



Inheritance of resistance to yellow vein mosaic virus disease in interspecific crosses of *Abelmoschus*

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ABSTRACT

Okra [*Abelmoschus esculentus* (L.) Moench] is an important vegetable crop grown in tropical and subtropical regions of the world. The total production of okra is drastically reduced due to various biotic and abiotic stresses. Among the biotic stresses, viral disease is of major concern. In this context, the present investigation has been carried out to understand the inheritance of yellow vein mosaic virus (YVMV) disease resistance in okra using segregating populations of *A. esculentus* cv. Punjab Padmini $\times$ *A. moschatus* and *A. esculentus* cv. Punjab Padmini $\times$ *A. tuberculatus* and to study the chromosome number of the three species involved in the *A. esculentus* cv. Punjab Padmini, *A. moschatus*, *A. tuberculatus* and their inter-specific F_1 hybrids. The results of crossability studies revealed that, the wild species *A. moschatus* and *A. tuberculatus* are compatible with cultivated specie *A. esculentus* cv. Punjab Padmini. Inheritance of YVMV disease has been observed to be simple and governed single dominant gene in case of *A. esculentus* $\times$ *A. moschatus* cross and a single recessive gene in *A. esculentus* cv. Punjab Padmini $\times$ *A. tuberculatus* cross.

Key words: *A. esculentus*, *A. moschatus*, *A. tuberculatus* Dominant, Recessive

Okra [*Abelmoschus esculentus* (L.) Moench] cultivation in India is seriously threatened by YVMV disease. The causal virus complex is spread by an insect vector white fly (*Bemisia tabaci*). The disease was first identified by Kulkarni (1924) in India and later studied by Capoor and Varma (1950) and Varma (1952). Further Uppal *et al.* (1940) established the viral origin of the disease and coined the name yellow vein mosaic. The disease not only reduces the yield substantially but also affects marketability of the fruits. The evolution of new viral strains seems to be one of the major factor responsible for the breakdown of tolerance/resistance, as in the majority of the cases it is location specific. The genetic resistance is most effective, economic and environmental friendly means to reduce losses due to this disease. Presently, no YVMV disease resistant lines are available in the cultivated okra germplasm. Thus, intervarietal transfer of YVMV resistance is unlikely. On the other hand, wild *Abelmoschus* species are highly valuable sources of disease resistance genes. Infection of 100% plants in a field is very usual and yield loss ranges between 50 and 94% depending on the stage of crop growth at which infection occurs

(Ali *et al.* 2005). Although, the production constraints can be controlled up to some extent through insecticide treatment but insect control by chemical means adds to the cost of production and is hazardous to the human health and ecosystem. So, cultivation of resistant varieties is one of the practicable options for the management of disease because genetic resistance is safe, effective, reliable and cost-free alternative to ensure a healthy crop. In the past, *A. manihot* ssp. *manihot* and *A. manihot* ssp. *tetraphyllus* have been used for the development of YVMV disease resistant okra cultivars such as Punjab-7, Punjab Padmini, Parbhani Kranti, Arka Abhay and Arka Anamika. Presently, all these varieties have become susceptible, thus necessitating a constant search and transfer of newer sources/accessions of YVMV disease resistance. Several attempts have been made to study the inheritance of YVMV disease by number of research workers since 1962. All the reports are contradictory to each other and there is no definite report providing information for inheritance of resistance to YVMV disease. In India, the first attempt to understand the nature of inheritance was made by Singh *et al.* (1962) and they found that two recessive alleles at two loci conferred resistance in inter-varietal crosses of okra. Later, Thakur (1976) implicated two complementary genes to govern the resistance to YVMV under natural epiphytotic conditions. Dhillon (1978) agreed with these results and further revealed that the additive component of variance was predominant compared with dominant gene effects. They

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also concluded that the genes governing YVMV resistance were influenced by environmental conditions and were temperature sensitive. Thus, there might be a possibility of polygenic control. Sharma *et al.* (1987) also found that temperature has significant effect on the incidence of YVMV in okra. Arumugam and Muthukrishnan (1980) and Jambhale and Nerkar (1981) revealed that the resistance to YVMV was controlled by a single dominant gene. But Sharma and Dhillon (1983) suggested that there are two complementary genes governing the resistance to YVMV. Vashisht *et al.* (2001) revealed that there is complex genetic control of resistance to YVMV through higher order interactions and they reported that the inheritance of YVMV was governed by epistatic gene action. Dhankhar *et al.* (2005) again confused the situation as they reported that the inheritance of resistance to YVMV is under the control of two complementary genes following Mendelian segregation. Therefore, the present research work was planned to investigate the inheritance of YVMV disease resistance in okra.

MATERIALS AND METHODS

The plant material for the research experiment consisted of two wild species of okra namely *A. moschatus* and *A. tuberculatus* along with cultivated okra species *A. esculentus* cultivar Punjab Padmini. Both the wild species were used as the YVMV resistant source (donor) and each one is crossed with susceptible okra cultivar Punjab Padmini. To generate interspecific F_1 hybrids separately with both wild species, seeds of all the three species including two wild and one cultivated were sown during October, 2016. The October was chosen as the time for seed sowing as both the wild species flowered from November to February under our conditions. On the other hand, cultivated okra is highly sensitive to low temperature. Therefore, to get the synchronization of flowering period for both the wild species and cultivated okra species for crossing purpose, staggered sowing for cv. Punjab Padmini was done under polyhouse conditions at an interval of one month from September to December. For crossing purpose, wild species *A. moschatus* and *A. tuberculatus* were used as male parents and *A. esculentus* cv. Punjab Padmini was used as female parent.

Crosses with each of the wild species namely *A. moschatus* and *A. tuberculatus* were attempted with Punjab Padmini to generate F_1 hybrid seed during 2016 under the polyhouse conditions. The seed of interspecific F_1 hybrids were obtained in March, 2017. During the same period the self seed for all the three parents involved in the development of crosses were also produced. In May 2017, F_1 seed for both the hybrids were grown to get the interspecific F_1 plants and further utilized to produce BC_1F_1 generation for cross *A. esculentus* $\times$ *A. moschatus* and BC_1F_2 for cross *A. esculentus* $\times$ *A. tuberculatus*.

The data for total number of pollinations made, number of healthy fruits produced on the female parent, number of seeds per fruit (range) and number of seeds get germinated were recorded to work out the fruit setting per

cent, crossability index and per cent seed germination for interspecific hybrids.

During June 2018, sowing of seed for all the generations generated through the use of wild species *A. moschatus* and *A. tuberculatus* was done as set-I and set-II. Set-I consisted of parents namely *A. moschatus* and Punjab Padmini, their interspecific F_1 hybrid and BC_1F_1 generation. Similarly, set-II comprised parents *A. tuberculatus* and Punjab Padmini their F_1 hybrid, BC_1F_1 and BC_1F_2 generation. Row to row and plant to plant spacing for the plant material was kept as 60×45 cm. For set-I and set-II data for YVMV disease susceptible and resistant plants were recorded 90 days after sowing. Highly susceptible okra cultivar Pusa Sawani was planted as every 7th row of the experiment and border row of the experimental plot. This material was kept unsprayed to maintain the whitefly population for the spread of YVMV disease under natural epiphytotic conditions. All the recommended cultural practices for okra crop were followed as per the package of practices of Punjab Agricultural University, Ludhiana. The numbers of YVMV disease resistant and susceptible plants were recorded in the segregating generations of both the crosses at 90 days after sowing.

The observed data was classified according to the expected groups and the deviations of the observed numbers from the calculated numbers were determined. The Chi-square value was computed, and the corresponding probability value obtained.

The Chi-square (X^2) test for goodness of fit was made by subjecting the data to the following formula:

$$x^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

O_i = Observed value, E_i = Expected value

RESULTS AND DISCUSSION

The two different sets of crosses were made by crossing *A. esculentus* cv. Punjab Padmini with *A. moschatus* and *A. tuberculatus*. Data for crossability index (%age) and germination %age for set-I (*A. esculentus* cv. Punjab Padmini, *A. moschatus* and their inter-specific F_1 hybrid) and set-II of *A. esculentus* cv. Punjab Padmini, *A. tuberculatus* and their inter-specific F_1 hybrid has been presented in Table 1. The results of experiment of set-I indicated that the wild specie *A. moschatus* is compatible to generate the crosses with Punjab Padmini. Fifty flowers Punjab Padmini were selfed, out of which 48 produced healthy fruits. The percent fruit set for *A. esculentus* cv. Punjab Padmini was 96%. In wild parent *A. moschatus* thirty flowers were selfed, out of which 27 flowers set fruits and fruit setting percentage was 90%. Whereas, for inter-specific F_1 hybrid involving *A. esculentus* cv. Punjab Padmini $\times$ *A. moschatus* out of 184 crossed flowers, only 84 actually produced fruits, thus marking the fruit setting percentage to be 45.7%. Joseph *et al.* (2013) reported 47.06 % fruit setting for interspecific F_1 hybrid involving *A. esculentus* $\times$ *A. moschatus* parents. In the present study it was observed that the percentage of

Table 1 Crossability index (%age) and germination (%age) for interspecific crosses *A. esculentus* cv. Punjab Padmini×*A. moschatus* and *A. esculentus* cv. Punjab Padmini×*A. tuberculatus*

	Parents/Crosses	No. of flowers selfed/crossed	Fruit set (number)	Fruit set %age	Crossability index of fruits (%)	Number of seeds/fruit	100 seed weight (g)	Seed germination % age
Set I	<i>A. esculentus</i> cv. Punjab Padmini	50	48	96	-	26-39	5.97	97.00
	<i>A. moschatus</i>	30	27	90	-	64-83	1.57	35.00
	<i>A. esculentus</i> cv. Punjab Padmini × <i>A. moschatus</i>	184	84	45.7	49.14	0-6	3.02	20.5
Set II	<i>A. esculentus</i> cv. Punjab Padmini	50	48	96	-	26-39	5.97	97.00
	<i>A. tuberculatus</i>	25	24	95	-	31-39	1.43	43.00
	<i>A. esculentus</i> cv. Punjab Padmini × <i>A. tuberculatus</i>	111	63	56.76	35.22	9-17	5.10	76.0

fruit setting is less in interspecific F_1 hybrid as compared to its parents. Prabu and Warade (2013) also reported that the fruit setting percentage of interspecific F_1 hybrid is less as compared to its parents. The number of seeds per fruit in *A. esculentus* cv. Punjab Padmini was ranged from 26-39 seeds. In *A. moschatus* the number of seeds per fruit was ranged from 64-83 seeds.

The inter-specific F_1 hybrid *A. esculentus* cv. Punjab Padmini×*A. moschatus* had 0-6 number of seeds per fruit. The 100 seed weight of parent *A. esculentus* cv. Punjab Padmini was 5.97 g, while for the wild parent *A. moschatus* it was 1.57 g. The inter-specific F_1 hybrid *A. esculentus* cv. Punjab Padmini×*A. moschatus* had 3.02 g of 100 seed weight. In this cross some shrivelled and non-viable seeds were also reported which indicates involvement of post-zygotic sterility during inter-specific hybridization. Similar results were obtained by Prabu and Warade (2013) and Sheela (1986). Among the parents, the higher number of seeds per fruit observed in parent *A. moschatus* was due to the lower 100 seed weight, while the lowest number of seeds per fruit was observed in parent *A. esculentus* cv. Punjab Padmini due to higher 100 seed weight. In the interspecific hybrid the 100 seed weight was intermediate (3.02g) between both the parents indicating the hybrid nature of the F_1 plant. The germination percentage of parent *A. esculentus* cv. Punjab Padmini was 97%, while the germination percentage for wild parent *A. moschatus* was 35%. The inter-specific F_1 hybrid *A. esculentus* cv. Punjab Padmini×*A. moschatus* had 20.5% germination percentage, which was lower than both of the parents.

The results of experiment of set-II exhibited that the wild specie *A. tuberculatus* is compatible to generate the crosses with *A. esculentus* cv. Punjab Padmini. Fifty flowers were selfed in parent *A. esculentus* cv. Punjab Padmini out of which 48 were set fruits. The percentage of fruit set in *A. esculentus* cv. Punjab Padmini was 96%. In wild parent *A. tuberculatus* twenty five flowers were selfed, out of which twenty four flowers set fruits and fruit setting percentage was 96%. In their inter-specific F_1 hybrid *A. esculentus* cv. Punjab Padmini×*A. tuberculatus*, 111 number of flowers

were crossed, out of which 63 flowers set the fruit resulted in the 56.8% of fruit setting. The fruit setting percentage of the F_1 hybrid was lower than both of the parents. The crossability index of *A. esculentus* cv. Punjab Padmini×*A. tuberculatus* was 59.17%. The number of seeds per fruit in *A. esculentus* cv. Punjab Padmini was ranged from 26-39 seeds. In species *A. tuberculatus* the number of seeds per fruits was ranged from 31-39 seeds. The inter-specific F_1 hybrid *A. esculentus* cv. Punjab Padmini×*A. tuberculatus* had 9-17 number of seeds per fruit. The 100 seed weight of parent *A. esculentus* cv. Punjab Padmini was 5.97 g, while for the wild parent

A. tuberculatus it was 1.43g. The inter-specific F_1 hybrid *A. esculentus* cv. Punjab Padmini×*A. tuberculatus* had 5.10g of 100 seed weight. Similar results were obtained by Prabu and Warade (2013). Among the parents, the higher number of seeds per fruit observed in parent *A. tuberculatus* was due to the lower 100 seed weight, while the lowest number of seeds per fruit was observed in parent *A. esculentus* cv. Punjab Padmini due to higher 100 seed weight. In the interspecific hybrid, the 100 seed weight was intermediate (5.10g) between both the parents indicating the hybrid nature of the F_1 plant. The germination percentage of parent *A. esculentus* cv. Punjab Padmini was 97%, while the germination percentage for wild parent *A. moschatus* was 43%. The inter-specific F_1 hybrid *A. esculentus* cv. Punjab Padmini×*A. tuberculatus* had 76% germination percentage, which was intermediate between both of the parents indicating the hybrid nature of the F_1 plant. Kuwada (1964) also reported 74% seed germination in cross combination involving *A. esculentus*×*A. tuberculatus*.

The resistant parent *A. moschatus* and *A. tuberculatus* and susceptible parent *A. esculentus* cv. Punjab Padmini were crossed to study the inheritance pattern of YVMV resistance. The Parents, F_1 and backcrosses of both the parents were screened for YVMV reaction. The plants showing disease symptoms up to 90 days of sowing were considered as susceptible, whereas the plants with no symptoms of disease upto 90 days of sowing were considered as resistant. The segregation pattern of YVMV resistance of inter-specific F_1 hybrid of *A. esculentus* cv. Punjab Padmini×*A. moschatus*

Table 2 Frequency of segregants showing resistance/ susceptible reaction to YVMV using wild species *A. moschatus* at 90 days of sowing

Generation	Resistant	Susceptible	Total	Ratio	χ^2	p
F ₁ (<i>A. esculentus</i> cv. Punjab Padmini × <i>A. moschatus</i>)	34	-	34	-	-	-
BC ₁ F ₁ (<i>A. esculentus</i> cv. Punjab Padmini / <i>A. moschatus</i> / <i>A. esculentus</i> cv. Punjab Padmini)	32	28	60	1:1	0.27	0.60

Table 3 Frequency of segregants showing resistance/ susceptible reaction to YVMV at 90 days of sowing for donor *A. tuberculatus*

Generation	Resistant	Susceptible	Total	Ratio	χ^2	p
F ₁ (<i>A. esculentus</i> cv. Punjab Padmini × <i>A. tuberculatus</i>)	-	30	30	-	-	-
BC ₁ F ₁ (<i>A. esculentus</i> cv. Punjab Padmini / <i>A. tuberculatus</i> / <i>A. esculentus</i> cv. Punjab Padmini)	-	55	55	-	-	-
BC ₁ F ₂ (<i>A. esculentus</i> cv. Punjab Padmini × <i>A. tuberculatus</i>)	20	67	87	3:1	0.19	0.67

is presented in Table 2 and segregation pattern of YVMV resistance of inter-specific F₁ hybrid of *A. esculentus* cv. Punjab Padmini × *A. tuberculatus* is presented in Table 3.

All the plants of *A. esculentus* cv. Punjab Padmini were found to be susceptible, whereas out of total 25 plants of *A. moschatus*, no plant was found to depict any symptoms of YVMV disease up to 90 days of sowing. All the inter-specific F₁ hybrid plants involving *A. esculentus* cv. Punjab Padmini × *A. moschatus* were found to be resistant. In the backcross generation (BC₁F₁) total number of plants were 60 and out of which 32 plants were found to be resistant and 28 plants were susceptible and showed a good fit to expected ratio of 1:1 (Resistant : Susceptible), indicating that YVMV disease resistance is controlled by single dominant gene.

In parent *A. esculentus* cv. Punjab Padmini all the plants were found to be susceptible were as in case of *A. tuberculatus*, all the plants were found to be resistant, no plant was found to depict any symptom of YVMV disease

upto 90 days of sowing. In F₁ and BC₁F₁ generation, all plants were susceptible, whereas in BC₁F₂ generation 20 plants were found to be resistant and 67 susceptible, thus, showing a good fit to expected ratio of 3:1 (Susceptible: Resistant) suggesting single recessive gene involved to control resistance for YVMV disease. Earlier, studies on the inheritance pattern have suggested varied inheritance patterns indicating the role of two complementary dominant genes in susceptible × susceptible (S × S) and susceptible × resistant (S × R) crosses, and two duplicate dominant genes in resistant × resistant (R × R) crosses (Pullaiah *et al.* 1998). However, in interspecific crosses between *A. manihot* and *A. tetraphyllus*, a single dominant gene is reported to control resistance for YVMV disease (Jambhale and Nerker 1981, Dutta 1984). On the other hand, resistance to YVMV in *A. manihot* subsp. *manihot* was reported to be controlled by two dominant genes (Sharma and Dhillon 1983).

Thus the present investigation provided the understanding of YVMV disease inheritance and will serve as a step toward the mapping of genes conditioning resistance and their subsequent transfer/pyramiding in okra varieties and elite lines through marker aided selection.

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