pattern.

Name of the genotype	Parentage	No. of. plants scored	Reaction to MYMV	Place of origin	Putative gene symbol
ADT 3	Pureline selection from Thirunelveli local	15	Susceptible	Aduthurai	$r_1r_1r_2r_2$
MDU 1	ADB 2003 × VBG 66	15	Susceptible	Madurai	$r_1r_1r_2r_2$
Mash 114	Blackgram × Rice bean (<i>Vigna umbellata</i>)	15	Highly resistant	PAU, Ludhiana	$R_1R_1R_2R_2$
PU 31	UPU-1 × UPU-2	15	Highly resistant	Pantnagar	$R_1R_1R_2R_2$

pollinated during the *Summer* of 2021, with F_2 seeds collected for MYMV resistance screening.

MYMY screening and trait analysis

MYMV resistance screening was carried out in June–July 2021 (S_1) and 2022 (S_2) at a farmer's field in Panpoli, (a recognized MYMV hotspot in the Tenkasi district, Tamil Nadu). Utilizing an infector row method with CO 5 blackgram variety, seeds of test genotypes were raised in eight rows of 3 meter length, spaced at 30 cm × 10 cm under unprotected conditions. Disease incidence data were recorded (Fig 1) when the susceptible check displayed symptoms surpassing 80% through the methodology outlined by Singh and Singh (1988). Simultaneously, an extensive evaluation of eleven yield-contributing traits in the F_2 population was undertaken.

Scale	Percentage of plant foliage affected	Reaction
1	0.1 to 5.0% leaf area mottling	Resistant (R)
3	5.1 to 10.0% leaf area mottling	Moderately resistant (MR)
5	10.1 to 25.0% leaf area with mottling and yellowing	Moderately susceptible (MS)
7	25.1 to 50.0% leaf area with mottling	Susceptible (S)
9	> 50.0% and up to 100% of the leaf area with mottling and yellowing on severe case	Highly Susceptible (HS)

Singh and Singh (1988)

Statistical analysis

Expected deviation between MYMV-resistant and susceptible plants in the F_2 segregating population was assessed using a goodness-of-fit test. Coefficient of variation estimates, such as phenotypic coefficient of variation (PCV %) and genotypic coefficient of variation (GCV %) were calculated following Burton's method (1952). Broad-sense heritability (h^2) was determined using Lush's method (1940) and expressed as a percentage. Genetic advance as per cent of the mean (GAM) were calculated according to Johnson *et al.* (1955). Simple correlations between various yield components were estimated following Al-Jibouri *et al.* (1958) and path coefficients were determined using the method outlined by Dewey and Lu (1959).

RESULTS AND DISCUSSION

MYMV Inheritance

The outcomes of the Chi-square test for Cross I (ADT 3 × PU 31) and Cross II (MDU 1 × Mash 114) are detailed in Table 2. The investigation into the inheritance of Mungbean Yellow Mosaic Virus (MYMV) resistance (Fig 2) revealed the absence of disease symptoms in the F_1 generations of both crosses across two seasons, indicating the presence of a dominant gene governing resistance. The observed-to-expected ratio of F_2 segregants for MYMV inheritance in

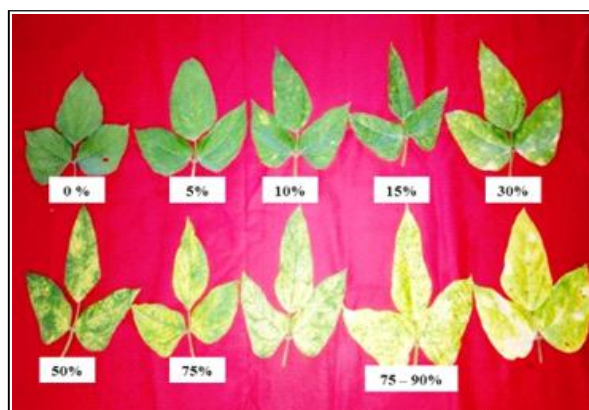


Fig 1: YMV disease scoring based on percentage leaf infection.



Fig 2: Screening F_2 segregants for MYMV disease using the infector row.

both crosses demonstrated a well-fitted 15:1 ratio (resistant:susceptible), supported by non-significant chi-square values. Female parents ADT 3 and MDU 1 showed disease symptoms, confirming their susceptibility, while male parents PU 31 and Mash 114 remained symptom-free, reinforcing their resistance. The dominant gene's expression in both homozygous and heterozygous conditions aligns with our findings and previous studies by Murugan and Nadarajan (2012) in urdbean genotypes. However, Reddy and Singh (1995) in urdbean reported a monogenic recessive gene and additive gene for MYMV resistance, highlighting the complexity and variability in MYMV resistance genetics across legume varieties and necessitating a nuanced approach to breeding strategies.

Variability analysis

Genetic variability is a cornerstone in successful plant breeding programs, directly influencing the potential for trait improvement. Our study delved into the estimation of PCV (%) and GCV (%) for various traits related to MYMV resistance. Significant differences ($P < 0.01$) among F_2 genotypes for 11 yield attributes were elucidated through ANOVA (Table 3). The variability parameters, detailed in Tables 4, 5, shed light on the genetic variation within the F_2 population of two crosses. Notably, high GCV (%) was observed for number of branches per plant (40.4% and 45.1%) in Cross I, yield per plant (36.3% and 40.1%) and number of pods per plant (30.5% and 33.4%) in Cross II. Conversely, low GCV (%) values of 1.1% and 1.6% (Cross I) and 2.1% and 2.6% (Cross II) for days to maturity and 3.0% and 3.3% (Cross I) and 3.2% and 3.0% (Cross II) for the days to 50% flowering was recorded. Regarding PCV (%), Cross I showcased high values for number of branches per plant (83.8% and 76.1%), while Cross II exhibited

elevated PCV for yield per plant (57.5% and 53.7%) and number of pods per plant (54.2% and 52.3%). Lower PCV values of 6.6% and 7.1% for days to maturity and 6.8% and 7.3% for 100 seed weight in Cross I, along with 7.0% and 7.3% for the number of days for 50% flowering in Cross II, underscored relatively lower variability in these traits. Although the estimated PCV (%) exceeded GCV (%) for all traits, high GCV (%) values were observed for number of branches per plant, yield per plant and number of pods per plant under MYMV conditions. This result aligns with findings of Thirumalai and Murugan (2020) in blackgram F_2 segregants.

The analysis of heritability (h^2) provided insights into the degree of genetic influence on the observed traits. The estimates highlighted predominantly low heritability across all traits, with some exceptions. Notably, moderate heritability surfaced in yield per plant (40% and 38%), plant height (34% and 31%) and number of pods per plant (32% and 33%) in Cross II, while Cross I registered moderate heritability for 100 seed weight (32% and 30%) and number of clusters per plant. Conversely, Cross I exhibited low heritability for number of pods per cluster (-2% and 1%), whereas Cross II showcased low heritability for number of branches per plant (7% and 9%) and days to maturity (8% and 6%). No traits had high heritability, showing significant environmental influence. Moderate heritability was seen in yield per plant, plant height, number of pods and 100 seed weight. Notably, higher GAM was observed for yield per plant (47.1% and 44.5%) and number of pods per plant (35.4% and 33.3%) in Cross II and for number of branches per plant (40.1% and 42.3%) in Cross I. Conversely, low GAM was registered for number of pods per cluster (-1.3% and -1.7%) and days to maturity (0.4% and 1.3%) in Cross

Table 2: Goodness of fit test for MYMV inheritance.

Generation	Observed values				Expected ratio		x ² values		Probability	
	Resistant		Susceptible							
	2021	2022	2022	2022	2022	2022	2022	2022	2021	2022
ADT3 × PU 31 (Cross I)										
F ₁	15	16	-	-	-	-	-	-	-	-
F ₂	292	269	17	14	15:1	15:1	0.69	0.83	0.80- 0.70	0.40 - 0.30
MDU1 × MASH 114 (Cross II)										
F ₁	17	15	-	-	-	-	-	-	-	-
F ₂	272	272	15	14	15:1	15:1	0.50	0.56	0.50- 0.40	0.50 - 0.40

Table 3: Analysis of Variance (ANOVA) for yield components in blackgram.

Source	df	Mean squares										
		Plant height (cm)	Number of branches per plant	Days to 50% flowering	Days to maturity	Number of clusters per plant	Number of pods per cluster	Number of pods per plant	Number of seeds per pod	Pod length (cm)	100 seed weight (g)	Yield per plant (g)
Replications	1	1.07	0.32	3.12	5.38	0.04	0.01	1.48	0.01	0.05	0.01	0.02
Genotypes	40	12.45**	0.38**	4.30**	21.56**	1.84**	0.33**	21.37**	0.85**	0.28**	0.31**	0.33**
Error	40	3.51	0.04	1.05	2.75	0.39	0.06	6.51	0.36	0.06	0.02	0.02

* $p > 0.05$.

I. The GAM analysis emphasized that traits such as number of pods per cluster, yield per plant and number of branches per plant exhibit additive gene action and are highly responsive to selection. This finding was consistent with Rana *et al.* (2022); Gnanasekaran *et al.* (2024) reported high h^2 and GAM (%) for above traits in blackgram.

Table 4: Variability estimates for yield components in F_2 generation of cross I.

Traits	Mean		Range		GCV (%)		PCV (%)		ECV (%)		h^2 (Broad sense) %		GA as % of mean	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
PH	21.0	19.8	13.6-32.5	15.3-36.7	8.2	7.3	16.6	17.3	14.4	13.7	24	26	8.3	8.7
NBPP	1.4	1.7	0.0-3.5	2.3-4.5	40.4	45.1	83.8	76.1	73.5	69.4	23	21	40.1	42.3
DFF	34.4	31.3	31.5-41.5	27.5-39.5	3.0	3.3	7.1	6.9	6.4	7.1	18	15	2.7	3.1
DM	74.3	65.4	67.5-86.0	71.3-83.2	1.1	1.6	6.6	7.1	6.6	6.3	03	07	0.4	1.3
NCPP	4.8	3.1	2.0-7.5	2.3-7.5	22.4	19.3	38.3	40.2	31.0	28.1	34	31	26.9	28.0
NPPC	3.1	4.3	1.5-5.0	2.1-4.3	4.9	5.1	37.5	33.6	37.8	36.1	-2	-1	-1.3	-1.7
NPPP	14.4	12.5	3.0-35.0	2.7-28	20.6	23.1	50.6	53.1	46.2	45.2	17	21	17.3	15.7
NSPP	6.7	8.3	4.5-9.0	3.9-9.7	8.5	9.1	15.7	17.3	13.2	12.7	29	27	9.5	8.7
PL	4.3	3.9	3.7-4.8	4.7-5.1	3.4	3.9	8.4	7.9	7.7	7.9	16	19	2.8	3.1
HSW	3.4	4.1	2.3-3.8	3.2-3.6	3.8	2.9	6.8	7.3	5.6	4.8	32	30	4.5	4.9
SPY	3.2	4.0	0.5-7.9	0.6-6.9	23.7	26.4	50.8	48.1	44.3	42.1	22	24	22.7	21.7

S_1 - 2021, S_2 - 2022, PH: Plant height, NBPP: Number of branches per plant, DFF: Days to 50% flowering, DM: Days to maturity, NCPP: Number clusters per plant, NPPC: Number of pods per cluster, NPPP: Number pods per plant, NSPP: Number of seeds per pod, PL: Pod length, HSW: 100 seed weight, YPP: Yield per plant.

Table 5: Variability estimates for yield components in F_2 generation of cross II.

Traits	Mean		Range		GCV (%)		PCV (%)		ECV (%)		h^2 (Broad sense) %		GA as% of mean	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
PH	28.1	29.3	17.5-40.6	15.8-38.5	14.3	15.7	24.4	23.7	19.9	18.1	34	31	17.1	16.7
NBPP	1.0	1.3	0.0-3.0	1.3-3.5	27.7	25.1	34.5	37.6	47.8	43.3	7	9	15.1	14.8
DFF	35.0	31.7	32.0-42.5	33.0-39.3	3.2	3.0	7.0	7.3	6.2	6.7	21	19	3.0	3.3
DM	76.4	78.2	68.5-88.0	59.1-62.6	2.1	2.6	7.5	8.3	7.2	7.8	8	6	1.2	1.6
NCPP	4.7	3.9	2.5-11.0	2.7-9.1	13.4	14.3	43.1	38.9	41.0	39.1	10	8	8.6	9.1
NPPC	3.0	3.3	1.0-5.0	0.7-6.1	16.8	15.1	36.2	31.2	32.1	27.8	21	23	16.0	15.6
NPPP	13.9	12.3	2.5-28.5	2.7-25.6	30.5	33.4	54.2	52.3	44.8	45.1	32	33	35.4	33.3
NSPP	6.5	5.6	4.0-8.5	3.9-8.9	5.6	6.3	18.0	17.6	17.1	16.7	10	12	3.6	3.9
PL	4.6	5.1	3.1-6.6	3.5-7.3	5.5	5.7	12.2	13.5	10.9	11.3	20	17	5.1	6.0
HSW	4.3	4.1	3.5-5.2	3.3-6.1	6.2	6.6	12.0	11.7	10.2	12.5	26	23	6.5	6.7
YPP	3.9	3.7	0.8-9.6	1.3-11.1	36.3	40.1	57.5	53.7	44.6	39.3	40	38	47.1	44.5

Table 6: Simple correlation for yield components in F_2 generation of cross I (S_1).

Traits	PH	NBPP	DFF	DM	NCPP	NPPC	NPPP	NSPP	PL	HSW	YPP
PH	1.00	0.36*	-0.02	0.01	0.22*	0.16*	0.33*	0.01	-0.09	0.06	0.30*
NBPP		1.00	0.01	-0.01	0.07	0.09	0.17*	-0.07	-0.12	-0.05	0.15
DFF			1.00	0.88*	0.01	-0.05	-0.02	-0.04	0.03	-0.01	-0.01
DM				1.00	0.03	-0.04	-0.01	-0.10	0.01	-0.03	-0.01
NCPP					1.00	-0.24*	0.56*	0.06	-0.04	0.08	0.52*
NPPC						1.00	0.56*	0.08	0.06	-0.04	0.48*
NPPP							1.00	0.05	-0.09	0.00	0.81*
NSPP								1.00	0.59*	0.06	0.32*
PL									1.00	-0.02	0.09
HSW										1.00	0.15
YPP											1.00

* $p < 0.05$.

Association analysis

The correlation analysis explored relationships between yield and its components, offering insights for targeted breeding. Selection for yield can be more effective when considering its components alongside overall yield (Grafius, 1960). Tables 6-9 presented relative magnitudes of simple and phenotypic correlations among various yield-contributing traits. In Cross I, yield per plant correlated

positively with number of pods per plant (0.81 and 0.86), number of clusters per plant (0.52 and 0.62), number of pods per cluster (0.48 and 0.41) and number of seed per pod (0.32 and 0.42). Similarly, in Cross II, positive correlation was observed for number pods per plant (0.87 and 0.89) number of pods per cluster (0.64 and 0.74), number of clusters per plant (0.60 and 0.70) and plant height (0.51 and 0.63). These associations highlight the

Table 7: Simple correlation matrixes of yield components in F_2 generation of cross I (S_2).

Traits	PH	NBPP	DFF	DM	NCPP	NPPC	NPPP	NSPP	PL	HSW	YPP
PH	1.00	0.01	0.16*	0.09	0.08	-0.04	-0.05	0.06	-0.01	0.15	-0.05
NBPP		1.00	0.07	0.13	0.34*	0.06	0.22*	0.19*	0.16*	0.01	0.23*
DFF			1.00	0.80*	0.13	0.03	0.18*	0.30*	-0.02	0.11	0.19*
DM				1.00	0.09	0.08	0.27*	0.35*	-0.11	-0.10	0.26*
NCPP					1.00	0.02	0.72*	0.32*	0.18*	-0.14	0.62*
NPPC						1.00	0.42*	0.08	0.16*	-0.06	0.41*
NPPP							1.00	0.42*	0.11	-0.30*	0.86*
NSPP								1.00	0.57*	-0.29*	0.42*
PL									1.00	0.01	0.18*
HSW										1.00	-0.18*
YPP											1.00

*p<0.05.

Table 8: Simple correlation matrixes of yield components in F_2 generation of cross II (S_1).

Traits	PH	NBPP	DFF	DM	NCPP	NPPC	NPPP	NSPP	PL	HSW	YPP
PH	1.00	0.45*	0.17*	0.16*	0.48*	0.34*	0.52*	0.24*	0.11	0.06	0.51*
NBPP		1.00	0.01	0.01	0.49*	0.28*	0.49*	0.12	0.06	-0.03	0.42*
DFF			1.00	0.82*	-0.01	0.01	0.03	0.12	0.04	0.08	0.13
DM				1.00	-0.02	-0.08	-0.05	0.06	-0.03	0.04	0.01
NCPP					1.00	0.18*	0.71*	0.16*	0.09	0.09	0.60*
NPPC						1.00	0.72*	0.24*	0.16*	-0.08	0.64*
NPPP							1.00	0.21*	0.12	0.01	0.87*
NSPP								1.00	0.72*	-0.09	0.42*
PL									1.00	-0.09	0.27*
HSW										1.00	0.16*
YPP											1.00

*p<0.05.

Table 9: Simple correlation matrixes of yield components in F_2 generation of cross II (S_2).

Traits	PH	NBPP	DFF	DM	NCPP	NPPC	NPPP	NSPP	PL	HSW	YPP
PH	1.00	0.37*	0.19*	0.21*	0.53*	0.29*	0.54*	0.34*	0.13	0.05	0.63*
NBPP		1.00	0.03	0.02	0.57*	0.34*	0.39*	0.13	0.09	-0.02	0.43*
DFF			1.00	0.64*	-0.03	0.03	0.01	0.14	0.06	0.09	0.16
DM				1.00	-0.01	-0.04	-0.04	0.03	-0.01	0.03	0.03
NCPP					1.00	0.16*	0.74*	0.19*	0.07	0.07	0.70*
NPPC						1.00	0.68*	0.19*	0.18*	-0.06	0.74*
NPPP							1.00	0.23*	0.13	0.03	0.89*
NSPP								1.00	0.74*	-0.05	0.41*
PL									1.00	-0.08	0.30*
HSW										1.00	0.18*
YPP											1.00

*p<0.05.

importance of considering these traits in selecting high-yielding genotypes in the F_2 generation was consistent with Veeranjanyulu *et al.* (2007) findings in blackgram.

Path coefficient analysis, potent indices for revealing cause-and-effect relationships, identified traits with direct and indirect impacts on yield. Illustrated in Fig 3 and 4, the path coefficients for the 11 yield components provided valuable insights. In Cross I, yield per plant had notably high direct effects on number of

Pods per plant (1.17), number of seeds per pod (0.87), number of branches per plant (0.71) and 100 seed weight (0.67). Conversely, moderate direct effects were observed for number of pods per cluster (0.21), with a low effect for the days to 50% flowering (0.13). Cross II revealed a high direct effect for 100 seed weight (0.54), number of pods per cluster (0.47) and number of seeds per pod (0.41). Traits such as number of pods per plant, number of seeds per pod and 100 seed weight

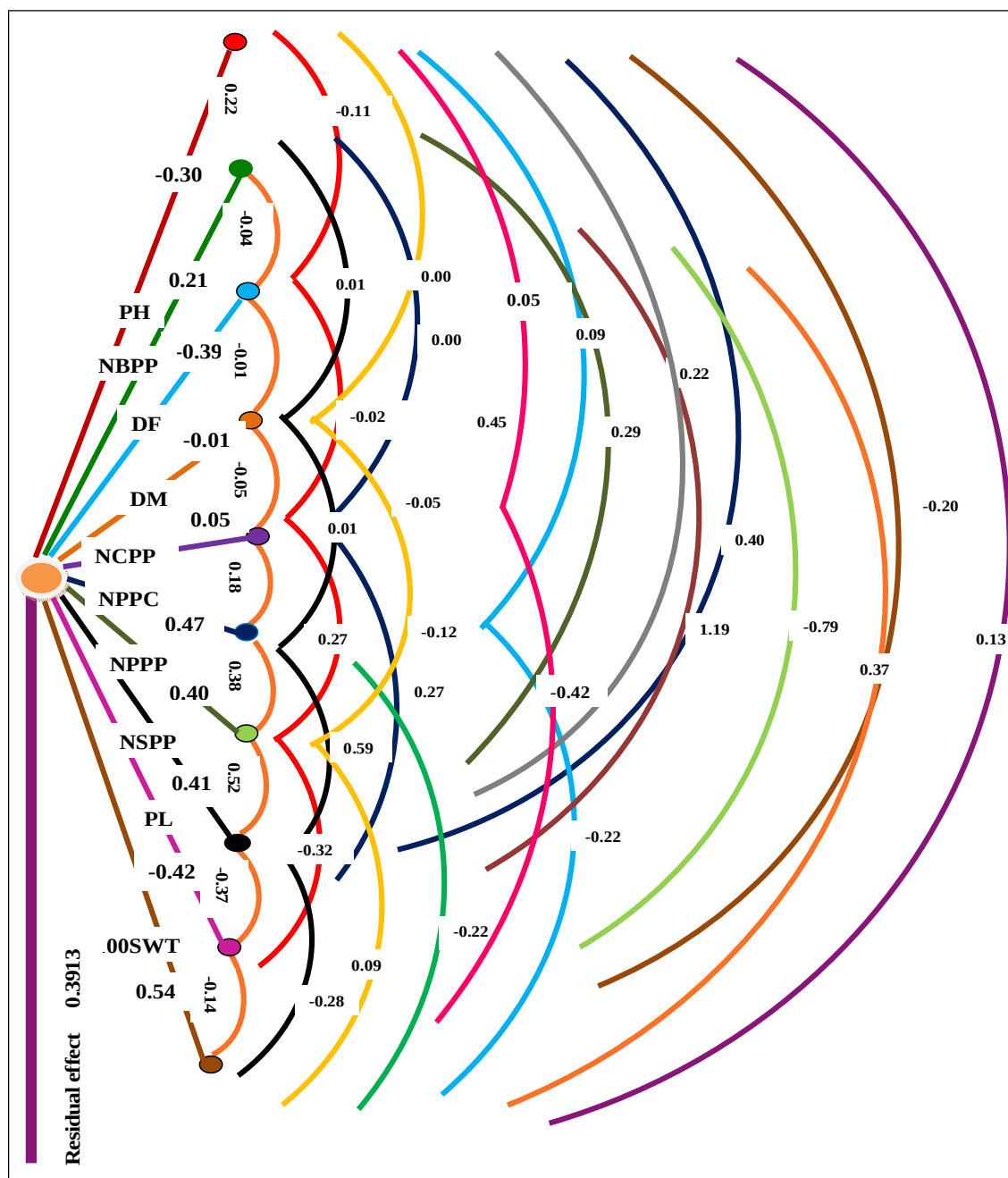


Fig 3: Direct and indirect effect of yield components in F_2 of cross "MDU 1x Mash 114".

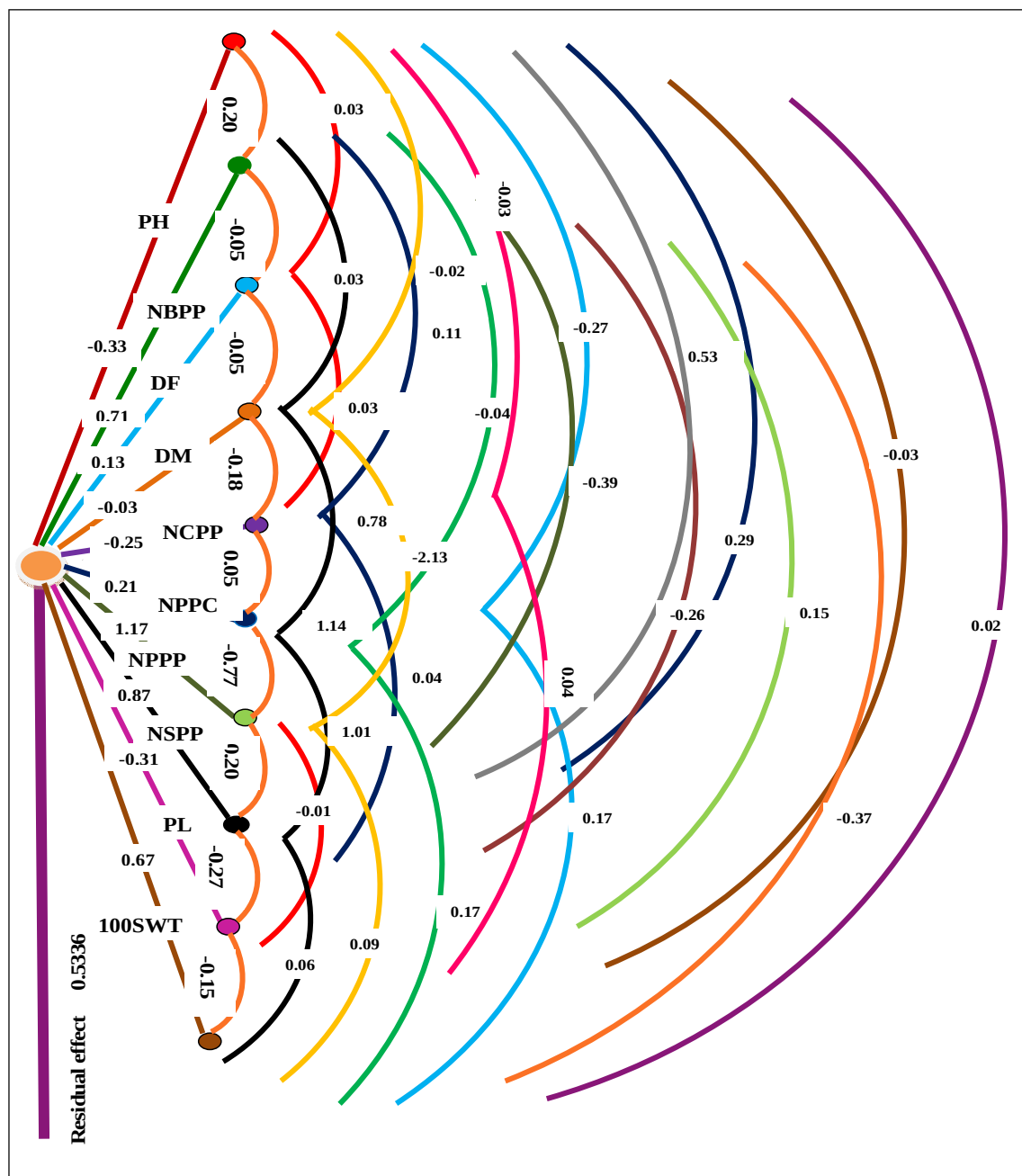


Fig 4: Direct and indirect effect of yield components in F_2 of cross "ADT 3 x PU 31".

exhibited higher direct effects on yield, suggesting limited influence from MYMV disease. This highlights the potential effectiveness of selecting high-yielding genotypes based on these specific yield components, consistent with similar findings reported by Sathees *et al.* (2019); Surendhar *et al.* (2024) in blackgram.

CONCLUSION

The study reveals that MYMV resistance in blackgram is influenced by duplicate dominant gene action, as indicated

by the 15:1 (resistant: susceptible) segregation ratio in the F_2 generation, complicating selection strategies. Traits such as number of pods per cluster, number of clusters per plant, number of seeds per pod and 100 seed weight demonstrate stable additive gene action, showing resilience to environmental influences and offering potential for targeted improvement in early segregating generations.

Conflict of interest

All authors declare that they have no conflict of interest.

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