SCREENING AND BIOCHEMICAL CHARACTERIZATION OF TOMATO GENOTYPES FOR RESISTANCE TO BACTERIAL WILT

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Screening and biochemical characterization of tomato genotypes for resistance to bacterial wilt"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled "**Screening and biochemical characterization of tomato genotypes for resistance to bacterial wilt**" is a record of research work done independently by **Mr. S. Subash Chandra Bose,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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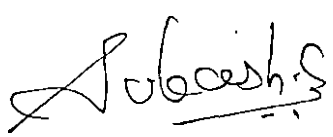


S.Subash Chandra Bose

To my beloved parents, brother & friends

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

1. INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is the second most important vegetable crop next to potato. It is grown all over the world for its edible fruits. Tomato is used in several forms and is widely used as a food ingredient. The fruits are consumed raw, cooked or processed as juice, ketchup, sauce, paste, puree, etc. It is a good source of vitamin C, vitamin A and vitamin B_1. Tomato has almost become a part of our daily diet.

FAO estimates show a world tomato production of 84.6 million tonnes from an area of 31 lakh hectares during 1996 and in India the annual production of tomato is 48 lakh tonnes from an area of 3.2 lakh hectares during the same period.

The area under tomato cultivation in Kerala is very limited. The main reason being the incidence of bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi *et al.* The warm humid tropical climate and acidic soil conditions in Kerala favour the incidence of bacterial wilt. The losses accounted due to this disease in tomato vary from 20-100 per cent.

The disease is manifested at all stages of crop growth with the maximum severity occurring during the flowering stage. Persistence of the pathogen in different types of soils for a longer period and its wider host range often hamper the effectiveness of management measures. As compared to other bacterial diseases, it is very difficult and expensive to control by using chemicals. The variable reaction of cultivars to the causal organisms and differential behaviour of the pathogen itself make breeding programme arduous. Breeding for wilt resistance is likely to remain the most widespread control measure as well as the core item in the integrated control strategies. Hence the knowledge of source(s) of resistance and their biochemical background are important pre-requisites.

At present, only limited information is available on various aspects of defence mechanisms against bacterial wilt in tomato. The inherent potential of a

genotype to impart resistance is determined by the resistance mechanism operating within it. The resistant varieties possess various biochemical barriers to restrict growth of the pathogen in host cell. The plants' defence mechanism against invasion of pathogen includes, hypersensitive reaction, antimicrobial proteins and metabolites such as phytoanticipins and/or phytoalexins (Collinge *et al.*, 1996).

Recently isozyme variations are used as a powerful tool to complement and to supplement conventional biochemical studies. Isozymes are direct products of single locus and relating of phenotypic variations with genotypic characters is relatively easier. The zymograms pertaining to various genotypes will divulge the exact genomic position of different varieties and hence screening of tomato genotypes through isozyme markers will be more reliable. These markers will also serve as a tool for rapid screening in tomato disease resistance breeding programme.

Due to increasing importance and enlivened demand by the consumers, there is more enthusiasm to grow tomato in the state. This had made it necessary to evolve resistant varieties with high productivity and quality. Besides resistance breeding to bacterial wilt there is a need to unravel the biochemical mechanisms of wilt resistance. Devising effective tools to screen and identify potential sources of resistance pave way for development of resistant varieties. Use of isozyme markers for effective screening of wilt resistant varieties is one of the methods in this dimension.

Against this background, the present investigation was carried out with the following objectives,

1 Screening and characterization of tomato genotypes for resistance / susceptibility against *R. solanacearum*.

2 To study the biochemical bases of resistance to bacterial wilt.

3 To find the possibility of biochemical cataloguing of tomato genotypes into resistant / moderately resistant and susceptible using isozyme patterns.

2. REVIEW OF LITERATURE

Bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi *et al.* is one of the most destructive diseases in the warm humid regions of the world. Smith (1896) first described the pathogen and its symptomology. Over 4000 papers have been published since then on this disease in different crop plants. Even before the identification of this pathogen, occurrence of this disease was familiar to the farmers in tropical, subtropical and warm temperate zones of the world.

In India Hedayathullah and Saha (1941) first reported the occurrence of bacterial wilt in tomato from West Bengal. This was followed by a detailed study of bacterial wilt in brinjal by Das and Chattopadhyay (1956). They reported a yield reduction of 54.6 to 62.3 per cent in brinjal. In tomato the yield reduction ranged from 20 to 100 per cent (Sadhankumar, 1995).

The review of literature on the causal organism of bacterial wilt, host range, resistance mechanisms, screening for bacterial wilt resistance and isozymes are covered in this chapter.

2.1 The pathogen and host range

The bacterium *Ralstonia solanacearum* (Smith) Yabuuchi *et al.* was first characterized by Smith (1896) and he reported its occurrence in tomato, brinjal and potato. The pathogen is a complex species consisting of several races differing in many characters. Okabe (1937) had described four colony types of *P.Solanacearum*, viz. F = wild types which were fluidal, irregular milky colony; OP = opalescent, circular, homogenous; C = circular and SS = pale. The last three were isolated from advanced stages of the disease.

Kelman (1954) distinguished two colony variants on tetrazolium medium. The one most common being the normal or wild type which were irregularly round, entire, white, or white with light pink centre and the mutant or

butyrous type which were round, translucent, smooth, deep red with a narrow light bluish border. He also reported that wild types are highly virulent and produced wilting in 14 days.

Khan *et al.* (1979) reported that the tomato isolates on TZC medium were convex to flat, fluidal, slimy with pink centre. The existence of variation among the isolates of the pathogen had been well demonstrated (Smith, 1896; Kelman, 1954; Buddenhagen and Kelman, 1964; Addy *et al.* 1980).

Cross infectivity of isolates of *Pseudomonas solanacearum* E.F.Smith from different host plants was studied by many workers. Buddenhagen *et al.* (1962) had reported that the causal organism from many solanaceous plants like tobacco, tomato and brinjal were capable of cross infecting each other.

Buddenhagen *et al.* (1962 and 1985) differentiated strains of *Pseudomonas solanacearum* E.F.Smith based on host range, pathogenicity and colony appearance on TZC medium. They distinguished them into three different races.

1. Race 1 (Solanaceous strain) - wide host range distributed throughout the lowlands of tropics and subtropics. They affect tomato, tobacco, many solanaceous and other weeds and certain diploid bananas.
2. Race 2 (Musaceous strain) - affecting triploid bananas, heliconia or both ; initially limited to American tropics and spreading to Asia.
3. Race 3 (Potato strain) - affecting potato and tomato, but highly virulent on other solanaceous crops.
4. Race 4 (Ginger strain) - from Philipines
5. Race 5 (Mulberry strain) - from China

Hayward (1964) classified a collection of 185 isolates of *Pseudomonas solanacearum* E.F. Smith into four biotypes based on their capacity to oxidise

three disaccharides (lactose, maltose, cellobiose) and three hexose alcohols (Manitol, sorbitol and dulcitol).

1. Biotype I - Oxidised neither group
2. Biotype II - Oxidised only disacchrides
3. Biotype III - Oxidised both disacchrides and alcohols
4. Biotype IV - Oxidises only hexahydric alcohols

Biotype II had a restricted host range and was obtained from two host plants, potato and tomato whereas the other biotypes were obtained from many families in addition to *Solanaceae*.

According to Persley *et al.* (1985) the bacterial wilt pathogen could be grouped into five races which differ in host range, geographic distribution and ability to survive under different environmental conditions. This classification was similar to the earlier classification by Buddenhagen *et al.*

Kumar *et al.* (1993) differentiated twelve isolates of *P. solanacearum* E.F.Smith from solanaceous hosts into biovars following Haywards classification. All the isolates from tomato, potato, aubergine (brinjal) and bell pepper (Capsicum) were identified as biovar III or a sub-type in biovar III. An isolate from chilli (Capsicum) that differed from others was tentatively identified as biovar V. All the isolates utilized glucose, fructose, sucrose, galactose and glycerol.

Yabuuchi *et al.* (1992) transferred several species of the rRNA homology group II Pseudomonads including *P. solanacearum* to the genus *Burkholderia*. Later work based on sequencing of 16S rRNA genes and polyphasic taxonomy led to the proposal of genus *Ralstonia* and the pathogen has been renamed as *Ralstonia solanacearum* Yabuuchi *et al.* in 1995.

Tomato, capsicum and aubergine cultivars with varying level of resistance to *R. solanacearum* were tested for disease incidence to an aggressive

strain of the bacterium (Grimault *et al.*, 1994). Wilted plants of all host species contained similar bacterial populations at the hypocotyl and mid-stem. All plant species showed latent infection at lower population levels. Resistant tomato and aubergine cultivars showed a decrease in bacterial populations between taproot and mid-stem.

2.2 Ecology of the pathogen

The behaviour of the pathogen in infested soil is poorly known. Chester (1950) reported that the pathogen survives saprophytically in the soil for as long as six years under natural conditions. It is inferred that the primary inoculum came from the soil but there was no conclusive evidence that the pathogen is a ubiquitous inhabitant in the soil (Buddenhagen and Kelman, 1964).

As *Ralstonia solanacearum* Yabuuchi *et al.* is a poor competitor it does not survive in soil for long time. Plant roots of infected plants serve as storehouse for the pathogen. Granda and Sequeira (1993) have studied survival of *P. solanacearum* in rhizosphere clearly. According to them the pathogen invades the root of presumed non-hosts such as bean and maize. Localized or systemic infection of the susceptible plants was responsible for the survival of the pathogen.

Sequeira (1993) reported that the bacterium survives continually infecting the roots of susceptible plants or by colonizing the rhizosphere of non-host plants.

2.3 Symptomatology

According to Walker (1952), the first expression of the disease is wilting of the lower leaves of the plants. This wilting is usually accompanied with yellowing of older leaves. Sometimes dwarfing and stunting may also occur.

The entry of pathogen into plant is mainly through roots and it was believed that a wound is necessary for entry (Walker, 1952; Kelman, 1953; Chupp and Sherf, 1960). Hilderbrant (1950) has also reported that the entry of the pathogen is through natural openings. He reported that root contact with infected plants was not necessary for infection. Bacteria find its way through the points of origin of secondary roots.

The pathogen first enters the intercellular spaces of cortex, from where it moves to pith and xylem vessels (Walker, 1952). On entry of the pathogen into susceptible host plant, visible symptoms occur within two to eight days (Kelman, 1953; Chupp and Sherf, 1960).

Hussain and Kelman (1958) reported that wilting of affected plants is attributed to the presence of heat sensitive enzymes like cellulase and poly galacturonase respectively.

According to Maine (1960) the wilting of the plant is attributed to hydrolytic enzymes and toxins (extra cellular polysaccharides) produced by *P. solanacearum* affected the structural integrity and essential physiological process of the host tissue respectively.

The roots and lower parts of the stem show a browning of vascular bundles and water soaked appearance in the root (Chupp and Sherf, 1960). Later dark brown to black areas develops due to decay of root system and whole plant dies off. A very distinct and characteristic indication of bacterial wilt is the appearance of bacterial ooze from the injured vascular regions (Ashrafuzzaman and Islam, 1975).

Cytological evidence is sparse to elucidate how the bacterium reaches the vascular system. According to Sequeira (1993) it is presumed that the bacteria digests its way through the primary wall of the weakened cortical cells as well as the tracheiary elements where it is exposed between spiral thickenings.

2.4 Genetics of resistance

Schaub and Baver (1944) pioneered the resistant breeding work in tomato at North Carolina in USA. Lousiana Pink and T414 showed field level resistance to the wilt. The most and viable way of control of this organism is reported to be by the development of resistant varieties (Kelman, 1953).

Abeygunawardena and Siriwardena (1963) reported *Lycopersicon pimpinellifolium* as a source of resistance. The resistance was partially dominant at seedling stage. In mature plants, recessive genes controlled the resistance. The expression of the resistant variety is a function of the age of the plant and changes in temperature (Acosta *et al.*, 1964). They also reported that a single pair of gene (spt) controls the resistance in *L. pimpinellifolium*. They also reported a linkage between spt and wilt resistance. Suzuki et al. (1964) reported quantitative inheritance for wilt resistance.

Henderson and Jenkins (1972) reported resistance in Venus, Saturn and Beltisville 3814 to bacterial wilt. Rao *et al.* (1975) tested 23 wilt resistant cultivars and lines from USA and Philippines for their reaction to an Indian isolate of *Pseudomonas solanacearum* E.F. Smith and among them only one line, CRA 66 selection A from Hawaii was resistant.

Based on the crosses between wilt resistant PI 126408 with susceptible Bonny Best, Ferrer (1976) suggested polygenic inheritance for wilt resistance. The genes involved were additive and no dominance was observed.

Mew and Ho (1976) found that the line VC-8-1-2-1 was resistant to *Pseudomonas solanacearum* regardless of the inoculum density. They also observed that susceptible varieties were not significantly affected by changes in inoculum density but resistant lines became less resistant at high inoculum densities. Hsu (1976) found that varieties A95-6 and UP 1167 were comparatively

resistant following root inoculation, but were susceptible following inoculation of stem or top leaf.

Devi (1978) reported wilt incidence of less than 30 per cent in Venus, Saturn and CRA 66 Selection A. Goth *et al.* (1983) tested selected tomato lines and cultivars against eight isolates of *P.solanacearum* collected from different locations and found that CL 32d-0-1-19 GS was resistant to three isolates, viz. K60, 126408-1 and Tifton 80 of race 1.

Tikoo *et al.* (1983) reported the presence of two independent gene systems for wilt resistance. The resistance was governed by multiple recessive genes in CRA 66 Sel A from Hawaii and by single dominant gene in 663-12-3 from Taiwan. Sreelathakumari (1983) reported a complimentary and hypostatic type of digenic recessive gene system for wilt resistance.

Rajan and Peter (1986) reported a monogenic incompletely dominant gene action in the resistant line LE-79. NirmalaDevi (1987) reported that resistance to bacterial wilt in CRA 66 Sel A was under polygenic control. Monma and Sakata (1993) reported that bacterial wilt resistance in D-9 and Hawaii 7998 was recessive, as there was incomplete dominance towards susceptibility.

Many workers have reported the sources of resistance in tomato. The variety TSS1 (highly resistant) by Hoque *et al.* (1981); Scorpio by Peterson *et al.* (1983); Redlands Summertaste by Herrington and Saranah (1985); Redlander by Herrington and Brown (1988); Hawaii 7997; GA 1565, GA 1405 and GA 219 by Scott *et al.* (1993).

Sadhankumar (1995) evaluated 66 tomato genotypes against bacterial wilt for three seasons and reported that Sakthi and LE 79-5 were consistently resistant to bacterial wilt. He also located four additional sources of resistance, viz., LE 214, CAV-5, LE 415 and LE 382-1. Gonzalez and Summers (1995)

reported resistance to bacterial wilt in cultivars like CATIE 17331, 17334, 17349, 17739, 17740 and VC 821B.

The resistant source to bacterial wilt in tomato has been reviewed and evaluated worldwide by Wang *et al.* (1997). Paul (1998) reported resistance to wilt in tomato cultivars like BT 18, LE 79-5, LE 296, Sakthi and LE 453.

2.5 Biochemical basis of resistance

2.5.1 Phenolics

Plants have a wide range of chemicals, which show protective action against pathogens. Among these an aromatic ring bearing a hydroxyl substituent called phenolic substituent show antifungal, antibacterial and anti viral activities. Kuc (1964) reported that in some cases inhibition of a microorganism might result from the cumulative effect of two or more compounds. Non-diffusable chemicals like tomatine, phenols etc., have a key role in plant defence mechanism as reported by Tapliyal and Nene (1967). Mahadevan (1973) reported that resistance to parasitic micro-organisms like bacteria, fungi and viruses is not only due to structural barriers like thick epidermis, leaf hairs and thick cuticle, but also sugar content, osmotic pressure, pH and other features.

Catechol, procatechuic acids, phenols, flavanoids and tomatine are the main pre-infectional inhibitiors present in plants (Stoessel, 1969; Langecake *et al.*, 1972 and Roddick, 1974). Phenols when present in high concentrations are toxic to plant cells themselves (Tepper and Anderson, 1984). Therefore phenols are present only in small amounts and this may be insignificant in the suppression of pathogen. But of late many reports suggest an increased synthesis of phenols in plants at very large quantities due to plant-pathogen interactions (Vidhyasekaran, 1990).

Phenolics do have a decisive role in fusarium wilt resistance in tomato. An increased synthesis of both total and orthodihydric phenols have been reported in *Fusarium oxysporum* f. sp. *lycopersici* infected tomato plants. Matta *et al.*

(1967) reported an increased synthesis of these compounds in resistant varieties when compared to susceptible ones. Peroxidase and polyphenoloxidase are capable of oxidising phenolic compounds to quinones, which are more toxic to fungi.

Phenolics and its protective role against bacterial wilt disease was reported by many scientists (Patil *et al.*, 1964 and Tapliyal and Nene, 1967 in potato; Rajan, 1985 and Sadhankumar, 1995 in tomato; Gangappa, 1986 and Gopinath and Madalageri, 1986 in brinjal; Markose, 1996 in chilli and Paul (1998) in tomato, chilli and brinjal).

Gopinath and Madalageri (1986) and Sadhankumar (1995) observed a significant correlation of phenol with resistance and suggested a possible role of phenols in the mechanism of wilt resistance in brinjal and tomato respectively. Similarly Paul (1998) observed highly significant positive correlation of phenols with resistance in chilli, tomato and brinjal. But Sitaramaiah and Sinha (1984) and Geetha (1989) were unable to correlate the total phenol content to resistance/susceptibility to bacterial wilt in brinjal. However, exceptionally, Kuc (1964) and Rajan (1985) observed a negative correlation between resistance and total phenol content in tomato and inferred that lower levels of phenolics in roots of resistant variety may be due to the increased rate of oxidation of phenolics. Markose (1996) reported that the resistant variety Ujwala exhibited significant increase in total phenol content in roots.

Ortho dihydroxy phenols (OD phenols) are known to be highly toxic and play a major role in disease resistance (Mahadevan, 1966). They get easily oxidised by polyphenol oxidase and peroxidase to highly reactive quinones which are effective inhibitors of sulphydryl enzymes thereby preventing the metabolic activity of host and parasitic cells (Mahadevan, 1970). Bajaj (1988) reported that chlorogenic acid and caffeic acid are the most important phenolic compounds involved in disease resistance mechanisms.

Ortho dihydroxy phenolic compounds such as caffeic acid, chlorogenic acid, orthoquinones and tannins were shown to strongly inhibit the activities of extra cellular enzymes produced by microorganisms in addition to growth inhibition (Hunter, 1978).

A positive association between OD phenol content in roots and bacterial wilt resistance has been established. The resistant lines had higher OD phenol content compared to susceptible lines in tomato, brinjal and chilli (Rajan, 1985; Gangappa, 1986; Geetha, 1989; Sadhankumar, 1995; Markose, 1996 and Paul, 1998).

2.5.2 Host enzymes

Retig (1974) has reported the role of polyphenol oxidase in fusarium wilt resistance in tomato. They observed increased activity in both roots and stems of resistant plants after inoculation. In susceptible plants there was no increase in activity as reported by them.

Host enzymes like peroxidase and polyphenol oxidase play an important role in disease resistance. These enzymes are responsible for synthesis and degradation of phenolics and quinones respectively. Quinones are highly bactericidal and fungitoxic (Rama and Dunleavy, 1975). Hence sometimes the increased activity of these enzymes might be responsible for disease resistance.

Obukowicz and Kennedy (1981) also opined the importance of polyphenol oxidase enzyme in resistance against *P. solanacearum* in tobacco.

Felton *et al.* (1989) reported that the foliage and fruit of tomato plant contains polyphenol oxidase (PPO) and peroxidase (PRX) that are compartmentally separated from orthodihydroxy phenolic substrates *in situ*. But on damage to leaf tissues by insect feeding, the enzyme and phenolic substrates come in contact, resulting in rapid oxidation of phenolics to orthoquinones.

Karwasra and Parashar (1989) reported higher polyphenol oxidase activity in Kufri Lalima, a potato variety resistance to bacterial soft rot, compared to Kunfri Badshah, a susceptible potato variety.

Increased activity of polyphenol oxidase was reported by Singh and Singh (1989) in leaves of two resistant varieties of *Capsicum annuum*. On infection with pathogen the activity of the enzyme increased markedly in resistant varieties leading to the formation of more quinones and other oxidation products, resulting in reduced multiplication and inactivation of the pathogen.

Markose (1996) reported that polyphenol oxidase activity was higher in bacterial wilt resistant variety of chilli in all plant parts at various growth stages. The enzyme activity increased upon infection, largely in the resistant genotype. Paul (1998) also confirmed similar type of behavior for polyphenol oxidase activity with respect to wilt resistance in chilli, brinjal and tomato.

2.5.3 Screening techniques for bacterial wilt resistance

2.5.3.1 Field testing - spot planting

Narayanankutty and Peter (1986) reported that spot planting is most effective in eliminating susceptible plants while screening for resistance. They reported that planting a susceptible check along with the test plant helps in confirming the presence of inoculum of the pathogen in the soil.

2.5.3.2 Inoculation studies

This involves the artificial inoculation of the pathogen to test disease resistance in plants. Vawdrey (1993) and Boiteux and Monma (1994) reported that root dip inoculation techniques with bacterial suspension of 5×10^8 c.f.u. /ml was effective in screening for resistance to bacterial wilt in tomato. Martin and French (1995) suggested the use of stem inoculation method for judging resistance reactions.

Kumar *et al.* (1995) assessed various inoculation methods for disease incidence and disease development of *R. solanacearum* (Smith) Yabuuchi *et al.* in tomato seedlings. They reported that hypodermic syringe inoculation and root dips at transplanting were effective than leaf clip method.

2.5.3.3 Isozyme analysis

Since the discovery by Hunter and Market in 1957, isozymes have played an essential role in many branches of biology like taxonomy, host pathogen interaction studies and evolutionary genetics. Today, it has become the most widely recognized links between the organismal and molecular approach to our science. Isozymes are different variants of the same enzymes, having identical or similar functions and present in the same individual (Market and Moller, 1959).

Farkas and Stahmann (1966) reported that presence of two new peroxidase isozymes II and III in peroxidase zymogram pattern of infected leaves. Uninfected leaves exhibited peroxidase isozymes IV and I. Stavely and Hanson (1967) detected qualitative and quantitative differences in isozymes like glucose-6-phosphate dehydrogenase, phosphatase, peroxidase and polyphenol oxidase. Hwang et al.(1982) classified barley cultivars into highly resistant, moderately resistant and highly susceptible to powdry mildew based on esterase zymograms.

Molecular markers, such as isozymes, have a number of inherent properties that allow the theoretical approaches pioneered by these earlier scientists to be used very effectively for dissecting and manipulating quantitative variation.

Studies conducted by Bashan *et al.* (1987) on the relation of enzymes and resistance against *Pseudomonas syringae* pv. *tomato* revealed presence of four dibased peroxidase isozymes in extracts from diseased plants, while only one was present in healthy plants.

Liu *et al.* (1988) reported that, shoots and upper leaves of smut resistant millet cultivars showed more number of peroxidase and polyphenol oxidase bands compared to susceptible cultivars. They suggested the possible use of above observation as a marker for selecting smut resistant cultivars in maize.

Ganguly and Dasgupta (1988) studied the polyphenol oxidase isoenzyme from healthy roots of tomato variety Pusa Ruby infected by *M. incognita*. They reported the absence of a band with Rm value of 0.52 in healthy or apparently healthy tissues.

Isozyme variations are used as a powerful tool to compliment conventional biochemical and genetic studies (Yndgard and Hoskuldson, 1989). Pavlov (1989) used peroxidase isozyme spectra for identification of remote hybrids in tomato. Bournival *et al.* (1989) detected GOT II locus on chromosome seven as a selectable marker to expedite the transfer of bacterial wilt race 3 resistance to commercial tomato cultivars.

Patterson and Payne (1989) have briefed the preparation of zymograms of plant extracts using isoelectric focusing on ultra thin layers. They discussed methodology of extraction, focusing on thin gels and importance of ampholytic chemical interference.

Kudryakova and Kalloo (1991) have reviewed isozymes in the genus *Lycopersicion* and elucidated the use of isozymes as markers in genetic mapping, introgression and other breeding work. They suggested that *L. peruvianum* and *L. chilense* showed the maximum polymorphism.

Indian tomato cultivars were distinguished into different groups based on the protein-banding pattern by Chakrabarthi *et al.* (1992). Henn *et al.* (1992) used six isozyme systems for identification of tomato cultivars by Polyacrylamide electrophoresis (PAGE). They distinguished nine alcohol dehydrogenase phenotypes and 3 acid phosphatase phenotypes.

Mather *et al.* (1993) used cellulose-acetate electrophoresis of individual tomato seeds for identification of random cultivars. Thirteen out of 29 isozyme loci were polymorphic and suggested the same can be used as an ideal tool for tomato genetic improvement.

Wang *et al.* (1994) carried out genetic analysis of a complex hypersensitive reaction to bacterial spot in tomato using eighteen isozymes and a morphological marker. They reported significant heterosis score as linkage between the marker locus and a hypersensitive reaction factor in cv. Hawaii 7998.

Lindhout (1995) have reviewed extensively the use of markers in tomato for identification of Cultivars against various pathogen and pest. About 25 genes have been reported so far.

Agong (1995) evaluated 23 tomato land races for salt and drought tolerance. According to him electrophoresis study revealed limited polymorphism.

Gupta *et al.* (1995) studied the levels of total phenol, polyphenol oxidase and peroxidase in leaves of alternaria leaf blight resistant and susceptible cultivars of *Brassica* spp. They reported an increased level of total phenol and more number of bands for polyphenol oxidase in resistant cultivars.

A specific peroxidase isoenzyme (Rf=0.47) was identified from *H. bulbosum* roots by Deyu *et al.* (1995). They related this band to BaYMV resistance and suggested the use of it as a marker in barley disease resistance breeding.

Siraly *et al.* (1995) used 7 different PAGE gel systems and screened tomato genotypes for resistance to *Melidogyne incognita*. They reported clear and reproductive band resolution of Aps-7 allele products conferring resistance.

Zymograms of poly galacturonase enzyme revealed special bands in stems of tomato plant resistant to *F. oxysporum* f. sp. *lycopersici* as reported by Pietro *et al.* (1996).

Peroxidase induction and their isozyme patterns in leaves of *Alternaria solani* resistant tomato cultivar (NCEBR-1) and susceptible (HC 3880) were studied by Fernandez *et al.* (1996). They reported an increase in number of bands and enzyme activity in resistant cultivars and suggested that the possibility of peroxidase being one of the defence mechanism against the pathogen. Similar results were reported by Solorzano *et al.* (1996) for peroxidase and polyphenol oxidase in tomato.

Ramesh *et al.* (1996) screened 38 tomato cultivars/accessions against *Meloidogyne incognita* using peroxidase isoenzymes. The percentage of reliability of this method in predicting resistance and susceptibility ranged from 75-100 per cent. Quantitative trait linked loci (QTL) conferring resistance to bacterial wilt of tomato was identified by Thoquet *et al.* (1996). The most important QTL is located at chromosome 4 and chromosome 6 in cv. Hawaii 7996.

Barcelo *et al.* (1996) reported the differential expression of a basic peroxidase isoenzyme B3 (pI=8.9) in leaves and stems of *P. viticola* resistant *vitis* hybrids. They suggested the possible use of this isoform as a marker of disease resistance in *vitis* spp. to *P. viticola* as it was confined to only resistant varieties.

Existence of ternary complex comprising peroxidase, IAA and oxygen was reported by Gazaryan *et al.* (1996). They suggested a specific interaction between plant peroxidase and IAA oxidation. This is of importance because IAA plays a crucial role in shikimate pathway resulting in production of secondary metabolites like phenols.

Fan-YanPing *et al.* (1996) reported an increased content of total phenol, polyphenol oxidase and peroxidase isoenzymes in leaves of *Venturia nashicola*

resistant pear c.v. Yali. They also reported an additional band of peroxidase isoenzyme in the fast band area of the resistant cultivar.

Soybean rust resistant cultivars had four additional bands for peroxidase isoenzyme than susceptible cultivars as reported by Fei *et al.* (1997).

Morales *et al.* (1997) studied the ability of grapevine peroxidase isoenzyme B5 to oxidise trans-resveratrol. They suggested that this isoenzyme enabled a specific metabolic function, which act as a marker of disease resistance in grapevine leaves and shoots for Gamay Rouge grape berries.

Differential expression and turnover of the tomato polyphenol oxidase gene family during vegetative and reproductive development was reported by Thipyapong *et al.* (1997). They reported that, the accumulation of polyphenol oxidase in specific idioblast cells of stems, leaves and fruits varied with age.

3. MATERIALS AND METHODS

The present study was undertaken from August 1998 to March 1999 in the Vegetable Research Farm, Department of Olericulture and Biochemistry Laboratory, College of Horticulture, Vellanikkara, Thrissur. The area where field experiments was carried out enjoys a warm humid tropical climate. The experimental site is considered as hot spot for bacterial wilt (Plate 1). The details of the materials and methods used in the present studies are elaborated here under.

3.1 Evaluation of tomato genotypes for resistance /susceptibility against *R. solanacearum* (Smith) Yabuuchi *et al.*

Materials

Twenty-four tomato genotypes requisitioned from India and abroad were used as experimental materials. The list of these genotypes is given in Table 1. The evaluation was done during August 1998 to January 1999.

Method

Spot planting technique was resorted to screen out resistant/susceptible genotypes. Variety Pusa Ruby was used as the susceptible check. One seedling of Pusa Ruby was planted with test variety in the same hill to confirm the presence of pathogen in the spot (Plates 2a and 2b).

The field was thoroughly prepared first. Trenches of 15-cm width were taken at a spacing of 60 cm between trenches. Twenty seedlings were planted for each genotype giving a spacing of 60 x 60 cm. The experiment was laid out in Randomized Block Design (RBD) with two replications. Thirty day old seedlings were transplanted. The crop was raised as per the Package of Practices Recommendations for Crops (KAU, 1993). Wilt incidence was recorded at fortnightly intervals. Incidence of bacterial wilt was confirmed by Ooze test. The

Plate I . Field view of tomato genotypes in wilt sick plot

Plate II a. Spot planting technique

Plate II b. Spot planting technique

Table 1. Tomato genotypes used in the study.

Genotypes	Source
1. Sakthi	KAU, Vellanikkara
2. Mukthi	KAU, Vellanikkara
3. LE- 214	AVRDC, Taiwan
4. LE- 474	Gulf Coast Research & Education Center, Florida
5. LE- 415	Heinz, U.S.A.
6. LE- 470	KAU, Vellanikkara
7. LE- 421 (CAV-5)	Portblair
8. LE- 457	AVRDC, Taiwan
9. BT-1	OUAT, Bhuvaneswar
10. LE- 455	KAU, Vellanikkara
11. LE- 526	NBPGR, New Delhi
12. LE- 619	AVRDC, Taiwan
13. LE- 615	AVRDC, Taiwan
14. LE- 616	AVRDC, Taiwan
15. LE- 617	AVRDC, Taiwan
16. LE- 613	AVRDC, Taiwan
17. LE- 614	AVRDC, Taiwan
18. LE- 618	AVRDC, Taiwan
19. BT-101-22	OUAT, Bhuvaneswar
20. CO-1	TNAU, Coimbatore
21. CO-3	TNAU, Coimbatore
22. Pant-T_1	GBTUAT, Patnagar
23. Pant-T_3	GBTUAT, Pantnagar
24. Pusa Ruby	IARI, New Delhi

scoring for resistance/susceptible reaction was made as per Mew and Ho (1976), as depicted below:

R - Resistant (<20 per cent plants wilted)

MR - Moderately Resistant (>20 <40 per cent plants wilted)

MS - Moderately Susceptible (>40 <60 per cent plants wilted)

S - Susceptible (>60 per cent plants wilted)

3.2 Characterization of tomato genotypes

To study the morphological and biochemical characters, all the above twenty-four genotypes/lines were raised in sterilized pots. A Completely Randomized Design (CRD) design with three replications was laid out to study the biochemical characters. The following observations were recorded.

A. Morphological characters

1. Growth habit (Indeterminate/determinate/semi-determinate)
2. Plant height (cm)
3. Number of branches

B. Biometric characters

1. Days to first flowering

This was calculated as the number of days taken from germination to first flowering.

2. Days to 50 per cent flowering

Days taken from germination to 50 per cent of the plants to flower were taken in this case.

3. Days to first harvest

This was calculated as the number of days taken from germination to first harvest.

4. Days to last harvest

Number of days taken from germination to last harvest was taken.

5. Crop duration (days)

Number of days from germination to last harvest of the crop.

6. Fruits/plant

Total number of fruits harvested per plant was taken for this observation

7. Yield/plant (kg)

Fruit yield obtained from a plant at different harvests were added together.

8. Fruit size

The fruits were classified as per the IBPGR descriptor for tomato, viz.

i. Very small (< 3 cm)

ii. Small (3-5cm)

iii. Medium (5-8cm)

iv. Large (8-10cm)

v. Very large (>10cm)

Shape:

i. Flattened

ii. Slightly flattened

iii. Round

iv. High round

v. Heart shaped

vi. Cylindrical

vii. Plum shaped

9. Fruit weight (g)

Average individual weight of the fruit.

10. Seed content (g)

Calculated by taking seed weight per fruit taken for recording the fruit weight.

All observations on fruits were taken from second or third truss at full maturity stage.

3.3 Biochemical basis of bacterial wilt resistance

All the twenty-four genotypes included in the field screening trial were used for biochemical studies to assess the biochemical status, which determine the defence mechanism.

The experimental materials raised in the pots mentioned above were used for this part of experiment also. Plant samples were drawn from leaves and roots of each genotype at 45 days and 60 days after germination and used for assaying the biochemical parameters. The estimations were carried out in the Biochemistry Laboratory of College of Horticulture, Vellanikkara. The following biochemical factors were studied.

1. Total phenols
2. O.D. phenol
3. Isozyme banding pattern
   a. Polyphenol oxidase
   b. Peroxidase

3.3.1 Estimation of total phenols

Sample preparation

The samples (roots and leaves) were collected from plants using sharp scissors and washed thoroughly with tap water. They were then washed with distilled water and rinsed with the same for three times. These samples were then wiped with blotting paper in order to remove the moisture, and used for preparation of alcohol extract.

One gram of plant tissue (either root / leaf) was homogenized in a mortar and pestle with 10 ml methanol. The homogenized material was centrifuged at 3000 rpm for ten minutes. The supernatant was collected in a separate test tube. The sediments were reground in a mortar and pestle with five ml methanol. Centrifuged as above and pooled together to form a total volume of 15 ml.

Estimation

Total phenols were estimated by Folin Ciocalteau method. (Mahadevan and Sridhar, 1982). The intensity of colour was read at 650 nm in Spectronic 20® spectrophotometer. The total phenol content was calculated from a standard curve of catechol and was expressed as ppm of fresh weight of sample.

3.3.2 Estimation of O.D. phenol

The same extract used in estimation of total phenol was used for estimation of O.D. phenol also.

Arnow's method was followed for the estimation of ortho-dihydric phenols (Mahadevan and Sridhar, 1982). The absorbance of the pink coloured solution was read in Spectronic 20® spectrophotometer at 515 nm. Catechol was used as standard and O.D. phenol content was expressed as ppm of fresh weight of sample.

3.3.3 Isozyme analysis

All the twenty-four genotypes/lines used in previous estimations were taken for isozyme analysis. Polyphenol oxidase and Peroxidase isoenzymes were analysed as depicted below:

3.3.3.1 Electrophoresis

Polyacrylamide gel electrophoresis (PAGE) using Hoefer Mighty Small™ II gel system was used for preparing zymograms of polyphenol oxidase and peroxidase isoenzymes. Acrylamide monomers were polymerised with N-N methylene bis acrylamide [CH_2(NH CONH = CH_2)$_2$ bis] to obtain the gel. Freshly prepared ammonium per sulphate acted as catalyst and N,N,N',N' - tetramethyl ethylene diamine (TEMED) as chain initiator.

Polyacrylamide gel was preferred because of its chemical inertness, high resolution, easiness in handling, transparency of the gel and easiness in preparation.

Gel preparation

The following stock solutions were prepared.

1. Monomer stock solution (30% Acry. 2.7% Bis)

Acrylamide	- 30.0 g
Bis acrylamide	- 0.8 g
Distilled water	- 100 ml

Store at 4°C away from light

2. 4x Resolving gel buffer (1.5 M Tris-cl, pH 8.8)

Tris base	- 18.5 g
Adjust the pH to 8.8 with 1N HCl	
Distilled water to	- 100 ml

3. 4x Stacking gel buffer (0.5 M Tris-cl, pH 6.8)

Tris base	- 0.6 g
Adjust the pH to 6.8 with 1N HCl	
Distilled water to	- 10 ml

4. Initiator (10% APS)

Ammonium per sulphate	- 0.1 g
Distilled water to	- 1 ml
(Prepared freshly)	

5. Destaining solution (40% Methanol, 7% Acetic acid)

Acetic acid	- 70 ml
Methanol	- 400 ml
Distilled water to	- 1000 ml

Preparation of gel column

The Hoefer Mighty Small™ II Gel system of Hoefer Pharmacia Biotech Inc, California was used. The size of the gel was 8.0 cm x 9.4 cm. The gel was prepared by using the following gel recipe.

Gel Recipe used for standardization

	7.5%		8.5%		10%	
	10 ml	20 ml	10 ml	20 ml	10 ml	20 ml
Monomer	2.49	4.98	2.83	5.66	3.33	6.66
Resolving Buffer	2.50	5.00	2.50	5.00	2.50	5.00
Distilled water	4.94	9.88	4.60	9.20	4.10	8.20
10% APS	50 µl	100 µl	50 µl	100 µl	50 µl	100 µl
TEMED	5 µl	10 µl	5 µl	10 µl	5 µl	10 µl

Of the above, 10 per cent strength gel was selected because of better molecular sieving. The amount of various stocks given for 10 per cent gel strength

were mixed serially. Thoroughly stirred and injected into the gel caster with the help of gel caster syringe of Hoefer® make. The combs were pushed in between the caster plates for making wells and allowed to polymerise in the caster (15-20 min). Care was taken such that the gel was devoid of gas bubbles.

Electrophoretic run

The following two solutions were prepared.

1. Electrophoresis Buffer (0.025 M Tris, pH 8.3, 0.192 M glycine)

Tris base	- 1.5125 g
Glycine	- 7.2 g
Distilled water to	- 500 ml

2. 2x Treatment Buffer (0.125 M Tris-cl, pH 6.8, 20% glycerol, 10% 2-Mercapto ethanol)

4x Tris-cl, pH 6.8	- 2.5 ml (= Stock 3)
Glycerol	- 2.0 ml
2-Mercapto ethanol	- 0.2 ml
Bromophenol blue	- 0.2 mg (100 µl of 1% solution)
Distilled water to	- 10.0 ml

Divided into 1-ml aliquots, and store at -4°C.

After polymerisation, the gels were transferred to electrophoretic apparatus. The upper and lower tanks were filled with pre-chilled electrode buffer of pH 8.3. Fifteen µl of treatment buffer was mixed with 15µl of enzyme extract in separate eppendorf's tubes. The mixture was vortexed with the help of transfer pipette of E.Merck®. The above operation was carried out at 5°C. From this mixture 15µl was loaded to the well after removing the combs. Upper tank was connected to cathode and lower one to anode. The enzyme extracts were subjected to electrophoresis under the alkaline system of Davis.(1964).

The run was carried out at 5°C for peroxidase and room temperature for polyphenol oxidase till the tracker dye (Bromophenol blue) reached the anode end of the gel column. Cooling system was used to circulate cold water for maintaining 5°C for peroxidase and room temperature for polyphenol oxidase as a means for heat dissipation and also to prevent the enzyme from denaturation. A current of 10 mA was maintained per plate and it took 50-75 min for completion of the run.

3.3.3.2 Polyphenol oxidase

Sample preparation

Leaf and root samples were cut with sharp scissors and collected in refrigerated chests. Then these samples were washed thoroughly with distilled water for three times and wiped with filter paper to absorb moisture. The samples were taken from 45 days and 60 days old plants.

The following extraction buffer was used for polyphenol oxidase enzyme extract preparation.

Composition of Extraction buffer

0.05 M Tris-HCl
0.1% ascorbic acid
0.1% cystein-HCl
0.002% Magnesium chloride
pH - 8.0
Stored at 4°C

Homogenization and centrifugation

This part of the experiment was carried out at 5°C. Leaf and root samples (2g each) were chopped into small pieces. To this 2ml of extraction buffer containing 17 per cent sucrose and 0.1% Tween 80 were added at the time of

extraction. The sample was homogenized by grinding well with a pre-chilled mortar and pestle placed in a tray containing ice.

The slurry was centrifuged at 13,500 rpm for 20 min at 5°C in Kobato® 6900 make refrigerated centrifuge. The supernatant was used as enzyme source for polyphenol oxidase isoenzyme analysis.

The electrophoretic run was carried out at room temperature, as the enzyme was more active.

Staining for polyphenol oxidase

The staining solution composed of the following:

0.1 M potassium phosphate buffer (pH 7.0)	- 200 ml
p-phenylene diamine	- 0.2 g
Catechol	- 600 mg

Equilibrate the gel for 30-60 min in the staining solution until yellow bands appear. The bands were fixed using fixing and destaining solution. Photographs were taken and zymograms were drawn.

3.3.3.3 Peroxidase

All the steps mentioned below were carried out at 5°C. The plant samples were prepared in the same way as done for polyphenol oxidase.

The following extraction buffer was used for peroxidase enzyme extract preparation.

Composition of Extraction buffer

Tris	- 21.1995 g
Citric acid	- 2.62675 g
Vitamin C	- 0.52839 g
Cystiene HCl	- 0.52689 g
Distilled water to	- 500 ml
pH	- 7.0

Homogenization and centrifugation

Leaf and root samples of each variety (0.5 g) were chopped into small pieces in pre-chilled extraction buffer (1 ml) in a pre-chilled mortar and pestle. To this 17 per cent sucrose and 0.04g of insoluble poly vinyl pyrolidone (PVP) were added at the time of extraction. The mortar and pestle were placed in a tray containing ice in order to maintain the grinding temperature at 5°C.

The homogenized material was centrifuged at 16000 rpm for 15 minutes in Kobota® 6900 refrigerated centrifuge at 5°C. The supernatant was used as enzyme source for peroxidase isoenzyme electrophoresis.

The electrophoretic running was carried out at 5°C as mentioned earlier.

Staining for peroxidase

The staining solution composed of the following:

0.2 m Acetate buffer (pH 5.6) - 200 ml	
Benzidine	- 0.2 g
H_2O_2 3%	- 0.8 ml

Fresh stain was prepared each time. Acetate buffer and benzidine were mixed, heated to boil, cooled and filtered. Hydrogen peroxide was added at the time of staining. The gels were immersed in staining solution till brown bands

appeared and destained in destaining solution mentioned under preparation of stock solutions. As the bands faded on standing for long time, photographs were taken on the same day of staining and zymograms were drawn immediately.

The relative electrphoretic mobility of polyphenol oxidase and peroxidase were calculated as the ratio of the movement of the band to that of the tracking dye.

3.3.3.4 Nomenclature of isozymes adopted in the present study

The enzymes were designated by the following abbreviations.

1. Polyphenol oxidase - PPO
2. Peroxidase - PRX

Numbering

For numbering of enzymes, all the bands of an enzyme in the plant part studied were taken together. The slow moving cathodal band was numbered 1 (eg. PPO-1), the second one numbered 2 and so on. The faster ones were given subsequent numbers ascendingly.

3.4 Statistical analysis

Analysis of variance of data was carried out using MSTATC package. Treatments were compared using DMRT whenever necessary.

4. RESULTS

Results of the investigation are presented under the following heads.

4.1 Evaluation of tomato genotypes for bacterial wilt resistance

4.2 Biochemical bases of resistance to bacterial wilt

4.2.1 Total phenols

4.2.2 O.D. phenols

4.3 Isoenzyme analysis in tomato genotypes

4.3.1 Polyphenol oxidase

4.3.2 Peroxidase

4.1 Evaluation of tomato genotypes for bacterial wilt resistance

Twenty four tomato genotypes were evaluated for resistance to bacterial wilt in a wilt sick plot during August 1998-January 1999. These 24 genotypes comprised resistant, moderately resistant and susceptible varieties to bacterial wilt. There was 100 per cent disease incidence in the susceptible check, Pusa Ruby confirming the virulent form of bacterial inoculum in the field. The results of disease reaction of these tomato genotypes are presented in Table 2. Out of the twenty four genotypes screened against the pathogen three were rated as resistant, four moderately resistant, four moderately susceptible and thirteen as susceptible.

The variety Sakthi, Mukthi and LE-474 were found to be resistant with disease incidence of 10 per cent, 12 per cent and 20 per cent respectively. The lines LE-214, LE-415, LE-470 and LE-421 were moderately resistant with disease incidence of 22.5 per cent, 32.5 per cent, 22.5 per cent and 25 per cent respectively. LE-457, BT-1 and LE-455 with disease incidence of 50 per cent, 52.5 per cent and 57.5 per cent respectively were moderately susceptible. The other

Table 2. Reaction of varieties/lines of tomato for resistance/susceptibility to *R. solanacearum*

Sl.No.	Varieties/lines	Wilt (%)	Reaction
1	Sakthi	10.0	R
2	Mukthi	12.0	R
3	LE-214	22.5	MR
4	LE-474	20.0	R
5	LE-415	32.5	MR
6	LE-470	22.5	MR
7	LE-421	25.0	MR
8	LE-457	50.0	MS
9	BT-1	52.5	MS
10	LE-455	57.5	MS
11	LE-526	80.0	S
12	LE-619	100.0	S
13	LE-615	90.0	S
14	LE-616	95.0	S
15	LE-617	95.0	S
16	LE-613	95.0	S
17	LE-614	97.5	S
18	LE-618	100.0	S
19	BT-101-22	82.5	S
20	CO-1	70.0	S
21	CO-3	95.0	S
22	Pant T_1	100.0	S
23	Pant T_3	100.0	S
24	Pusa Ruby	100.0	S

R - Resistant MR - Moderately Resistant
S - Susceptible MS - Moderately Susceptible

varieties/lines screened were highly susceptible to bacterial wilt (> 60 percent disease incidence) (Table 2).

4.2 Growth and yield attributes of tomato genotypes screened

The Table 3 depicts the morphological and yield attributes of the tomato genotypes used in the study and were grown in pots. Analysis of variance and comparison of treatment means using DMRT indicated significant differences among the twenty-four genotypes for all the characters studied.

4.2.1 Morphological characters

4.2.1.1 Growth habit

Varieties/lines, viz. Sakthi, LE-214, LE-474, LE-415, LE-470, LE-421, LE-457, CO-1, CO-3, BT-1, LE-457, and Pant T_1 were semi-determinate in growth habit. The lines LE-619, LE-615, LE-615, LE-617, LE-613, LE-614, LE-618 Pusa Ruby and Pant T_3 were indeterminate in growth habit. The variety Mukthi was of determinate growth habit.

4.2.1.2 Plant height

Plant height ranged from 45.7 cm to 112.0 cm. The line LE-455 recorded the lowest (45.7 cm) followed by LE-421 (45.7 cm) and LE-474 (45.7 cm). Pant T_1 was the tallest (112.0 cm) followed by LE-617 (107.7 cm) and LE-615 (99.3 cm).

4.2.1.3 Number of branches

The variety, Sakthi produced the minimum number of branches (7) followed by CO-1 (8). The maximum number of branches was produced by LE-617 (16) followed by LE-613 (15).

Table 3. Mean performance of tomato genotypes evaluated for resistance to bacterial wilt

Sl. No.	Variety	Growth habit	Plant height (cm)	No. of branches	Days to first flowering	Days to 50% flowering	Days to first harvest	Days to last harvest	Crop duration days	Fruits/ plant*	Yield/ plant (kg)	Fruit weight	Fruit size and shape	Seed content (g)
1	Sakthi	SD	56.33^{ij}	7.00^{i}	59.00^{ef}	60.67^{hijk}	93.33^{hi}	120.0^{ef}	123.0^{efg}	10.00^{fghi}	0.278^{fghijk}	27.77^{cdef}	Medium, round	0.710^{c}
2	Mukthi	D	63.67^{ghi}	8.68^{ghi}	58.00^{ef}	60.33^{hijk}	93.00^{hij}	118.3^{ef}	121.7^{fg}	13.00^{efgh}	0.405^{cde}	31.13^{cde}	Medium, highly rounded	0.830^{c}
3	LE-214	SD	69.67^{fg}	10.33^{efgh}	67.00^{bc}	69.33^{cde}	103.30^{cde}	118.3^{ef}	120.0^{g}	9.33^{ghij}	0.299^{fghi}	33.08^{cde}	Small, round	0.193^{efgh}
4	LE-474	SD	46.67^{k}	8.33^{hi}	60.00^{def}	62.00^{ghij}	93.00^{hij}	133.3^{c}	135.0^{c}	7.33^{hijk}	0.208^{jkl}	29.0^{cdef}	,,	0.700^{c}
5	LE-415	SD	69.33^{fg}	12.33^{cde}	67.67^{b}	70.00^{cd}	103.70^{cde}	116.7^{ef}	118.3^{g}	6.67^{ijk}	0.203^{kl}	30.5^{cde}	,,	0.150^{fgh}
6	LE-470	SD	60.67^{ghij}	14.00^{abc}	67.33^{b}	66.00^{ef}	98.00^{f}	128.3^{cd}	133.3^{cd}	11.00^{fgh}	0.470^{bc}	41.63^{c}	Medium, round	1.200^{b}
7	LE-421	SD	45.67^{k}	9.00^{ghi}	60.00^{def}	62.67^{fghi}	94.67^{ghi}	118.3^{ef}	123.3^{efg}	8.67^{hij}	0.192^{l}	24.3^{def}	Small, highly pointed	0.133^{gh}
8	LE-457	SD	58.67^{hij}	9.67^{fgh}	56.33^{f}	58.00^{k}	89.33^{k}	123.3^{de}	128.3^{de}	17.33^{b}	0.329^{efg}	19.94^{efg}	Medium, rounded	0.230^{efgh}
9	BT-1	SD	67.33^{gh}	10.67^{efg}	59.33^{def}	61.33^{ghijk}	95.33^{fgh}	133.3^{c}	133.3^{cd}	26.67^{b}	0.711^{a}	26.8^{cdef}	Small, cylindrical, oblong, with pointed tip	1.133^{b}
10	LE-455	SD	45.67^{k}	10.67^{efg}	58.00^{ef}	60.67^{hijk}	92.00^{ijk}	118.3^{ef}	121.7^{fg}	9.67^{ghi}	0.356^{def}	36.83^{cd}	Medium High round	0.800^{c}
11	LE-526	SD	62.33^{ghi}	11.33^{def}	62.00^{de}	63.67^{fgh}	96.67^{fg}	118.3^{ef}	120.0^{g}	13.00^{efg}	0.431^{bcd}	33.54^{cde}	Small, rounded	0.317def
12	LE-619	ID	85.67^{cd}	15.67^{a}	75.00^{a}	77.00a	107.00^{b}	146.7^{b}	153.3^{b}	35.33^{a}	0.291^{fghij}	8.263^{g}	Small, plum shaped, ellipsoid	0.080^{h}
13	LE-615	ID	99.33^{b}	15.00^{ab}	74.67^{a}	76.00^{ab}	106.00^{bc}	151.7^{b}	156.7^{ab}	20.67^{c}	0.495^{b}	24.07^{def}	Small, heart shape, pointed tip	1.067^{b}
14	LE-616	ID	86.00^{cd}	15.00^{ab}	73.67^{a}	76.00^{ab}	106.00^{bc}	148.3^{b}	153.6^{b}	10.00^{fghi}	0.286^{fghijk}	28.55$^{cde f}$	,,	0.210^{efgh}
15	LE-617	ID	107.70^{a}	14.33^{abc}	73.67^{a}	72.67^{bc}	104.00^{cd}	158.3^{a}	160.0^{a}	13.67^{ef}	0.333^{efg}	24.34^{def}	Small round with nipple tip	0.270^{defg}
16	LE-613	ID	93.00^{bc}	13.00^{bcd}	72.33^{a}	74.33^{ab}	107.30^{b}	158.3^{a}	160.0^{a}	10.33^{fghi}	0.291^{fghi}	28.17^{cdef}	,,	0.350^{de}
17	LE-614	ID	96.00^{b}	13.67^{abc}	73.33^{a}	75.67^{ab}	108.00^{ab}	158.3^{a}	160.0^{a}	29.00^{b}	0.433^{bcd}	14.92^{fg}	Very small, round	0.157fgh
18	LE-618	ID	99.00^{b}	13.00^{bcd}	75.33^{a}	77.67^{a}	110.30^{a}	158.3^{a}	161.7^{a}	10.00^{fghi}	0.267^{ghijkl}	26.81^{def}	,,	0.247^{efgh}
19	BT-101-22	SD	67.00^{gh}	11.33^{def}	66.33^{bc}	68.33^{de}	98.00^{f}	121.7^{e}	126.7^{ef}	5.67^{ijk}	0.492^{b}	86.97^{a}	Large, slightly flattened	0.420^{d}
20	CO-1	SD	53.00^{ij}	8.33^{hi}	57.00^{f}	59.00^{ijk}	90.33^{jk}	121.7^{e}	126.7^{ef}	14.67^{de}	0.325^{efgh}	22.19^{def}	Small, round	0.330^{de}
21	CO-3	SD	55.67ij	10.00^{fgh}	56.67^{f}	58.67^{jk}	102.00^{de}	118.3^{ef}	123.3^{efg}	6.67^{ijk}	0.268^{ghijkl}	56.79^{b}	Medium, High rounded	0.297^{defg}
22	Pant T1	ID	112.00^{a}	14.00^{abc}	68.67^{b}	70.67^{cd}	102.70^{de}	118.3^{ef}	121.7^{fg}	7.00^{ijk}	0.211^{jkl}	30.23^{cde}	Small, highly flattened	0.240^{efgh}
23	Pant T3	SD	84.00^{de}	11.33^{def}	57.00^{f}	58.33^{jk}	101.0^{e}	121.7^{e}	126.7^{ef}	4.00^{k}	0.240^{hijkl}	63.0^{b}	Medium, slightly flattened	1.833^{a}
24	Pusa Ruby	ID	76.67^{ef}	12.33^{cde}	63.33^{cd}	64.67^{fg}	96.67^{fg}	11.3^{f}	118.3^{g}	9.00^{hij}	0.231^{ijkl}	24.64^{def}	Medium, flattened with deep ridges	0.750^{c}

Treatment means having same alphabets in superscript do not differ significantly at 5% level
D - Determinate, SD - Semi Determinate, ID - In Determinate, * - Plants were grown in pots

4.2.2 Yield characters

4.2.2.1 Days to first flowering

LE-457 was first to flower in 56 days followed by CO-3 (57 days) and Pant T_1 (57 days). LE-618 (75 days) was last to flower followed by LE-619 (75 days) and LE-615 (75 days). Resistant lines like Sakthi, Mukthi and LE-474 took 59 days, 58 days and 60 days to flower respectively.

4.2.2.2 Days to 50 per cent flowering

The line LE-618 took maximum number of days (78 days) to complete 50 per cent flowering followed by LE-619 (77 days). The line LE 457 was the earliest for 50 per cent flowering (58 days) followed by Pant T_3 (58 days). The resistant ones like Sakthi, Mukthi and LE-474 took 61 days, 60 days and 62 days respectively.

4.2.2.3 Days to first harvest

The minimum number of days for first harvest was taken by LE-457 (89 days) followed by CO-1 (90 days after germination). LE-618 took the maximum number of days to harvest (110 days) followed by LE-614 (108 days). The varieties Sakthi, Mukthi and LE-474 took 90 days each for first harvest.

4.2.2.4 Days to last harvest

LE-613, LE-614 and LE-617 recorded the longest duration for last harvest (158 days). Pusa Ruby took the shortest duration for last harvest (113 days) followed by LE-415 (116 days). Resistant lines like Sakthi, Mukthi and LE-474 yielded up to 120 days, 118 days and 133 days respectively.

4.2.2.5 Crop duration

LE-618 had the longest duration of 162 days followed by LE-617 with 160 days after germination. Pusa Ruby had the shortest duration (118 days) followed by LE-415 (118 days). Resistant lines like sakthi, Mukthi and LE-474 had a duration of 123 days, 122.7 days and 135 days respectively.

4.2.2.6 Fruits/plant

Pant T_3 produced the minimum number of fruits (4) followed by BT-101-22 (5). The maximum fruits were produced by LE-619 (35) followed by LE-614 (29). Sakthi, Mukthi and LE-474 produced 10, 13 and 9 fruits respectively.

4.2.2.7 Yield/plant

The yield/plant ranged from 0.192 kg to 0.711 kg. The lowest yield was recorded in LE-421 (0.192 kg/plant) followed by LE-415 (0.203 kg/plant). BT-1 recorded the highest yield of 0.711 kg/plant followed by LE-615 (0.495 kg/plant) and BT-101-22 (0.492 kg/plant). Resistant lines like Sakthi, Mukthi, LE-474 yielded 0.278 kg/plant, 0.405 kg/plant and 0.208 kg/plant respectively.

4.2.2.8 Fruit size

BT-101-22 recorded the large sized fruits, which were slightly flattened. LE-470, LE-457, LE-455 and CO-1 were having medium sized fruits. The remaining varieties had small fruits while LE-619 had very small fruits.

4.2.2.9 Fruit weight

The average fruit weight ranged from 8.26 g in LE-619 to 86.97 g in BT-101-22.

4.2.2.10 Seed content

The average seed content ranged from 0.08 g in LE-619 to 1.83 g in Pant T_3.

4.2 Biochemical bases of resistance to bacterial wilt

This study was carried out to assess the biochemical constituents of the twenty four tomato genotypes which were used in the previous experiment. The leaf and root samples of all the twenty four varieties at 45 days and 60 days were analysed. The results of the biochemical analysis are given in Table 4 and 5.

4.2.1 Total phenol

Total phenol content of root samples was significantly higher in LE-474 (533.5 ppm) at 60 days and at 45 days (512.3 ppm), compared to other varieties (Table 4). The lowest total phenol content was in LE-615 (111.2 ppm) and Pant T_1 at 60 days (127.8 ppm) which showed susceptibility to bacterial wilt. Resistant varieties like Sakthi, Mukthi, and LE-474 had significantly higher amounts of total phenols at both the stages as compared to susceptible varieties.

The total phenol content in leaf samples varied significantly among the varieties. The phenol content in leaf samples increased with age except in LE-455. Maximum phenol content in leaves was observed in Mukthi (1708 ppm) at 60 days and LE-415 (1593 ppm) at 45 days. Resistant varieties like Sakthi, Mukthi and LE-474 had significantly high total phenol content compared at 45 and 60 days old leaf samples when compared to susceptible varieties. The lowest level of total phenols in leaf samples was observed in LE-618 (705.5 ppm) at 45 days and LE-455 (627.4 ppm) at 60 days. In general, the total phenols increased with age of plants in majority of the plants analysed. The leaf samples had high amounts of total phenols compared to roots at 45^{th} and 60^{th} day.

Table 4. Total phenol content in tomato genotypes at various growth stages (ppm)

Sl.No.	Varieties	D.R.	45th day		60th day	
			Leaf	Root	Leaf	Root
1	Sakthi	R	1541.0^{b}	315.7^{b}	1625.0^{a}	381.7^{b}
2	Mukthi	R	1300.0^{e}	250.8^{cd}	1708.0^{a}	322.8^{d}
3	LE-214	MR	1386.0^{c}	223.8^{d}	1314.0^{b}	250.3^{g}
4	LE-474	R	1289.0^{e}	512.3^{a}	1353.0^{b}	533.5^{a}
5	LE-415	MR	1593.0^{a}	233.7^{d}	959.5^{b}	279.4^{f}
6	LE-470	MR	1290.0^{e}	315.8^{b}	1381.0^{b}	372.0^{c}
7	LE-421	MR	1345.0^{d}	271.7^{c}	1462.0^{b}	292.2^{e}
8	457	MS	1079.0^{g}	153.7^{fgh}	1570.0^{a}	172.6^{jkl}
9	BT-1	MS	1027.0^{h}	169.1^{ef}	1231.0^{b}	198.8^{i}
10	LE-455	MS	981.6^{i}	148.9^{fgh}	627.4^{d}	178.8^{j}
11	LE-526	S	826.3^{l}	190.2^{e}	1306.0^{ab}	212.4^{h}
12	LE-619	S	872.3^{k}	127.8^{hi}	1167.0^{b}	146.3^{mn}
13	LE-615	S	801.6^{lm}	111.2^{i}	1479.0^{ab}	128.8^{op}
14	LE-616	S	777.7^{mn}	155.8^{fgh}	1495.0^{ab}	177.8^{jk}
15	LE-617	S	785.3^{m}	129.9^{hi}	930.8^{bc}	152.6^{m}
16	LE-613	S	739.4^{no}	132.6^{ghi}	931.2^{bc}	168.5^{kl}
17	LE-614	S	765.8^{mn}	135.7^{ghi}	908.2^{bc}	135.2^{op}
18	LE-618	S	705.5^{o}	127.4^{hi}	1306.0^{ab}	131.2^{op}
19	BT-101-22	S	920.8^{j}	135.3^{ghi}	1271.0^{b}	168.4^{kl}
20	CO-1	S	1153.0^{f}	139.0^{fghi}	1386.0^{ab}	154.4^{m}
21	CO-3	S	875.7^{k}	129.3^{hi}	1110.0^{b}	138.8^{no}
22	Pant T_1	S	879.1^{k}	129.2^{hi}	976.3^{bc}	127.8^{p}
23	Pant T_3	S	826.0^{l}	128.8^{hi}	924.7^{bc}	135.6^{op}
24	Pusa Ruby	S	841.9^{kl}	162.7^{efg}	990.7^{bc}	165.8^{l}

The figures with same alphabets in superscript do not differ significantly

4.2.2 O.D. phenol

In roots, O.D. phenol was maximum in LE-470 (42.97 ppm) and the lowest in Pant T_1 (8.125 ppm) at 45 days (Table 5). The variety Sakthi had the highest O.D. phenol content in roots (35.26 ppm) at 60 days while BT-101-22 recorded the lowest (11.66 ppm) at the same stage. In general, the resistant and moderately resistant lines had significantly higher amounts of O.D. phenol as compared to susceptible varieties at the same growth stages (Table 5).

The line LE-474 had the highest O.D. phenol content in leaves at 45 days (172.6 ppm). The lowest was recorded in leaves of LE-457 (60.71 ppm) at 45 days. Resistant and moderately resistant varieties like Sakthi, Mukthi, LE-474, LE-214, LE-415, LE-470 had significantly higher amount of O.D. phenol in comparison with susceptible varieties (Table 5). LE-470 had higher content of O.D. phenol in leaves at 60 days (179.2 ppm). The lowest was in LE-613 (76.36 ppm) at 60 days. The 60 days old leaf samples had higher O.D. phenol content than 45 days old leaf samples in resistant and moderately resistant varieties. The trend was same in susceptible varieties also but the content was relatively lower when compared to resistant varieties/lines.

4.3 Isoenzyme Analysis

Twenty four varieties/genotypes of tomato which were earlier included in field screening for disease reaction were analysed for variation in isozyme pattern for polyphenol oxidase and peroxidase.

Root and leaf samples at 45 days and 60 days were taken for the study. The banding pattern had variation in most of the samples analysed.

Table 5. O.D. phenol content in tomato genotypes at various growth stages (ppm)

Sl.No.	Varieties	D.R.	45th day		60th day	
			Leaf	Root	Leaf	Root
1	Sakthi	R	132.10^{bc}	23.75^{bc}	138.20^{c}	35.26^{a}
2	Mukthi	R	128.60^{c}	19.83^{def}	132.00^{d}	23.64^{cd}
3	LE-214	MR	127.70^{c}	20.40^{de}	131.50^{d}	23.18^{cd}
4	LE-474	R	172.60^{a}	20.79^{cde}	178.60^{a}	18.92^{efg}
5	LE-415	MR	170.00^{a}	25.71^{b}	175.50^{a}	22.99^{cd}
6	LE-470	MR	171.00^{a}	42.97^{a}	179.20^{a}	24.34^{c}
7	LE-421	MR	161.40^{ab}	21.03^{cd}	165.90^{b}	18.22^{fgh}
8	LE-457	MS	60.71^{f}	18.65^{defg}	117.20^{ef}	22.99^{cd}
9	BT-1	MS	107.30^{cde}	16.49^{gh}	112.80^{f}	30.66^{b}
10	LE-455	MS	112.00^{cd}	17.72^{efg}	120.40^{e}	16.95^{fghi}
11	LE-526	S	84.26^{def}	10.64^{ijkl}	90.51^{gh}	16.38^{ghi}
12	LE-619	S	82.11^{def}	8.47^{kl}	86.16^{ghij}	14.88^{hij}
13	LE-615	S	74.76^{ef}	16.86^{fg}	84.32^{ijk}	20.37^{def}
14	LE-616	S	76.31^{ef}	19.08^{defg}	81.22^{jklm}	22.39^{cde}
15	LE-617	S	81.65^{def}	10.64^{ijkl}	88.02^{ghi}	13.73^{ij}
16	LE-613	S	72.16^{ef}	12.90^{ij}	76.36^{m}	14.10^{ij}
17	LE-614	S	74.67^{ef}	8.66^{kl}	78.26^{lm}	13.18^{ij}
18	LE-618	S	76.67^{ef}	11.14^{ijkl}	78.01^{lm}	14.84^{hij}
19	BT-101-22	S	132.10^{bc}	10.96^{ijkl}	134.30^{cd}	11.66^{j}
20	CO-1	S	82.07^{def}	9.86^{jkl}	90.19^{gh}	14.89^{hij}
21	CO-3	S	80.46^{def}	10.02^{jkl}	83.76^{ijkl}	15.31^{ghij}
22	Pant T_1	S	90.11^{def}	8.13^{l}	91.13^{g}	12.11^{j}
23	Pant T_3	S	92.21^{def}	11.65^{ijk}	84.71^{hijk}	12.31^{ij}
24	Pusa Ruby	S	83.66^{def}	13.78^{hi}	80.15^{klm}	15.01^{hij}

The figures with same alphabets in superscript do not differ significantly

4.3.1 Polyphenol oxidase

Root sample

As many as fifteen protein bands have been resolved in root samples. The set of protein bands PPO-4 to PPO-8 with Rm values 0.180, 0.265, 0.372 respectively were dense. PPO-4 (Rm=0.180) was absent in all susceptible varieties (Table 6, Fig.1 and Plate 3). The same trend was exhibited with PPO-7 (Rm=0.265) and PPO-10 (Rm=0.372) respectively. PPO-11 (Rm=0.393) was absent among moderately susceptible genotypes and majority of the susceptible genotypes. The isozyme bands like PPO-2, PPO-5, PPO-6, PPO-8, PPO-13, PPO-14 with Rm values 0.159, 0.212, 0.244, 0.319, 0.444 and 0.468 respectively were more frequent among susceptible genotypes. PPO-3 (Rm=0.159) and PPO-15 (Rm=0.50) respectively were not found in any of the root samples at 45 days.

In 60 days old roots PPO-1, 3, 6 and 12 with Rm values 0.074, 0.159, 0.244 and 0.414 respectively were absent (Table 7, Fig.2 and Plate 4). PPO-5 (Rm=0.212) was present in majority of the varieties except in LE-613, LE-614 and LE-618. The most densely stained protein bands were in PPO-5 to PPO-10 region. The fast moving PPO-12 to PPO-15 regions were feeble with light bands. PPO-4 (Rm=0.180) and PPO-9 (Rm=0.351) were absent in susceptible genotypes. The protein bands like PPO-7, PPO-8, PPO-13, PPO-14 and PPO-5 with Rm values 0.265, 0.319, 0.446, 0.468 and 0.50 were confined only to susceptible genotypes. The less variant form PPO-2 with Rm value 0.106 was present only in LE-421.The protein bands like PPO-1 (Rm=0.074), PPO-3 (Rm=0.159), PPO-6 (Rm=0.244) and PPO-12 (Rm=0.414) were absent in 60 days old root samples of all genotypes tested.

Table 6. Rm value of different bands of polyphenol oxidase in 45 days old roots of tomato

Sl. No.	Variety	D.R.	Rm															Total No.of bands
			Banding from origin															
			PPO 1	PPO 2	PPO 3	PPO 4	PPO 5	PPO 6	PPO 7	PPO 8	PPO 9	PPO 10	PPO 11	PPO 12	PPO 13	PPO 14	PPO 15	
1	Sakthi	R	0.074	-	-	0.180	-	-	0.265	-	-	0.372	0.393	0.414	-	-	-	6
2	Mukthi	R	0.074	-	-	0.180	-	-	0.265	-	-	0.372	0.393	0.414	-	-	-	6
3	LE-214	MR	0.074	-	-	0.180	-	-	0.265	-	-	0.372	0.393	0.414	-	-	-	6
4	LE-474	R	0.074	-	-	0.180	-	-	0.265	-	-	0.372	0.393	0.414	-	-	-	6
5	LE-415	MR	-	-	-	0.180	-	-	0.265	-	-	0.372	0.393	0.414	-	-	-	5
6	LE-470	MR	-	-	-	0.180	-	-	0.265	-	-	0.372	0.393	0.414	-	-	-	5
7	LE-421	MR	-	-	-	0.180	-	-	0.265	-	-	0.372	0.393	0.414	-	-	-	5
8	LE-457	MS	-	-	-	0.180	-	-	0.265	-	-	0.372	-	-	-	-	-	3
9	BT-1	MS	-	-	-	0.180	-	-	0.265	-	-	0.372	-	-	-	-	-	3
10	LE-455	MS	-	-	-	0.180	-	-	0.265	-	-	0.372	-	-	-	-	-	3
11	LE-526	S	-	-	-	-	0.212	-	-	0.319	-	-	-	0.414	-	-	-	3
12	LE-619	S	0.074	-	-	-	0.212	-	-	0.319	-	-	-	0.414	-	-	-	4
13	LE-615	S	0.074	-	-	-	0.212	-	-	0.319	-	-	-	-	-	-	-	3
14	LE-616	S	-	-	-	-	0.212	-	-	0.319	-	-	-	-	-	-	-	2
15	LE-617	S	-	-	-	-	0.212	-	-	0.319	-	-	-	0.414	-	-	-	3
16	LE-613	S	-	-	-	-	0.212	-	-	0.319	-	-	-	0.414	-	-	-	3
17	LE-614	S	-	-	-	-	-	0.244	-	0.319	-	-	-	0.414	0.446	0.468	-	5
18	LE-618	S	-	-	-	-	-	0.244	-	0.319	-	-	-	0.414	0.446	0.468	-	5
19	BT-101-22	S	-	-	-	-	-	0.244	-	0.319	-	-	-	0.414	0.446	0.468	-	5
20	CO-1	S	-	-	-	-	-	0.244	-	0.319	-	-	-	0.414	-	-	-	3
21	CO-3	S	-	-	-	-	0.212	-	-	-	-	-	0.393	0.414	0.446	0.468	-	5
22	Pant T_1	S	-	-	-	-	0.212	-	-	-	-	-	0.393	0.414	0.446	0.468	-	5
23	Pant T_3	S	-	0.159	-	-	0.212	-	-	-	-	-	0.393	0.414	0.446	0.468	-	6
24	Pusa Ruby	S	-	0.159	-	-	0.212	-	-	-	-	-	0.393	0.414	0.446	0.468	-	6

DR - Disease Reaction; R - Resistant; MR - Moderately Resistant; MS - Moderately Susceptible; S - Susceptible

Fig.1. Zymogram of polyphenol oxidase in tomato roots at 45 days

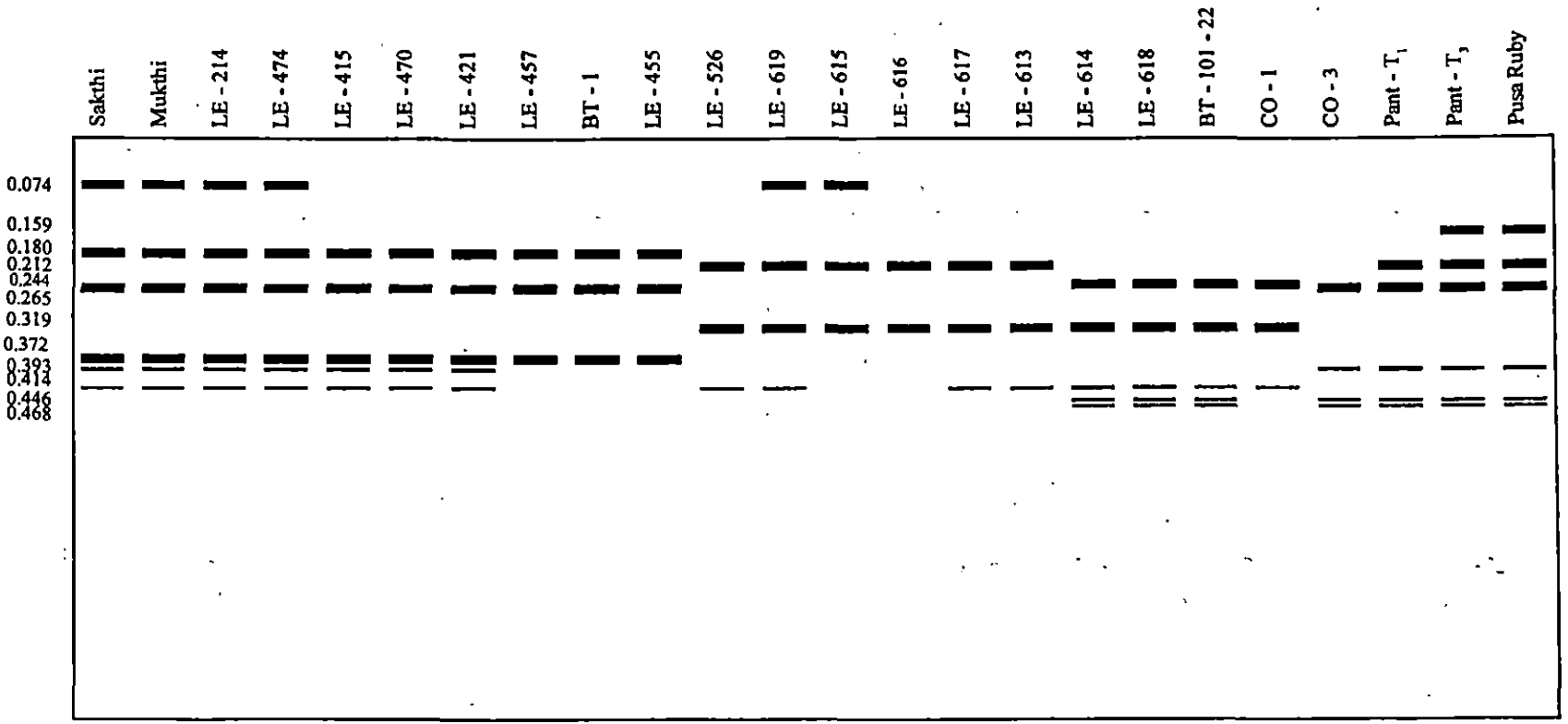


Table 7. Rm value of different bands of polyphenol oxidase in 60 days old roots of tomato

Sl. No.	Variety	D.R.	Rm														Total No.of bands
			Banding from origin														
			PPO 1	PPO 2	PPO 3	PPO 4	PPO 5	PPO 6	PPO 7	PPO 8	PPO 9	PPO 10	PPO 11	PPO 12	PPO 13	PPO 14	PPO 15	
1	Sakthi	R	-	-	-	0.180	0.212	-	-	-	0.351	0.372	0.393	-	-	-	-	5
2	Mukthi	R	-	-	-	0.180	0.212	-	-	-	0.351	0.372	0.393	-	-	-	-	5
3	LE-214	MR	-	-	-	0.180	0.212	-	-	-	0.351	0.372	0.393	-	-	-	-	5
4	LE-474	R	-	-	-	0.180	0.212	-	-	-	0.351	0.372	0.393	-	-	-	-	5
5	LE-415	MR	-	-	-	0.180	0.212	-	-	-	0.351	0.372	0.393	-	-	-	-	5
6	LE-470	MR	-	-	-	0.180	0.212	-	-	-	0.351	0.372	0.393	-	-	-	-	5
7	LE-421	MR	-	0.106	-	0.180	0.212	-	-	-	0.351	-	-	-	-	-	-	4
8	LE-457	MS	-	-	-	0.180	0.212	-	-	-	0.351	0.372	0.393	-	-	-	-	5
9	BT-1	MS	-	-	-	0.180	0.212	-	-	-	0.351	0.372	0.393	-	-	-	-	5
10	LE-455	MS	-	-	-	0.180	0.212	-	-	-	0.351	0.372	0.393	-	-	-	-	5
11	LE-526	S	-	-	-	-	0.212	-	0.265	0.319	-	-	-	-	0.446	0.468	0.50	6
12	LE-619	S	-	-	-	-	0.212	-	0.265	0.319	-	-	-	-	0.446	0.468	0.50	6
13	LE-615	S	-	-	-	-	0.212	-	0.265	0.319	-	-	-	-	0.446	0.468	0.50	6
14	LE-616	S	-	-	-	-	0.212	-	0.265	0.319	-	-	-	-	0.446	0.468	0.50	6
15	LE-617	S	-	-	-	-	0.212	-	0.265	0.319	-	-	-	-	0.446	0.468	0.50	6
16	LE-613	S	-	-	-	-	-	-	0.265	0.319	-	-	-	-	-	0.468	-	3
17	LE-614	S	-	-	-	-	0.212	-	0.265	0.319	-	-	-	-	-	0.468	-	4
18	LE-618	S	-	-	-	-	-	-	-	0.319	-	-	-	-	0.446	-	-	2
19	BT-101-22	S	-	-	-	-	-	-	0.265	0.319	-	-	-	-	-	0.468	-	3
20	CO-1	S	-	-	-	-	0.212	-	0.265	0.319	-	-	-	-	0.446	0.468	0.50	6
21	CO-3	S	-	-	-	-	0.212	-	0.265	-	-	0.372	0.393	-	0.446	-	-	5
22	Pant T_1	S	-	-	-	-	0.212	-	0.265	-	-	0.372	0.393	-	0.446	-	-	5
23	Pant T_3	S	-	-	-	-	0.212	-	0.265	-	-	0.372	0.393	-	0.446	-	-	5
24	Pusa Ruby	S	-	-	-	-	0.212	-	0.265	-	-	0.372	0.393	-	0.446	-	-	5

DR - Disease Reaction; R - Resistant; MR - Moderately Resistant; MS - Moderately Susceptible; S - Susceptible

Fig. 2. Zymogram of polyphenol oxidase in tomato roots at 60 days

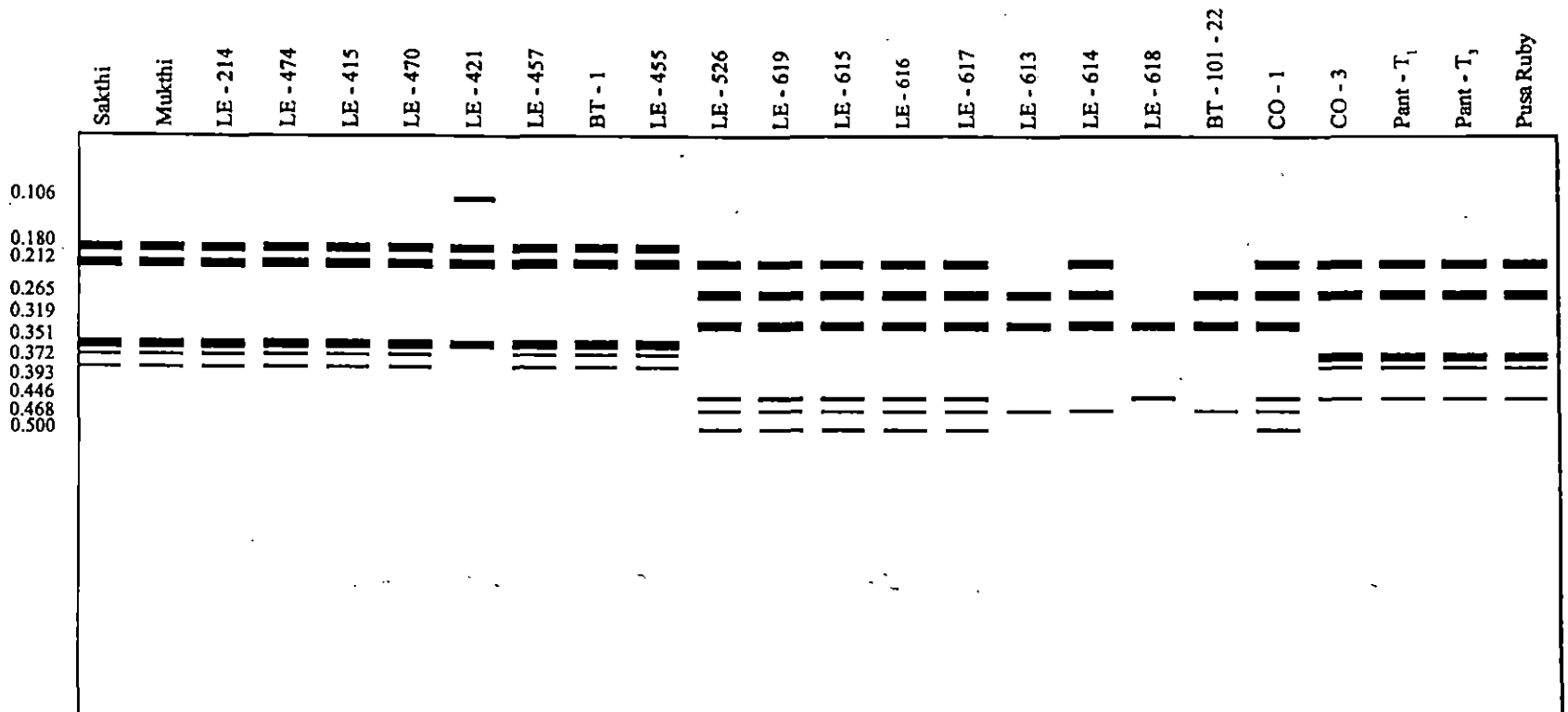


Plate III. Polyphenol oxidase banding pattern in tomato roots at 45 days

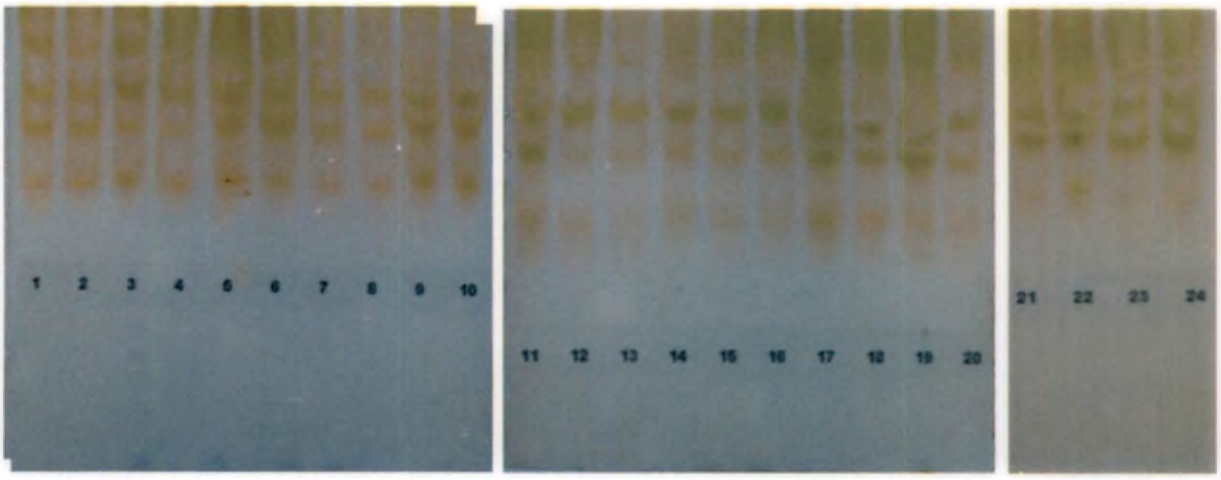
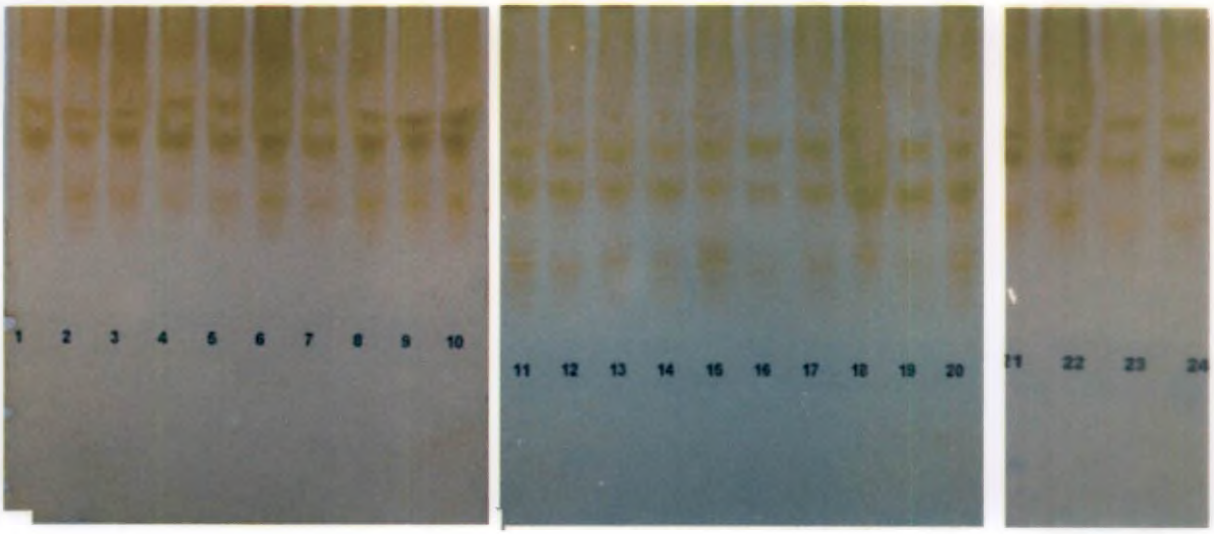


Plate IV. Polyphenol oxidase banding pattern in tomato roots at 60 days



Legend : 1-Sakthi, 2-Mukthi, 3-LE-214, 4-LE-474, 5-LE-415, 6-LE-470, 7-LE-421, 8-LE-457, 9-BT-1, 10- LE-455, 11-LE-526, 12-LE-619, 13-LE-615, 14- LE-616, 15- LE-617, 16- LE-613, 17- LE-614, 18-LE-618, 19- BT-101-22, 20-CO-1, 21-CO-3, 22-Pant-T_1 , 23-Pant-T_3, 24-Pusa Ruby.

Leaf sample

Nine bands were resolved for leaf samples. The most densely stained bands were observed in 45 days old leaf sample (Table 8, Fig.3 and Plate 5). The isozyme band PPO-6 (Rm=0.372) was sharp in case of resistant, moderately resistant and moderately susceptible varieties which include Sakthi, Mukthi, LE-214, LE-474, LE-415, LE-470, LE-421, LE-457, BT-1 and LE-455 respectively. However, in other varieties this band was broader and dispersed. The isozyme band PPO-6 (Rm=0.372) was followed by a singlet PPO-7 (Rm=0.394) in ten varieties. The protein bands like PPO-1, PPO-2, PPO-4, PPO-7 (Rm values 0.159, 0.180, 0.265 and 0.394 respectively) were common among resistant, moderately resistant and moderately susceptible genotypes. PPO-3 (Rm=0.212), PPO-5 (Rm=0.319) and PPO-8 (Rm=0.468) were found only among susceptible genotypes (Table 8). Among these, PPO-8 (Rm=0.498) was present predominantly in susceptible genotypes. The less variant form PPO-9 (Rm=0.489) was present only in a single genotype, LE-618. The slow moving bands like PPO-1 to PPO-4 bands and fast moving bands like PPO-7 to PPO-9 were light and feeble. Most of the resistant and moderately resistant genotypes had a doublet of protein bands at PPO-6 and PPO-7 with Rm values 0.372 and 0.394 respectively.

In 60 days old leaf samples PPO-6 (Rm=0.372) was common for all varieties (Table 9). This band was dense and thick compared to other bands. Polyphenol oxidase activity in leaf samples at 60 days was low and there was only seven bands compared to nine bands in 45 days old leaf samples. PPO-2 (Rm=0.180) was present only in moderately resistant and moderately susceptible varieties like LE-470, LE-421, LE-457, BT-1 and LE-455. PPO-7 (Rm=0.394) was seen among resistant, moderately resistant and moderately susceptible genotypes. The same protein band was not seen in susceptible genotypes. Isozyme bands like PPO-2, PPO-3, PPO-5 and PPO-8 with Rm values 0.212, 0.319 and 0.468 were

Table 8. Rm value of different bands of polyphenol oxidase in 45 days old leaves of tomato

Sl. No.	Variety	D.R.	Rm									Total No.of bands
			Banding from origin									
			PPO 1	PPO 2	PPO 3	PPO 4	PPO 5	PPO 6	PPO 7	PPO 8	PPO 9	
1	Sakthi	R	0.159	0.180	-	0.265	-	0.372	0.394	-	-	5
2	Mukthi	R	0.159	0.180	-	0.265	-	0.372	0.394	-	-	5
3	LE-214	MR	0.159	0.180	-	0.265	-	0.372	0.394	-	-	5
4	LE-474	R	0.159	0.180	-	0.265	-	0.372	0.394	-	-	5
5	LE-415	MR	0.159	0.180	-	0.265	-	0.372	0.394	-	-	5
6	LE-470	MR	0.159	-	-	0.265	-	0.372	0.394	-	-	4
7	LE-421	MR	0.159	0.180	-	0.265	-	0.372	0.394	-	-	5
8	LE-457	MS	0.159	0.180	-	0.265	-	0.372	0.394	-	-	5
9	BT-1	MS	0.159	0.180	-	0.265	-	0.372	0.394	-	-	5
10	LE-455	MS	-	0.180	-	0.265	-	0.372	0.394	-	-	4
11	LE-526	S	-	-	-	-	0.319	0.372	-	0.468	-	3
12	LE-619	S	-	-	-	-	0.319	0.372	-	-	-	2
13	LE-615	S	-	-	0.212	-	0.319	0.372	-	0.468	-	4
14	LE-616	S	-	-	0.212	-	0.319	0.372	-	0.468	-	4
15	LE-617	S	-	-	0.212	-	0.319	0.372	-	0.468	-	4
16	LE-613	S	-	-	0.212	-	0.319	0.372	-	0.468	-	4
17	LE-614	S	-	-	0.212	-	0.319	0.372	-	0.468	-	4
18	LE-618	S	-	-	0.212	-	0.319	0.372	-	0.468	0.489	5
19	BT-101-22	S	-	-	-	-	0.319	0.372	-	0.468	-	3
20	CO-1	S	-	-	0.212	-	0.319	0.372	-	0.468	-	4
21	CO-3	S	-	-	-	0.265	-	0.372	-	0.468	-	3
22	Pant T_1	S	-	-	-	0.265	-	0.372	-	0.468	-	3
23	Pant T_3	S	-	-	-	0.265	-	0.372	-	0.468	-	3
24	Pusa Ruby	S	-	-	0.212	0.265	-	0.372	-	0.468	-	4

DR - Disease Reaction; R - Resistant; MR - Moderately Resistant; MS - Moderately Susceptible; S - Susceptible

Fig. 3. Zymogram of polyphenol oxidase in tomato leaves at 45 days

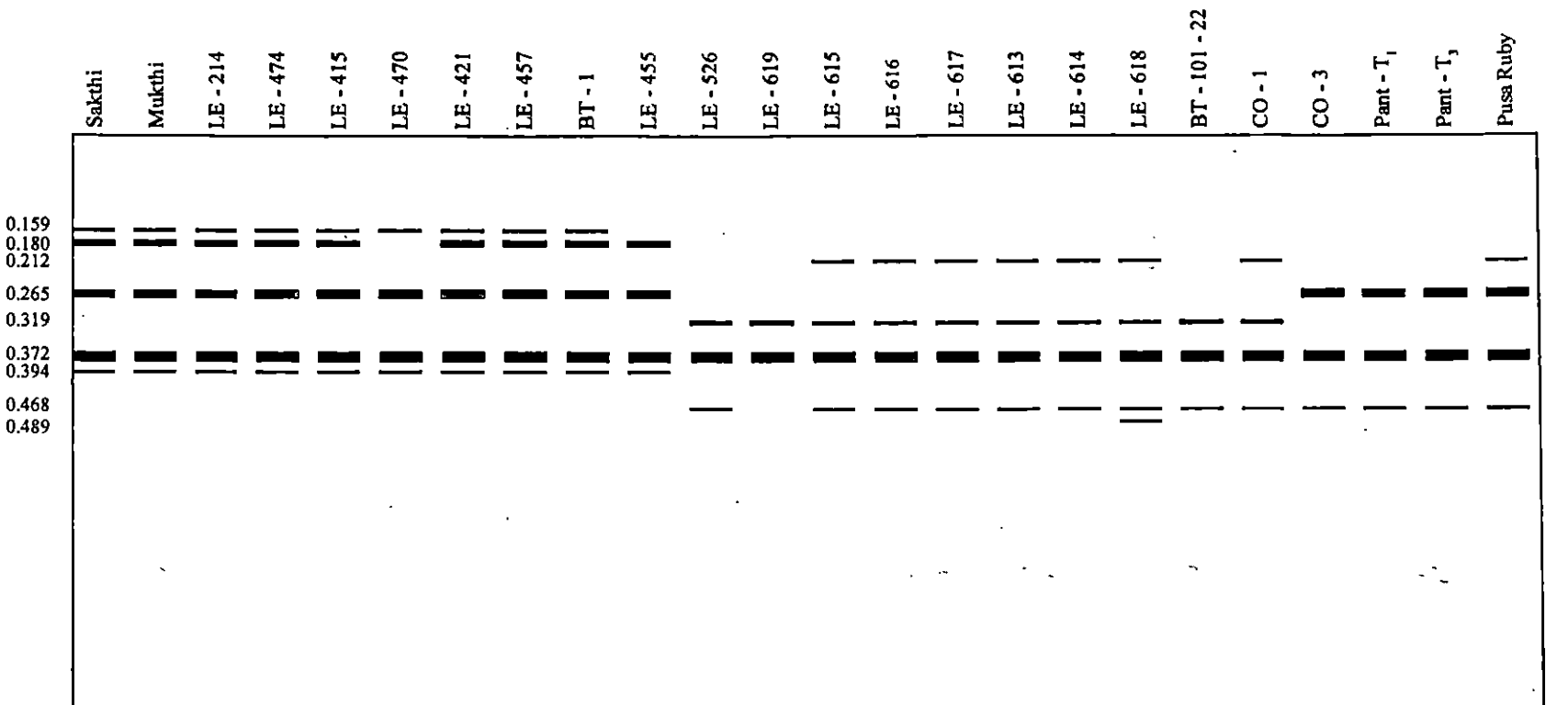


Table 9. Rm value of different bands of polyphenol oxidase in 60 days old leaves of tomato

Sl. No.	Variety	D.R.	Rm									Total No.of bands
			Banding from origin									
			PPO 1	PPO 2	PPO 3	PPO 4	PPO 5	PPO 6	PPO 7	PPO 8	PPO 9	
1	Sakthi	R	-	-	-	0.265	-	0.372	0.394	-	-	3
2	Mukthi	R	-	-	-	-	-	0.372	0.394	-	-	2
3	LE-214	MR	-	-	-	0.265	-	0.372	0.394	-	-	3
4	LE-474	R	-	-	-	0.265	-	0.372	0.394	-	-	3
5	LE-415	MR	-	-	-	0.265	-	0.372	0.394	-	-	3
6	LE-470	MR	-	0.180	-	0.265	-	0.372	0.394	-	-	4
7	LE-421	MR	-	0.180	-	0.265	-	0.372	0.394	-	-	4
8	LE-457	MS	-	0.180	-	0.265	-	0.372	0.394	-	-	4
9	BT-1	MS	-	0.180	-	0.265	-	0.372	0.394	-	-	4
10	LE-455	MS	-	0.180	-	0.265	-	0.372	0.394	-	-	4
11	LE-526	S	-	-	-	-	0.319	0.372	-	0.468	-	3
12	LE-619	S	-	-	0.212	-	0.319	0.372	-	-	-	3
13	LE-615	S	-	-	-	-	0.319	0.372	-	0.468	-	3
14	LE-616	S	-	-	0.212	-	0.319	0.372	-	0.468	-	4
15	LE-617	S	-	-	0.212	-	0.319	0.372	-	0.468	-	4
16	LE-613	S	-	-	0.212	-	0.319	0.372	-	-	-	3
17	LE-614	S	-	-	0.212	-	0.319	0.372	-	-	-	3
18	LE-618	S	-	-	0.212	-	0.319	0.372	-	0.468	-	4
19	BT-101-22	S	-	-	-	-	0.319	0.372	-	0.468	-	3
20	CO-1	S	-	-	0.212	-	0.319	0.372	-	0.468	-	4
21	CO-3	S	-	-	-	0.265	-	0.372	0.394	-	-	3
22	Pant T_1	S	-	-	-	0.265	-	0.372	0.394	-	-	3
23	Pant T_3	S	-	-	-	0.265	-	0.372	0.394	-	-	3
24	Pusa Ruby	S	-	-	-	0.265	-	0.372	0.394	-	-	3

DR - Disease Reaction; R - Resistant; MR - Moderately Resistant; MS - Moderately Susceptible; S - Susceptible

Fig. 4. Zymogram of polyphenol oxidase in tomato leaves at 60 days

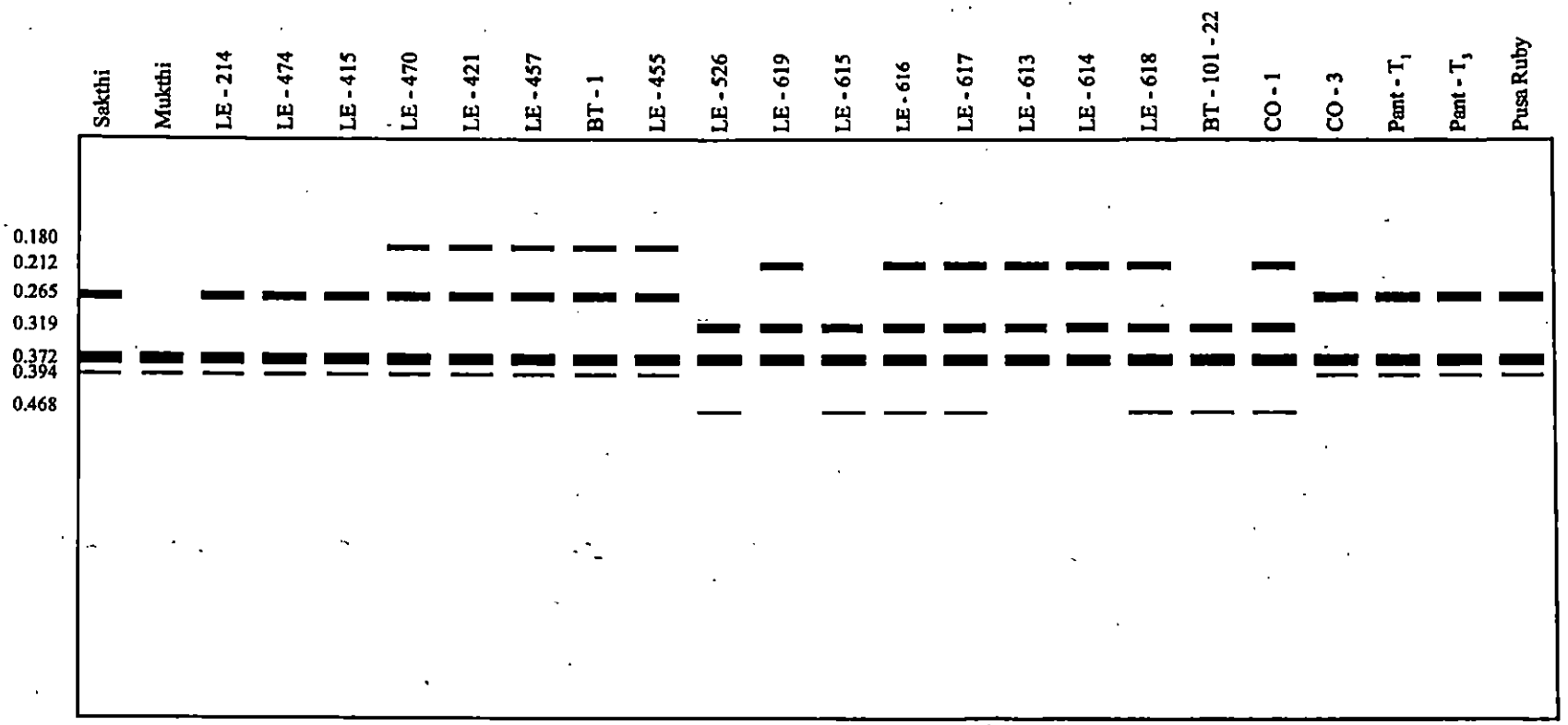


Plate V. Polyphenol oxidase banding pattern in tomato leaves at 45 days

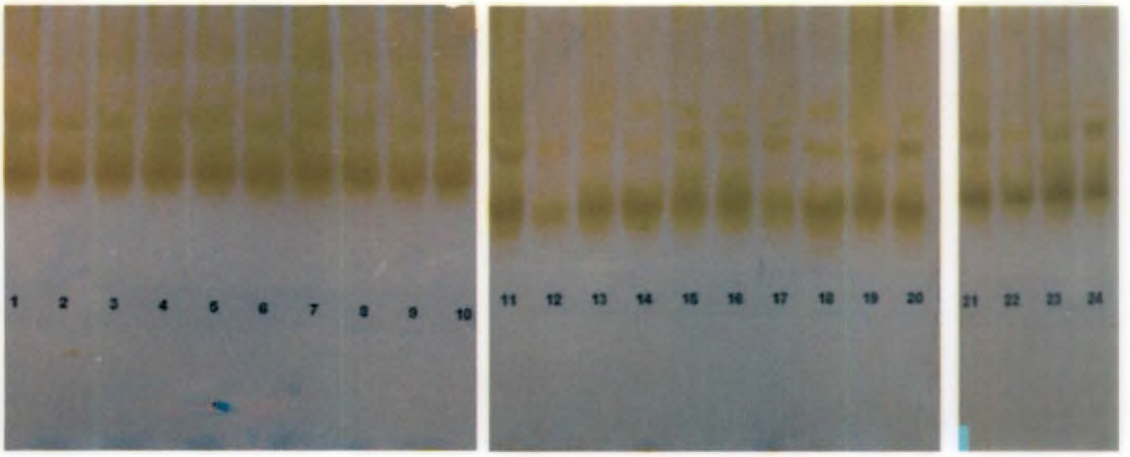
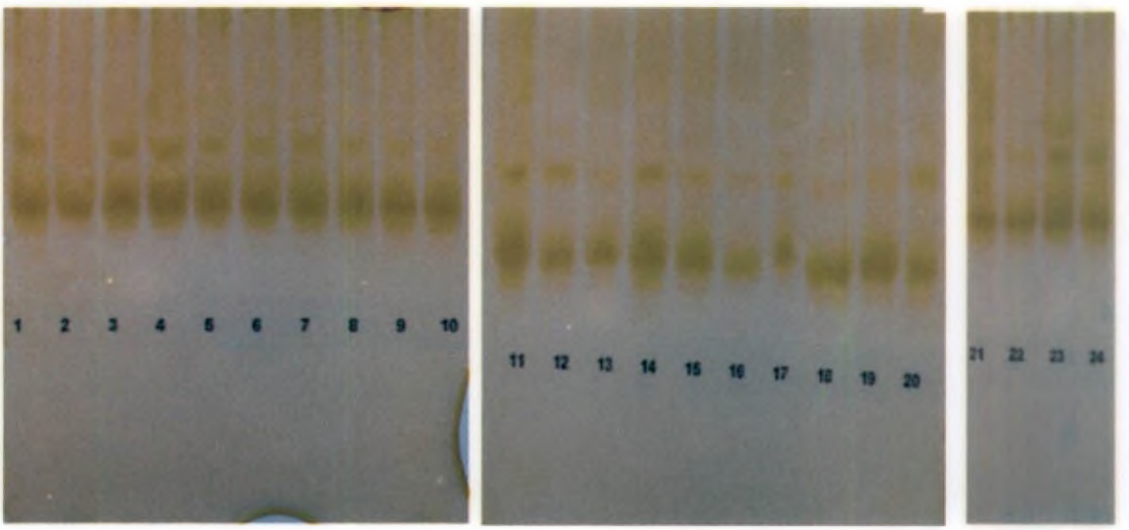


Plate VI. Polyphenol oxidase banding pattern in tomato leaves at 60 days



Legend : 1-Sakthi, 2-Mukthi, 3-LE-214, 4-LE-474, 5-LE-415, 6-LE-470, 7-LE-421, 8-LE-457, 9-BT-1, 10- LE-455, 11-LE-526, 12-LE-619, 13-LE-615, 14- LE-616, 15- LE-617, 16- LE-613, 17- LE-614, 18-LE-618, 19- BT-101-22, 20-CO-1, 21-CO-3, 22-Pant-T_1 , 23-Pant-T_2, 24-Pusa Ruby.

present only in resistant varieties. The set of isozyme bands with intermediate mobility in PPO-6 and 7 region formed a doublet in resistant, moderately resistant and moderately susceptible genotypes and were absent in highly susceptible genotypes (Fig.4 and Plate 6).

4.3.2 Peroxidase

Root samples

In roots, peroxidase activity was more and 13 bands were resolved. The bands were clear, thin and sharp in most of the varieties analysed.

In 45 days old root samples, PRX-3 (Rm=0.191) and PRX-11 (Rm=0.404) were present in all varieties, with exceptions that LE-474 had only PRX-3 while LE-421 had only PRX-11. PRX-2 (Rm=0.106) was absent in BT-101-22, CO-3, Pant T_1, Pant T_3 and Pusa Ruby. Similarly PRX-4 (Rm=0.223) was absent in LE-526, LE-619, LE-615 and LE-617 respectively. PRX-3 (Rm=0.191), PRX-4 (Rm=0.223) and PRX-11 (Rm=0.191) were intense and dark (Table 10, Fig. 5 and Plate 7).

PRX-7 (Rm=0.287) was present only in highly resistant, moderately resistant and moderately susceptible varieties. PRX-8 (Rm=0.329) was present only in resistant and moderately resistant varieties like Sakthi, Mukthi, LE-214, LE-474, LE-415, LE-470, and LE-421 respectively with an exception to LE-457.

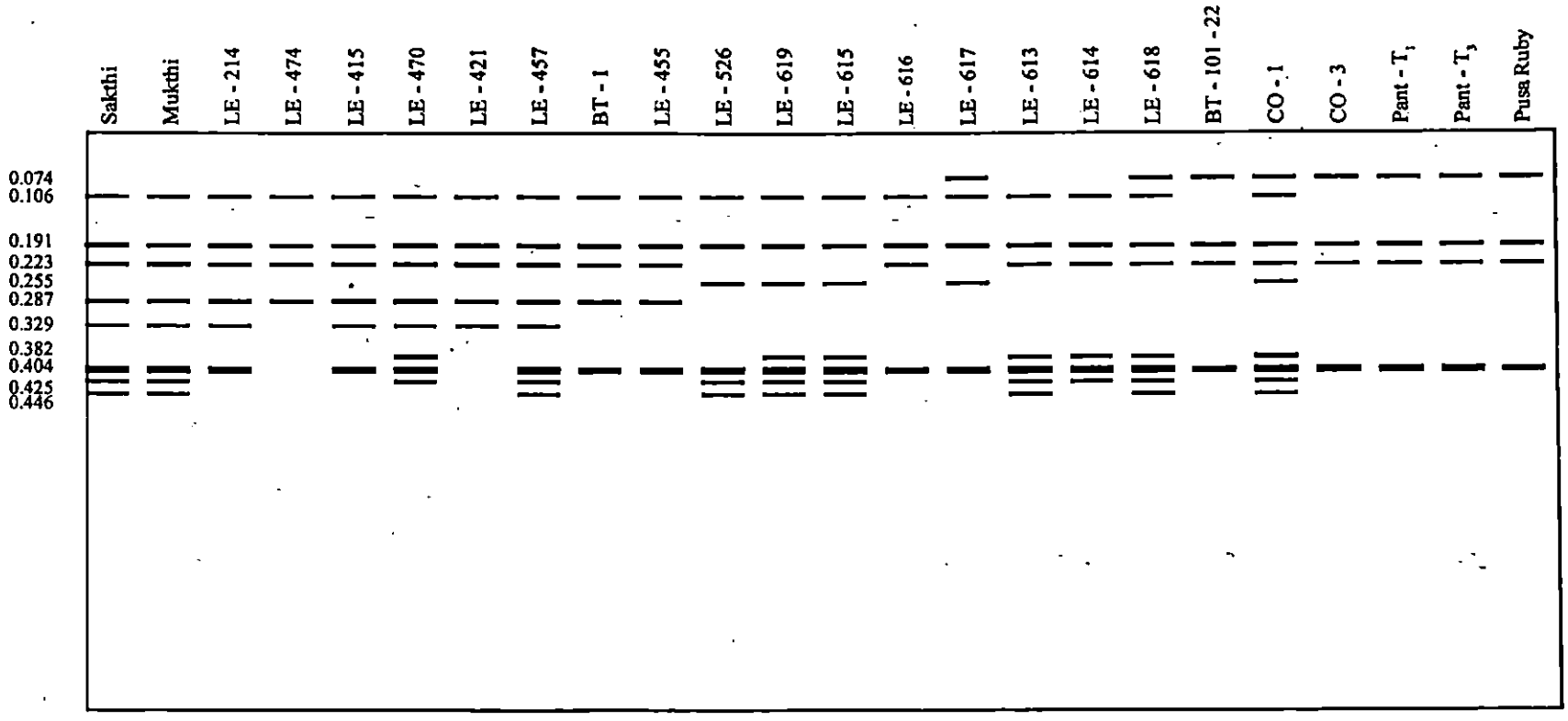
Other isozyme bands like PRX-1, PRX-5, PRX-6, PRX-10 and PRX-12 were variably distributed among different genotypes. PRX-9 (Rm=0.340) and PRX-5 (Rm=0.244) were absent in 45 days old root samples. The presence of doublet of bands with Rm 0.404, 0.425 was observed in 12 of the 24 genotypes and triplet of bands with Rm 0.404, 0.425 and 0.446 was observed in 10 of the genotypes analysed.

Table 10. Rm value of different bands of peroxidase in 45 days old roots of tomato

Sl. No.	Variety	D.R.	Rm													Total No.of bands
			Banding from origin													
			PRX-1	PRX-2	PRX-3	PRX-4	PRX-5	PRX-6	PRX-7	PRX-8	PRX-9	PRX-10	PRX-11	PRX-12	PRX-13	
1	Sakthi	R	-	0.106	0.191	0.223	-	-	0.287	0.329	-	-	0.404	0.425	0.446	8
2	Mukthi	R	-	0.106	0.191	0.223	-	-	0.287	0.329	-	-	0.404	0.425	0.446	8
3	LE-214	MR	-	0.106	0.191	0.223	-	-	0.287	0.329	-	-	0.404	-	-	6
4	LE-474	R	-	0.106	0.191	0.223	-	-	0.287	-	-	-	-	-	-	4
5	LE-415	MR	-	0.106	0.191	0.223	-	-	0.287	0.329	-	-	0.404	-	-	6
6	LE-470	MR	-	0.106	0.191	0.223	-	-	0.287	0.329	-	0.382	0.404	0.425	-	8
7	LE-421	MR	-	0.106	0.191	0.223	-	-	0.287	0.329	-	-	-	-	-	5
8	LE-457	MS	-	0.106	0.191	0.223	-	-	0.287	0.329	-	-	0.404	0.425	0.446	8
9	BT-1	MS	-	0.106	0.191	0.223	-	-	0.287	-	-	-	0.404	-	-	5
10	LE-455	MS	-	0.106	0.191	0.223	-	-	0.287	-	-	-	0.404	-	-	5
11	LE-526	S	-	0.106	0.191	-	-	0.255	-	-	-	-	0.404	0.425	0.446	6
12	LE-619	S	-	0.106	0.191	-	-	0.255	-	-	-	0.382	0.404	0.425	0.446	7
13	LE-615	S	-	0.106	0.191	-	-	0.255	-	-	-	0.382	0.404	0.425	0.446	7
14	LE-616	S	-	0.106	0.191	0.223	-	-	-	-	-	-	0.404	-	-	4
15	LE-617	S	0.074	0.106	0.191	-	-	0.255	-	-	-	-	0.404	-	-	5
16	LE-613	S	-	0.106	0.191	0.223	-	-	-	-	-	0.382	0.404	0.425	0.446	7
17	LE-614	S	-	0.106	0.191	0.223	-	-	-	-	-	0.382	0.404	0.425	-	6
18	LE-618	S	0.074	0.106	0.191	0.223	-	-	-	-	-	0.382	0.404	0.425	0.446	8
19	BT-101-22	S	0.074	-	0.191	0.223	-	-	-	-	-	-	0.404	-	-	4
20	CO-1	S	0.074	0.106	0.191	0.223	-	0.255	-	-	-	0.382	0.404	0.425	0.446	9
21	CO-3	S	0.074	-	0.191	0.223	-	-	-	-	-	-	0.404	-	-	4
22	Pant T_1	S	0.074	-	0.191	0.223	-	-	-	-	-	-	0.404	-	-	4
23	Pant T_3	S	0.074	-	0.191	0.223	-	-	-	-	-	-	0.404	-	-	4
24	Pusa Ruby	S	0.074	-	0.191	0.223	-	-	-	-	-	-	0.404	-	-	4

DR - Disease Reaction; R - Resistant; MR - Moderately Resistant; MS - Moderately Susceptible; S - Susceptible

Fig. 5. Zymogram of peroxidase in tomato roots at 45 days



In 60 day old roots, the protein bands such as PRX-2, PRX-5, PRX-9 and PRX-11 (Rm values 0.106, 0.244, 0.340 and 0.404) were present in all cultivars. While the protein bands like PRX-1, PRX-3, PRX-6, PRX-7 and PRX-8 (with Rm values 0.075, 0.191, 0.255, 0.287 and 0.329 respectively) were absent (Table 11, Fig.6 and Plate 8). The bands PRX-5, PRX-9 and PRX-11 (Rm values 0.244, 0.340 and 0.404 respectively) were dense and prominent. PRX-4 (Rm=0.223) was less frequent and found only in LE-526. PRX-10, PRX-12, PRX-13 (Rm value 0.382, 0.425, 0.446 respectively) were absent CO-1, CO-3, Pant T_1, Pant T_3 and Pusa Ruby.

In 60 days old root samples there was no significant difference in the banding pattern of resistant and moderately resistant varieties. Doublets and Triplets of bands were common in most of the varieties analysed.

Leaf samples

In leaf samples a total of eight different peroxidase bands were obtained both at 45^{th} day and 60^{th} day. Leaf samples at 45 days had six bands. Two bands, viz. PRX-3 and PRX-4 were absent in 45 days old samples.

The bands PRX-2 (Rm=0.212) and PRX-6 (Rm=0.329) were found in 45 days old leaf samples of all varieties. The protein band PRX-6 (Rm=0.329) was dense in all varieties (Table 12, Fig.7 and Plate 9). PRX-6 in the case of Sakthi, LE-214, LE-470 and LE-421 were broader than other varieties. PRX-1 (Rm=0.160) was found in Sakthi (R), Mukthi (R), LE-214 (MR), LE-474 (R), LE-415 (MR), LE-470 (MR), LE-421 (MR), LE-457 (MS), BT-1 (MS) and LE-455 (MS).

Varieties belonging to both resistant and moderately resistant groups, viz. Sakthi, Mukthi, LE-474, LE-214, LE-415, LE-470 and LE-421 had a protein

Table 11. Rm value of different bands of peroxidase in 60 days old roots of tomato

Sl. No.	Variety	D.R.	Rm													Total No.of bands
			Banding from origin													
			PRX-1	PRX-2	PRX-3	PRX-4	PRX-5	PRX-6	PRX-7	PRX-8	PRX-9	PRX-10	PRX-11	PRX-12	PRX-13	
1	Sakthi	R	-	0.106	-	-	0.244	-	-	-	0.340	0.382	0.404	0.425	0.446	7
2	Mukthi	R	-	0.106	-	-	0.244	-	-	-	0.340	0.382	0.404	0.425	-	5
3	LE-214	MR	-	0.106	-	-	0.244	-	-	-	0.340	0.382	0.404	0.425	0.446	7
4	LE-474	R	-	0.106	-	-	0.244	-	-	-	0.340	0.382	0.404	0.425	-	6
5	LE-415	MR	-	0.106	-	-	0.244	-	-	-	0.340	0.382	0.404	0.425	-	6
6	LE-470	MR	-	0.106	-	-	0.244	-	-	-	0.340	0.382	0.404	0.425	0.446	7
7	LE-421	MR	-	0.106	-	-	0.244	-	-	-	0.340	0.382	0.404	0.425	-	6
8	LE-457	MS	-	0.106	-	-	0.244	-	-	-	0.340	0.382	0.404	0.425	0.446	7
9	BT-1	MS	-	0.106	-	-	0.244	-	-	-	0.340	0.382	0.404	0.425	0.446	7
10	LE-455	MS	-	0.106	-	-	0.244	-	-	-	0.340	0.382	0.404	0.425	0.446	7
11	LE-526	S	-	0.106	-	0.223	0.244	-	-	-	0.340	-	0.404	-	-	5
12	LE-619	S	-	0.106	-	-	0.244	-	-	-	0.340	0.382	0.404	0.425	0.446	7
13	LE-615	S	-	0.106	-	-	0.244	-	-	-	0.340	0.382	0.404	0.425	0.446	7
14	LE-616	S	-	0.106	-	-	0.244	-	-	-	0.340	0.382	0.404	0.425	0.446	7
15	LE-617	S	-	0.106	-	-	0.244	-	-	-	0.340	0.382	0.404	0.425	0.446	7
16	LE-613	S	-	0.106	-	-	0.244	-	-	-	0.340	0.382	0.404	0.425	-	6
17	LE-614	S	-	0.106	-	-	0.244	-	-	-	0.340	0.382	0.404	0.425	0.446	7
18	LE-618	S	-	0.106	-	-	0.244	-	-	-	0.340	0.382	0.404	0.425	0.446	7
19	BT-101-22	S	-	0.106	-	-	0.244	-	-	-	0.340	0.382	0.404	0.425	0.446	7
20	CO-1	S	-	0.106	-	-	0.244	-	-	-	0.340	-	0.404	0.425	0.446	6
21	CO-3	S	-	0.106	-	-	0.244	-	-	-	0.340	-	0.404	-	-	4
22	Pant T_1	S	-	0.106	-	-	0.244	-	-	-	0.340	-	0.404	-	-	4
23	Pant T_3	S	-	0.106	-	-	0.244	-	-	-	0.340	-	0.404	-	-	4
24	Pusa Ruby	S	-	0.106	-	-	0.244	-	-	-	0.340	-	0.404	-	-	4

DR - Disease Reaction; R - Resistant; MR - Moderately Resistant; MS - Moderately Susceptible; S - Susceptible

Fig. 6. Zymogram of peroxidase in tomato roots at 60 days

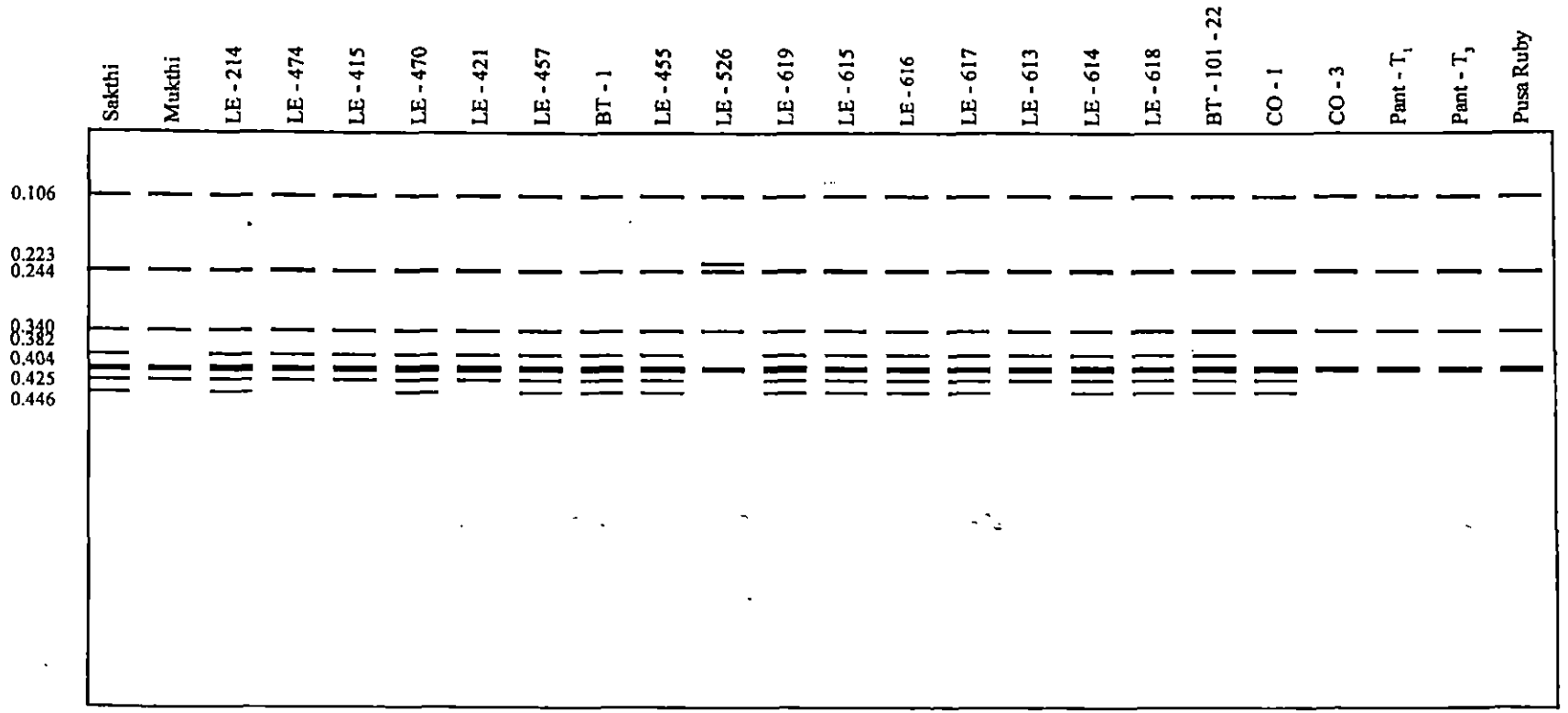


Plate VII. Peroxidase banding pattern in tomato roots at 45 days

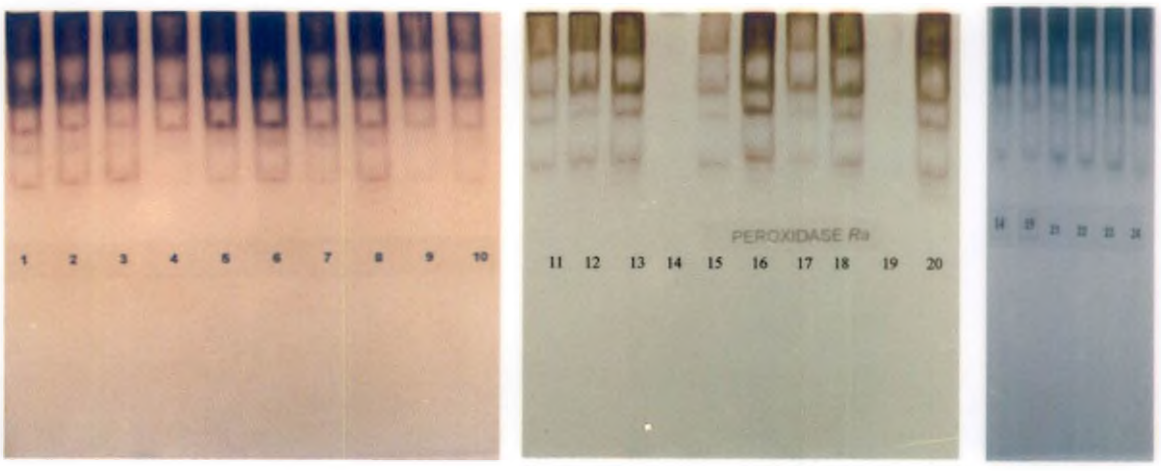
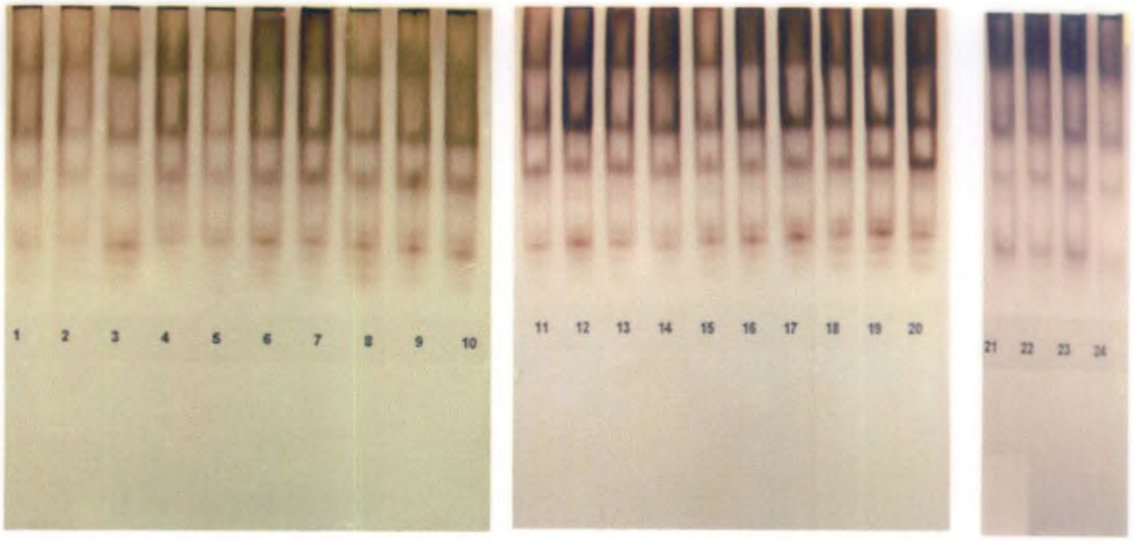


Plate VIII. Peroxidase banding pattern in tomato roots at 60 days



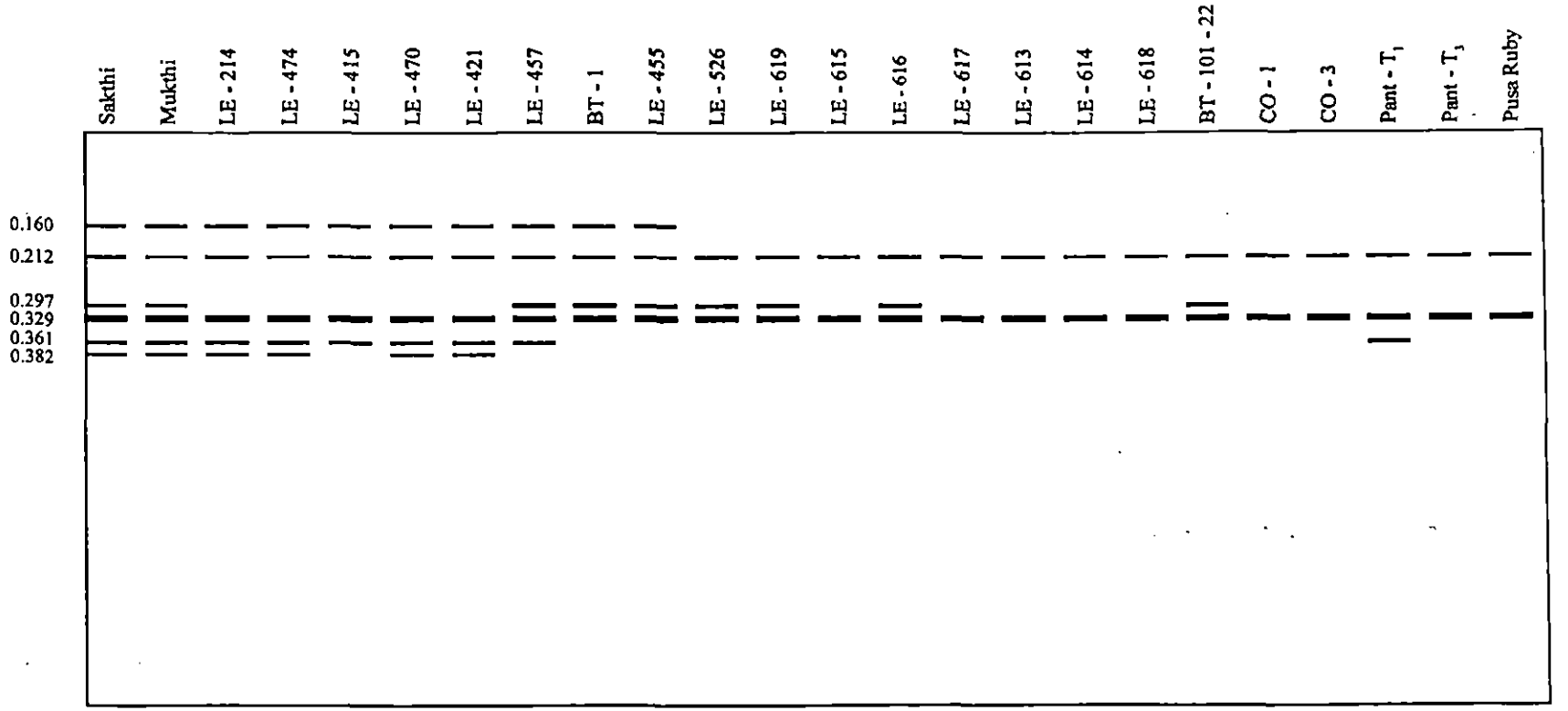
Legend : 1-Sakthi, 2-Mukthi, 3-LE-214, 4-LE-474, 5-LE-415, 6-LE-470, 7-LE-421, 8-LE-457, 9-BT-1, 10- LE-455, 11-LE-526, 12-LE-619, 13-LE-615, 14- LE-616, 15- LE-617, 16- LE-613, 17- LE-614, 18-LE-618, 19- BT-101-22, 20-CO-1, 21-CO-3, 22-Pant-T_1 , 23-Pant-T_3, 24-Pusa Ruby.

Table 12. Rm value of different bands of peroxidase in 45 days old leaves of tomato

Sl. No.	Variety	D.R.	Rm								Total No.of bands
			Banding from the origin								
			PRX-1	PRX-2	PRX-3	PRX-4	PRX-5	PRX-6	PRX-7	PRX-8	
1	Sakthi	R	0.160	0.212	-	-	0.297	0.329	0.361	0.382	6
2	Mukthi	R	0.160	0.212	-	-	0.297	0.329	0.361	0.382	6
3	LE-214	MR	0.160	0.212	-	-	-	0.329	0.361	0.382	5
4	LE-474	R	0.160	0.212	-	-	-	0.329	0.361	0.382	5
5	LE-415	MR	0.160	0.212	-	-	-	0.329	0.361	-	4
6	LE-470	MR	0.160	0.212	-	-	-	0.329	0.361	0.382	5
7	LE-421	MR	0.160	0.212	-	-	-	0.329	0.361	0.382	5
8	LE-457	MS	0.160	0.212	-	-	0.297	0.329	0.361	-	5
9	BT-1	MS	0.160	0.212	-	-	0.297	0.329	-	-	4
10	LE-455	MS	0.160	0.212	-	-	0.297	0.329	-	-	4
11	LE-526	S	-	0.212	-	-	0.297	0.329	-	-	3
12	LE-619	S	-	0.212	-	-	0.297	0.329	-	-	3
13	LE-615	S	-	0.212	-	-	-	0.329	-	-	2
14	LE-616	S	-	0.212	-	-	0.297	0.329	-	-	3
15	LE-617	S	-	0.212	-	-	-	0.329	-	-	2
16	LE-613	S	-	0.212	-	-	-	0.329	-	-	2
17	LE-614	S	-	0.212	-	-	-	0.329	-	-	2
18	LE-618	S	-	0.212	-	-	-	0.329	-	-	2
19	BT-101-22	S	-	0.212	-	-	0.297	0.329	-	-	3
20	CO-1	S	-	0.212	-	-	-	0.329	-	-	2
21	CO-3	S	-	0.212	-	-	-	0.329	-	-	2
22	Pant T_1	S	-	0.212	-	-	-	0.329	0.361	-	3
23	Pant T_3	S	-	0.212	-	-	-	0.329	-	-	2
24	Pusa Ruby	S	-	0.212	-	-	-	0.329	-	-	2

DR - Disease Reaction; R - Resistant; MR - Moderately Resistant; MS - Moderately Susceptible; S - Susceptible

Fig. 7. Zymogram of peroxidase in tomato leaves at 45 days



band at Rm=0.361. This band (PRX-7) was specific to the above varieties and was not found in moderately susceptible and susceptible varieties. Same was the case with PRX-8 (Rm=0.382) which was present only in resistant and moderately resistant genotypes with exception to LE-415. The protein band PRX-5 (Rm=0.297) was present in both resistant and susceptible cultivars.

In 60 days old leaf samples also a total of 8 bands were resolved. PRX-3 (Rm=0.225) and PRX-6 (Rm=0.329) were present in all varieties irrespective of their disease reaction. PRX-6 was denser and thicker in all varieties except LE-619. PRX-7 (Rm=0.361) was common among all varieties except LE-619, LE-613 and LE-614.

The protein band PRX-5 (Rm=0.297) was found specific to only resistant and moderately resistant varieties. Variant forms like PRX-1 was present in LE-415, LE-470 and LE-457, while PRX-4 was present in LE-474 and LE-415 and PRX-4 in LE 526 and LE-616 (Table 13, Fig.8 and Plate 10).

PRX-2 (Rm=0.212) which was present in all varieties at 45th day were not found in most of the varieties at 60th day. PRX-3 (Rm=0.255) was found in all varieties at 60 days. PRX-7 (Rm=0.329) was invariably present in all varieties at all stages of analysis.

Table 13. Rm value of different bands of peroxidase in 60 days old leaves of tomato

Sl. No.	Variety	D.R.	Rm								Total No.of bands
			Banding from the origin								
			PRX-1	PRX-2	PRX-3	PRX-4	PRX-5	PRX-6	PRX-7	PRX-8	
1	Sakthi	R	-	-	0.255	-	0.297	0.329	0.361	-	4
2	Mukthi	R	-	-	0.255	-	0.297	0.329	0.361	-	4
3	LE-214	MR	-	-	0.255	-	0.297	0.329	0.361	-	4
4	LE-474	R	-	-	0.255	0.276	0.297	0.329	0.361	-	5
5	LE-415	MR	0.160	-	0.255	0.276	0.297	0.329	0.361	-	6
6	LE-470	MR	0.160	-	0.255	-	0.297	0.329	0.361	-	5
7	LE-421	MR	-	-	0.255	-	-	0.329	0.361	-	3
8	LE-457	MS	0.160	-	0.255	-	-	0.329	0.361	-	4
9	BT-1	MS	-	-	0.255	-	-	0.329	0.361	-	3
10	LE-455	MS	-	-	0.255	-	-	0.329	0.361	-	3
11	LE-526	S	-	0.212	0.255	-	-	0.329	0.361	0.382	5
12	LE-619	S	-	0.212	0.255	-	-	0.329	-	-	3
13	LE-615	S	-	0.212	0.255	-	-	0.329	0.361	-	4
14	LE-616	S	-	0.212	0.255	-	-	0.329	0.361	0.382	5
15	LE-617	S	-	0.212	0.255	-	-	0.329	0.361	-	4
16	LE-613	S	-	0.212	0.255	-	-	0.329	-	-	3
17	LE-614	S	-	0.212	0.255	-	-	0.329	-	-	3
18	LE-618	S	-	0.212	0.255	-	-	0.329	0.361	-	4
19	BT-101-22	S	-	0.212	0.255	-	-	0.329	0.361	-	4
20	CO-1	S	-	0.212	0.255	-	-	0.329	0.361	-	4
21	CO-3	S	-	-	0.255	-	-	0.329	0.361	-	3
22	Pant T_1	S	-	-	0.255	-	-	0.329	0.361	-	3
23	Pant T_3	S	-	-	0.255	-	-	0.329	0.361	-	3
24	Pusa Ruby	S	-	-	0.255	-	-	0.329	0.361	-	3

DR - Disease Reaction; R - Resistant; MR - Moderately Resistant; MS - Moderately Susceptible; S - Susceptible

Fig. 8. Zymogram of peroxidase in tomato leaves at 60 days

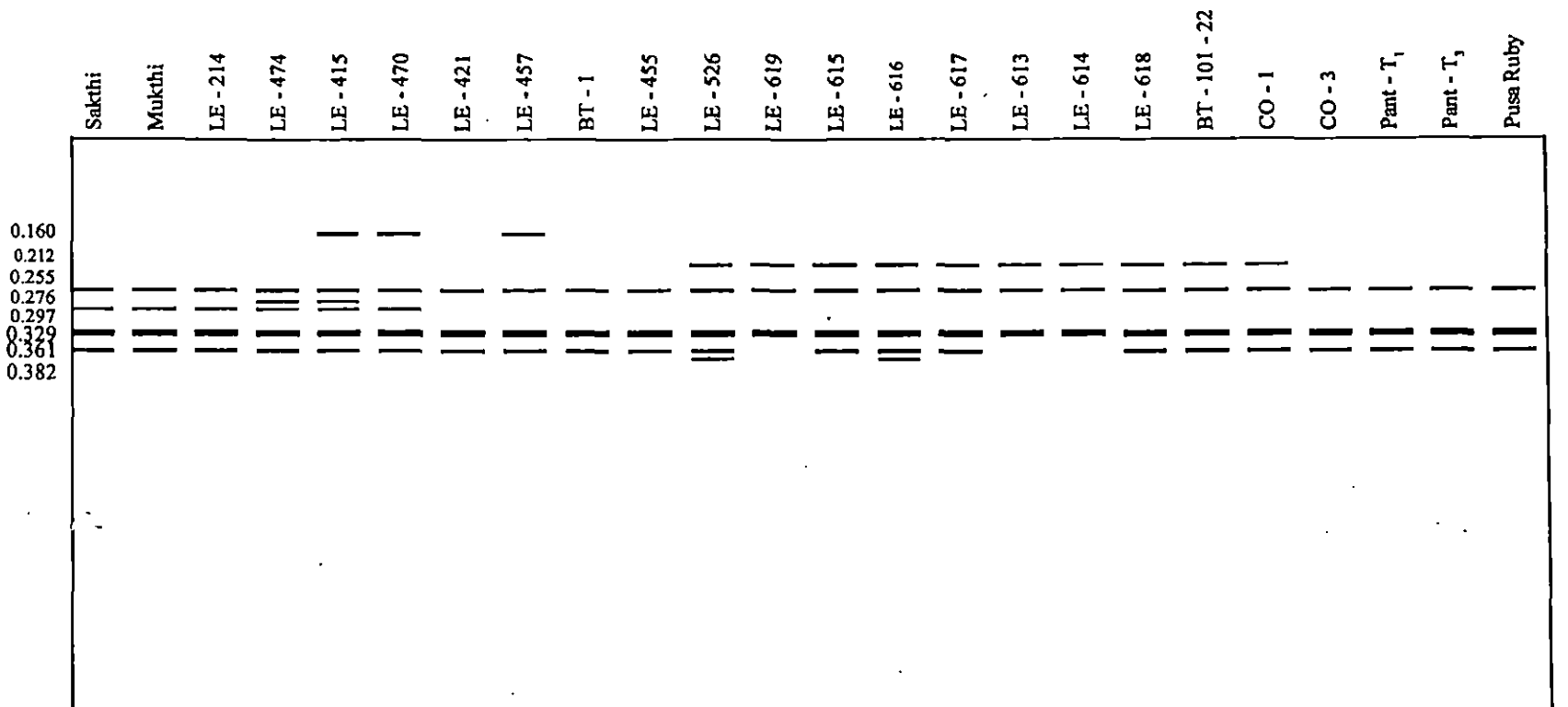


Plate IX. Peroxidase banding pattern in tomato leaves at 45 days

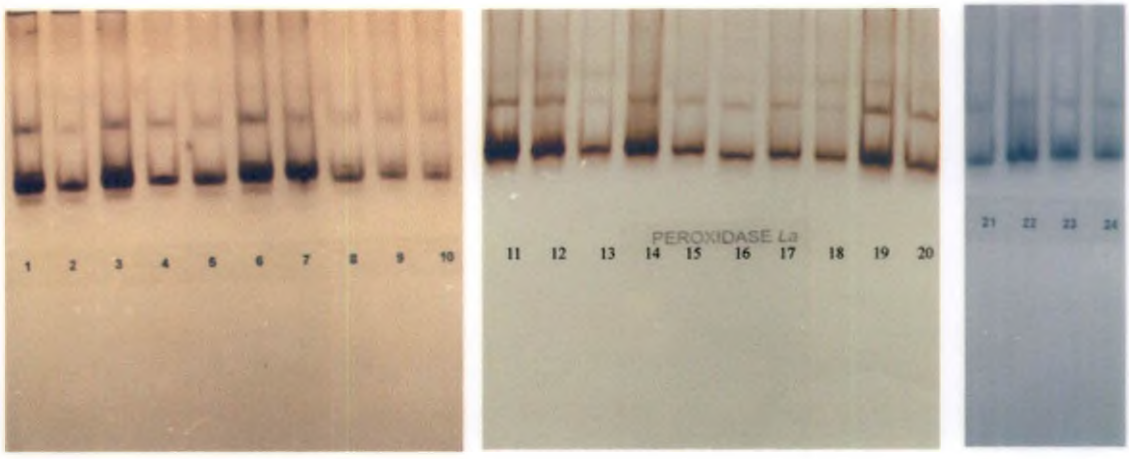
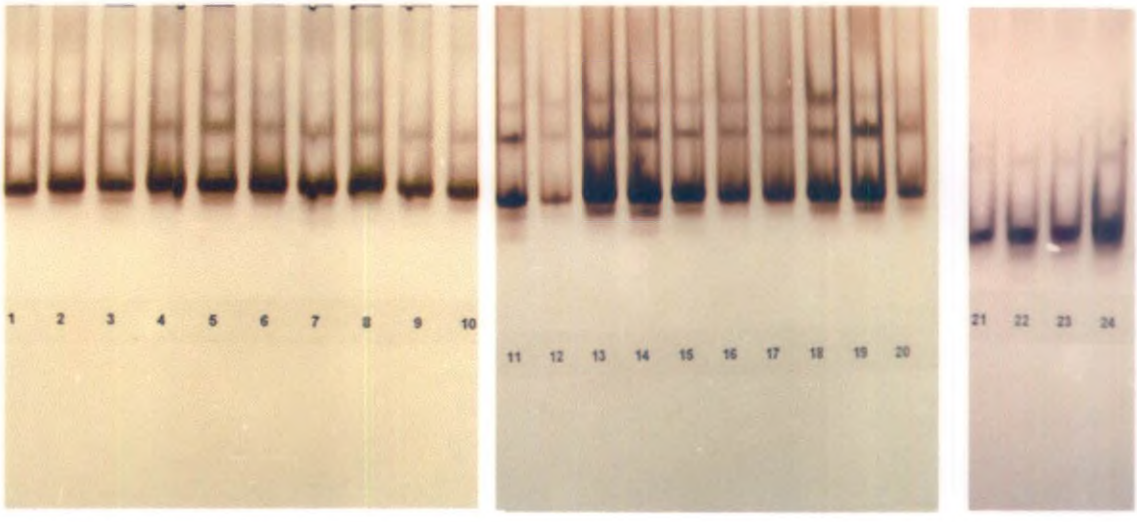


Plate X. Peroxidase banding pattern in tomato leaves at 60 days



Legend : 1-Sakthi, 2-Mukthi, 3-LE-214, 4-LE-474, 5-LE-415, 6-LE-470, 7-LE-421, 8-LE-457, 9-BT-1, 10- LE-455, 11-LE-526, 12-LE-619, 13-LE-615, 14- LE-616, 15- LE-617, 16- LE-613, 17- LE-614, 18-LE-618, 19- BT-101-22, 20-CO-1, 21-CO-3, 22-Pant-T_1 , 23-Pant-T_3, 24-Pusa Ruby.

5. DISCUSSION

Tomato cultivation in tropical countries confronts the major problem of bacterial wilt disease caused by *Ralstonia solanacearum* (Smith) Yabuuchi *et al.* In Kerala the acidic soil condition favours the incidence of bacterial wilt. Chemical control of this pathogen is not effective or economically feasible. The most effective and easy way of controlling this disease is the development of resistant varieties.

A thorough knowledge on the sources of resistance and biochemical factors influencing this malady is very essential to tackle the problem. In this context biochemical markers especially isozymes may contribute in the selection procedure while screening the varieties for wilt resistance. At present, the information on this aspect is rather scanty, especially in tomato. The present study was undertaken to characterize biochemically and to screen tomato genotypes for resistance to bacterial wilt so as to evolve biochemical markers.

The response of different tomato genotypes to bacterial wilt, their performance and the biochemical factors influencing the incidence of bacterial wilt are discussed in this chapter.

5.1 Field evaluation of tomato genotypes for resistance to bacterial wilt

Twenty four tomato genotypes were evaluated for its reaction to bacterial wilt during August 1998 to January 1999. The variety Sakthi with 90 per cent survival rate was found to be consistent for resistant reaction. Mukthi followed this with 88 per cent survival. LE-474 recorded a survival rate of 80 per cent. According to classification suggested by Mew and Ho (1976), these three genotypes can be grouped under resistant genotypes. The resistance of these genotypes to bacterial wilt has been reported earlier (Rajan, 1985; Sadhankumar, 1995 and Paul, 1998).

The lines LE-214, LE-415, LE-470 and LE-421 with survival percentage of 77.5, 67.5, 77.5 and 75.0 respectively were moderately resistant. The moderately resistant nature of these varieties have already been reported by many workers (LE-214 and LE-415 by Sadhankumar, 1995 and Paul, 1998). These lines can well form additional sources of resistance to this disease.

The lines LE-457, BT-1, LE-455 and CO-1 were moderately susceptible to bacterial wilt with survival percentage of 50.0, 27.5, 42.5 and 30.0 respectively. Paul (1998) has earlier reported same type of disease reaction in LE-455 and CO-1.

All other varieties included in the study were highly susceptible with survival rate ranging from 0 to 18.5 per cent. LE-619, LE-618, Pant T_1, Pant T_3 and Pusa Ruby recorded 100 percent susceptibility to this disease. This is in accordance with earlier reports of Sadhankumar (1995) and Paul (1998) in Pusa Ruby.

5.2 Salient features of tomato genotypes screened for wilt resistance

The line LE-455 with semi-determinate growth habit was the dwarfest among the 24 genotypes screened in this study. Pant T_1 was the tallest having indeterminate growth habit which was but susceptible to bacterial wilt. This shows that growth habit cannot be taken as a criterion as to indicate resistance or susceptibility.

Regarding number of branches, LE-619 had the maximum number of branches (16) and Sakthi (7) with minimum number of branches.

The genotype LE-618 was the first to flower while LE-457 was the last in this respect. The same trend was shown for days to 50 per cent flowering and days to first harvest. LE-613 had the longest duration for last harvest while Pusa Ruby had the shortest duration for last harvest. Both genotypes showed the same trend with respect to duration of the crop.

LE-619 produced the maximum number of fruits and Pant T_3 had the minimum. BT-1 was the highest yielder (0.711 kg/plant) followed by LE-615 and BT-101-22. The lowest yield was recorded by LE-421 followed by LE-415. Although LE-619 produced the maximum number of fruits, its yield was low because of very low average fruit weight (8.26 g). The per plant yield was low because of adverse weather condition like heavy wind which prevailed during January and February 1999. Eventhough the susceptible variety like BT-1 recorded higher yield when grown in pots, the same could not survive in the field due to extreme susceptibility to bacterial wilt. BT-101-22 had the maximum fruit weight (86.97 g). Pant T_3 (1.83 g/fruit) had the highest seed content while LE-619 had the lowest seed content (0.08 g/fruit).

These findings indicate the absence of any linkage or pleiotropic action of the genes conferring bacterial wilt resistance on those influencing the morphological or biometrical characters. However, a detailed investigation on this aspect involving a wide array of characters would tell about possible linkage with disease resistance and other characters.

In general, the bacterial wilt resistant genotypes viz., Sakthi, Mukthi and LE-474 and moderately resistant genotypes like LE-214, LE-415, LE-470 and LE-421 are good yielders offering good scope for large scale cultivation in wilt prone areas.

5.3. Biochemical bases of resistance to bacterial wilt

The plants' defence in response to injury and/or infection are dynamic which culminate in the sealing off the injury or the invading parasite. It is to the host's advantage to evolve mechanisms to inhibit pathogen growth (resistance mechanism) and to pathogens advantage to evolve mechanisms which overcome these defences (pathogenicity factors) (Collinge *et al.*, 1996).

Different defence mechanisms are attributed by resistant varieties for its survival. Hence, it is pertinent to study the biochemical factors contributing to resistance or susceptibility. Based on the above facts, a comparative study was carried out in the selected 24 genotypes at two growth stages to assay the content of total phenols, O.D. phenol and isozymes. These selected varieties had different disease reaction to *Ralstonia solanacearum*.

5.3.1 Total phenols

The total phenol content in all the bacterial wilt resistant as well as moderately resistant genotypes studied was higher than the susceptible ones in roots and leaves at two growth stages studied. High content of phenols in resistant plants suggests the role of phenols in imparting resistance to bacterial wilt. Walker (1923 and 1926) has already reported protective role of phenolics against disease incidence. Menon and Schachinger (1957) illustrated the role of phenolics in combating diseases in tomato. Sadhankumar (1995) has already reported increased level of phenolics in resistant genotypes compared to susceptible genotypes in tomato and Paul (1998) in solanaceous vegetables.

5.3.2 O.D. Phenol content

The roots of resistant genotypes, viz. Sakthi, Mukthi, LE-214, LE-474, LE-415, LE-470 and LE-421 had higher O.D. phenol content compared to susceptible genotypes at 45 and 60 days. The findings of Rajan, 1985; Gangappa, 1986; Geetha, 1989; Sadhankumar, 1995; Markose, 1996 and Paul, 1998 are in line with the present observation.

The O.D. phenol content in leaf samples of bacterial wilt resistant genotypes was higher than susceptible genotypes except in the case of BT-101-22 which was on par with resistant genotypes. The resistant varieties like Sakthi and Mukthi with exceptions to LE-474 had less O.D. phenol content in leaves compared to the moderately resistant varieties like LE-470, LE-415 and LE-421

besides having the total phenols content at a higher level. This suggests that lower O.D. phenol level in leaves of 45th and 60th day can be correlated with wilt resistance in tomato when the total phenols was high. The results of BT-101-22, a susceptible variety with high O.D. phenol and low total phenol content at 45 and 60 days old leaves are supporting the above statement (i.e.low total phenol). The reports of Paul (1998) in tomato, brinjal and chilli stem for O.D.phenol content were in line with the present results. Comparatively low O.D. phenol content in resistant varieties may be contributed by the immediate oxidation of O.D. phenol to more toxic compounds like quinones by the oxidising enzymes like polyphenol oxidase and peroxidase (Mahadevan, 1970).

The O.D. phenol content increased with age in roots and leaves except in the leaves of Pant T_3 and Pusa Ruby. Similarly the total phenol content was increasing with age exceeding the level of O.D. phenol. The low O.D. phenol content in plant at a particular point of time as compared to total phenols might be due to the conversion of O.D. phenols into other substances. The reaction of the genotypes as resistant or susceptible would be having a bearing on the relative content of the total phenols and O.D. phenols in plants. The difference in level is also affected by the enzyme activity in the plant.

In resistant genotypes as the level of total phenol and O.D. phenols are high, the multiplication rate of the pathogen is less and hence the pathogen is not able to invade the plant. In susceptible genotypes, as the level of these chemicals are low, the pathogen multiplies quickly and plant succumbs.

5.3.3 Isozyme analysis

Isozyme analysis by electrophoresis provides a well-defined and effective method to detect genetic differences among individuals. Among the organic molecules, isozymes are very useful aids to compare genotypes, though

they are used only as a supplementary tool along with morphological, genetical or other biochemical methods.

The banding pattern is an expression of the particular enzyme system assayed and its mode of inheritance. Many enzymes are coded by more than a single gene. Additional bands or shifts in migration may arise from post-translational modification of enzymes.

In the present study, isozyme pattern of polyphenol oxidase and peroxidase were studied in 24 tomato genotypes at 45 days and 60 days in leaves and root samples. The bands were clear in both leaves and roots. With respect to the number of bands, root sample had more number of bands.

5.3.3.1 Polyphenol oxidase

In root samples a total of 15 bands were resolved based on the electrophoretic banding pattern at 45 and 60 days old plants. The presence of PPO-7 (Rm=0.265) and PPO-5 (Rm=0.212) was considered as the base protein for polyphenol oxidase in root samples. Base bands differ in 45th and 60th day old root samples. This result is in agreement with the reports of Thipyapong *et al.* (1997) on the differential expression of polyphenol oxidase during vegetative and reproductive phase in tomato.

In the 45th day, the root samples expressed the protein bands PPO-1 (Rm=0.074) along with PPO-4 (0.180), PPO-7 (Rm=0.265), PPO-10 (Rm=0.372), PPO-11 (Rm=0.393) and PPO-12 (Rm=0.414) indicating a combination of protein bands for highly resistant nature of genotypes like Sakthi, Mukthi, LE-214 and LE-474. The above isozyme pattern along with high total phenol and OD phenol in 45th day root can be considered as a marker for resistance to bacterial wilt in tomato. These results are in confirmation with reports of Solorzano *et al.* (1996) in tomato for resistance to *Alternaria solani*, Gupta *et al.* (1995) in *Brassica* spp. for

resistance to Alternaria leaf blight and Fan-YanPing *et al.* (1996) in pear for *Venturia nashicola* resistance.

The moderately resistant tomato varieties like LE-415, LE-470 and LE-421 had the above protein bands except PPO-1 (Rm=0.074). Hence the role of PPO-1 (Rm=0.074) in combination with PPO-7 (Rm=0.265), PPO-10 (Rm=0.372), PPO-11 (Rm=0.393) and PPO-12 (Rm=0.414) and high total phenols in 45 days old roots can be considered as marker for highly resistant varieties of tomato. This result was in confirmation with earlier reports by Bournival *et al.* (1989) for GOT-2 loci as a marker for selecting fusarium wilt resistant tomato varieties.

In 60 days old plants, the root samples of resistant varieties like Sakthi, Mukthi and LE-474 expressed only the following protein bands PPO-4 (Rm=0.180), PPO-6 (Rm=0.244), PPO-9 (Rm=0.351), PPO-10 (Rm=0.372) and PPO-11 (Rm=0.393). The absence of PPO-1 (Rm=0.074), PPO-7 (Rm=0.265) and PPO-12 (Rm=0.414) in the 60 days old samples indicated the differential expression of polyphenol oxidase gene with growth stages. This observation is in agreement with Thipyapong *et al.* (1997) in tomato.

The roots had additional protein bands at both the stages compared to leaves for polyphenol oxidase. This may be an indication of the biochemical constituents provided by the polyphenol oxidase in tomato roots. The protein bands PPO-1 (Rm=0.074), PPO-2 (Rm=0.106), PPO-6 (Rm=0.244), PPO-9 (Rm=0.351) and PPO-12 (Rm=0.414) were expressed in the root samples of the selected varieties irrespective of age.

In 45 days old plants, the root samples showed the protein bands, PPO-1 (Rm=0.074) and PPO-12 (Rm=0.414) which were present in resistant varieties only. Therefore, it can be presumed that both bands were complimentary to resistance in tomato varieties. Whereas PPO-9 (Rm=0.851) was the only protein band specific to resistant varieties in the 60 days old root samples. The 45 day old

roots expressed two protein bands PPO-1 (Rm=0.074) and PPO-12 (Rm=0.414) and 60th day roots expressed one band PPO-9 (Rm=0.851) which can be considered as markers for bacterial wilt resistance. This indicated the variation of polyphenol oxidase at different stages of growth and development. The total phenol content in root was high in the resistant varieties ranging from 250-550 ppm. This is in confirmation with earlier reports in tomato by Fan-YanPing *et al.* (1996) and Solorzano *et al.* (1996) in tomato and Gupta *et al.* (1995) in *Brassica* spp.

In leaf samples at 45th day, the band PPO-6 (Rm=0.372) can be considered as the base band which was recorded for all leaf samples. Most of the resistant and moderately resistant varieties had a total of 5 bands while the highly susceptible varieties had only 3 to 4 bands (Table 14). This is in confirmation with reports of Ganguly and Dasgupta (1988) in tomato roots for nematode resistance and Fan-YanPing *et al.* (1996) in leaves of pear cultivars for resistance to *Venturia nashicola*. Protein bands PPO-4 (Rm=0.265) and PPO-7 (Rm=0.394) may have a relation for the accumulation of high total phenols. The above results were in line with the reports of Gupta *et al.* (1995) in *Brassica* spp. for resistance to *Alternaria* leaf blight. The resistant and moderately resistant genotypes had a combination of 5 protein bands namely PPO-1 (Rm=0.159), PPO-2 (Rm=0.180), PPO-4 (Rm=0.265), PPO-6 (Rm=0.372) and PPO-7 (Rm=0.394) in 45th day leaf samples.

Eight bands were resolved in 60 days old leaf samples. Protein band PPO-6 (Rm=0.372) was present in all varieties and hence considered as base band. The nature and properties of proteins available at 45th and 60th day can also be related with total phenols and O.D. phenol content in plant parts. The electrophorogram of 60 days old leaf samples showed that the presence of protein band PPO-6 (Rm=0.372) with PPO-2 (Rm=0.180), PPO-4 (Rm=0.265) and PPO-7 (Rm=0.394) which may be related for the high O.D. phenol content in moderately resistant genotypes like LE-214, LE-415 and LE-470. The presence this four bands (PPO-2, PPO-4, PPO-6 and PPO-7) with high O.D. phenols in 60 days old leaves

can be used as a marker for screening the moderately resistant genotypes for bacterial wilt. This result is in line with Gupta *et al.* (1995) in tomato for alternaria leaf blight resistance.

5.3.3.2 Peroxidase

A total of 13 protein bands were resolved for root samples at 45 day old roots. The bands PRX-3 (Rm=0.191), PRX-4 (Rm=0.223) and PRX-11 (Rm=0.404) can be considered as base bands for root samples at 45 days since they were recorded in the selected varieties. From the zymograms of the samples, it can be inferred that there were no specific bands for resistance. In other ways the peroxidase can be considered have an important role in the anabolic process of producing secondary products i.e. phenolics. This result was in line with the report of Gazaryan *et al.* (1996). They provided the first evidence for a ternary complex comprising peroxidase, IAA and Oxygen, which play an important role in Shikimate pathway. It is responsible for the production of secondary products such as phenolics as reported by them.

In 60 days old root samples a group of protein bands such as PRX-2 (Rm=0.106), PRX-5 (Rm=0.244), PRX-9 (Rm=0.340) and PRX-11 (Rm=0.404) can be considered as base bands. At this stage there was no specific bands for resistant varieties.

In leaf samples a total of eight protein bands were observed. Protein band PRX-7 (Rm=0.361) in combination with PRX-8 (Rm=0.382) was seen in resistant and moderately resistant varieties. Hence this combination along with PRX-1 (Rm=0.160), PRX-2 (Rm=0.212), PRX-5 (Rm=0.297) and PRX-6 (Rm=0.329) can be considered as marker for resistance in 45 days old leaf samples rather than in roots. Peroxidase may act as biological catalyst for the production of high phenol content rather than oxidation/ degradation of phenols to more toxic quinones or other organic molecules as carried out by polyphenol oxidase. The

phenolics in the selected varieties showed a positive trend in support of the above statement. This result is in agreement with results of DeYu *et al.* (1995) in Barley, Lebeda and Dolezal (1995) in millets, Barcelo *et al.* (1996) and Morales *et al.* (1997) in grapes. The zymograms of 60 days old leaf samples were not expressing any relation with resistance in the genotypes.

The number of peroxidase bands in root samples of selected tomato varieties recorded a decreasing trend from resistant to susceptible varieties at 45 days. Same was the case with polyphenol oxidase also. This confirms that at a particular stage of growth (45 days), the roots of resistant varieties had more number of bands than in susceptible varieties. This result is in confirmation with reports of Fei *et al.* (1997) in soyabean and Liu *et al.* (1988) in millet.

The findings clearly indicate that the presence of PPO-1 (Rm=0.074) and PPO-7 (Rm=0.265) in 45 days old roots can be used as a marker for bacterial wilt resistance tomato genotypes. Similarly the presence of bands PPO-6 (Rm=0.372), PPO-2 (Rm=0.180), PPO-4 (Rm=0.265) and PPO-7 (RM=0.394) can be used as a marker for moderately resistant varieties. PRX-7 (Rm=0.361) and PRX-8 (Rm=0.381) in 45 day old leaf samples can also be considered as marker for resistant and moderately resistant varieties.

Table 14. Comparison of peroxidase and polyphenol oxidase bands in tomato genotypes

Enzymes	Growth stage and plant part	Number of bands in different resistant groups			
		R	MR	MS	S
Peroxidase	Leaf at 45 days	5-6	4-5	4	2-3
	Root at 45 days	6-8	6-8	5-8	4-7
	Leaf at 60 days	4-5	4-6	3-4	3-4
	Root at 60 days	6-7	6-7	7	4-7
Polyphenol oxidase	Leaf at 45 days	5	4-5	4-5	2-4
	Root at 45 days	6	5	3	2-3
	Leaf at 60 days	2-3	3-4	4	2-4
	Root at 60 days	5	5	4-5	2-6

R - Resistant; MR - Moderately Resistant; MS - Moderately Susceptible
S - Susceptible

Summary

6. SUMMARY

The investigations on "Screening and biochemical characterization of tomato genotypes for resistance to bacterial wilt" was undertaken in the Department of Olericulture and Biochemistry Laboratory, College of Horticulture, Vellanikkara, Thrissur during 1998-1999. The findings of the experiments are summarised here under.

Twenty four genotypes were evaluated in the field for bacterial wilt resistance during August 1998 to January 1999. The variety Sakthi, Mukthi and LE-474 recorded a survival of 90 per cent, 88 per cent and 80 per cent respectively followed by LE-214, LE-470, LE-421 and LE-415 with average survival of 77.5 per cent, 77.5 per cent, 75 per cent and 67.5 per cent respectively.

The morphological studies revealed that, LE-617 had more number of branches. LE-618 was the first to complete 50 per cent flowering and days to first harvest. LE-613 had the longest yielding period and duration. LE-619 produced the maximum number of fruits (35/plant) while BT-1 had the highest yield of 0.711 kg/plant when the plants were grown in pots. BT-101-22 had the maximum fruit weight (86.87 g). These studies on morphological characters of tomato lines revealed that resistance to bacterial wilt is neither related with growth habit nor with other parameters studied.

Investigations on biochemical bases of resistance revealed that the total phenol content of all the bacterial wilt resistant as well as moderately resistant genotypes were higher than susceptible genotypes in leaves and roots at two growth stages, viz. 45th and 60th day after germination.

In moderately resistant varieties the O.D.phenol content in leaves was higher when compared to resistant and susceptible genotypes at 45 and 60 days whereas in roots the trend was vice versa.

Electrophoretic studies of isozymes revealed that the root samples had more number of protein bands compared to leaf samples at two growth stages, viz. 45th and 60th day.

Regarding polyphenol oxidase a total of 15 bands were resolved in root samples. The base protein band for polyphenol oxidase differed with age in root samples. In 45 days old root samples a combination of protein bands PPO-1 (Rm=0.074), PPO-4 (Rm=0.180), PPO-7 (Rm=0.265), PPO-10 (Rm=0.372), PPO-11 (Rm=0.393) and PPO-12 (Rm=0.414) were resolved only in highly resistant varieties like Sakthi, Mukthi, LE-214 and LE-474. This combination was not seen in other varieties. In 60 days old root samples the highly resistant varieties like Sakthi, Mukthi and LE-474 expressed the protein bands PPO-4 (Rm=0.180), PPO-6 (Rm=0.244), PPO-9 (Rm=0.351), PPO-10 (Rm=0.372) and PPO-11 (Rm=0.393) which were not present in 45 days old root samples. In 45 days old root samples the protein bands, PPO-1 (Rm=0.074) and PPO-12 (Rm=0.414) were present only in resistant varieties. Similarly PPO-9 (Rm=0.851) was the only band specific to resistance in 60 days old root samples.

A total of eight different bands were seen in root samples for polyphenol oxidase at 45th and 60th day. The resistant and moderately resistant varieties had a total of five bands while the highly susceptible varieties had only 3-4 bands. The resistant and moderately resistant genotypes had a combination of 5 protein bands namely PPO-1 (Rm=0.159), PPO-2 (Rm=0.180), PPO-4 (Rm=0.265), PPO-6 (Rm=0.372) and PPO-7 (Rm=0.394) in leaf samples at 45th day.

In 60 days old leaf samples isozyme band PPO-6 was present in all varieties. Protein bands like PPO-6 (Rm=0.372) along with PPO-2 (Rm=0.180), PPO-4 (Rm=0.265) and PPO-7 (Rm=0.394) were resolved only among moderately resistant genotypes like LE-214, LE-415 and LE-470.

In case of peroxidase, a total of 13 protein bands were resolved in root samples at 45 days. The bands PRX-3 (Rm=0.191), PRX-4 (Rm=0.223) and PRX-11 (Rm=0.404) were present in most of the varieties in 45 days old roots. There were no specific bands resolved for resistant varieties at 45 days in root samples.

In 60 days old roots, protein bands such as PRX-2 (Rm=0.106), PRX-5 (Rm=0.244), PRX-7 (Rm=0.340) and PRX-11 (Rm=0.404) were present in most of the varieties which can be considered as base band. From the peroxidase zymograms of 60 days old root samples no specific bands could be considered for resistance in the experimental plants.

In leaf samples at 45 days, the protein bands like PRX-7 (Rm=0.361) and PRX-8 (Rm=0.382) were recorded only in resistant and moderately resistant varieties. In 60 days old leaves there was no specific bands for resistance as in the case of 60 days old roots. The number of peroxidase bands in root samples in selected tomato varieties recorded a decreasing trend from resistant to susceptible varieties at 45 days. Same was the case with polyphenol oxidase.

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SCREENING AND BIOCHEMICAL CHARACTERIZATION OF TOMATO GENOTYPES FOR RESISTANCE TO BACTERIAL WILT

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ABSTRACT OF A THESIS

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ABSTRACT

Investigation on 'Screening and biochemical characterization of tomato genotypes for resistance to bacterial wilt' was carried out in the Department of Olericulture, and Biochemistry Laboratory, College of Horticulture, Vellanikkara during 1997-99. The objectives of this study were to identify tomato genotypes resistant to bacterial wilt and to find the possibility for biochemical cataloguing of bacterial wilt resistant tomato genotypes.

Evaluation for bacterial wilt resistance revealed that Sakthi, Mukthi and LE-474 were consistently resistant to bacterial wilt. Four additional sources of bacterial wilt resistance were identified, viz. LE-214, LE-415, LE-470 and LE-421. Based on the percentage wilting the twenty four genotypes included in this study were classified into four groups, viz. Resistant, Moderately Resistant, Susceptible and Highly susceptible.

All the bacterial wilt resistant and moderately resistant genotypes had a higher content of total phenols in roots and leaves at 45th and 60th day of plant growth.

O.D. phenol content in roots of resistant varieties were higher than susceptible varieties. In leaf, the O.D. phenol content was high in moderately resistant genotypes at 45th and 60th day.

All the genotypes were studied for isozyme variation with respect to two enzymes, viz. polyphenol oxidase and peroxidase. In general the roots had more number of bands compared to leaves at both the stages.

The root samples at 45^{th} day showed two polyphenol oxidase bands, viz. PPO-1 (R_m=0.074) and PPO-12 (Rm=0.414) which were specific to resistant varieties alone. This combination along with high total phenols may be considered as a biochemical marker for resistance to bacterial wilt in tomato. In 60 days old

roots of resistant and moderately resistant genotypes the protein band PPO-9 (Rm=0.851) was predominantly present.

Regarding peroxidase, 45 days old leaf samples had a combination of protein bands, viz. PRX-7 (Rm=0.361) and PRX-8 (Rm=0.382) in resistant and moderately resistant varieties. But at 60 days the roots and leaves did not show any specific band for resistance.

In general at 45 days both leaf and root samples had more number of bands for polyphenol oxidase and peroxidase in resistant varieties compared to susceptible varieties. The study revealed that it was possible to arrive at a combination of specific isozyme bands at a particular growth stage, which can be used as a marker for bacterial wilt resistant tomato genotypes.