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# DISEASE RESISTANCE AND GENETIC VARIATION OF WILD RELATIVES OF OKRA (*Abelmoschus esculentus* L.)

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## ABSTRACT

**Yellow vein mosaic virus (YVMV) is the major disease in okra cultivation, while powdery mildew is a fungal disease affecting okra when there is high humidity and low level of sunlight. No sources of resistance for these two diseases have been identified in the local germplasm collection. Three wild relatives of okra, three cultivated types and two introduced okra varieties were screened in pots in a farmer field for YVMV infection. The same germplasm was screened by bud grafting and insect transmission techniques in a greenhouse. Among wild relatives, *Abelmoschus angulosus* showed resistance to YVMV consistently under all experimental conditions. *Abelmoschus ficulneus* and *Abelmoschus moschatus* were susceptible to virus infection. Several lines of the variety Haritha was infected by graft inoculation although it showed resistance when the plants were grown in pots in the field. When the germplasm was screened for powdery mildew *Abelmoschus angulosus* showed complete resistance while, *A. moschatus* and *A. ficulneus* were identified to be less resistant. Genetic variation between the accessions was also established by using RAPD markers.**

**KEYWORDS:** Okra Germplasm, Disease Resistance, Yellow Vein Mosaic Virus, Powdery mildew, RAPD.

## INTRODUCTION

Okra (*Abelmoschus esculentus* L.) is a major vegetable crop in Sri Lanka as well as in many Asian and African countries. It is popular mainly as a home garden crop and, due to wide adaptability okra is grown in many parts of the wet, intermediate and dry zones of the country. Fruits of okra are very rich in calcium (90 mg/100 g fresh wt.) (Markose and Peter, 1990) and provide a valuable supplementary nutrition in human diet. Among the production constrains affecting local okra cultivation several disease problems have been identified. Yellow vain mosaic virus (YVMV) disease is the most serious problem for okra cultivation, especially in the wet zone. Heavy incidence of the disease causes loss in marketable yield up to 50% – 94%, depending on the stage of crop growth at which the infection occurs (Sastri and Singh, 1974). This virus is spread by a vector (*Bemisia tabasi*). All locally grown recommended varieties of okra are susceptible to this disease except "Haritha"(Giritharan and Arulandhy, 1994, 1995).

Powdery mildew is another disease in okra caused by a fungal pathogen, *Erysiphe cichoracearum* (Anon, 2001). Although this disease has not been reported as a serious problem in okra cultivation in Sri Lanka, it may appear in significant scale when the environmental conditions are conducive to the spread of the disease such as warm temperature, high humidity and shade.

Infected leaves gradually turn completely yellow, die, and fall off thus, shortening of productive life of the plant and reducing the yield. To-date not a single source of resistance has been reported for powdery mildew.

Wild relatives of crops have been recognized as an important source of useful genes for breeding programs (Stalker, 1980). A number of characters present in crop wild relatives have been transferred to cultivated types through wide hybridization at the interspecific or intergeneric levels (Nomura and Makara, 1993). Three wild species related to okra namely, *Abelmoschus angulosus, A. ficulneus* and *A. moschatus* have been collected and conserved at the Plant Genetic Resources Center (PGRC). The extent of sexual compatibilities of these species with okra has been reported previously (Samarajeewa *et al.*, 1999), but no systematic evaluation of disease resistance in this germplasm has been made.

In the present study, we have screened three wild species together with five other okra cultivars for yellow vein mosaic virus and powdery mildew resistance. This report provides information on the existence of genetic resistance in wild relatives of *A. esculentus* for the two diseases and discuses the potential of transferring these two traits into cultivated okra *via* breeding. We also present data on the genetic differences of wild relatives and okra cultivars at DNA level.

## MATERIALS AND METHODS

### Plant materials

The *Abelmoschus* genotypes, MI-7, MI-5, Haritha, Pusa Sawani, Prabhani kranthi, *A. angulosus, A. ficulneus,* and *A. moschatus* were used in the experiments (table 1). Seeds were obtained from the Gene Bank at PGRC and grown in a greenhouse for seed multiplication under standard pollination conditions (Serge, 1991).

**Table 1. Germplasm used in the study.**

| *Species name* | *Common name* | *Status* |
|---|---|---|
| *A. angulosus* | --- | Wild |
| *B. ficulneus* | --- | Wild |
| *A. moschatus* | --- | Wild |
| *A. esculentus* | MI – 5 | Cultivated |
| *A. esculentus* | MI – 7 | Cultivated |
| *A. esculentus* | Haritha | Cultivated |
| *A. esculentus* | Prabani kranthi | Introduction |
| *A. esculentus* | Pusa Sawani | Introduction |

**Screening of *Abelmoschus* spp. for resistance to yellow vein mosaic virus**

The *Abelmoschus* germplasm was screened for YVMV infection using three different procedures *i.e.* in the field in pots, by graft inoculation and by vector transmission. Field screening was carried out in a farmer field at Beruwela in the Kalutara district where okra is cultivated extensively and the disease incidence is very high. The selected farmer field had established okra cultivation with the variety, Athupaha. The experiments were started when the plants were about two-months-old and catching YVMV infection. Two-week-old potted plants (table 1) were placed in between infected plants. Twenty plants from each genotype were kept in a CRD with 4 replicates. Four weeks later, presence and absence of YVMV disease symptoms were recorded for each plant. The experiment was conducted in two locations using the same cultivars, in one location from August – September and the other from October – November, 2003.

Graft inoculation was carried out in a planthouse at PGRC. Virus infected materials necessary for inoculations were maintained on infected plants of cultivar, Athupaha collected from the farmer field in Beruwela. Wedge graft method was used. Twenty one-month-old plants from each selected genotype were graft inoculated using about 2 cm long scion obtained from YVMV infected Athupaha plants. Four weeks after grafting, the plants were observed for disease symptoms, and development of yellowing in veins was recorded.

Vector transmission experiment was also done in the greenhouse. Fifteen plants from each genotype (table 1) were used for this experiment. First, 20 YVMV infected potted plants of cultivar Athupaha were maintained in the greenhouse prior to the release of whiteflies. Whiteflies were reared by introducing 200 insects collected from field to ten one-month-old eggplants (var. SM 130) maintained in a cage (170 Lx 76 W x 77 H cm) with an insect proof net. The whiteflies were released to the planthouse one month later and when the okra plants were three-weeks-old. The YVMV disease symptoms were observed four weeks after releasing the insects. Observations were made every other day for two weeks.

**Screening for powdery mildew resistance**

**Microscopic examination**

First, for confirmation of the fungus, mature okra leaves of var. MI-7 growing in greenhouse in November 2002, which had white spots on the leaf surface were taken for microscopic examination. The spots were scratched with a needle and transferred on to a microscopic slide for observation under a light microscope. Presence of conidia was observed and photographed for

identification of the fungus. Plants with such infection provided inoculums for screening experiments.

**Screening of genotypes**

The inoculation experiment was carried out inside the planthouse. The same genotypes used to evaluate for YVMV resistance (table 1) were used. Forty potted plants, five from each genotype, were placed in a bed of greenhouse. Three such beds were maintained. When the plants were four-weeks-old, three 5 – 10 days old leaves after bud burst were brush-inoculated using spores obtained from the infected plants (Anon, 2001). One week after inoculation, the leaves were observed for disease symptoms. The observations were carried out for three weeks. The plants that showed no symptoms on inoculated leaves were considered as resistant.

**Molecular analysis of genotypes**

Total plant DNA was extracted from young fresh leaf samples of *Abelmoschus* genotypes (table 1) using "miniprep" method (Abeysinghe, 2000). However, the extraction buffer was prepared as described by Jose and Usaha (2000). The DNA was quantified by using Shimadzu UV-1201 spectrophotometer. Random Amplified Polymorphic DNA (RAPD) method was used to fingerprint the genotypes and DNA was amplified using PCR protocol described by Williams *et al.*, (1990) using Perkin-Elmer DNA thermocycler. Ten Operon random primers, OPA-09, OPA-10, OPA-16, OPC-02, OPC-10, OPC-19, OPD-09, OPK-05, OPK-07 and OPK-15 were used for PCR amplification. Amplified DNA sample were electrophoresed on 1.4% agarose gels, and then the gels were stained with ethidium bromide and observed under UV light. Gel pictures were taken using Gel Doc EQ gel documentation system, Quantity 1 package. Data analysis was done based on SPSS (Version 10) computer software program. Jacard matrix was constructed using present and absent of DNA bands and cluster analysis was based on average linkage method.

## RESULTS AND DISCUSSION

**Screening of *Abelmoschus* germplasm for YVMV disease resistance**

In the field experiments the potted plants were used. They were not grown in the farmer field since it is uneconomical to the farmer. Number of plants that did not show typical virus infection was counted. The data of both locations were combined and presented in table as a percentage (table 2).

**Table 2. Number of plants that did not show virus infection under different virus transfer methods.**

| *Genotype* | *Percentage* | | |
|---|---|---|---|
| | *Field screening* | *Grafting* | *Vector transmission* |
| MI - 7 | 15 | 0 | 13 |
| MI - 5 | 0 | 0 | 20 |
| Haritha | 100 | 90 | 100 |
| Pusa Sawani | 90 | 10 | 46 |
| Prabhani kranthi | 60 | 10 | 40 |
| *A. angulosus* | 100 | 100 | 100 |
| *A. ficulneus* | 15 | 0 | 46 |
| *A. moschatus* | 70 | 65 | 33 |

According to the results from field screening, MI-7, MI-5 and *A. ficulneus* were highly susceptible while *A. moschatus,* Prabhani kranthi and Pusa Sawani were partially resistant to the disease. Haritha and *A. angulosus* were resistant to the disease. When graft inoculated, MI-7, MI-5, Prabhani kranthi, Pusa Sawani and *A. ficulneus* were highly susceptible to the disease while *A. moschatus* showed partial resistance, and *A. angulosus* showed complete resistance. However, two plants of Haritha had infection. When the vector transmission technique was employed, MI-7, MI-5, Pusa Sawani, Prabhani kranthi, *A. ficulneus* and *A. moschatus* were susceptible to the disease while Haritha and *A. angulosis* were totally resistant.

Results of all the screening experiments suggest that *A. angulosus* was consistently resistant to YVMV disease. Haritha, which is considered to have field resistance, did not do so under all conditions suggesting that the variety is either not completely resistant or the original germplasm used in the study is not been genetically uniform with respect to YVMV resistance. Therefore, further studies are necessary to isolate resistant lines of Haritha and confirm resistance. The present study indicates that *A. angulosus* is the sole source of YVMV resistance available within the local germplasm tested so far. This study is completely based on visual observation of symptoms, and we have assumed that the plants are resistant if they do not produce symptoms characteristic to the diseases. However, the possibility that the symptoms are not expressed despite virus colonization of tissues cannot be excluded, and serological or molecular detection may warrant further confirmation (Joyce, 2000). It is also worthwhile to note that our observations are based on a virus infecting the okra plants in the wet zone.

## Screening for powdery mildew resistance

### Microscopic examination of the fungus

Although powdery mildew infects a number of crop varieties including okra (Hamir, 2000) no report is available in Sri Lanka on the incidence or the epidemiology of the disease. Our observations confirmed that the fungus is producing typical conidia (figure 1) of powdery mildew (Anon, 2001).

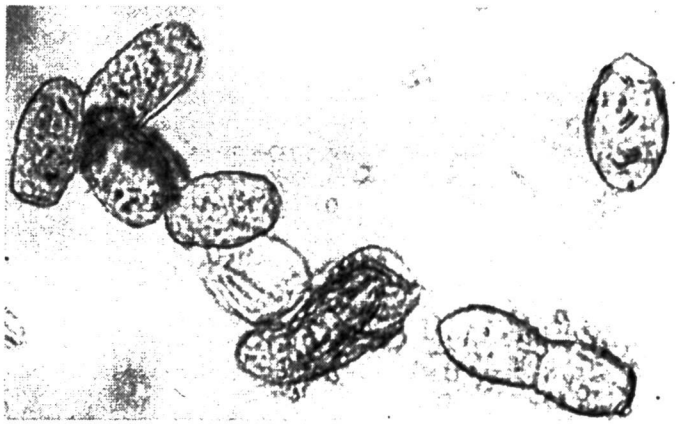

**Figure 1.Conidiospores of fungi *Erysiphe cichoracearum* (10 x 2. 5).**

### Screening of germplasm

**Table 3. Results of screening for powdery mildew resistance.**

| *Genotype* | *No. of plants inoculated* | *No. of resistant plants* | *Degree of resistance* |
|---|---|---|---|
| MI-7 | 15 | 00 | -- |
| MI-5 | 15 | 00 | -- |
| Haritha | 15 | 00 | -- |
| Pusa Sawani | 15 | 00 | -- |
| Prabhani kranthi | 15 | 00 | -- |
| *A. angulosus* | 15 | 15 | Highly resistant |
| *A. ficulneus* | 15 | 15 | Resistant |
| *A. moschatus* | 15 | 15 | Resistant |

Results of the experiment based on inoculation (table 3) revealed that all cultivars of okra tested are susceptible to the disease, while all the three wild species used in this study were resistant to the disease, *A. angulosus* being highly resistant to the disease while other two species showed resistance. Survey of literature indicated that the present study could be the first confirmed report on resistance to powdery mildew in okra germplasm.

## Molecular analysis of okra germplasm

The ten primers used for the analysis gave rise to polymorphic bands. The PCR products generated with primer OPA 7 is shown in figure 2.

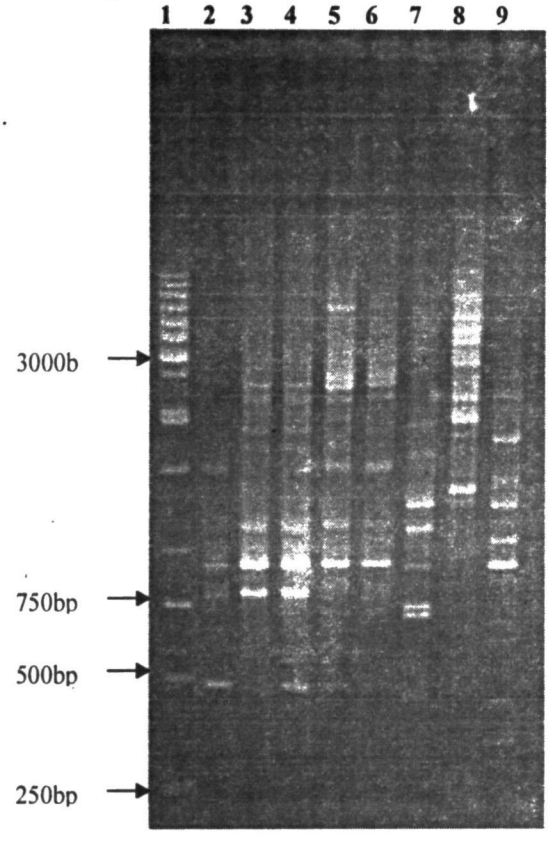


**Figure 2. DNA of okra germplasm amplified with primer OPK-07. (1. 1kb DNA Ladder; 2. MI-7; 3. MI-5; 4. Haritha; 5. Pusa Sawani; 6. Prabhani kranthi; 7. *A. angulosus;* 8. *A. ficulneus;* 9. *A. moschatus*).**

A total of 130 bands were recorded for all eight genotypes with OPA-09, OPA-10, OPA-16, OPC-02, OPC-10, OPC-19, OPD-09, OPK-05, OPK-07 and OPK-15 primers. Number of bands produced by each primer is given in table 4.

A high level of polymorphism in okra germplasm was observed with ten primers. This may be due to the diverse nature of germplasm used in the analysis. As expected all the cultivated varieties of *A. esculentus* formed one cluster (figure 3). Of this, the two introduced varieties, Pusa sawami and Prabani kranthi, formed a sub cluster suggesting that they are originating from genetically different background. Formation of a sub group by *A. ficulneus* and *A. angulosus* suggests that they are genetically more related to each other than with *A. moschatus*. Our previous crossability studies of wild species with

okra have indicated similar observation where *A. angulosus* and *A. ficulneus* crossed with okra more easily than with *A. moschatus* (Samarajeewa, 2001).

**Table 4. Classification of banding patterns based on the primers.**

| Primer | *Sequence* | *Polymorphic bands* | | *Monomorphic bands* | *Total no. of bands* |
|---|---|---|---|---|---|
| | | *Shared* | *Unique* | | |
| OPA-09 | 5'GGGTAACGCC3' | 06 | 00 | 03 | 09 |
| OPA-10 | 5'GTGATCGCAG3' | 09 | 01 | 02 | 12 |
| OPA-16 | 5'AGCCAGCGAA3' | 05 | 00 | 06 | 11 |
| OPC-02 | 5'GTGAGGCGTC3' | 11 | 00 | 05 | 16 |
| OPC-10 | 5'TGTCTGGGTG3' | 09 | 00 | 04 | 13 |
| OPC-19 | 5'GTTGCCAGCC3' | 08 | 01 | 02 | 11 |
| OPD-09 | 5'CTCTGGACAC3' | 07 | 00 | 05 | 12 |
| OPK-05 | 5'TCTGTCGAGG3' | 12 | 00 | 03 | 15 |
| OPK-07 | 5'AGCGAGCAAG3' | 14 | 04 | 00 | 18 |
| OPK-15 | 5'CTCCTGCCAA3' | 07 | 02 | 04 | 13 |
| Total | | 88 | 08 | 34 | 130 |

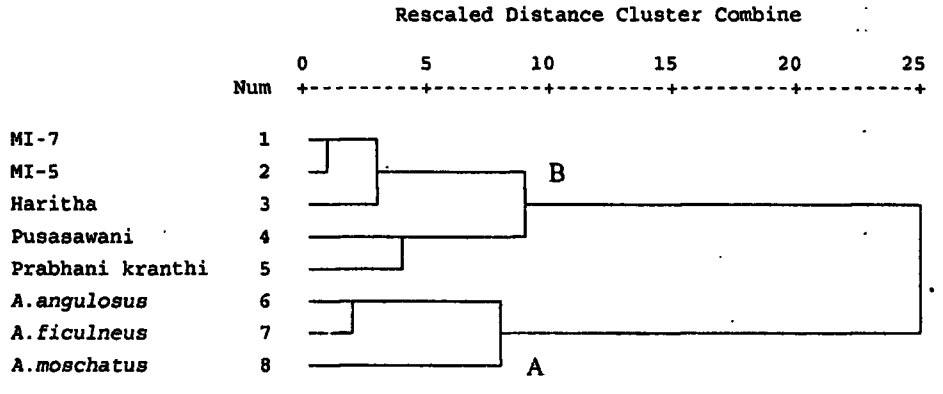


**Figure 3. Dendogram of eight *Abelmoschus* accessions based on RAPD markers subjected to cluster analysis using average linkage between groups.**

**Wild *Abelmoschus* species as a potential source for powdery mildew and YVMV resistance**

In order to utilize the wild relatives of okra that are resistant to the tested diseases in the crop improvement programme, fertile progenies should able to be produced by crosses between the wild *Abelmoschus* species and okra. Our DNA analysis by RAPD indicated that the wild species are related distantly to locally cultivated okra. Thus the transferring of the characters to okra can possibly be made only through wide hybridization or any other biotechnological means. Our previous crossability studies suggested that *A. ficulneus* as a male parent can be crossed with okra to produce fertile progenies. Therefore, powdery mildew resistance in this genotype will enable to be transferred to okra without much sterility problems. Although *A. angulosus* was less compatible it was able to produce $F_1$ hybrids with okra, which formed fertile seeds occasionally. Therefore, *A. angulosus* may also serve as a potential source with resistance trait to improve okra against both YVMV and powdery mildew diseases. A comprehensive wide hybridization program is needed to incorporate resistant traits into okra successfully.

## CONCLUSION

Among wild relatives of okra, *A. angulosus* showed complete resistance to YVMV and powdery mildew diseases as confirmed by all the screening methods. *A. ficulneus* and *A. moschatus* accompany a high degree of resistance only to powdery mildew These germplasm can be potential genetic resources in breeding of okra for YVMV and powdery mildew disease resistance. Further studies are needed to recover any resistant line from the variety, Haritha.

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