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Fusarium Wilt of Muskmelon: Breeding Approaches for Resistance

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SUMMARY

Muskmelon is a subtropical cucurbitaceous crop which belongs to family Cucurbitaceae. It serves key roles in the field of plant molecular biology and plant physiology as an excellent model plant for investigating the phenomenon of sex determination and ripening processes. Varieties of melons having good yield and quality traits; and tolerance to abiotic stress and diseases have been developed. Plant-breeding methods-conventional/molecular breeding and transgenic technology are the only tools left, with which we can develop varieties resistant to detrimental disease like Fusarium wilt. The development of plant biotechnological tool offers the prospect to rapid crop improvement. In this article, we have discussed some breeding approaches for resistance to the wilt disease.

INTRODUCTION

Muskmelon (*Cucumis melo* L.) is cultivated on 1.2 million ha area with 27.34 million MT production and 26.14 MT/ha productivity on the globe as of 2018. China is the largest producer with share followed by Turkey, Iran, Egypt and India (FAO, 2020). In India, it is cultivated on 54 thousand ha with 1231 thousand MT production (NHB, 2019). It is extensively cultivated in hot and dry areas of Uttar Pradesh, Punjab, Rajasthan, Madhya Pradesh, Bihar and Karnataka. Riverbeds of Yamuna, Ganga, Narmada, Kavery, Krishna and Godavari are well known for muskmelon cultivation in India. Breeding for disease resistance has been one of the most important objectives in muskmelon from the last few decades. Muskmelon is susceptible to a number of fungal diseases which affect its yield and quality. Among these, the most devastating disease is Fusarium wilt which is caused by a soil borne pathogen, *Fusarium oxysporum* Schlechtend: Fr. f. sp. *melonis* W C Snyder & H N Hansen (FOM). FOM is prevalent in temperate and tropical regions and is a problem worldwide. The pathogen was first reported as fundamental agent of muskmelon wilt in Rajasthan (Mathur and Shekhawat, 1992) and later on from other parts of North India. The control of fusarium wilt diseases with fungicides is an insignificant method and has several disadvantages, particularly the cost of fungicides and their residual effects. Therefore, it is imperative to develop varieties that are resistant to diseases.

Pathogen races and resistance genes of host

FOM can survive in the soil for extended periods of time as chlamydospores, and is capable of colonising crop residues and the roots of most crops grown in rotation with melon. The only effective control is the use of resistant cultivars. Four races of FOM have been identified, namely 0, 1, 2 and 1.2 (Risser *et al*., 1976). Two resistance genes (Fom-1 and Fom-2) have been identified in melons. Fom-1 confers resistance to FOM races 0 and 2, and Fom-2 confers resistance to races 0 and 1. These two genes are extensively used in breeding programmes, which can be assisted by marker assisted selection using markers linked to these resistance genes. However, no genes have been identified that confer resistance to race 1.2.

Resistant cultivar development methods

The success of breeding programs for wilt resistance in melon is influenced by many factors, including: the nature of the pathogen and diversity of virulence in the population; availability, diversity and type of genetic resistance; or the effectiveness of methods and tools, such as molecular markers, used for assessing plant resistance.

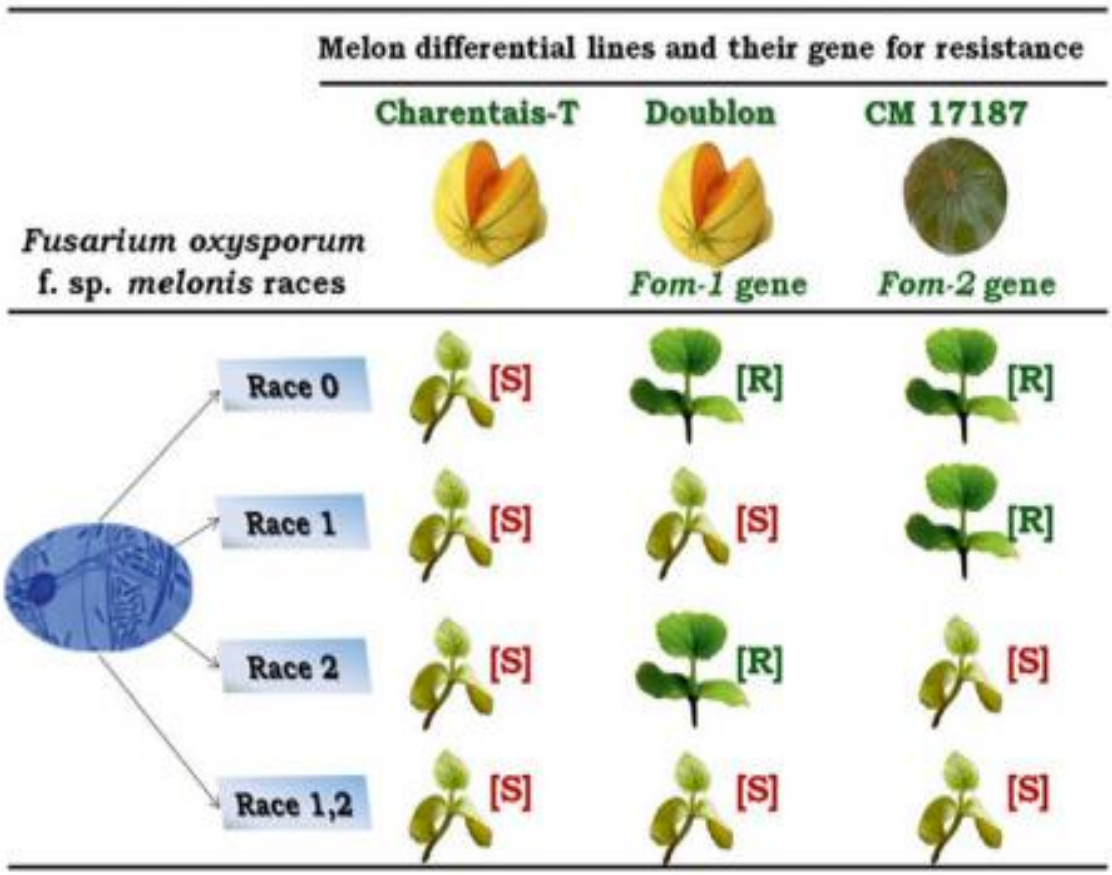

Fig: Classification of *Fusarium oxysporum* f. sp. *melonis* races based on Risser *et al*. (1976), S susceptible, R Resistance (Source: Oumouloud, 2008)

In vitro methods

In vitro techniques became widely spread during the 20th century, and their potential use in plant breeding was quickly realized and adapted. *In vitro* techniques for crop improvement first consisted of micropropagation and plant regeneration, and then *in vitro* methods were also found to be useful for eliminating disease and selecting for resistant cells or explants. *In vitro* tissue culture techniques have been used to generate genetic changes via somaclonal variations (by organogenesis or somatic embryogenesis) that can be used for breeding purposes. Also, *in vitro* selection using specific chemical compounds and pathogens is useful aspect of tissue culture. Researchers now use fungal culture filtrates or toxins to investigate the response of susceptible and resistant genotypes of different plant species or cultivars to disease factors. In comparison with field screening and other biotechnological methods, selection techniques are more cost and labour effective, and do not require large experimental fields. More recently, *in vitro* techniques have been combined with mutatagenesis for generating genetic variation, including novel disease-resistant mutants. Treatment of *in vitro* explants with physical or chemical mutagens may increase the frequency of genetic variation considerably and *in vitro* mutation frequencies are much higher than for somaclonal variation. *In vitro* selection using FOM filtrates can be effectively used for the selection of melon. This method can also be applied for the selection of various mutants, which are disease resistant. Steps in a method to

screen for resistance in melon, from both irradiated and non-irradiated explants by using FOM culture filtrates of various races in *in vitro* conditions is as follows-

- Preparation of fungal culture filtrate and selection medium
- Preparation of *in vitro* plant material
- *In vitro* mutagenic treatment
- *In vitro* screening and evaluation
- Validation and *in vivo* establishment

If successfully applied, this methodology can lead to the identification of new melon cultivars resistant to FOM within a relatively short period of time.

Molecular approaches

To understand the flow of resistance gene(s) in population, a rigid genetic study along with robust phenotyping must be done. A durable resistance can be developed by combing major and minor gene into one background. Identification of such resistant gene(s) in short period may help breeder to speed-up breeding programme with molecular approach.

Resistance (R) Gene Linked Markers and Marker Assisted Selection

Identification of molecular markers linked with resistant genes served a valuable tool during selection of resistant genotypes. It helps in screening during period of disease escape or uncertain symptom expression under artificial inoculation tests. Developed polymorphic markers simplify the way to detect resistant genes from different species. Before its introgression into commercial variety, a complete characterization of these resistance genes is necessary. Further resistance conferring genes can be transferred through biotechnological approaches. Method for marker assisted backcrossing for introgression of resistant gene is mentioned as follows-

- Plant material- Several hybrids are developed using a resistant line and a susceptible but popular variety. These hybrid genotypes are employed for validation of functional markers.
- *Fusarium* inoculation- Isolates of FOM are cultured and mass production is obtained. The genotypes are inoculated with spore suspension. A non inoculated control is maintained per accession.
- DNA extraction and cloning of Leucine Rich Repeat (LLR) domain of R gene- The Genomic DNA is extracted and its quality and quantity is evaluated through spectrophotometry and electrophoresis. According to available information in NCBI (National Centre for Biotechnology Information), primer pairs are designed and consecutively PCR amplification is performed. The purified PCR product is then cloned into suitable vector (plasmid). The nucleotide sequences from the resistant and susceptible melon germplasms as well as protein sequences were analyzed by BioEdit program. Based on the difference in sequences of resistant and susceptible genotypes, allele specific primers are designed to amplify resistant and susceptible alleles, respectively. The genotypes are then screened using these primer pairs.
- Phenotyping and genotyping of backcross generations- A donor parent with the genotype RR is crossed with a recurrent parent with the genotype rr and their F_1s are backcrossed to recurrent parents to produce BC_1F_1 generation. Using artificial inoculation, resistant genotypes are detected. To verify the resistant plants via molecular markers, the survived plants after inoculation are genotyped by the developed functional markers. Verified resistant plants are crossed again with recurrent parents to produce BC_2F_1. In this generation also, the resistant plants are detected using both artificial inoculation and molecular markers.

- Markers thus validated can be used for phenotypic selection from homogenous BC_3 population and selfed genotypes obtained from selected plants might give resistant isogenic plants.

CONCLUSION

Since preventive measure for FOM wilt is not effective all the time, breeding for resistance is one of the most important breeding objectives. Since melon fusarium wilt is a complex disease, criteria for determining the virulence of the pathogen needs to be worked out. Different NBS-LRR (Nucleotide binding site-leucine rich repeat) class of resistance (R) gene should be searched throughout melon accessions to overcome this problem. A role of various biological agents needs to be studied further. There are many unknown aspects to the study of fusarium wilt in melon. It is expected that additional advances will be made, building on knowledge of the characteristics of the pathogen and the physiology and genetics of plant resistance leading to development of wilt resistant high yielding varieties.

REFRENCES

Risser, G., Banihashimi, Z. & Davis, D. W. (1976). A proposed nomenclature of Fusarium oxysporum f. sp. melonis races and resistance genes in Cucumis melo. Phytopathology 66: 1105-1106.

Mathur, K. & Shekhawat, K. S. (1992). Varietal screening of muskmelon against wilt Indian Journal of Mycology and Plant Pathology 22, 78-79.

FAO (2020). Production and Production Indices Data. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy. http://faostat.fao.org/faostat/collections?subset=agriculture

Sousaraei, N., Ramshini, H., Lotfi, M., & Sharzei, A. (2018). Marker assisted backcrossing for introgression of Fusarium wilt resistance gene into melon. Euphytica, 214(1), 7.

Oumouloud, A., Arnedo-Andres, M. S., Gonzalez-Torres, R., & Alvarez, J. M. (2008). Development of molecular markers linked to the Fom-1 locus for resistance to Fusarium race 2 in melon. Euphytica, 164(2), 347-356.

Beckman, C. H., & Roberts, E. M. (1995). On the nature and genetic basis for resistance and tolerance to fungal wilt diseases of plants. Advances in Botanical Research, 21, 36-77.