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Heterosis for yield and resistance to mosaic disease in vegetable cowpea (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt)

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Abstract
To assess the heterosis over mid, better, and standard parents, fifteen F_1 hybrids from a cross between three mosaic-resistant cowpea accessions as testers and five high yielding yard long bean varieties as lines alongside their parents and a standard check were tested for 19 yield characters. Field scoring for the black eye cowpea mosaic virus (BlCMV) was performed to determine mosaic resistance. All the parameters under study showed significant desirable heterosis, except for pod length and girth. The crosses KAU Deepika x Manjari and KAU Mithra x Manjari registered significant positive heterosis over mid, better, and standard parents for pod yield and high mid-parent heterosis and standard heterosis for pods per plant. The highest magnitude of standard heterosis was identified for pods per plant (101.14%), followed by yield per plant (75.30%). Besides the heterotic potential, the hybrid KAU Mithra x Manjari, KAU Deepika x Manjari and Githika x Manjari were resistant to mosaic disease.

Keywords: Heterosis, cowpea, line x tester, mosaic resistance

Introduction
Cowpea (*V. unguiculata*) is a popular dual-purpose legume grown in the tropics and subtropics. It is the critical dietary staple for many underdeveloped and developing countries in Africa, Latin America, and Asia. In India, vegetable cowpea, both bush type (*Vigna unguiculata* (L.) Walp.) and trailing vegetable cowpea or yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) has emerged as a remunerative and preferred vegetable crop cultivated for its tender pods. Various biotic stresses limit yield in cowpea. Of this, viral diseases are a big constraint in the yield of vegetable cowpea, especially in the tropical region, and a disease incidence of 0-66% has been reported under natural conditions (Bashir *et al*., 2002) [2]. In various regions of India, there have been severe cases of the aphid-transmitted potyvirus BlCMV. In Kerala, most of the promising cultivars have seen an increased incidence of mosaic disease in recent years. Several studies have confirmed that mosaic disease of cowpea in Kerala is a result of different isolates of both cowpea aphid-borne mosaic virus (CABMV) and black eye cowpea mosaic virus (BlCMV) (Radhika, 1999; Krishnapriya 2015) [22, 14]. The development of resistant varieties is the most effective and sustainable approach to manage virus diseases. Being a self-pollinated crop, hybridisation can often create desired variability in cowpea. Many researchers have identified resistant sources against cowpea mosaic disease that can be used in the hybridization programme to generate vegetable cowpea varieties with high yield and mosaic resistance. (Pavithra *et al*., 2013; Iqbal *et al*., 2021; Krishnan *et al*., 2021) [20, 9, 13]. Hence, the current study aims to explore the extent of heterosis in vegetable cowpea for selected yield traits utilising cowpea accessions having mosaic resistance in the breeding programme.

Materials and Methods
The experimental material comprised five high-yielding but mosaic susceptible yard long bean accessions and three resistant cowpea accessions selected after artificial inoculation. The lines as female parents and testers as pollen parents were laid in two crossing blocks in Kharif 2022 in the open field and in a rain shelter at Pepper Research Station, Panniyur, KAU, to produce 15 F_1 hybrids. The 15 F_1 hybrids and their eight parents were raised with a standard variety (Lola) in a randomized block design with three replications in Rabi 2022. The seedlings grown in portrays were transplanted to the main field one week after sowing at a spacing of 1 m x 1 m with a plot size of 10 m^2 in each replication. Anaswara, a mosaic disease susceptible variety,

was planted in the border rows to ensure sufficient inoculum in the field. Observations on 19 important yield characters *viz*, vine length, branches per plant, terminal leaf length, terminal leaf width, lateral leaf length, lateral leaf width, days to flowering, pod length, pod girth, pod weight, pods per plant 100 seed weight, seeds per pod, yield per plant, yield per plot, days to harvest, crop duration, protein content, keeping quality and mosaic disease incidence were recorded. The analysis of variance was performed using the treatment data to determine the genotypic variability. Heterosis was determined as the deviance of the mean of F_1s ($\overline{F}_1$) from their mid parent (MP), better parent (BP), and standard parent (SP) as formulated by Hayes *et al*. (1965) [8] and Briggle (1963) [4]. Disease scoring was done 45 days after planting based on the 0-5 scale (Bos, 1982) [3] developed for numerical scoring of black eye cowpea mosaic virus (BlCMV). Based on the numerical scoring, the disease or vulnerability index was also calculated using the suitable formula:

$$VI = \frac{(0n_0 + 1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5)}{nt(n_c-1)} \times 100$$

where;
VI- Vulnerability Index; n_0, $n_1 \ldots n_5$- Number of plants in categories 0,1,2,3,4,5;
n_t - Total number of plants; n_c- Total number of categories

Results and Discussion

The analysis of variance for parents, crosses, and parents Vs crosses evaluated for 19 quantitative traits studied is given in Table 1. The mean squares due to genotypes, parents, and hybrids were highly significant for all the characters except for pod girth and yield per plot, for which combinations were found to be non-significant. Significant variations were noticed for parents Vs crosses for all the characters except vine length, terminal leaf width, pod girth, and 100 seed weight This revealed ample variability among the genotypes, parents, and crosses for the traits assessed. Therefore, there is potential for improvement through suitable breeding techniques. For 15 characters under study, the variations resulting from parents Vs crosses proved that the means of crosses were significantly distinct from the means of the parents, implying considerable heterosis for these characters. Significant positive heterosis was observed for vine length, branches per plant, leaf length, leaf width, 100 seed weight, pod weight, pods per plant, seeds per pod, yield per plant, yield per plot, crop duration, pod protein content and keeping quality (table 2.). The mid parent, better parent, and standard parent heterosis were significant either separately or in combination in a negative direction for days to first flowering and days to first harvest.

Table 1: Analysis of variance (mean squares) of heterosis for different traits in yard long bean

Characters	Replication	Treatments	Parents	Parents Vs Crosses	Crosses	Error
DF	**2**	**22**	**7**	**1**	**14**	**44**
Vine length	8485.174**	12858.204**	32392.804**	747.001	3955.990*	1599.113
Branches per plant	10.167**	2.729**	2.770**	8.706**	2.281**	0.670
Terminal leaf length	1.261	7.495**	15.620**	14.671**	2.920*	1.425
Terminal leaf width	0.435	7.718**	17.144**	0.592	3.514**	0.709
Lateral leaf length	1.350	7.267**	14.673**	16.358**	2.914**	1.449
Lateral leaf width	0.777	8.310**	18.663**	0.329	3.704**	0.743
Days to flowering	38.758**	37.866**	19.459**	149.98**	39.061**	4.309
Pod length	6.374	6.386**	1.137**	1.986**	2.930**	4.636
Pod girth	0.028	0.113*	0.225**	0.036	0.062	0.057
Pod wt	0.5289	244.289**	486.692**	508.043**	104.248**	3.454
Pods per plant	5209.821**	3271.103**	1918.761*	32434.979**	1864.139**	688.486
Seeds per pod	0.113	15.671**	9.017**	15.169**	19.035**	1.120
100 seed wt	0.369	20.044**	42.874**	0.500	10.025**	0.456
Yield per plant	898389.49**	369799.33**	322788.86**	1884628.51**	285102.49**	84673.39
Yield per plot	52.081*	29.682**	33.370*	125.833**	20.969	11.683
Days to harvest	33.201**	39.434**	32.573**	87.924**	39.401**	2.774**
Crop duration	320.824**	21.868**	22.750*	79.905**	17.282*	8.078
Protein content	0.044	1.459**	1.434**	0.606**	1.533**	0.024**
Keeping quality	6.529	76.808**	62.302**	43.199**	86.462**	8.687

* **Significant at p= 0.05 and p= 0.01 levels respectively

Among vegetative characters, significant relative heterosis, heterobeltiosis, and standard heterosis were found for leaf width and number of branches per plant. Superior heterobeltiosis and relative heterosis were exhibited for leaf length, and relative heterosis alone for vine length. For the number of branches per plant, four hybrids displayed high standard heterosis (31.26% to 44.66%), and hybrid Vyjyanthi x Manjari showed good heterosis over the mid, better, and standard parent. Three hybrids were superior to the better and standard parent for terminal leaf width. The three types of heterosis were manifested for terminal leaf width in the hybrid Vellayani Jyothika x EC 18734 and lateral leaf width in the hybrid KAU Mithra x EC 18734. Superior relative heterosis was reported in eight hybrids for leaf length and five hybrids for vine length. Jithesh (2009) [10] and Lakshmi (2016) [15] observed similar results for vegetative characters in yard long bean.

Earliness, defined by negative heterosis estimations, is an important goal of any breeding program since it aids the farmer in obtaining higher crop returns. The standard heterosis for days to flowering was substantially negative in all the hybrids. (-28.57% to -7.02%), and 14 hybrids exhibited significant standard heterosis for days to harvest (-21.35% to -5.16%). KAU Mithra x EC 18734 displayed maximum and significant negative average heterosis, heterobeltiosis, and standard heterosis (-16.18%, -15.70, and -28.57%,

respectively) for days to first flowering. Four hybrids revealed significant negative heterosis concerning this trait's mid, better, and standard parents. The hybrids, KAU Mithra x VU 7, Vellayani Jyothika x VU7, and Vellayani Jyothika x EC 18734, showed the highest standard heterosis (-21.35%), heterobeltiosis (-11.27%) and relative heterosis (-10.73%) respectively for days to first harvest. Seven hybrids expressed negative relative, better parent, and standard heterosis for days to first harvest. Considerable hybrid vigour in traits related to earliness in cowpea has been observed by Kadam *et al*. (2013) [12], Dinakar *et al*. (2019) [5], and Joshi *et al*. (2022) [11].

Table 2: Range of heterosis percent for different characters and best crosses in yard long bean

Characters	Range of heterosis over			No. of heterotic crosses	Best cross (Over SP/BP)
	MP	BP	SP		
Vine length	-19.27 to 22.04	-26.12 to -10.21	-23.38 to -12.38	6	Githika x EC 18734
No. of branches/ plant	27.55 to 63.93	-41.34 to 38.89	31.26 to 44.66	7	Vellayani Jyothika x VU 7
Terminal leaf length	10.40 to 28.47	-15.06 to 14.29	-11.95 to -12.92	7	KAU Deepika x VU 7
Terminal leaf width	-19.12 to 37.74	-40.17 to 28.18	-21.16 to 22.59	7	V. Jyothika x EC 18734
Lateral leaf length	11.57 to 29.57	-15.07 to 14.94	-14.97 to -11.89	8	KAU Deepika x VU 7
Lateral leaf width	-21.85 to 43.43	-44.16 to 29.68	-27.47 to 15.01	6	KAU Mithra x EC 18734
Days to first flowering	-16.18 to -5.79	-15.70 to -10.06	-28.57 to -7.02	15	KAU Mithra x EC 18734
Pod length	-36.99 to -10.91	-60.15 to -7.82	-48.53 to -11.11	0	Nil
Pod girth	Nil	-13.29 to -9.63	-14.79 to -12.60	0	Nil
Pod weight	-42.60 to -9.56	-66.15 to -14.65	-50.25 to 38.75	3	KAU Mithra x Manjari
Pods per plant	41.52 to 127.26	35.00 to 95.36	61.17 to 101.14	12	Vyjayanthi x VU7
Seeds/pod	-26.43 to 16.66	-32.08 to 8.92	-29.46 to -22.91	4	V. Jyothika x Manjari
100 seed weight	-11.56 to 33.77	-28.26 to 11.96	-11.58 to 24.35	9	KAU Deepika x Manjari
Yield/ plant	43.75 to 129.55	50.27 to 60.47	43.29 to 75.30	10	KAU Deepika x Manjari
Yield per plot	49.31 to 116. 82	49.39 to 50.27	68.10 to 75.28	7	KAU Deepika x Manjari
Days to harvest	-10.73 to 4.43	-11.27 to 15.25	-21.35 to -5.16	14	KAU Mithra x VU7
Crop duration	3.41 to 5.89	-4.08 to 4.95	-7.56 to -5.34	5	KAU Mithra x EC 18734
Pod protein content	4.32 to 13.23	-16.67 to -4.82	-4.59 to 39.17	8	KAU Deepika x Manjari
Keeping quality	-22.83 to 34.36	-20.49 to 80.18	26.53 to 62.87	8	KAU Mithra x Manjari

Among 15 hybrids, none displayed beneficial heterosis for pod length and girth. Negative heterosis may be due to the lower mean values of all the testers for these traits than the lines in the crosses. Gudadhe *et al*. (2015) [7], Sharath and Joseph (2016) [23] and Verma *et al*. (2020) [25] have also observed relatively low or negative heterosis for these characters. Three hybrids registered positive standard heterosis for pod weight, but none was superior to the mid and better parent. The range of this trait was -50. 25% to 38.75%, and the best-performing hybrid was KAU Mithra x Manjari (38.75%). Twelve crosses manifested significant mid parent heterosis, while seven recorded high heterobeltiosis and economic heterosis for pods per plant. Average heterosis, heterobeltiosis, and standard heterosis ranged from 41.52% to 127.26%, 35.00% to 93.56%, and 61.44% to 101.14%, respectively. While Vyjayanthi x VU 7 recorded the highest standard heterosis for this attribute, Deepika x EC 18734 exhibited the highest significant heterosis for the mid and better parent. Beneficial heterosis for pods per plant in cowpea was also identified by Pandey and Singh [17] (2015), Get *et al*. (2021) [6], and Joshi *et al*. (2022) [11].

Three hybrids registered significant relative heterosis for seeds per pod, and cross KAU Mithra x EC 18734 was the best-performing hybrid (16.66%). The cross Vellayani Jyothika x Manjari (8.92%) manifested positive heterosis over the better parent for this trait. Nine combinations displayed significant standard heterosis; eight hybrids recorded significant relative heterosis, and one hybrid, Githika x VU 7 (11.96%), exhibited positive heterobeltiosis for 100 seed weight. KAU Deepika x Manjari (24.35%) and Githika x VU 7 (37.77%) were the better-performing hybrids over the standard and mid parent, respectively. This aligns with the findings of Patel *et al*. (2009) [18] and Pallavi *et al*. (2020) [16]. Five hybrids recorded positive average heterosis for crop duration, and only one cross exhibited significant positive heterobeltiosis. Above the mid and better parent, KAU Mithra x EC 18734 was the superior hybrid.

Ten hybrids displayed significant heterosis for mid parent, while three hybrids each registered significant heterosis over better parent and standard parent for yield per plant. Maximum relative heterosis, heterobeltiosis, and standard heterosis were observed for the cross Vyjayanthi x EC, Vyjayanthi x VU 7, and KAU Deepika x Manjari, respectively. For yield per plot, significant heterosis was exhibited by seven hybrids over the mid parent and two crosses over the better parent and standard parent. KAU Mithra x Manjari exhibited maximum relative heterosis (59.01%) and better parent heterosis (50.27%). The highest standard heterosis was displayed by KAU Deepika x Manjari (75.28%), followed by KAU Mithra x Manjari (68.10%). Heterosis for yield per plant has been recorded by Sharma *et al*. (2013) [24], Gudhadhe *et al*. (2015) [7], and Pethe *et al*. (2017) [21].

Quality characters are essential attributes in the breeding of new varieties. Significant relative heterosis was observed in six hybrids, and positive standard heterosis in seven hybrids for pod protein content. The best hybrid for pod protein content was Githika x Manjari over mid parent and KAU Deepika x Manjari over the standard parent. Anitha *et al*. (2016) [1] and Pathak *et al*. (2017) [19] also observed considerable heterosis for pod protein content. Only four hybrids registered significant average heterosis in the negative direction, and two hybrids displayed significant negative heterosis for physiological weight loss. The highest significant desirable heterosis for this trait over mid and better parent was observed in KAU Mithra x Manjari. The results confirmed the heterosis study in yard long bean conducted by Lakshmi (2016) [15].

The disease reaction of different hybrids toward mosaic incidence was another critical aspect of this study (fig. 1.). The vulnerability index of parents ranged from 0 to 77.33, and hybrids ranged from 6.66 to 89.33. Among the 15 crosses, three were resistant, nine were medium susceptible, one susceptible, and two highly susceptible to mosaic disease. The check for yield characters, Lola, was highly susceptible, but the parent, Manjari (resistant check), was highly resistant to the disease. The three crosses, Githika x Manjari, KAU Deepika x Manjari, and KAU Mithra x Manjari, which involved Manjari as the tester parent, were resistant to mosaic disease. The vulnerability index of these crosses was 14.66, 12.00, and 6.66, respectively. The results indicate that the parent Manjari is a good source of resistance to mosaic disease, and the resistant crosses obtained from the study can be further utilized for crop improvement programmes for mosaic resistance in cowpea. Krishnan *et al.* (2021) [13] investigated the resistance of the variety Manjari against cowpea aphid-borne mosaic virus (CABMV) and stated that the variety is tolerant to mosaic disease.

In the present study, desirable significant standard heterosis has been identified for 12 characters studied *viz;* no. of branches per plant, terminal leaf width, lateral leaf width, days to first flowering, days to harvest, pod weight, pods per plant, yield per plant, yield per plot, 100 seed weight, pod protein content and keeping quality. Significant heterosis was observed for 16 characters concerning the mid parent and 14 characters over the better parent. Significant positive heterosis could not be identified for pod length and pod girth. It may be because all three testers included in the crosses had lesser *per se* value than the lines for these characters, and two testers were bush type vegetable cowpea. Different crosses were superior for yield characters over the better or standard parent. The cross, KAU Deepika x Manjari, registered heterosis for 11 characters, and KAU Mithra x Manjari showed heterosis for 10 characters. The highest standard heterosis for yield per plant and yield per plot was shown by KAU Deepika x Manjari, followed by KAU Mithra x Manjari. These crosses also demonstrated heterosis for major yield traits including pods per plant and earliness. In addition to the potential of heterosis, the above two crosses were found resistant to mosaic disease based on the results of the vulnerability index. Another cross, Githika x Manjari, was also resistant to the disease, but the cross exhibited significant heterosis for only three characters.

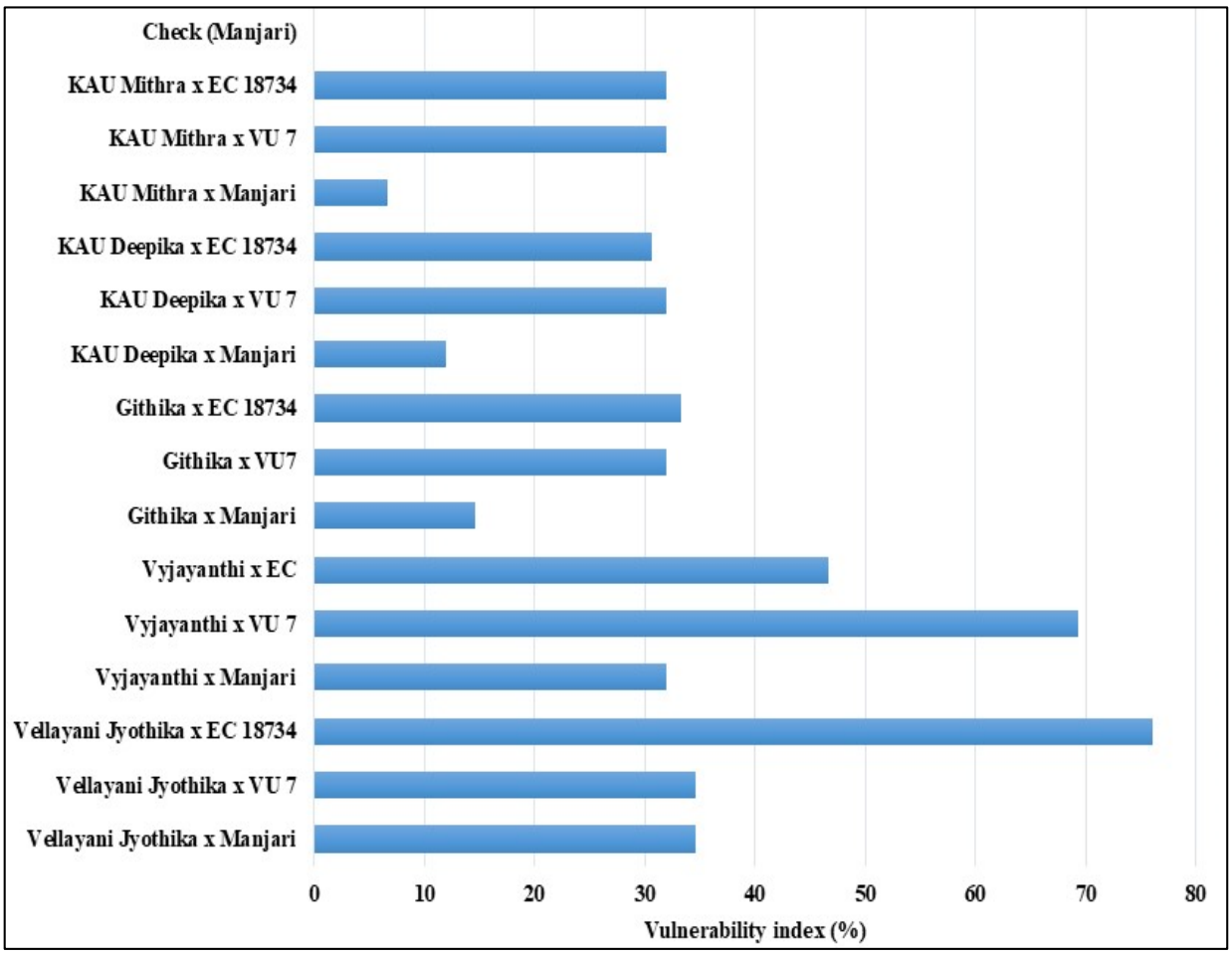

Fig 1: Vulnerability index (%) of cowpea hybrids for mosaic disease

Conclusion

It can be concluded that based on the heterosis of important yield characters and resistance to mosaic disease, two hybrids, KAU Deepika x Manjari and KAU Mithra x Manjari, displayed the best performance among the hybrids. The findings depict that the superior performance of these two hybrids results from significant heterosis of these crosses for the maximum number of yield-contributing traits. Therefore, these hybrid combinations can be further utilised in breeding programmes to develop vegetable cowpea varieties with high yield and mosaic resistance.

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