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# Molecular Identification of *I-2* Gene, a *Fusarium* Wilt Resistant Marker in Selected Varieties of Tomato

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## Abstract

*Fusarium wilt is a well-known disease of tomatoes all over the world. The use of resistant varieties is the most efficient approach to control this disease. The current study was carried out to investigate the presence of marker gene(s) resistant to Fusarium wilt in tomato germplasm. This research subsequently sets out which one of the resistant varieties is suitable to be used to contain the disease. Thirteen varieties namely Pusa ruby, Pant bahar, Punjab chhahara, Arka alak, Arak abha, Ratan, CLN-2116-B, CLN-1767-238-2Y, CLSN-2123-A, CLN-1621-E, CLN-1621-T, CLN-1621-L, and CLN-2026-C were screened for the presence of I-2 resistance gene, a marker known to confer resistance to the race 2 of F. oxysporum f. sp. Lycopersici, using PCR-based molecular identification. Two varieties namely Arak abha and Ratan were tested positive for the presence of I-2 against race 2 of the pathogen.*

**Keywords:** *Fusarium oxysporum*, resistant gene, tomato germplasm

## Introduction

Tomato, scientifically known as '*Solanum lycopersicum',* belongs to Solanaceae family and is said to be the most essential fruit vegetable in the world [1-4]. The cultivation of tomatoes is a source of employment for many people, significantly farmers, and it remains a major source of income for them. Moreover, it is the second most important agricultural crop throughout the world after potato, as well as a valuable crop in the local market. Being an essential part of food and diet, it also increases the source of revenue for urban and rural populations [5, 6]. Calories, vitamins, and

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minerals involved in the improvement of the nutrient quality of stews and salads are present in tomato fruit [2, 7]. It is widely used in a variety of dishes (foods) made in different hotels and homes all over the world [7, 8].

Tomatoes are highly effective in curing various injuries owing to their antibiotic properties [9]. They contain a high amount of vitamin A and C in addition to lycopene, a food constituent, known to decrease the occurrence of prostate cancer, heart, and age along with other interrelated diseases [10]. The production of tomatoes (both quality and quantity) is affected by many diseases [11]. Due to the risk of climate change and an extensive spread of pathogens, improvement in crop production while circumventing the use of chemical pesticides is the main concern for the agricultural industry [12]. However, Fusarium wilt poses a serious threat to tomato production at all its growing stages [13].

*Fusarium* wilt is a well-known disease of tomatoes found in greenhouse and fields all over the world [14, 15]. This disease is caused by *Fusarium oxysporum f.* sp. *lycopersici*, a significant wilting pathogen found in tomatoes [3]. This disease causes around 80% reduction in tomato yield. There have been partial achievements to handle and control this disease. The emergence of new variants of pathogens and their growth is a continuous problem in this regard [16]. The pathogen has three physiological races (1, 2, and 3, referred to as r1, r2, and r3) distinguished by their specific pathogenicity on tester plants carrying dominant race-specific resistance genes [17, 18]. *Fusarium oxysporum* is a soil resident pathogen and infects plants through their roots. It attacks the tomato plants by entering their roots and emerges inside the plant through the cortex towards the stele [19].

A number of fungicides have been used to control the disease caused by Fusaria species [20, 21]. However, the use of chemical fungicides is associated with adverse effects on the environment including run off into fresh water reservoirs and executing non-target eco-friendly organisms [22]. The presence of a limited number of fungicides has a considerably smaller effect on the environment. The incidences of wilt diseases in tomatoes can be decreased by treating the seed with artificial fungicides. These fungicides are usually expensive and the net income provided to the growers is not economically sustainable, particularly in the developing regions of the world [23].

Thus, using resistant varieties is the best proven method to control the Fusarium head blight of tomato. Resistance to Fusarium head blight is of special importance, particularly considering the accumulation of Fusarium toxins in the grains, though breeding for root disease resistance has not been given much importance. Adopting integrated management of the pest coupled with breeding for resistance is, therefore, of utmost importance to contain the pathogen and its associated crop loss [24]. A pre-requisite for developing resistant varieties, however, is to identify the source of resistance along with the cloning of resistance genes [25]. DNA-based molecular markers have been commercially used in plant breeding programs since the early 1980s. This approach has been time- and labor-efficient for transferring useful traits into agronomical and desirable varieties and hybrid plants, including tomatoes [26]. Extensive research has been conducted to identify DNA-based molecular markers linked to disease resistance genes. To date, around 40 genes conferring resistance to tomato pathogens have been mapped onto the tomato genome. Markers linked to resistance genes may be useful for cloning and sequencing these genes. In tomatoes, several resistance genes have been sequenced so far, including *Cf 2*, *Cf 4, Cf 5, Cf 9, Pto, Mi, I2,* and *Sw-5* [27].

In the current study, genes for resistance to *F. oxysporum* race 1 were mapped onto short arms and those for resistance to race 2 were mapped onto the long arms of chromosome 11 [28]. Through high resolution mapping, it was demonstrated that *I-2* was positioned between the markers 6-16 and TG36 in a way that it was 0.23 cm from 6-16 and 1.3 cm from TG36 [29]. The DNA sequence of the *I-2* gene cluster is available in Gene Bank National Center for Biotechnology Information [30]. The *I-2* marker was used to screen its presence in 13 tomatoes germplasms. These were tested in the current study to be subsequently used in breeding for resistance programs against the *Fusarium* wilt disease of tomatoes.

## Materials and Method

### Plant Material

Thirteen tomatoes varieties, for instance, Pusa ruby, Pant bahar, Punjab chhahara, Arka alak, Arak abha, Rattan, CLN-2116-B, CLN-1767-238-2Y, CLSN-2123-A, CLN-1621-E, CLN-1621-T, CLN-1621-L, and CLN-2026-C from the National Agricultural Research Center Islamabad were used in

the current study for experimental purposes. These were grown in separate soil pots.

## DNA Extraction

Young leaves of three-week old fresh tomato plants were used to extract the DNA. After grinding the leaves with pestle and mortar in liquid nitrogen, DNA extraction solution was added to grind the samples. This method was implemented by using CTAB protocol [2% CTAB, 1.4M NaCl, 20mM EDTA, 1% polyvinylpyrrolidone (PVP), 100mM Tris HCl, pH 8.0, and 0.2% mercaptoethanol] [31]. Following the rounds of centrifugations and precipitation, DNA pellet was formed. It was subsequently washed in 75% ethanol before air drying at room temperature. The air-dried DNA pellet was re-suspended in 50µl of double distilled sterile water. For PCR, DNA sample was diluted 20 times to be used as a template [30].

## Primer

The following specific primers (for *I-2* gene), designed by Mohtar [30] for *Fusarium oxysporum*-resistance fragment, were used for PCR amplification. These primers served as molecular identifiers to select the germplasm/genotype, harboring the resistance *I-2* marker gene.

Forward primer 5’ CTGA AAA CTC TCC GTA TTT 3’

Reverse primer 3’ TAG AGG TTA GTG AGA AGC 5’

## Polymerase Chain Reaction

The amplification reaction volume of 24µl was used which contained 14µl $ddH_2O$, 0.5µl of forward primer, 0.5µl of reverse primer, 1.6µl of dNTPs, 0.5µl of Taq DNA polymerase, 2.5µl of Taq polymerase buffer, 3.4µl of $MgCl_2$, and 1µl of template DNA. The PCR protocol consisted of an initial denaturation step at 94˚C for 5 mins, followed by 35 cycles at 94˚C for 60 seconds, 55˚C for 60 seconds, 72˚C for 90 seconds, and the final extension step of 72˚C for 7 mins.

## Agarose Gel Electrophoresis

PCR products of 15.0µL were electrophoresed on 1% agarose gel stained with ethidium bromide and photographed under UV light using Gel Doc 2000 system (Alpha imager).

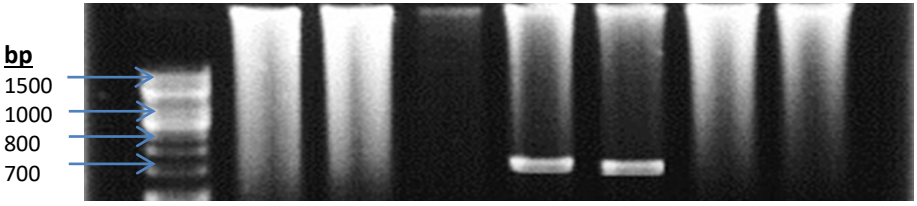
## Results

Thirteen Pakistani tomatoes germplasms were screened to detect the presence of *Fusarium oxysporum* resistance *I-2* gene via polymerase chain reaction-based detection. The size of fragment was 700 bp as reported by Mohtar [30]. Two varieties, namely Arak abha and Ratan, were detected positive for the resistance marker *I-2* gene. While, no band for *I-2* gene was detected in the rest of the 11 varieties (Table 1).

**Table 1.** Tomato genotypes were tested using the PCR method for the presence (+) and absence (-) of *I-2* gene, for resistance to *Fusarium oxysporum f. sp. lycopersici* race 2.

| S. No. | Name of variety | *I-2* Gene | S. No. | Name of Variety | *I-2* Gene |
|---|---|---|---|---|---|
| 1 | Pusa ruby | - | 8 | CLN-1767-238-2Y | - |
| 2 | Pant bahar | - | 9 | CLSN-2123-A | - |
| 3 | Punjab chhahara | - | 10 | CLN-1621-E | - |
| 4 | Arak abha | + | 11 | CLN-1621-T | - |
| 5 | Ratan | + | 12 | CLN-1621-L | - |
| 6 | Arka alak | - | 13 | CLN-2026-C | - |
| 7 | CLN-2116-B | - | | | |

**Figure 1.** The banding pattern of 13 tomato varieties has been depicted to exhibit the presence or absence of targeted fragments.




Note. M=Molecular Marker, 1=Pusa ruby, 2=Pant bahar, 3=Punjab chhahara, 4=Arak abha, 5=Ratan, 6=Arka alak, 7=CLN-2116-B, 8=CLN-1767-238-2Y, 9=CLSN-2123-A, 10=CLN-1621-E, 11=CLN-1621-T, 12=CLN-1621-L, 13=CLN-2026-C. Ladder marker on the left shows the approximate sizes of the bands in base pairs.

## Discussion

Developing tomato varieties resistant to *F. oxysporum* f. sp. *lycopersici* is both environmentally safe and one of the most durable approaches to manage *Fusarium* wilt. In breeding, bioassays are often required to assess the inheritance and efficiency of the introgressed gene(s) to perform resistance programs. Attaining resistance against an array of the same or different pathogens is often a sought after objective of any breeding for resistance program. This procedure often involves the introgression of heterogeneous genes, simultaneously resistant against two or more diseases [32]. Furthermore, subjecting plants to infection by several pathogens is burdensome and may even lead to the loss of essential breeding material. The scenario necessitates the development of reliable molecular markers as a rapid protocol to screen for resistance genes in the progeny lines.

In the current study, PCR-based analysis confirmed the presence of the molecular marker for *I-2* resistance gene against race 1 of *F. oxysporum* in 2 out of 13 genotypes, namely Arak abha and Ratan. Its detection implies that these varieties harbor the *I-2* gene and can be transferred to different varieties via crossing and selection procedures. Ideally, the bioassay-based resistance efficacy of the marker genes should have been evaluated for the *I-2* gene. However, due to resources and time limitations, the task remains in pending. It is significant to note that 600-bp fragment for the *I-2* gene has never been detected in a race 2 vulnerable genotype of tomatoes. Furthermore, since the primers specific to the *I-2* gene were designed from the coding region of the DNA sequence, there is a high possibility that the resistance conferred to the race 2 of *F. oxysporum* is conferred by the *I-2* protein, although immunological evidence is still required to validate the hypothesis for better results. Regardless, PCR-based method can be successfully incorporated in breeding for *I-2*-based resistant tomato germplasms.

Marker Assisted Selection (MAS) may prove helpful in screening the presence or absence of resistant genes in a more efficient way. MAS, in arrangement with conventional breeding, has become a well-organized method. The production of more resistant varieties would yield better crops and help to address the food protection problem facing the developing world. The current study is the first in Pakistan which has identified two varieties that have the genes for resistance to *Fusarium oxysporum.* It implies that these two varieties are the source of + gene, which could be

transferred to other varieties during the crossing and selection procedure. Furthermore, bioassay against and screening for more *F. oxysporum* resistance genes is needed to establish the distribution of *F. oxysporum* resistance allels.

## Conclusion

Out of the 13 tomato varieties tested molecularly, two varieties namely Arak abha and Ratan were tested positive for the presence of *I-2* resistance gene against race 2 of the *F. oxysporum* f. sp. *Lycopersici* pathogen. PCR analysis is a straight-forward and labor- and time-saving approach to screen for resistant genotypes. The approach should occupy a permanent place in screening for resistance protocols before the validation of the varieties at greenhouse and field levels via biological assays.

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