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REVIEW ARTICLE **OPEN ACCESS**

A Review on Role of Hevein Gene in Production of Fungal Resistant Tomato Plant

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ABSTRACT
Tomato (Lycopersicon esculentum) is the common name for a wine-like herb of Solanaceae family. To overcome the problem of diseases current genetic engineering and plant transformation techniques have been used to incorporate specific fungal resistance genes into plants. With some aspect of recombinant DNA technique to arrive at genetically engineered tomato plant with fungal resistant Hevein gene incorporated into them. Thereby, a tomato plant gained antifungal activity. In this review article, we described the role of Hevein gene in production of fungal Resistant tomato plant.
Keywords*: Hevein gene, Lycopersicon esculentum*

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INTRODUCTION

Tomato is technically a fruit not a vegetable but they belong to the vegetable garden. These warm-season perennials have a variety of cultivars which vary in size and shape from the tiny and sweet cherry style tomatoes to big juicy and meaty beefsteak tomatoes. Tomatoes are consumed either cooked or raw and are low in calories and an excellent source of Vitamin A and C. Uncooked tomatoes also provide vitamin E. Characteristically tomato plants are erect, branched, and aromatic and covered with glandular hairs. The base may become woody. A compound leaf has arrangements of leaflets or pinnae in two rows along the axis and is coarsely toothed on the edges. Yellow flowers borne in terminal inflorescences appearing extra-axillary or lateral. After flowering tomatoes require 50-60 days to reach fruit maturity. The fruit is botanically classed as a berry. Although the full size of the fleshy fruit, called berry, is attained in half that time, the later stage of maturity is marked by external color change with coinciding internal chemical changes [1-4].

VARIETIES

Tomatoes vary in size, taste and growth patterns. Some are determinate, they grow only to a certain height and they flower and bear fruits only within a certain period of time. Indeterminate varieties grow, flower and set fruit over the entire growing season. Some varieties are small red cherry, large red cherry, red pear, yellow pear, etc. which have large vines small fruits, lemon boy, jubilee and sunray with yellow and orange fruits and so on.

PROBLEM AND CARE

Tomatoes that grow in wet, humid conditions are susceptible to plant diseases and leaf diseases. Dark brown or blackish spots will appear, followed by yellowing or browning usually to those leaves on the lower branches. Remove infected leaves as soon as they are noticed to help reduce spread. Tomatoes are also susceptible to some insect problems with cutworms and a few other garden pests. Mulching will help to prevent weeds, reduce leaf diseases; it will also help the distribution of water and will generally make

the tomato plants stronger. Mulches such as leaves, grass clippings, compost, straw, can be spread around to enrich the soil once the plants are set.

DISEASES OF TOMATOES

1. *Damping off* (caused by fungi *Pythium, Rhizoctonia* or *Phytophthorathat*)*:* This is one of the worst diseases of tomato occurring in the nursery. Damping off of tomato occurs in two stages, i.e. the pre-emergence and the post emergence phase. In the pre-emergence phase the seedlings are killed just before they reach the soil surface. The young radical and the plumule are killed and there is complete rotting of the seedlings. The post-emergence phase is characterized by the infection of the young, juvenile tissues of the collar at the ground level. The infected tissues become soft and water soaked. The seedlings topple over or collapse [2].
2. *Early blight*(one form of tomato blight, is caused by a fungus, *Alternariasolani*)*:* This is a common disease of tomato occurring on the foliage at any stage of the growth. The fungus attacks the foliage causing characteristic leaf spots and blight. Early blight is first observed on the plant as small, black lesions mostly on the older foliage. Tissues surrounding the spots may turn yellow. If high temperature and humidity occur at this time, much of the foliage is killed. Lesions on the stems are similar to those on leaves, sometimes girdling the plant if they occur near the soil line. Transplants showing by the late blight fungus often die when set in the field. The fungus also infects the fruit, generally through the calyx or stem attachment. Lesions attain considerable size, usually involving nearly the entire fruit; concentric rings are also present on the fruit.
3. *Buck eye rot (*caused by the soil-borne fungus *Phytophthora parasitica*)*:* Fruit rot or buck eye rot is a serious disease in all the tomato growing areas. The disease causing the fruit to rot initially affects the fruits near the ground level. The pathogen does not affect the foliage and thus the disease is distinct from late blight. The disease appears as greyish green or brown water soaked spot that usually occurs where the fruit touches the soil. As the spot enlarges the surface of lesion assumes a pattern of concentric rings of narrow, dark brown and wide, light brown bands. When young green fruits are infected, they usually become mummified.
4. *Late Blight (*caused by fungus, (*Phytophthorainfestans*)*:* Late blight occurs when humid conditions coincide with mild temperatures for prolonged periods. If conditions are ideal for diseases development, disease development is rapid causing severe economic losses. Lesions produced on the leaves are at first irregular, rather large, greenish-black and water-soaked. These areas enlarge rapidly, becoming brown under humid conditions. The disease attacks the fruit as well as the leaves of the plant.
5. *Fusarium Wilt*(caused by *Fusariumoxysporum*sp. *lycopersici*.)*:*This is one of the worst tomato diseases of tomato occurring mostly in the nurseries. The first symptom of the disease is clearing of the veinlet and chlorosis of the leaves. The younger leaves may die in succession and the entire may wilt and die in a course of few days. In young plants, symptom consists of clearing of veinlet and dropping of petioles. At later stage, browning of vascular system occurs. Plant becomes stunted and dies.
6. *Septoria leaf spot*(caused by fungus *Septorialycopersici*)*:* The plant may be attacked at any stage of its growth. The disease is characterized by numerous, small, grey, circular leaf spots having dark border.
7. *Powdery Mildew (*caused by the fungus*Leveillulataurica*.)*:*The disease occurs severely during dry seasons. A white powdery coating of the fungal growth appears on the leaf surface. Infected leaves may be dwarfed, stiff and narrow. The fungus progressively attacks new leaves, spreading over leaf stems, twigs and even the fruit. Terminal growth of the affected shoot is stunted or killed. The fruit yield and size are reduced.
8. *Bacterial Wilt*(caused by the bacterium *Ralstonia solanacearum*, previously known as *Pseudomonas solanacearum):* This is one of the serious diseases of tomato crop. Relatively high soil moisture and soil temperature favor disease development. Characteristic symptoms of bacterial wilt are the rapid and complete wilting of normal grown up plants. Lower leaves may drop before wilting. Pathogen is mostly confined to vascular region; it may invade the cortex and pith causing yellow brown discoloration of tissues. An infected plant part when cut and immersed in clear water, a white streak of bacterial ooze is seen coming out from cut ends [9].
9. *Bacterial leaf spot*(caused by *Xanthomonas campestris*pv. *Vesicatoria*)*:* Moist weather and splattering rains are conductive to disease development. Infected leaves show small, brown, water soaked, circular spots surrounded with yellowish halo. The infection is mostly on older leaves and may cause serious defoliation. Ripe fruits are not susceptible to the disease. Surface of the seed becomes contaminated with bacteria [7].
10. *Bacterial Canker*(caused by *Clavibacter michiganensis* subsp. *michiganensis*.)*:*Temporary and later on permanent wilting of leaflets of affected plants is observed during the diseases in the field. Light

streaks appear at the juncture of petiole and stem extending down the internode and up the petiole. At a later stage canker like opening may appear in stem, petiole and midrib. When the stem of diseased plants is cut longitudinally, a creamy white, yellow or brown line follows the phloem. The disease appears on the green fruit as water soaked spots with a white halo. Halo is the distinguishing character of bacterial leaf spot of tomato.

11. *Tomato mosaic virus:* Tomato mosaic virus (ToMV) is a member of family *tobamoviridae* and belongs to the genus *tobamovirus*, which is a plant pathogenic virus. The disease is characterized by light and dark green mottling on the leaves often accompanied by wilting of young leaves in sunny days when plants first become infected. The leaflets of affected leaves are usually distorted, puckered and smaller than normal. Sometimes the leaflets become indented resulting in 'fern leaf' symptoms. The affected plant appears stunted and pale green. The virus is spread by contact with clothes, hand of working labor, touching of infected plants with healthy ones, plant debris and implements [18].
12. *Tomato leaf curl virus:* Tomato yellow leaf curl virus (TYLCV) is a DNA virus from the genus *Begomovirus* and the family *Geminiviridae*.This disease is transmitted by whitefly (*Bemisiatabaci).* It is one of the most devastating diseases of tomato. Leaf curl disease is characterized by severe stunting of the plants with downward rolling and crinkling of the leaves. The newly emerging leaves exhibit slight yellow coloration and later they also show curling symptoms. Older leaves become leathery and brittle. The nodes and internodes are significantly reduced in size. The infected plants look pale and produce more lateral branches giving a bushy appearance. The infected plants remain stunted [19].
13. *Tomato spotted wilt virus*(belongs to the genus *Tospovirus* of the family *Bunyaviridae*)*:* The spotted wilt virus is transmitted through thrips(*Thripstabaci, Frankliniellaschultzi and F. occidentalis).* This disease is similar to streak in that it causes streaking of the leaves, stems and fruits. Numerous small, dark, circular spots appear on young leaves. Leaves may have a bronzed appearance and later turn dark brown and wither. Fruits show numerous spots about one-half inch in diameter with concentric, circular markings. On ripe fruit, these markings are alternate bands of red and yellow.
14. *Tomato bunch top virus*(caused by a viroid, the tomato apical stunt viroid)*:* The infected plants show extensive abnormal growth with apical proliferation. The new leaves arising from the axillary buds give closely crowded bunchy appearance. The leaflet margin curl towards the tips and the surface show puckered conditions. Necrosis of leaves and stems are also characteristic symptoms. The diseased plants bear very few flowers and 1-2 very small fruit [1].
15. *Tomato big bud*(caused by *Candidatus Phytoplasma aurantifolia*)*:* The disease infects all the plant parts. The big bud of tomato is transmitted by leaf hopper (*Orosiusargenatatus).* The first indication of infection appears at the tips of the actively growing shoots. The youngest fruit truss, instead of becoming recurved as in normal plants, assumes an upright position. The buds on the truss also point in a vertical direction, the calyx segments remain united almost to the tips and whole calyx enlarges to a form like a bladder with toothed opening at the top. On pruned plants in the field, the growing points fail to develop normally. After a short time, the axillary buds grow out, forming shoots affected in the same way as the main shoot. Simultaneously, there is a gradual thickening of the stems of the affected parts due to the formation of an abnormal tissue [3].
16. *Anthracnose* (caused by several members of the genus *Colletotrichum):*At first, infected fruit show small, slightly sunken, water soaked spots. These spots enlarge, become darker in color, depressed and have concentric rings. Masses of the pink fruiting fungus can be seen on the surface of the lesions in moist weather. Under warm and humid conditions, the fungus penetrates the fruit completely destroying it. The fungus persists on infected plant. Fruits may be infected when green and small, but do not show any marked lesions until they begin to ripen. Fruit becomes more susceptible as they approach maturity [6].

HEVEIN GENE

Hevein is a small (4.7 kDa) cysteine-rich protein in the latex of the rubber tree (Heveabrasiliensis), and was originally described by Archer (1960).Hevein is a wound-induced and a lectin like protein from Heve abrasiliensis(rubber tree) where it is involved in the coagulation of latex. The gene HEV1 encodes the protein. The 187 amino-acid propeptide pro-hevein is cleaved in two fragments: a N-terminal 43 amino-acid Hevein bearing a chitin-binding type-1 domain (also known as CBM18 carbohydrate-binding module) that binds to chitin and a 138 amino-acid Win-like protein bearing a Barwin domain.It has antifungal properties [2, 20-].

Chitin binding lectins contain one or more hevein domain; the term 'hevein' refers to a chitin binding polypetide of latex of *Hevea brasiliensis*. They bind N-acetyl D-glucosoamine oligomers and polymers of N-acetyl D-glucosamine. Type-2-ribosome-inactivating proteins (RIPs) are lectins that catalytically

inactivate ribosomes or eukaryotes and therefore irreversibility shut down protein synthesis. They bind galactose, N-acetyl galactosamine and N-acetyl neuraminic acid. Monocot mannose-binding lectins bind only mannose and oligosaccharides of mannose and are found only in subgroups of monocot plants, alliaceae, amaryllidaceae, araceae, bromeliaceae, liliaceae and orchidaceae [11, 14, 18].
Proposed functions for plant lectins include a storage or transport role for carbohydrates in seed, binding of nitrogen fixing bacteria to root hairs and inhibition of fungal growth or insect feeding. Lectins have also been found to be effective against transmission of plant viruses.

PRODUCTION OF FUNGAL RESISTANT TOMATO PLANT

Plants are under constant invasion by pathogens which causes a reduction in yield, quality and growth rate. So, we have dealt with some aspect of recombinant DNA technique to arrive at genetically engineered tomato plant with fungal resistant Hevein gene incorporated into them. Thereby, a tomato plant gained antifungal activity [1-8].
Isolated plasmids from basic vectors pUC118/Hevein and binary vector pGPTV were subjected to EcoRI or XbaI digestion in order to arrive the release of the Hevein gene from pUC118 and also linearized pGPTV vector system. Then the gene Hevein was found to be 25ng/l and 5ng/l respectively. The hevein from pUC118 and pGPTV were ligated by performing ligation reaction. Appropriate concentration of insert and vector DNA (1:2) were maintained to get recombinant plasmid pGPTV/Hevein [4, 5, 12].
Competent cells were prepared by using calcium chloride and transferred the pGPTV containing Hevein in Tri-Parental Mating. pGPTV/Hevein was used as gene construct. Mixing *E.coli* strains having pGPTV-Hevein, pRK2013 and disarmed *Agrobacterium tumefaciens* strain MTCC431 on rich growth medium. Through the mobilization and transfer function provided by helper plasmid pRK2013, the recombinant pGPTV/Hevein in *Agrobacterium tumefaciens* (a natural genetic engineer) mediated transformation process with the help of helper strain, transfers Hevein gene into the system of *Agrobacterium* following transfection [16-19].
After co-cultivation on selection media callus were obtained. Genomic DNA isolation was done. With the help of PCR, it was confirmed that the gene Hevein got incorporated into tomato. Its amplification with specific primers confirmed the presence of the Hevein gene in the recombinants. Thus, indicating that the calli had gained fungal resistance.

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