GENETIC AND BIOCHEMICAL BASES OF RESISTANCE TO BACTERIAL WILT IN CHILLI

By

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THESIS

submitted in partial fulfilment of the
requirement for the degree of

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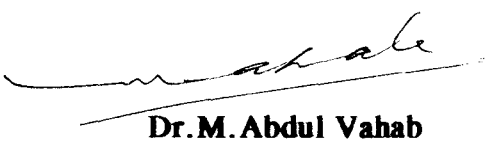

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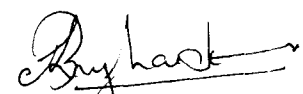

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Introduction

INTRODUCTION

Chilli (*Capsicum annuum* L.) is one of the most important vegetable cum spice crop grown for its pungency, spicy taste, aroma and the appealing colour it imparts to food. It is also a rich source of vitamins A and C. It is a strategic raw material for several of our speciality products. Green chilli, chilli powder, cayenne pepper, tabasco, paprika, sweet or bell pepper, pimentos and serrano pepper are all derived from the berries of *Capsicum* spp. Chilli is known in different names in different countries and even within the same country. Believed to be introduced by Portuguese to India, chilli has become almost an essential article of diet of both the rich and the poor.

Annual trade of chilli in the world is 55,000 to 65,000 tonnes which is 16.7 per cent of the total spice trade. India ranks first in area (9.17 lakh ha) and production (7.79 lakh t) in the world. During 1995-96, India exported 55,200 tonnes of chilli valued Rs.128 crores. Though chilli is grown throughout India, Andhra Pradesh leads in area and production. Andhra Pradesh, Maharashtra, Tamil Nadu, Karnataka, West Bengal, Bihar and Assam account for 96 per cent of total area under chilli in India. The cultivation of chilli in Kerala is limited to 638 ha with an annual production of 670 tonnes.

Chilli in India is threatened by myriad of biotic stresses, the most damaging being the bacterial wilt caused by *Pseudomonas solanacearum* (E.F.Smith) Smith. The warm humid tropical climate and acidic soil condition in Kerala are conducive for the incidence and severity of bacterial wilt. The destructiveness of the

disease is compounded by the wide host range of the wilt pathogen. Thus in areas where intercropping and continuous cropping are practiced, the severity of the problem increases by leaps and bounds.

The disease manifests at all stages of crop growth with the maximum severity at flowering stage. Persistence of the pathogen in different types of soils and its wide host range often hamper the effectiveness of the management measures. The chemotherapic and physicotherapic procedures used *in vivo* against the bacterium lack practical feasibility. The variable reaction of cultivars to the causal organism and the differential behaviour of the pathogen itself make breeding programmes arduous. The ideal strategy in this situation is breeding for disease resistance. Since host plant resistance is an important component of disease management, genetic resistance is regarded as an extremely feasible method for managing this disease. Therefore the knowledge on the sources of resistance, mode of inheritance and mechanism of host plant resistance is imperative for evolving effective disease control measures.

At present, only limited information is available on various aspects of defence mechanism against bacterial wilt of chilli. Although in the recent past disease resistant varieties were developed, the resistance was short lived posing greater challenges. The inherent potential of a genotype to impart resistance is determined by the resistance mechanism operating within it. It is the genetic control exercised through gene action that decides upon the manifestation of a particular trait in a genotype.

In solanaceous crops, the mode of inheritance of bacterial wilt resistance is governed by polygenic, monogenic dominant, monogenic recessive and partially

dominant systems. The resistant varieties possess various physical and biochemical barriers to restrict the entry and growth of the pathogen in host cell. The defence mechanisms which actually arrest pathogen penetration or development include the hypersensitive response, physical barriers, antimicrobial proteins and metabolites such as phytoanticipins and/or phytoalexins (Collinge *et al.*, 1994).

Resistance appears to manifest itself mainly through physiological defence mechanism of the host to disease development. Physiological adaptation occurs primarily through biochemical changes brought about by *de novo* transcription and resultant protein accumulation.

Recently isozyme variations are used as a powerful tool to complement and supplement conventional biochemical studies. These are the basic biochemical constituents which are not affected by environment. Moreover isoenzymes are usually direct products of single locus and relating the phenotypic variations with genotypic characters is relatively easier.

The increasing importance of chillies in the economy of our country has made it necessary to evolve resistant varieties with high productivity and good quality. Availability of such chilli varieties would open up newer vistas in chilli cultivation especially in the state of Kerala. Since resistance to bacterial wilt has been a high priority objective throughout the history of the crop improvement, concerted efforts are needed to elucidate the mechanisms and components of wilt resistance. Such strategic research is obviously imperative to utilize the available germplasm most effectively in breeding programmes. A comprehensive study in this direction has thus become inevitable.

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

1. To isolate and maintain pure culture(s) of *Pseudomonas solanacearum*.

2. To study the inheritance of bacterial wilt resistance in chilli.

3. To study the biochemical and anatomical bases of resistance to bacterial wilt.

4. To investigate the entry, movement, growth and multiplication of *P. solanacearum* by radiotracer techniques.

5. To develop a near isogenic line of green chilli for field resistance to bacterial wilt.

Review of Literature

REVIEW OF LITERATURE

Bacterial wilt caused by *Pseudomonas solanacearum* is one of the most destructive and wide spread plant diseases in the warm humid regions of the world. The disease was first reported from Italy in 1882 (Walker,1952). Burril (1890) made a report of bacterial wilt in connection with an unidentified bacterial disease of potato in USA. But it was Smith (1896) who first described the bacterial wilt disease of many solanaceous crops and identified the causal organism as *Pseudomonas solanacearum*.

2.1 Bacterial wilt disease of chilli

The bacterium *P. solanacearum* infects more than two hundred plant species belonging to thirty three families with the largest number of hosts in Solanaceae (Kelman, 1953). The occurrence of bacterial wilt of chillies was first recorded from USA (Rolfs, 1898). Subsequently, the disease was reported from Philippines (Reinking, 1919); Java (Schwarz, 1926); Central America (Cook, 1931); Ceylon (Park, 1932); South America (Deslandes, 1944) and Thailand (Chandrasrikal and Wannapee, 1972).

Shekhawat *et al.* (1978) observed that the causal organism of this disease is endemic in India throughout the West Coast, Central and Deccan Plateau of Karnataka, Western Maharashtra and Madhya Pradesh, Eastern plains of Assam, West Bengal, Orissa and Chotta Nagpur Plateau on potato, tomato, brinjal, chillies and wild datura, the incidence being 10-50 per cent.

In India, the first report on bacterial wilt of chilli was from Madhya Pradesh (ICAR, 1969). Later it was reported by Chattopadhyay and Mukherjee (1969) from West Bengal and Khan *et al.* (1979) from Karnataka. In Kerala, certain aspects on the management of bacterial wilt of chillies and its pathogen were studied by Rahim (1972); George (1973); Remadevi (1978); Peter *et al.* (1984); Gopalakrishnan and Peter (1991) and Jyothi (1992).

2.1.1 **The pathogen**

Pseudomonas solanacearum (E.F. Smith) Smith is a complex species consisting of several races differing in host range and pathogenicity. There occurs considerable geographical variation in the organism. Several races and strains occur in the same area although they usually attack different hosts.

The shape and size of the bacterium was first delineated by Smith (1896) as non-sporeforming, non-capsulate, gram negative small rods with polar flagella. Stanford and Wolf (1917) reported that the colonies of the bacterium on solid media were circular, glistening white, slightly raised with smooth margin and appeared within 36-48 h.

Two colony variants on Tetrazolium chloride medium (TZC) were distinguished by Kelman (1954). They were the normal or wild type which are irregularly round, entire, white or whitish with light pink centre and the mutant or butyrous type which are round, transluscent, smooth, deep red with a narrow light bluish border. He observed that the wild types were highly virulent and produced wilting in 14 days where as the mutant type was either weakly pathogenic or non-pathogenic and did not induce wilting even after 21 days.

Khan *et al.* (1979) reported that chilli isolates produced convex, slimy colonies with slight pinkish centre on TZC medium. Mathew *et al.* (1979) and Mathew and Nayar (1983) stated that *P. solanacearum* isolated on TZC medium yielded circular, smooth and greyish white colonies with light pink centre, while on potato dextrose agar, the colonies were small, circular, white, smooth and slimy. Similar growth characters of the bacterium on TZC medium were observed by other workers also (Samuel, 1980; He *et al.*, 1983; Swanepoel and Young, 1988 and Jyothi, 1992).

The pathogen lost its virulence very rapidly in culture due to transformation to avirulent forms and the virulence could be maintained by storing in potato dextrose agar cultures under sterile mineral oil at 25°C (Kelman and Jensen, 1951) or in sterile distilled water (Kelman and Person, 1961). Virulence in *P. solanacearum* is closely associated with *in vitro* production of viscous extracellular polysaccharide (EPS) (Pun and Addy, 1988). Yaowei *et al.* (1993) also conducted a genetic study and concluded that exopolysaccharide (EPS) is a virulence factor of *P. solanacearum* on potato.

The bacterial wilt pathogen recorded maximum growth between a pH range of 5.5 to 7.0 and a temperature range of 30 to 37°C (Smith, 1896; Kelman 1953; Remadevi, 1978; Hayward, 1979; Nayar, 1982 and Jyothi, 1992).

Several attempts have been made to group *P. solanacearum* isolates into biotypes or races on the basis of differences in physiological characteristics (Kelman, 1953; Buddenhagen and Kelman, 1964; Hayward, 1964); pathogenicity (Buddenhagen *et al.*, 1962); bacteriophage specificity (Hayward, 1964) and serological properties (Mortan *et al.*, 1965).

Buddenhagen *et al.* (1962)classified *P. solanacearum* isolates into three races based on host range, pathogenicity and colony appearance on TZC medium. Later two new races were proposed affecting ginger and mulberry from Philippines and China respectively (Buddenhagen, 1985). 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. The five groups are:

1. Solanaceous strain (Race 1): wide host range, distributed throughout the low lands of tropics and subtropics.
2. Musaceous strain (Race 2): restricted to *Musa* and a few perennial hosts initially limited to American tropics and spreading to Asia.
3. Potato strain (Race 3): restricted to potato and a few alternate hosts in tropics and subtropics.
4. Ginger strain (Race 4): from Philippines.
5. Mulberry strain (Race 5): from China.

Based on the ability to oxidise three disaccharides (lactose, maltose, cellobiose) and three alcohols (mannitol, sorbitol, dulcitol), Hayward (1964) classified a collection of 185 isolates of *P. solanacearum* into four biotypes or biochemical types as:

Biotype I - oxidised neither group

Biotype II - oxidised only disaccharides

Biotype III - oxidised both the groups

Biotype IV - oxidised only hexose alcohols

It is well established that biovar III is the dominant strain of *P. solanacearum* affecting solanaceous crops and ginger (Hayward, 1964; Remadevi, 1978; Tabei and Quimio, 1978; Samuel, 1980; Nayar, 1982; He *et al.*, 1983; Kumar *et al.*, 1993).

Cook and Sequeira (1988) used RFLP technique to study the relationship between biotypes I to IV of Hayward (1964) and races 1, 2 and 3 of Buddenhagen *et al.* (1962). The main conclusion was that *P. solanacearum* could be divided into two distinct groups. Group I includes strains of race 1, biovars III and IV and Group II includes strains of race 1, biovar I and races 2 and 3.

P. solanacearum is a heterogenous species and was divided into five races based on host range, and into five biovars based on biochemical properties (Hayward, 1991).

2.1.2 Ecology of the pathogen

The ecology of the pathogen in infested soil is poorly understood. It is inferred that the primary inoculum came from the soil but there was no conclusive evidence that the pathogen is an ubiquitous inhabitant in the soil. Under natural conditions, the pathogen was able to survive saprophytically in the soil for six years (Chestor, 1950). There are many reports of the survival of *P. solanacearum* in different soils (Graham *et al.*, 1979; Quimio and Chan, 1979 and Moffett *et al.*, 1981).

Infected plant debris and seed material were reported to serve as a source of survival of the pathogen (Graham *et al.*, 1979; Samuel, 1980; Chatterjee, 1994

and Singh, 1994). In most infested soils population were the highest in the top 15 cm, moderately high to high in 15-30 cm zone and low to nondetectable in the 30 to 45 cm zone (Jaworski, 1988).

Drying the seeds after harvest and keeping the water content below 8 per cent prevent seed transmission of the pathogen (Zhang *et al.*, 1993).

2.1.3 **Symptomatology**

The pathogen enters through the root system and it was believed that a wound is necessary for the entry (Walker, 1952; Kelman, 1953; Chupp and Sherf, 1960). Hildebrant (1950) reported entry of the bacterium through natural opening of the plant. The pathogen enters into the uninjured roots also (Libman *et al.*, 1964). Bacteria can enter at the points of origin of secondary roots. Insects also play a role in the spread of the disease (Vakili and Baldwin, 1966).

Visible symptom occur within 2 to 8 days of entry of the pathogen into the host plant (Walker, 1952; Kelman, 1953; Chupp and Sherf, 1960). The pathogen first enters into the intercellular spaces of cortex, from there it moves to pith and xylem vessels. Wilting of plants is due to vascular plugging (Walker, 1952).

Generally, the first expression of the disease is a wilting of the lower leaves of plants. This wilting is usually associated with yellowing of older leaves. Dwarfing and stunting of the plants may also occur. Adventitious roots appear on the stem of the diseased plants. Brown discolouration of the xylem tissues of the stem and roots may occur and a water soaked appearance in the root (Chupp and Sherf, 1960). Eventually dark brown to black areas develop due to decay of root

system and the whole plant dies off. A very distinct and characteristic indication of bacterial wilt is the appearance of bacterial ooze from the fresh cut surfaces of the stem and roots.

2.1.4 Methods of Artificial Inoculation

Several artificial inoculation methods are effective in distinguishing reaction of genotypes with different resistance levels to bacterial wilt. Dipping of seedlings in fresh bacterial ooze just before transplanting followed by inoculation of seedlings in the leaf axils resulted in 100 per cent infection in susceptible brinjal seedlings (Sheela, 1982).

Kishun and Chand (1988) conducted an experiment with seven inoculation methods by using 25 days old susceptible tomato cv. Pusa Ruby in nutrient solution alone as well as in sand and soil filled in pots. The results indicated that nutrient solution can be successfully used for evaluation of virulence of *P. solanacearum* and the best method was leaf axil inoculation. Of the several inoculation techniques tested, petiole clipping method was found to be the most effective for resistance screening (Butranu *et al.*, 1994).

Morgado *et al.* (1994) compared four methods of artificial inoculation in brinjal and recommended wounding method for simulating natural root infection. Tan *et al.* (1994) tried four inoculation methods viz., seed soaking, stem injection, leaf axil injection and root drenching in groundnut. The wilt incidence of the typical susceptible cultivar in the four methods were over 80 per cent but seed soaking and root drenching methods could be used to quantify level of resistance, was their conclusion.

2.2 Genetics of resistance

2.2.1 Source of resistance

Control of bacterial wilt by exclusion of pathogen, eradication of the organism by soil treatments or prevention of infection have proven to be impractical, ineffective or restricted in application. The most effective method of control of this organism is the development of resistant varieties (Kelman, 1953). Reports on this line are rather a few in chilli.

Empig *et al.* (1962) screened 11 varieties and two strains of pepper for bacterial wilt resistance and found that 'Pasites', 'All Big' and 'World Wonder' gave the lowest disease index. Rahim and Samraj (1974) screened nine chilli varieties for resistance against *P. solanacearum* and found variety 'Khandari' as highly resistant and 'Pungent Pride', 'Cherry Red', 'Vattal', 'Dark Purple' and 'Long Red' as moderately resistant.

Goth *et al.* (1983) reported that 'KAU Cluster' (Manjari) was resistant to four race 1 isolates and one race 3 isolate of *P. solanacearum*.

Rathiah (1983) studied the reaction of *Capsicum* cultivars to fruit rot, bacterial wilt and cercospora leaf spot of chilli and found that the variety 'Suryamukhi' was tolerant to all diseases and gave the highest mean yield compared with cluster. Peter *et al.* (1984) evaluated four Indian hot chillies ('Pant C-1', 'KAU Cluster', 'White Khandari' and 'Chuna') along with six US cultivars for reaction to nine isolates of *P. solanacearum* (race 1 and race 3) and found that Pant C-1 was most resistant one. Thomas (1985) also observed resistance to bacterial wilt in 'KAU Cluster'.

Jimenez *et al.* (1988) found that when four cultivars of sweet pepper were inoculated with *P. solanacearum*, 'Cholo' was the most resistant compared with traditional cultivars, 'Agronomico 10', 'Tacares PL' and '17245'. Matos *et al.* (1990) evaluated 50 genotypes of *Capsicum* sp. and found that *C. annuum* genotype NPH-143, NPH-144 and NPH-145 were highly resistant. Other six genotypes were rated as resistant and remainder as susceptible.

Gopalakrishnan and Peter (1991) screened 146 accessions of *Capsicum* sp. for resistance to bacterial wilt in a wilt sick soil after artificial inoculation and found that CA 219 (Ujawala) and CA 33 (Manjari) were high resistant with good dry chilli yields. Cluster fruited plants gave significantly better wilt resistance than solitary fruited types. Jyothi (1992) also revealed resistance in chilli variety Manjari against bacterial wilt.

Matsunaga and Monma (1995) evaluated 23 accessions of *Capsicum chinense*, 14 of *C. frutescens*, 25 of *C. baccatum*, two of *C. pubescence* and three of *C. annuum* eight weeks after inoculation with bacterial wilt pathogen. Ranche Khorsani (*C. chinense*); Heiser 6240, LS 2390 and LS 1840 (*C. frutescens*) and LS 1716, Casali BGH 1761 and Pickersgill (*C. baccatum*) were resistant.

2.2.2 **Factors affecting wilt incidence**

Resistance and susceptibility to disease are conditions with defined metabolic, environmental and genetic conditions. The weather and soil conditions influence the seasonal development and geographic distribution of plant disease. Ability of a micro-organism to cope with or repress a host resistance mechanism would be genetically controlled and subject to external influence.

A high temperature of 28 to 36°C and high soil moisture favour rapid development of wilt diseases (Gallegely and Walker, 1949; Remadevi, 1978 and Nayar, 1982). Hiryati *et al.* (1983) observed that severity of disease caused by *P. solanacearum* significantly increased with increased soil moisture from slightly, above wilting point to slight below saturation point for each soil type tested. Ho (1988) reported that high rainfall especially towards middle end of growing season favoured high bacterial wilt incidence in tomato. Hong *et al.* (1994) found that bacterial wilt developed most rapidly if the soil moisture and temperature are high during early crop growth. Heavy rains followed by a few sunny, hot days resulted in severe wilting. They also reported that disease incidence and severity were high in acidic soils (pH 5.0-5.5).

Increased resistance in resistant lines was apparently associated with age rather than plant size (Winstead and Kelman, 1952). Jenkins and Nesmith (1976) observed that tomato lines survived bacterial wilt, in the field better if eight weeks old seedlings are transplanted. Coyne and Schuster (1983) reported that resistant tomato plants became susceptible upto 21 days and became resistant again from 21 to 49 days. The disease incidence was the highest at peak flowering and fruiting stages although it appeared early in the season (Hong *et al.*, 1994).

Okabe (1969) studied the influence of inoculum concentration on the incidence of bacterial wilt. He showed that the inoculum potential of the pathogen to cause wilt in tomato was 6×10^4 cells/g of dry soil. Mew and Ho (1976) after studying the five inoculum levels (from 10^9 to 10^5 viable cells/ml), recorded that the susceptible varieties were not significantly affected by changes in inoculum concentration, while resistant varieties shifted from resistant to moderately resistant

when inoculum density was increased. Grimault and Prior (1994a) conducted a comparative study for finding out the invasiveness of *P. solanacearum* in tomato, brinjal and chilli. The cultivars differing in resistance were inoculated with similar bacterial population (10^9 - 10^{10} c.f.u/g FW) and reported that tomatoes and brinjal have similar mechanisms of resistance to *P. solanacearum* and that *Capsicum* was more tolerant towards high bacterial populations than the other plant species.

An inoculation concentration of 5 x 10^6 c.f.u/plant was suitable for separating resistant from susceptible potato cultivars (Montanelli *et al.*, 1995).

2.2.3 **Breeding for bacterial wilt resistance**

Mihov (1969) performed crosses between *Capsicum* sp. and observed that peppers produced from crosses between *C. annuum* and *C. fasciculatum* were wilt resistant and of good quality. Yazawa *et al.* (1980) compared the hybrids of three *C. annuum* cultivars with *C. chinense* '3341' from Bolivia and found that the vigorous Murasaki x 3341 was the best and resistant to *P. solanacearum*.

At Kerala Agricultural University, Girijadevi and Peter (1987) made crosses of two hot pepper lines viz., Manjari and Pant C-1 with five sweet pepper lines viz., Hungarian Wax, Sweet Red Cherry Pickling, Early Calwonder, Cubenelle and Yolowonder. All the F_1s were susceptible or moderately susceptible indicating the recessive nature of inheritance of resistance to bacterial wilt.

2.2.4 **Inheritance of resistance**

Information on inheritance of resistance is necessary for the choice of appropriate breeding programmes for developing a resistant variety. No worth while

attempt has been reported in chilli in this regard. In tomato and brinjal wilt resistance is a complex nature showing polygenic or digenic or a partially dominant or recessive inheritance of bacterial wilt resistance.

Tomato

Singh (1961) reported that resistance to bacterial wilt is controlled by multiple recessive genes, where as Akiba *et al.* (1972) observed it being governed by a pair of dominant genes. Reports from Asian Vegetable Research Development Centre, Taiwan (AVRDC, 1975) also revealed that multiple recessive genes acting additively controlled resistance to bacterial wilt. Graham and Yap (1976) conducted a variance component analysis of P_1, P_2, F_1, F_2, BC_1 and BC_2 generations of a cross between resistant and susceptible tomato cultivars. They estimated heritability of 42 per cent (narrow sense) and three per cent (broad sense) for wilt resistance with a degree of dominance of 75 per cent. Villareal and Lais (1978) endorsed the hypothesis of additive gene action for resistance to *P. solanacearum*.

Sreelathakumari (1983) reported a complementary and hypostatic type of digenic recessive gene system for wilt resistance. Tikoo (1983) reported the presence of two independent gene system for wilt resistance. Bosch *et al.* (1985) postulated a two gene model with epistasis for wilt resistance. Rajan and Peter (1986) reported a monogenic incompletely dominant gene action for wilt resistance in tomato. While Nirmaladevi (1987) reported that resistance to bacterial wilt was under polygenic control. Additive and epistatic gene action governed the resistance. Monma and Sakata (1993) reported that bacterial wilt resistance was partially recessive as there was incomplete dominance towards susceptibility.

By using RFLP technique, Danesh *et al.* (1994) studied genetics of resistance to bacterial wilt in tomato and reported partial resistance and revealed that the gene for partial resistance was on chromosome 6.

Brinjal

Kelman (1953) and Kuriyama (1975) reported that resistance to *Pseudomonas solanacearum* in brinjal was controlled by polygenes. Several workers reported that resistance to bacterial wilt in brinjal was controlled by a single dominant gene (Swaminathan and Sreenivasan, 1971; Vijayagopal and Sethumadhavan, 1974; Gopimony, 1983; Narayanan, 1984; Gangappa, 1986 and Gopinath and Madalageri, 1986). While Manjunath and Dutta (1987) observed the action of both additive and nonadditive genes in controlling bacterial wilt resistance and additive genetic variance was more than three times that of dominance variance indicating preponderance of additive genes in controlling resistance. They further confirmed that resistance to bacterial wilt in brinjal was controlled by recessive genes acting additively. Varghese (1991) reported monogenic recessive gene action for wilt resistance after evaluating six generations of the cross 'Surya' x 'Pant Rituraj'.

2.3 Biochemical basis of resistance

Disease resistance is the rule in plant kingdom, susceptibility being the exception. Plants have their own built-in defence mechanisms against all microorganisms. But in a few cases, the pathogens overcome this defence barrier with the offensive chemicals and cause diseases. The resistant varieties possess

certain defence mechanisms such as physical and biochemical barriers to restrict the entry and growth of the pathogen in host cells.

Muller (1959) and Cruickshank (1963) stated that a host might have two kinds of defence factors viz., prohibitins and phytoalexins. Prohibitins are passive chemical barriers, while phytoalexins are active biochemical barriers against infection (Mahadevan, 1970). Mahadevan (1973) reported that chemicals like prohibitins, phytoalexins and other post infectionally formed inhibiting substances appear to be important in the defence reaction.

Bell (1981) reported that levels of resistance in stem and root generally increased rapidly during the first two weeks of seedlings or when new shoot grows and slowly thereafter. Levels of resistance frequently decline with age.

2.3.1 **Defence mechanisms of the host**

Phenolics

A wide range of chemicals possessing an aromatic ring bearing a hydroxyl substituent called phenolic substances show antifungal, antibacterial and antiviral activities. Phenolics in high concentrations are toxic to plant cells themselves (Tepper and Anderson, 1984). Hence phenolics will normally be present in small quantities only in plants and these quantities may not be sufficient to suppress the development of pathogens. But in many plant pathogen interactions, the synthesis of phenolics is activated after infection and the high amount of phenolics synthesized rapidly suppress the pathogen development (Vidhyasekharan, 1990).

Phenolics play a key role in Fusarium wilt resistance in tomato. Tomato plants inoculated with *Fusarium oxysporum* f.sp. *lycopersici* synthesized increased

amounts, both of total and orthodihydroxy phenols. These compounds were synthesized rapidly in resistant than in susceptible plants (Matta *et al.*, 1967). Peroxidase and polyphenol oxidase are capable of oxidising phenolic compounds and oxidised phenolics (quinones) are more toxic to the fungi.

Protective role of phenolics 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). Gopinath and Madalageri (1986) and Sadhankumar (1995) indicated a high significant correlation of phenol with resistance and suggested a possible role of phenols in the mechanism of wilt resistance in brinjal and tomato respectively. But Sitaramaiah *et al.* (1985) and Geetha (1989) were unable to correlate the total phenol content to resistance/susceptibility to bacterial wilt in brinjal. However, Kuc (1964) and Rajan (1985) observed a negative correlation between resistance and total phenol content in tomato and they suggested that the lower levels of phenolics in the roots of the resistant line might be due to the increased rate of oxidation of phenolics.

Among the different phenolics, orthodihydroxy phenolics (OD phenolics) are known to be highly toxic and play a major role in disease resistance (Mahadevan, 1966). They are easily oxidised by polyphenol oxidase and peroxidase to highly reactive quinones which are effective inhibitors of sulphydryl enzymes thereby preventing the metabolic activities of host and parasitic cells (Mahadevan, 1970). Chlorogenic acid and caffeic acid were the most important phenolic compounds involved in disease resistance mechanisms (Bajaj, 1988).

Orthodihydroxy phenolic compounds such as caffeic acid and chlorogenic acid, and orthoquinones and tannins were shown to strongly inhibit the activities of extracellular enzymes produced by microorganisms, in addition to inhibition of its growth (Hunter, 1978), the importance of orthodihydroxy phenolic compounds in conferring disease resistance needed no further emphasis. However, since it is very difficult to measure the quantity of oxidised product i.e., quinones, suitable methods should be developed to quantify and localise their polymerisation products viz. melanins (Bajaj, 1988).

There was a positive association between OD phenol content in the roots and bacterial wilt resistance. The resistant lines had higher OD phenol content compared to the susceptible lines in tomato and brinjal (Rajan, 1985; Gangappa, 1986; Geetha, 1989 and Sadhankumar, 1995).

Rajan (1985) reported that after artificial inoculation, total phenols were higher in roots and shoots of susceptible variety, whereas OD phenol content was increased and remained at a higher level in resistant variety. Ahmed *et al.* (1994) reported that total phenols and OD phenols were found high in the yellow vein mosaic virus resistant varieties before inoculation while the phenolic compounds decreased in the resistant lines after inoculation in okra.

Proteins

Enhanced protein synthesis appears to be a universal phenomenon in compatible host pathogen interactions. *De novo* synthesis of new proteins was also reported (Tani and Yamamoto, 1979; De Wit and Bakkar, 1980). When the protein synthesis is inhibited by introducing inhibitors such as blasticidin S, puromycin and

cycloheximide, resistance of the varieties break down. This clearly suggests that the protein synthesis is an important factor in disease resistance. The synthesized proteins may not be inhibitory to the pathogens. They mostly activate the synthesis of defence chemicals. The preformed existing proteins may not be involved in the disease resistance process (De Wit and Bakkar, 1980; Gabriel and Ellingboe, 1982).

Changes in soluble protein constitution of leaves of near isogenic lines of tomato carrying resistance gene Cf_4 or Cf_5 to *Cladosporium fulvum* were investigated by Dewit and Bakkar (1980). A protein appeared more rapidly in the two incompatible combinations than in the compatible ones. In healthy uninoculated plants, it was not detectable. However, the protein did not inhibit hyphal growth of the fungus in tomato leaf. The exact role of this protein in disease resistance is not known.

Leach *et al.* (1983) detected entirely new protein which was different from Pathogenesis Related proteins (PR proteins). This protein could not be detected in healthy tissues. This protein appeared when lipo polysaccharide of *Pseudomonas solanacearum* was infiltrated into the leaves of tobacco cultivar. The accumulation of new protein as well as the increase in relative content of atleast two other proteins were correlated with the appearance of resistance to bacterial multiplication in tobacco tissues.

Biochemical properties associated with rust and powdery mildew resistance in pea were studied by Chander (1989) revealing that the healthy leaves of powdery mildew and rust resistant lines of peas had more total nitrogen and protein, while another study by Chander (1994) in chillies indicated that the resistant chilli line contained less total nitrogen and true protein.

Total protein and soluble protein were high in okra cultivars resistant to yellow vein mosaic virus. After inoculation the total proteins increased in both resistant and susceptible cultivars, to a greater extent in the latter (Ahmed *et al.*, 1994).

Malhotra and Singh (1994) reported negative correlation between coefficient of disease index of Fusarium wilt in tomato and total protein.

Ganguly (1995) suggested that the *Rhizoctonia solani* resistant rice variety had higher levels of protein than susceptible cultivar tested. Post infectional increase appeared to be associated with disease resistance. Protein content was high after 21 days.

Host enzymes

Some of the host enzymes play an important role in disease resistance. Peroxidase and polyphenol oxidases are the key enzymes which are responsible for synthesis of quinones from phenolics. Quinones are highly bactericidal and fungitoxic (Rama and Dunleavy, 1975). Hence sometimes the increased activity of these enzymes might be responsible for disease resistance.

Peroxidase

Peroxidase is a host enzyme which is frequently correlated with disease resistance. It is an important enzyme in the synthesis of lignins and it catalyses the oxidation of phenolics into more toxic quinones. The enzyme itself is inhibitory to some microorganisms (Rama and Dunleavy, 1975).

In French bean Rudolph and Stahmann (1964) reported increased peroxidase activity in the halo blight (*Pseudomonas phaseolicola*) resistant variety than in susceptible variety. Tissues of *Nicotiana tabacum* having high peroxidase activity were more resistant to the wild fire disease (*Pseudomonas tabaci*) than that of lower activity (Lovrekovich *et al.*, 1968). Commercial peroxidase injection resulted in induced resistance. They also detected a positive correlation between the level of peroxidase activity in tobacco leaves and resistance to *P. tabaci*.

Retig (1974) made detailed studies on the role of peroxidase in Fusarium wilt resistance in tomato. In the resistant plants, peroxidase activity in the roots increased significantly 24 hours after inoculation, where as in the susceptible plants, a similar increase was observed only 24 hours later. In the resistant plants alone the stem tissues showed high peroxidase activity, three days after inoculation.

Hammerschmidt *et al* (1982) reported that resistance to *Pseudomonas lachrymans* and *Colletotrichum lagenarium* was correlated with increased peroxidase activity in cucumber. Higher peroxidase activity was detected in potato varieties resistant to bacterial soft rot when compared to the susceptible variety based on the studies of Karwasra and Parashar (1989).

Ahmed *et al.* (1994) reported higher peroxidase activity in yellow vein mosaic resistant okra varieties. The activity of peroxidase was found to be high in powdery mildew resistant chilli variety IIHR 517 A (Chander, 1994).

Investigations on groundnut bacterial wilt resistance revealed that peroxidase activity was higher in resistant genotypes before and after inoculation (Kang and He, 1994; Liao *et al.*, 1994; Shan and Tan, 1994). Shan and Tan (1994)

also reported that roots were the most appropriate plant part for studying bio-chemical changes that occur after invasion by the wilt pathogen *Pseudomonas solanacearum*.

Polyphenol oxidase

Action of polyphenol oxidase is similar to that of peroxidase. This enzyme oxidises phenols to quinones and tannins which are highly toxic to microorganisms, resulting a major role in disease resistance. It is also toxic to viruses in certain cases. Maine and Kelman (1961) observed that polyphenol oxidase activity was much greater in infected than in healthy stem tissues and suggested that polyphenol oxidase activity may be involved directly or indirectly in resistance of host plants to pathogenic microorganisms including *Pseudomonas*.

Retig (1974) conducted studies on the role of polyphenol oxidase in *Fusarium* wilt resistance in tomato and observed a very high increased activity in both roots and stems of the resistant plants after inoculation. No increase in activity was found in susceptible plants.

Obukowicz and Kennedy (1981) also stressed importance of polyphenol oxidase enzyme in resistance against *P. solanacearum* in tobacco. In relation to resistance against *Pseudomonas syringae* pv. *tomato*, Bashan *et al.* (1987) also observed higher polyphenol oxidase enzyme activity in inoculated resistant cultivars than in inoculated susceptible ones.

In potato, Karwasra and Parashar (1989) reported higher polyphenol oxidase activity in Kufri Lalima, a potato variety resistant to bacterial soft rot, compared to Kufri Badshah, a susceptible potato variety. Ahmed *et al.* (1994)

reported higher polyphenol oxidase activity in yellow vein mosaic resistant okra varieties than susceptible ones. However, Chander (1994) detected lower polyphenol oxidase activity in powdery mildew resistant chilli line compared to that in susceptible one.

Duan *et al.* (1994) studied correlation between bacterial wilt resistance and polyphenol oxidase activity in groundnut and reported that no significant difference was observed between resistant and susceptible genotypes before inoculation. But after inoculation, differences were significant. Maximum enzyme activity was observed after six days of inoculation in resistant lines, while in susceptible ones, it took 10 days. Liao *et al.* (1994) also reported similar results in groundnut.

Esterase

Esterases are a complex and heterogeneous group of enzymes which hydrolyse the ester link of different metabolites. There is a direct correlation between the pattern of esterase and disease resistance. But the exact role they play in disease resistance is not known.

Xia *et al.* (1994) conducted studies on the role of esterase on *Verticillium* wilt resistance in cotton and reported higher esterase activity in resistant varieties than susceptible ones.

Shan and Tan (1994) studied the role of esterase activity in bacterial wilt disease resistance in groundnut and observed increased activity only in the susceptible cultivars. In resistant cultivars, esterase activity was inhibited.

2.3.2 Electrophoresis - A tool to study disease resistance

Gel electrophoresis of soluble proteins and enzyme create a powerful, well defined and effective method to detect the genetic differences among individuals. Isozymes, or multiple molecular forms of enzymes, are enzymes that share a common substrate but differ in electrophoretic mobility (Market and Moller, 1959). Isozyme variations are used as powerful tools to complement conventional biochemical and genetic studies (Yndgaard and Hoskuldsson, 1989).

Over the last 30 years, this technique is used in three distinct areas of plant disease research viz. (1) the taxonomy of pathogen, (2) the biochemical basis of host-pathogen interaction and (3) evolutionary genetics of pathogen population.

Farkas and Stahmann (1966) studied the effect of infection of Southern bean mosaic virus on *Phaseolus vulgaris*. The peroxidase zymogram pattern of infected leaves exhibited two new peroxidase isozymes II and III. Uninfected leaves exhibited peroxidase isozymes I and IV.

Different isozyme systems are investigated using gel electrophoresis of soluble enzymes involving fungal attack of maize. Both qualitative and quantitative differences are found in isozymes like glucose-6-phosphate dehydrogenase, phosphatase, peroxidase and polyphenol oxidase (Stavely and Hanson, 1967).

Gabriel and Ellingboe (1982) conducted protein gel electrophoresis in isogenic lines of wheat for resistance to *Erysiphe graminis* f.sp *tritici* and reported that existing proteins may not be involved in disease resistance.

Hwang *et al.* (1982) studied the electrophoretic patterns of proteins and esterase isozymes in noninfected leaves of six barley cultivars showing various levels of resistance to powdery mildew. The pattern of protein was similar for all the cultivars. But in the case of esterase, out of eight bands, genotypic differences were noticed in EST 4, EST 5 and EST 6. Based on these differences, they classified the cultivars into highly resistant, moderately resistant and highly susceptible.

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.

In tobacco plants inoculated with virulent and avirulent isolates of *Pseudomonas solanacearum* the isozyme patterns did not show any significant difference as observed by Yi *et al.* (1987).

Linde and Rhodes (1988) conducted isozyme studies with peroxidase enzymes in cucumber leaves and concluded that pre inoculation peroxidase activity in cucumber leaves were not associated with anthracnose resistance.

The genus *Capsicum* is less studied in isozyme genetic markers. Among 17 enzymes studied by Mathe and Wu (1988) the best result was expected by using the enzymes esterase, peroxidase, glutamate dehydrogenase, phosphoglucomutase, glutamate oxaloacetate transaminase and lactate dehydrogenase.

The peroxidase isozyme patterns of resistant and susceptible varieties of cotton to *Verticillium dahliæ* were compared by Xia *et al.* (1994). Both varieties showed the same pattern of peroxidase isozyme.

Alcazar *et al.* (1995) studied quantitative and qualitative changes in isoperoxidase patterns from stems of three cultivars of *Capsicum annuum* (susceptible, intermediate and resistant to *Phytophthora capsici*) and suggested that the increased peroxidase activity in conjunction with the appearance of an additional isoperoxidase band specific for the resistant cultivar could be the reason for the resistance of that cultivar against fungal attack.

Guldur and Yilmaz (1995) attempted for the rapid detection of tomato mosaic virus by agarose gel electrophoresis and reported that two main bands were produced from infected plants and no bands were detected in healthy plants.

2.4 Anatomical basis of bacterial wilt resistance

Anatomical evidence as to show how the bacterium reaches the vascular system is rather scanty. It is assumed that the bacterium has to digest its way through the primary wall of the weakened cortical cells as well as the tracheary elements where it is exposed between the spiral thickenings (Sequeira, 1993).

The pathogen first enters the intercellular space of the cortex. From there it moves to the pith and xylem vessels. Wilting of the plant is due to vascular plugging (Walker, 1952). Baldacci (1977) opined that besides EPS (Extra cellular polysaccharide) responsible for vascular plugging, a chemically unidentified fraction which alters the membrane permeability is produced by the pathogen. The bacterium also produced IAA which can initiate tylose formation and increase cell wall plasticity. Kolwal (1978) studied the histopathology of bacterial wilt of betel vine and noticed presence of bacteria in vascular bundles and parenchymatous cells.

Rapid deposition of compounds such as lignin (Friend *et al.*, 1973) and callose (Beckman *et al.*, 1982) on cell wall or near the point of penetration are certain mechanisms to prevent the entry of pathogen. Lignification resulted in the secondary cell wall thickening and acted as a physical barrier for the development of pathogen. The highly developed cells prevented colonization of the pathogen in host cells. Lignification occurred at the site of penetration of the pathogen and this barrier was resistant to cellulose and mascerating enzymes of the pathogen (Ride and Pearce, 1979).

Grimault *et al.* (1994) conducted histological studies in bacterial wilt resistant and susceptible tomato cultivars by light and electron microscopy, to investigate nature of barriers involved in the limitation of bacterial spread in resistant cultivars. They reported that in resistant cultivars, tyloses occluded the colonized vessels and the contiguous ones, limiting the bacterial spread. In the wilt susceptible cultivars, no tyloses were observed in colonized vessels and bacterial spread was not limited. Vascular colonization seemed generalized in the vascular bundles of the susceptible cultivar in contrast to the resistant one. Other reactions involved in resistance or susceptibility were observed such as gums, cell wall break down and modifications to the primary cell wall. They concluded that the limitation of bacterial spread associated with resistance of tomato to bacterial wilt was mainly attributed to an induced, nonspecific physical barrier.

Grafting experiments were carried out on tomato to investigate the mechanism of resistance to *P. solanacearum* (Grimault and Prior, 1994b). Colonization frequencies and bacterial densities observed in plants grafted on resistant or

susceptible root stock showed that resistance was correlated to the limitation of bacterial spread in the lower part of the stem.

Grimault and Prior (1994c) reported existence of a host defence mechanism for resistance to bacterial wilt after conducting studies using two avirulent mutant strains of *P. solanacearum* (8217 R - a spontaneous rough mutant and 8173 - a hrp engineered fluidal mutant) in resistant and susceptible cultivars of tomato.

Vasse *et al.* (1995) studied intercellular infection and protoxylem invasion of tomato roots by genetically defined mutant strains of *P. solanacearum* and explained the characterization of the process of tomato root infection. After colonization of exudation sites such as root extremities and axils of secondary roots the bacteria intercellularly infect the inner cortex and the vascular parenchyma. Following infection, the pathogenic strain invades protoxylem vessels degrading cell walls. They concluded that the colonization of approximately 25 per cent of xylem vessels in each vascular bundle of the hypocotyl just above the collar zone is sufficient to induce partial wilting of a tomato plant.

2.5 Use of radioisotopes in plant pathoglogical research

Information on the use of radioisotopes in bacteria especially *Pseudomonas solanacearum* (Smith) Smith is a few only. A general review on such studies in plant pathological research is presented.

The value of radioisotopes in research arises from the easiness and accuracy with which they can be identified and measured in extremely small amounts by use of highly sensitive methods. When a radioisotope has been

introduced into a system, its movement, behaviour and fate can be followed from source to ultimate destination. It can be done without recourse to normal chemical or physical methods of analysis which destroy and kill living tissues. Radioisotopes are therefore, of inestimable value in studying the complex dynamic biological system which also includes pathological aspects (Singh, 1989).

2.5.1 **Movement and distribution of pathogens in plant tissues**

Warren (1951) used ^{32}P to trace the movement and distribution of *Erwinia stewartii* within corn plants. Radioactivity of 0.37-1.00 μci/ml did not affect the development of disease or the growth rate of the bacteria, but concentrations above 4 μci/ml did. He used counters to determine the presence and radioautographs to determine the plant tissues involved and observed that there was no significant difference in distribution or rate of movement between susceptible and resistant varieties.

Suhayda and Goodman (1981) monitored the rate of migration of ^{32}P labelled virulent (E9 capsulated) and a virulent (E8 noncapsulated) strains of *Erwinia amylovora* in apple petiole and stem tissues. Irrespective of their capsulation, both were rapidly drawn into the vascular element of petiole and moved 5 mm within 20 min. The study provided evidence that virulent strain E9 moves quickly (34 mm/h) in xylem vessels and they concluded that the wounding of leaves that exposed xylem vessels to the pathogen could be an important factor in the infection process in nature.

Singh *et al.* (1983) reported that autoradiographs and ^{32}P activity of labelled pathogen showed that in cv. CO 312 eight internodes were infected while in

cv. CO 1148 it was restricted to either 1 or 1.5 internode. Mode of entry of *Colletotrichum falcatum*, the red rot pathogen of sugarcane, via. various portal entries was worked out using ^{32}P (Singh, 1989). The study revealed that lateral buds of sugarcane have minimum chances of pathogen entry in the presence of scales, however, infection increased when dried scales were removed, indicating that these acted as powerful mechanical barriers against the pathogen.

2.5.2 **Physiology of pathogen and host**

Radioisotopes are used extensively to study various aspects of physiology of parasitic fungi, bacteria, virus and nematodes.

Sequeira and Williams (1964) used radioactive precursors to investigate the differences in formation of indole-acetic acid by *Pseudomonas solanacearum*. They showed that the tobacco plant could convert tryptamine to IAA, where as the bacterium failed to do so. A wild type virulent strain of *P. solanacearum* and an avirulent mutant strain converted tryptophan to IAA, by different pathways.

Sequeira (1965) reinvestigated the inability of *P. solanacearum* to utilize tryptamine in synthesizing IAA to determine the origin of auxin in *P. solanacearum* infected tobacco. The amount of radioactive IAA was sufficiently higher during early phases of infection to suggest that IAA synthesis during the period by the host is correlated with host resistance.

Autoradiography showed the cellular accumulation of phenolic compounds during hypersensitive necrosis induced by *Puccinia graminis* f.sp. *tritici* in resistant wheat cultivars (Beardmore *et al.*, 1983). Ogram and Sayler (1988) used isotopic DNA probes in the ecological studies for rapid detection of microbes.

Wheat plants were inoculated with ^{35}S tagged *Erysiphe graminis* f.sp. *tritici*. The effect on the uptake and translocation to the epidermis of labelled S and its subsequent transfer to the pathogen was reported by Singh (1989). The amount of ^{32}P taken up and translocated to the epidermis of excised leaves was estimated by tagging *Erysiphe graminis* f.sp. *tritici* with ^{32}P.

2.6 Development of isogenic lines

Isogenic lines are those lines which differ for one gene (Allard, 1960; Mayo, 1980). They are of immense value in agricultural research. They are used to study the physiological effects, particularly in disease resistance. They play a great role to analyse the effect on major gene expression both of genetic and cytoplasmic background and of the physical environment (Evans, 1984).

For developing isogenic lines, F_1 and the successive generation from a suitable cross are selfed. In each generation, heterozygotes for the gene in question, are selected. After 8-10 generations of selfing, such plants may be expected to be homozygous for all the genes except the one maintained in heterozygous condition (Allard, 1960). Another method of producing isogenic lines consists of recurrent back crossing for 10 or more generations (Singh, 1990).

The appropriateness of the term "near isogenic line" was shown by Everson and Schuller (1955). Burton (1966) suggested that near isogenic lines could be created in less time and with less effort than isogenic lines. Zeven *et al.* (1986) confirmed the suspicion that near isogenic lines might indeed be different from isogenic lines in wheat.

Kalloo (1988) reported that generally four to five back crosses were required to produce near isogenic lines for resistance gene(s). In tomato, egg plant, cole crops and root crops, this method can be utilized to improve inbreds/varieties for production of multiple-resistant hybrids. The contribution of various growth attributes can be studied by involving isogenic lines.

Geetha (1989) used isogenic lines to work out the biochemical basis of resistance to bacterial wilt and heterosis in brinjal and reported that the isogenic lines of brinjal SM 6-2, SM 6-6 and SM 6-7 were evolved through pure line and single plant selection method giving emphasis on bacterial wilt resistance. The methods were practiced for eight years.

Mozzetti *et al.* (1995) developed a near isogenic line of *Capsicum*, resistant to *Phytophthora*. They crossed the susceptible variety to resistant variety followed by three back crosses on susceptible variety and these lines were used to find out the biochemical basis of disease resistance.

Materials and Methods

MATERIALS AND METHODS

The present investigations were carried out at the College of Horticulture, Kerala Agricultural University, Vellanikkara during 1992 to 1996. The crops were raised at the vegetable research farm of the Department of Olericulture, located at an altitude of 22.5 M above MSL and between 10° 32' N latitude and 76° 16' E longitude. The area enjoys a warm humid tropical climate. The experimental site has a sandy loam soil with a pH of 5.1.

The study consisted of the following experiments

1. Isolation and establishment of pure culture(s) of *Pseudomonas solanacearum*
2. Genetic basis of bacterial wilt resistance
3. Biochemical basis of bacterial wilt resistance
4. Anatomical basis of bacterial wilt resistance
5. Radiotracer studies on growth and infection of *P. solanacearum*
6. Development of near isogenic lines for resistance to bacterial wilt

3.1 Isolation and establishment of pure culture(s) of *Pseudomonas solanacearum*

This work was carried out at the Department of Plant Pathology, College of Horticulture, Vellanikkara.

3.1.1 Isolation of *P. solanacearum*

Chilli plants showing initial symptoms of bacterial wilt were collected and subjected to ooze test to detect presence of bacteria. Plant parts with profuse bacterial ooze were selected, cut into bits, surface sterilized with 0.1 per cent

mercuric chloride solution for one minute and serially passed through three changes of sterile distilled water. These bits were then placed on a sterile glass slide in a drop of sterile distilled water and teased apart to obtain a bacterial suspension. The suspension was streaked on Triphenyl Tetrazolium chloride Agar medium (TZC). Composition of TZC medium (Kelman, 1954) is as following.

Peptone	- 10.0 g
Casamino acid	- 1.0 g
Glucose	- 5.0 g
Agar agar	- 20.0 g
Distilled water	- 1000 ml
pH	- 6.8

The medium was sterilized at 15 lbs /sq. inch pressure for 15-20 minutes in an autoclave. Aqueous solution of TTC (1%) was also sterilized at 15 lbs pressure for 6-8 minutes by autoclaving and stored in the dark, 0.005 per cent of this solution was added to the above medium.

The inoculated plates were incubated at 30 $\pm$ 1 $^{\circ}$C for 48 h Characteristic isolated single colonies of round, fluidal, slimy and white with pin-point pink centres were identified and picked up into slants. This culture was purified by repeated cycles of purification by streaking on TZC medium.

3.1.2 **Pathogenicity tests**

Pathogenicity tests were conducted by inoculating a suspension of 24-48 h old culture of the bacterium prepared in sterile distilled water. The optical density (OD) of the solution was adjusted to 0.5 which is equivalent to 10^7 cfu per ml.

Two months old and vigorously growing chilli plants were transplanted in pots. Out of different methods of artificial inoculations like stem puncture, root dip and soil drenching tried, root dip method was the best and this method was used throughout the investigation. The pathogen was reisolated from the artificially inoculated host plants by the method already described and were compared and identified with the original isolate of the pathogen.

The reisolated culture was subjected to two cycles of purification and the pure culture was maintained. Stock cultures were maintained in PCA slants both at room temperature as well as at 10°C in refrigerated condition. Stock cultures were also maintained at 4°C by storing two or three loopful of the isolated bacteria in 5 ml of sterile distilled water in test tubes. Cultures were tested periodically for virulence and purity by streaking on TZC medium.

3.2 Genetic basis of bacterial wilt resistance

3.2.1 Experimental materials

The experimental materials comprised of selfed progenies of resistant variety Ujwala (CA 219) and the susceptible Pusa Jwala for developing F_1, F_2, B_1 and B_2 generations (Plate 1).

3.2.2 Development of F_1 hybrids

The experiment was laidout in the wilt prone area during September 1992 to January 1993. The variety Ujwala was grown in a bacterial wilt sick field. Spot planting was done with a known suscept Pusa Jwala to study the host reaction to the bacteria (Plate 2). The crop was raised as per package of practices of

Plate 1. Bacterial wilt susceptible (Pusa Jwala) and resistant (Ujwala) varieties in the wilt sick field

Plate 2. Spot planting technique

Agricultural University (KAU, 1989). When the plants started flowering, the roots of plants were injured with a spatula, and the soil was drenched with the bacterial suspension of *P. solanacearum* (0.5 OD) to ensure presence of sufficient inoculum in the soil.

The susceptible variety Pusa Jwala was grown in pots containing sterilized medium. The bacterial wilt resistant variety Ujwala (P_1) was crossed with the known suscept Pusa Jwala (P_2) to study genetics of resistance to bacterial wilt.

3.2.3 Development of segregating generations

During April to September 1993, the parents and F_1 hybrids were transplanted in the crossing block. The F_1's were backcrossed with both the parents to obtain B_1 and B_2 generation seeds and selfed to obtain F_2 generation seeds.

3.2.4 Evaluation of six generations for wilt resistance

The evaluation of F_1, F_2, B_1 and B_2 generations along with their parents (P_1 and P_2) was carried out in a wilt sick field during December 1993 to April 1994. Experiment was laid out in a randomised block design with five replications. There were 30 plants each in P_1, P_2 and F_1 and 100 plants each in F_2, B_1 and B_2 per replication. Spot planting with the known suscept Pusa Jwala was done to confirm presence of virulent strains of *Pseudomonas solanacearum* in the field. Incidence of bacterial wilt was recorded at 15 days interval from transplanting upto 105 days and was confirmed through ooze test.

3.2.5 Statistical analysis

Reaction of the six generations to bacterial wilt incidence

Percentage survival of plants in each generation in the field at intervals of 15, 30, 45, 60, 75, 90 and 105 days after transplanting were recorded. This period was divided into six stages viz. Stage I (15 to 30th day), Stage II (30 to 45th day), Stage III (45 to 60th day), Stage IV (60 to 75th day), Stage V (75 to 90th day) and Stage VI (90 to 105th day) and the percentage incidence of bacterial wilt in each stage was analysed statistically.

Inheritance of resistance to bacterial wilt

The data from segregating and non segregating populations were collected and analysed.

Mendalian basis of resistance

The chi-square test was done to test the fitness to appropriate genetic ratios in F_2, B_1 and B_2 (Panse and Sukhatme, 1978). The formula used was

$$X^2 = \frac{(l_2a_1 - l_1a_2)^2}{l_1l_2n}$$

where: a_1 and a_2 are the observed frequencies in classes expected to be in ratio l_1:l_2 and $n = a_1 + a_2$.

Penetrance of the gene for disease resistance was estimated as suggested by Avdeyev (1979).

Scaling tests and generation mean analysis

Cavalli's joint scaling test was done to test the model for generation means and to estimate components (Mather and Jinks, 1971).

Degree of dominance

The degree of dominance was calculated by using the formula 'h'/'d' (Mather and Jinks, 1971).

3.3 Biochemical basis of bacterial wilt resistance

The experiment was carried out in the Biochem lab of the College of Horticulture, Vellanikkara. Ujwala, the resistant variety and Pusa Jwala the susceptible variety were analysed for the biochemical status at four stages viz., 30 days, 45 days, 60 days and 75 days after sowing. Sixty days old seedlings of Ujwala and Pusa Jwala were inoculated through roots and analysed seven days after inoculation. Roots, stems, leaves and whole plant at different growth stages of healthy and 60 days old inoculated seedlings were used for the analysis.

The experiment consisted of five parts as under

1. Estimation of total phenols and OD phenols
2. Estimation of protein
3. Enzyme activities
4. Protein pattern by electrophoresis
5. Isoenzyme analysis

3.3.1 Estimation of total phenols and OD phenol

The resistant variety Ujwala and the susceptible variety Pusa Jwala were analysed for the biochemical status at four growth stages viz. 30 days, 45 days, 60 days and 75 days after sowing. To estimate the changes in the content, 60 days old seedlings of Ujwala and Pusa Jwala were inoculated through roots and estimated seven days after inoculation.

Alcoholic extracts of roots, stems, leaves and whole plant were used for estimation. The samples were separately cut into small pieces and placed in boiling ethyl alcohol (10 ml for each 2 g of tissue) for 10 minutes, cooled and the tissues were crushed thoroughly in a mortar and pestle. The homogenized material was centrifuged at 3000 rpm for 10 minutes.

Total phenols

Total phenols were estimated by Folin ciocalteu method (Mahadevan and Sridhar, 1982). The intensity of blue colour developed was read at 650 nm in a spectrophotometer. The total phenol content was calculated from a standard curve of tannic acid and was expressed as mg/g of sample.

$$\text{Factor x X} = \text{Y } \mu\text{g} = \frac{\mu\text{g standard}}{\text{Absorbance of standard}} \text{ x absorbance of sample}$$

$$= \frac{\text{Y}}{200} = \text{mg/g sample}$$

Ortho-dihydric phenol

Arnow's method was followed for the estimation of ortho-dihydric phenols (Mahadevan and Sridhar, 1982). The absorbance of the pink solution was read in a spectrophotometer at 515 nm. Catechol was used as the standard and OD phenol content was expressed as mg/g sample.

$$\text{Factor x X} = \text{Y } \mu\text{g} = \frac{\mu\text{g standard}}{\text{Absorbance of standard}} \text{ x absorbance of sample}$$

$$= \frac{\text{Y}}{200} = \text{mg/g}$$

3.3.2 **Estimation of protein**

Protein content of enzyme extract was determined by Lawry's method (Sadasivam and Manickam, 1992). Roots, stem, leaves and whole plant of the resistant variety Ujwala and susceptible variety Pusa Jwala at four growth stages viz., 30 days, 45 days, 60 days and 75 days after sowing and inoculated seedlings at 60 days were used for the study.

Reagents

A. 2% sodium carbonate in 0.1N sodium hydroxide

B. 0.5% copper sulphate in 1% potassium sodium tartrate

C. Mixed 50 ml A and 1 ml B prior to use

D. Substrate - Folin-Ciocalteau Reagent

E. Protein solution (stock standard)

Weighed accurately 600 mg bovine serum albumin and dissolved in 100 ml 0.1N NaOH i.e., 6000 μg/ml

F. Working standard

From the stock solution prepared 600 μg, 400 μg, 300 μg, 200 μg, 150 μg and 100 μg protein/ml.

Extraction buffer

Tris	- 21.1995 g
Citric acid	- 2.62675 g
Vitamin C	- 0.52839 g
Cystein HCl	- 0.52689 g
Water to	- 500 ml
pH	- 7

Enzyme extract

Extracted two g of fresh plant tissue in 10 ml of extraction buffer (pH 7) by grinding in a precooled mortar and pestle. The homogenised material was centrifuged at 18000 rpm for 15 minutes at 5°C. The supernatents were used as enzyme source.

Procedure

Pipetted out 1 ml of working standard solutions into series of test tubes and 0.1 ml and 0.2 ml of the sample extract in two other test tubes. Made up the volume to 1 ml with distilled water in all the test tubes. A tube with one ml of water

served as the blank. Added 5 ml of reagent C to each tube including blank. Mixed well and allowed to stand for 10 minutes. Then added 0.5 ml of reagent D, mixed well and kept for 30 minutes at room temperature for blue colour development and read at 60 nm. A standard graph was drawn and calculated the amount of protein in the sample and expressed as mg/g sample.

3.3.3 Enzyme activities

The activity of two enzymes viz., polyphenol oxidase and peroxidase were assayed. Roots, stems, leaves and whole plant of the resistant variety Ujwala and susceptible variety Pusa Jwala at four growth stages viz., 30 days, 45 days, 60 days and 75 days after sowing and inoculated seedlings at 60 days were used for the study.

Peroxidase

Peroxidase activity was assayed by the method suggested by Sadasivam and Manickam (1992). Guaicol was used as the substrate and the rate of formation of guaicol dehydrogenation product is a measure of the peroxidase activity.

$$\text{Guaicol} + H_2O_2 \xrightarrow{\text{Peroxidase}} \text{Oxidised guaicol} + 2\ H_2O$$

Extraction buffer

Same as for protein estimation. Ten ml of stock solution was diluted to 100 ml by cooled distilled water.

Substrate

Guaicol solution 20 mM - Dissolved 240 mg guaiacol in water and made up to 100 ml.

Hydrogen peroxide solution - Diluted 0.14 ml of 30 per cent H_2O_2 to 100 ml water. Prepared freshly.

Enzyme extract

Extracted one g of fresh plant tissue in three ml of extraction buffer (pH 7) along with 0.040 g of insoluble PVP in a precooled mortar and pestle. All operations were carried out at 4°C. The homogenised material was centrifuged at 18000 rpm for 15 minutes in a refrigerated centrifuge at 5°C. Used the supernatent as enzyme source with in 2-4 hours.

Procedure

Set the spectrophotometer at 436 nm with 3.1 ml buffer, 0.05 ml guaicol and 0.03 ml H_2O_2. Pipetted out 3 ml buffer solution, 0.05 ml guaiacol solution, 0.1 ml enzyme extract and 0.03 ml hydrogen peroxide solution in a cuvette, mixed well and placed in the spectrophotometer. Waited until the absorbance has increased by 0.05. Started a stopwatch and noted time required in minutes ($\blacktriangle t$) to increase the absorbance by 0.1.

Calculation

$$\text{Enzyme activity units/litre of extract} = \frac{3.18 \times 0.1 \times 1000}{6.39 \times 1 \times \blacktriangle t \times 0.1}$$

$$= \frac{500}{\blacktriangle t}$$

The extinction coefficient of guaiacol dehydrogenation product at 436 nm is 6.39 per micromole.

Polyphenol oxidase

Polyphenol oxidase activity was assayed by the method suggested by Malik and Singh (1980).

$$\text{Catechol} \xrightarrow[\text{Oxidase}]{\text{Catechol}} \text{Quinone}$$

Extraction buffer

Same as for protein estimation.

Buffer for assay

Monobasic sodium phosphate solution 0.2 M	- 87.7 ml
Dibasic sodium phosphate solution 0.2 M	- 12.3 ml
Water to	- 200 ml

Substrate

Buffer	- 100 ml
Catechol	- 0.01 M (0.11 g/100 ml)

Enzyme extract

Same as for protein estimation.

Procedure

Pipetted out 1 ml extraction buffer and 5.5 ml phosphate buffer in a cuvette and set the spectrophotometer at 495 nm. One ml enzyme extract and 5.5 ml phosphate buffer were taken in the cuvette and noted the reading. Pipetted out 1 ml enzyme extract, 5.4 ml buffer and 0.1 ml catechol in the cuvette, mixed immediately and started recording the changes in absorbance for every 30 seconds up to 5 minute.

Calculation

Plotted the increasing absorbance values and read the changes in absorbance per minute from the linear phase of the curve. Expressed enzyme activity in terms of rate of increased absorbance per unit time per mg protein.

$$\text{Specific activity} = \frac{\text{1 minute activity}}{\text{Protein (mg)}}$$

3.3.4 **Protein pattern by electrophoresis**

Polyacrylamide gel electrophoresis (PAGE) using vertical slab gel was carried out for electrophoretic pattern of proteins. Acrylamide monomers were polymerised with N-N methylene bis acrylamide [$CH_2(NH\ CO\ NH = CH_2)_2$ bis] to obtain the gel. Freshly prepared ammonium persulphate acted as catalyst and N, N, N', N' - tetra methyl ethylene diamine (TEMED) as chain initiator.

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

Preparation of the gel

The following stock solutions were prepared.

Solution A

Tris	- 38.3 g
TEMED	- 0.46 ml
IN HCl	- 48 ml
Distilled water to	- 200 ml
pH	- 9.0

Solution B

Acrylamide	- 30.0 g
Bisacrylamide	- 0.9 g
Distilled water to	- 100 ml

Solution C

Ammonium per sulphate	- 0.14 g
Distilled water to	- 100 ml

Preparation of gel column

The slab gel unit of 'Biochem' was used in the study. The size of slab gel was 16 cm x 14 cm x .01 cm. Solutions A and B were stored in amber coloured bottles at 0-4°C. Solution C was prepared fresh for each run. Stock A, B and C were pipetted out in the ratio 1:1:2, mixed well and the solution was gently injected

by a syringe in between glass plates kept in polymerisation stand. Pushed the combs in for making wells and allowed to polymerise in the electrophoretic column (45-60 min).

Electrophoretic run

Electrode buffer solution

Tris	- 6 g
Glycine	- 28.8 g
Distilled water to	- 1000 ml
pH	- 8.3

The stock buffer was diluted to 1:10 before use.

After polymerisation, the gels were transfered to electrophoretic apparatus. The upper and lower tanks were filled with pre-cooled electrode buffer. Removed the combs carefully and 10 μl of sample was applied to each well with a transfer pette of E. Merk. Upper tank was connected to the cathode and lower one to the anode. The analysis of protein was carried out in anionic system. Bromophenol blue (0.002%) was added to the upper tank as the tracer dye.

Electrophoresis was performed at 5 $^{\circ}$C for 4½ hours by using a cooling system to provide heat dissipation. Adjusted the current at 50 mA per slab and increased to 125 mA gradually and kept constant through out the running.

Preparation of the sample

Chilli seedlings were raised in pots sterilized with formaldehyde. The plant parts collected in ice baskets were washed under tap water and then rinsed with

distilled water. The adhering water was removed using blotting sheets. Roots and leaves of healthy and inoculated seedlings were used for the study.

Extraction buffer

Same as protein estimation.

Taken 0.1 g PVP and 17.15 g sucrose in 10 ml stock solution and made up to 100 ml. Insoluble PVP was used to chelate the polyphenols and thus prevent oxidation by polyphenol oxidase enzyme.

Chilled mortar and pestle was used for extraction. One gram sample and 5 ml of extraction buffer were used. All operations were done at 4°C. The homogenized material was centrifuged at 15000 rpm for 15 minutes in a refrigerated centrifuge at 5°C. After centrifugation, the clear supernatent was collected and stored below subzero temperature (refrigerator freezer chest).

Staining of gels

Immersed the gel in 0.01 per cent Coomassie brilliant blue R 250 in 15 per cent Trichloro acetic acid (TCA) overnight. Seven per cent glacial acetic acid was used for destaining at 50-60°C. Repeated the destaining till clear bands were obtained.

Qualitative analysis

The relative position of each visualised band in the gel were drawn schematically for easy reference and the Rm (relative mobility) value was calculated using the formula

$$Rm = \frac{\text{Distance migrated by the sample}}{\text{Distance migrated by the dye}}$$

3.3.5 Isoenzyme analysis

Polyacrylamide gel electrophoresis of two isoenzymes viz., peroxidase (PRX) and esterase (EST) were studied. Since the enzymes were unstable, fresh samples were used at low temperatures. The gel and buffer solution were prepared as in protein electrophoresis.

Extraction buffer

For peroxidase, protein extraction buffer was used. But for esterase,

Sodium phosphate buffer (5x conc.)	- 10 mM
EDTA Na^{2+}	- 1 mM
2-mercapto ethanol	- 1 mM
pH	- 9.5

Staining of gels

Peroxidase

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, filtered and then hydrogen peroxide was added. The gels were immersed in staining solution till brown bands appeared and destained in

seven per cent acetic acid. As the bands faded on standing for long time, photographs were taken on the same day of staining.

Esterase

Sodium dihydrogen phosphate	- 2.8 g
Disodium hydrogen phosphate	- 1.1 g
Fast blue RR salt	- 0.2 g
Alpha-naphthyl acetate	- 0.03 g
Water to	- 200 ml

Prepared fresh stain and incubated the gel in the staining solution at 37°C till light brown bands appeared. Stopped the enzyme action by adding a mixture of methanol: water:acetic acid:ethyl alcohol in the ratio 10:10:2:1 and photographed the zymogram and Rm values were calculated.

3.4 Anatomical basis of bacterial wilt resistance

The resistant variety Ujwala and the susceptible variety Pusa Jwala at 45 days after sowing were used for the comparative evaluation of the anatomy. Transverse hand sections of the stem from the middle of the first internode and taproot, 1 cm below ground level were taken, stained with safranin and temporary mounts were prepared and examined under the microscope. The arrangement of the epidermis, endodermis, vascular bundles and lignification of xylem vessels were observed and compared in the resistant and susceptible varieties.

3.5 Radiotracer studies on the growth and infection of *P. solanacearum*

The entry, movement, growth and multiplication, colonisation and infection of *P. solanacearum* were studied employing radiotracer techniques at the Radio Tracer Laboratory, College of Horticulture during May 1992 to May 1993. In these studies either ^{32}P or ^{35}S was used depending on the objective of the study. These radioisotopes were procurred from Board of Radiation and Isotope Technology, Bombay.

Virulent strain of *P. solanacearum* from the pure culture was grown in the peptone-casamino acid broth. After 48 hours, the above strain was cultured in medium containing ^{32}P or ^{35}S.

3.5.1 Tolerance of bacterium to ^{32}P and ^{35}S

The tolerance and growth of *P. solanacearum* to radioactivity concentrations was determined by two separate experiments viz. (a) the measurement of turbidity by using spectronic-20 and (b) serial dilution plate technique.

Measurement of turbidity

The broth was dispersed in 5 ml quantities and six concentrations of ^{32}P (0, 3, 6, 12, 21 and 30 μCi/5 ml or 0, 111, 222, 444, 777 and 1110 kBq/5 ml) or ten concentrations of ^{35}S (0, 10, 20, 40, 60, 80, 100, 120, 140 and 150 μCi/5 ml or 0, 370, 740, 1480, 2220, 2960, 3700, 4440, 5180 and 5550 kBq/5 ml) were added separately to each tube. Three replications were maintained. The tubes were inoculated with one loopful of a 24-48 hr old broth culture of *P. solanacearum*. After incubation for 48 hr, the bacterial growth was measured by using spectronic 20 with control tubes which contained no radioactive phosphorus and sulphur.

Serial dilution plate technique

The growth of the bacterium after irradiation at the above doses was also assayed using the serial dilution plate technique (10^{-4} dilution). The media used for this technique were TZC and the pink centred colonies were counted and expressed as the number of virulent colonies per ml of culture. The values were transformed into logarithemic transformation for statistical analysis. From these data, the effect of radioactivity on the growth of bacterium was examined.

Radioactivity concentration of 44.4 kBq/ml (1.2 μCi/ml) of ^{32}P or 740 kBq/ml (20 μCi/ml) of ^{35}S was chosen for the plant inoculation experiments since this amount of radiation did not affect the growth of *P. solanacearum*.

3.5.2 Distribution pattern of ^{32}P and ^{35}S tagged *P. solanacearum* in resistant and susceptible varieties

To determine the pattern of distribution of the bacteria within the host tissues sixty days old seedings of the resistant variety 'Ujwala' and susceptible variety 'Pusa Jwala' were used for inoculation.

Dose and duration of isotope treatment

Isotope	Concentration	Time allowed for absorption
^{32}P	44.4 kBq/ml	1 h and 24 h
^{35}S	740 kBq/ml	1 h and 24 h

For plant inoculation, radioactivity of 44.4 kBq/ml (1.2 μCi/ml) ^{32}P or 740 kBq/ml (20 μCi/ml) ^{35}S was added separately to sterilized broth. One ml of 48 h old inoculum was added to 50 ml of the above broth and incubated for 48 h .

Centrifugation was begun with 48 h old culture by transfering the contents aseptically to sterile centrifuge tubes and centrifuged at 7000 rpm. The clear supernatant was poured off, and the cells were resuspended in sterile medium and recentrifuged at 8000 rpm. Again two more centrifugations were done at 9000 and 10000 rpm by resuspending the pellets in sterile medium. In all these, centrifugation was done for 10 minutes at 30°C. All the procedures were done in an aseptic condition.

3.5.3 **Plant inoculation**

Plant inoculation with this labelled bacterium was done in small glass bottles (5 ml) containing 3 ml suspension and the root system of plant was inserted into the bottle after giving root injuries and kept in position by plugging it with cotton.

The inoculated plants were placed in the green house. After the prescribed period of absorption, the aerial plant parts were detached by cutting at the base of the stem. The plant parts were then arranged on an absorbent paper in their original position and secured with the aid of adhesive tape. The specimen sandwiched between absorbent sheets were then pressed in herbarium press and allowed to dry under room temperature.

One loopful of inoculum from the glass bottles was taken and streaked on TZC medium to confirm the viability and virulence of the bacteria.

3.5.4 Autoradiography

After drying, the pressed plant parts were autoradiographed by placing on X-ray films in dark and covered with smooth paper and pressed. The X-ray films were exposed for a period of 2 days for ^{32}P and 30 days for ^{35}S in the press. The plant parts were then removed and the film was developed using a commercial X-ray film developer solution.

3.5.5 Radioassay of plant samples

The determination of radioactive bacteria (^{32}P) in plant samples was done by following Cerenkov counting method developed by Wahid *et al.* (1985). The method consisted of wet digestion of dried and finely cut stem and leaves separately with 2:1 nitric-perchloric acid mixture (HNO_3 + $HClO_4$). Radioactivity was determined in the digest after transferring it quantitatively in to a 20 ml scintillation counting vial with distilled water. The radioactivity was determined in a computer controlled liquid scintillation system (Pharmacia LKB Wallac OY, Finland) adopting channel settings and computer programme for the liquid scintillation counting of tritium (^{3}H). The count rates (cpm) were corrected for background and decay.

The ^{35}S assays were performed by separating the aerial plant parts in to three sections viz. (1) the actively accumulated areas (nodes), (2) other parts of stem and (3) leaves. The samples @ 1 g each were digested using nitric-perchloric acid (2:1) mixture. The digested samples were made upto 5 ml and stored in sample bottles and kept for settling of silica. One millilitre of this digest was transferred into a 20 ml scintillation counting vial and mixed with 15 ml of a dioxane based

liquid scintillation mixture which is prepared by mixing Naphthalene 30 g, 2,5-Diphenyl oxazole (DPO) 2 g 1,4-bis (5-Phenyl Oxazolyl) Benzene (POPOP) 0.1 g, Methanol 50 ml and Ethylene glycol 10 ml and made up to 500 ml with Dioxane. The radioactivity was then determined by scintillation counting technique in a liquid scintillation system mentioned already.

3.6 Development of near isogenic lines for resistance to bacterial wilt

For the production of near isogenic resistant line of Pusa Jwala, the bacterial wilt resistant variety Ujwala was crossed with the susceptible variety Pusa Jwala. The F_1s were grown in a bacterial wilt sick field. Spot planting with Pusa Jwala was done to confirm the presence of virulent strains of bacteria in the field. The field resistant F_1 plants were crossed to Pusa Jwala to produce BC_1 generation which was raised again in the same bacterial wilt sick field with spot planting. The wilt resistant BC_1 plants were again back crossed to Pusa Jwala to produce BC_2 generation. BC_2 generation was evaluated again in the bacterial wilt sick field.

The wilt resistant plants from BC_2 were selfed to produce BC_2F_2. BC_2F_2 generation was evaluated again in the same wilt sick field and field resistant plants possessing characteristics of Pusa Jwala were selected. For this purpose, the survived plants were scored based on plant height, fruit orientation, clustered/ solitary fruit, fruit length and fruit colour.

Plant height

Plants having a height less than 52 cm were scored '1' and plants having a height more than 52 cm were scored '0'.

Fruit orientation

Plants with pendulous fruits resembling like Pusa Jwala were scored '1' and those with erect fruit characteristics of Ujwala was scored '0'.

Clustered/solitary fruit

Plants with solitary fruits as in Pusa Jwala were scored '1' and those with clustered fruits as in Ujwala was scored '0'.

Fruit length

Fruits of more than 8 cm length were scored '1' and those of less than 8 cm were scored '0'.

Fruit colour at maturity

Plants having light green fruits as in Pusa Jwala were scored '1' and plants having dark green fruits as in Ujwala were scored '0'.

Plants having a score of '1' for the above five characteristics were selected. They were again selfed to produce BC_2F_3. Scoring was done as in BC_2F_2 generation. The bacterial wilt resistant BC_2F_3 plants having characteristics of Pusa Jwala were selfed again to produce BC_2F_4. They were grown in a bacterial wilt sick field. The survived plants were scored for the traits of Pusa Jwala.

Results

RESULTS

Data from the present investigations were analysed and are presented under the following heads:

1. Isolation and establishment of pure culture(s) of *Pseudomonas solanacearum*
2. Genetic basis of bacterial wilt resistance
3. Biochemical basis of bacterial wilt resistance
4. Anatomical basis of bacterial wilt resistance
5. Radiotracer studies on the growth and infection of *Pseudomonas solanacearum*
6. Development of near isogenic lines for resistance to bacterial wilt

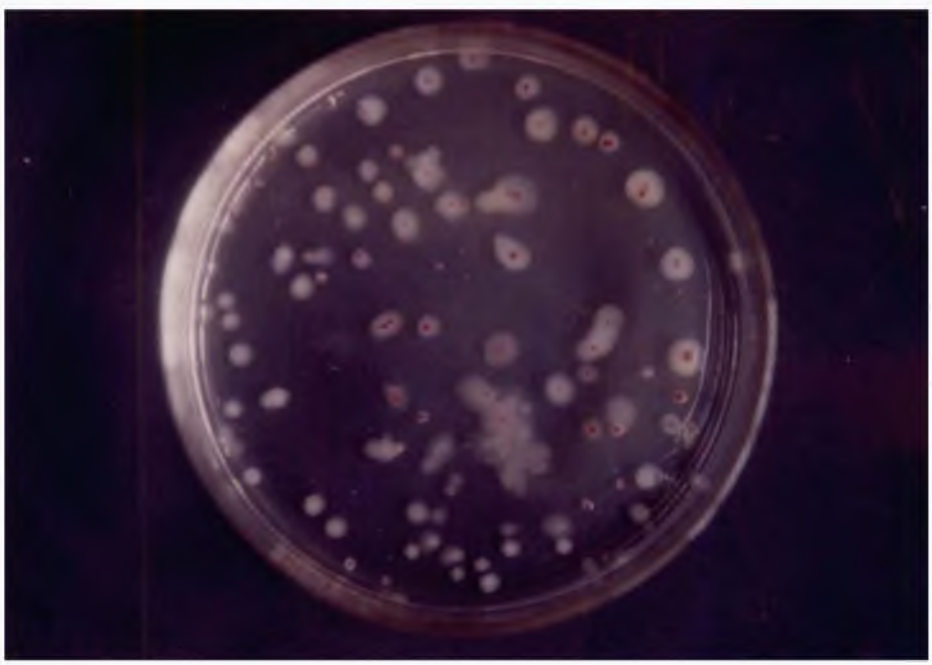
4.1 Isolation and establishment of pure culture(s) of *Pseudomonas solanacearum*

The bacterial wilt pathogen was isolated from just wilted chilli plants on Triphenyl Tetrazolium Chloride Agar medium (TZC). It produced circular, smooth, shiny, convex, greyish white, fluidal and slimy colonies with pin point pink centre after 24 to 48 h of incubation (Plate 3).

To test the pathogenicity of the bacterial isolates, different methods of artificial inoculation of chilli plants like stem puncture, root dip and soil drenching were tried. Root dip method gave better results.

Pathogenicity of the bacterial isolates were tested on healthy chilli seedlings artificially inoculated (root dip method) with the bacterial isolates. The inoculated plants started developing typical symptoms of wilt within a week. The plants wilted completely in two weeks. Bacteria isolated from such artificially

Plate 3. *Pseudomonas solanacearum* in TZC medium



inoculated plants yielded typical bacterial colonies resembling the original isolates. The pathogenicity of bacterial isolate was thus proved. These bacterial isolates were used throughout the course of this investigation.

4.2 Genetic basis of bacterial wilt resistance

The parents and progenies of F_1, F_2, B_1 and B_2 generation of the crosses involving the resistant variety 'Ujwala' and the susceptible variety 'Pusa Jwala' were evaluated to study genetic basis of bacterial wilt resistance.

4.2.1 Response of six generations to bacterial wilt incidence

The mean performance of six generations for survival(%) against bacterial wilt at intervals of 15, 30, 45, 60, 75, 90 and 105 days after transplanting were presented in Table 1 and in Fig.1.

There was significant difference in the performance between generations for incidence of bacterial wilt at all stages (Table 2). In P_1, P_2, F_1 and B_1, maximum wilt incidence was noticed in the first stage (15-30 days after transplanting). It got reduced gradually and by the end of fourth stage (60-75 DAP) no further incidence of bacterial wilt was noticed except in F_1 and B_2. In P_1 the incidence was only upto the third stage (45-60 DAP). In F_2 maximum wilt incidence was in the third stage (45-60 DAP) where as in B_2 it was in the second stage (30-45 DAP).

The differences in wilt incidence between stages were also highly significant in all the generations. At fifth and sixth stages, difference in the incidence of wilt was not significant and the survival of plants reached a plateau by 75th day.

Table 1. Mean performance of parents and its progenies for survival against bacterial wilt

Generations	Survival (%)						
	15th day	30th day	45th day	60th day	75th day	90th day	105th day
P_1	100.00	96.80	94.00	92.00	92.00	92.00	92.00
P_2	97.40	63.40	36.60	18.00	12.00	12.00	12.00
F_1	98.00	66.60	54.00	40.00	24.00	23.20	23.20
F_2	97.20	76.00	65.00	38.20	30.40	30.40	30.40
B_1	100.00	84.20	69.40	61.20	55.00	55.00	55.00
B_2	97.80	78.60	44.60	26.60	19.00	18.40	18.40

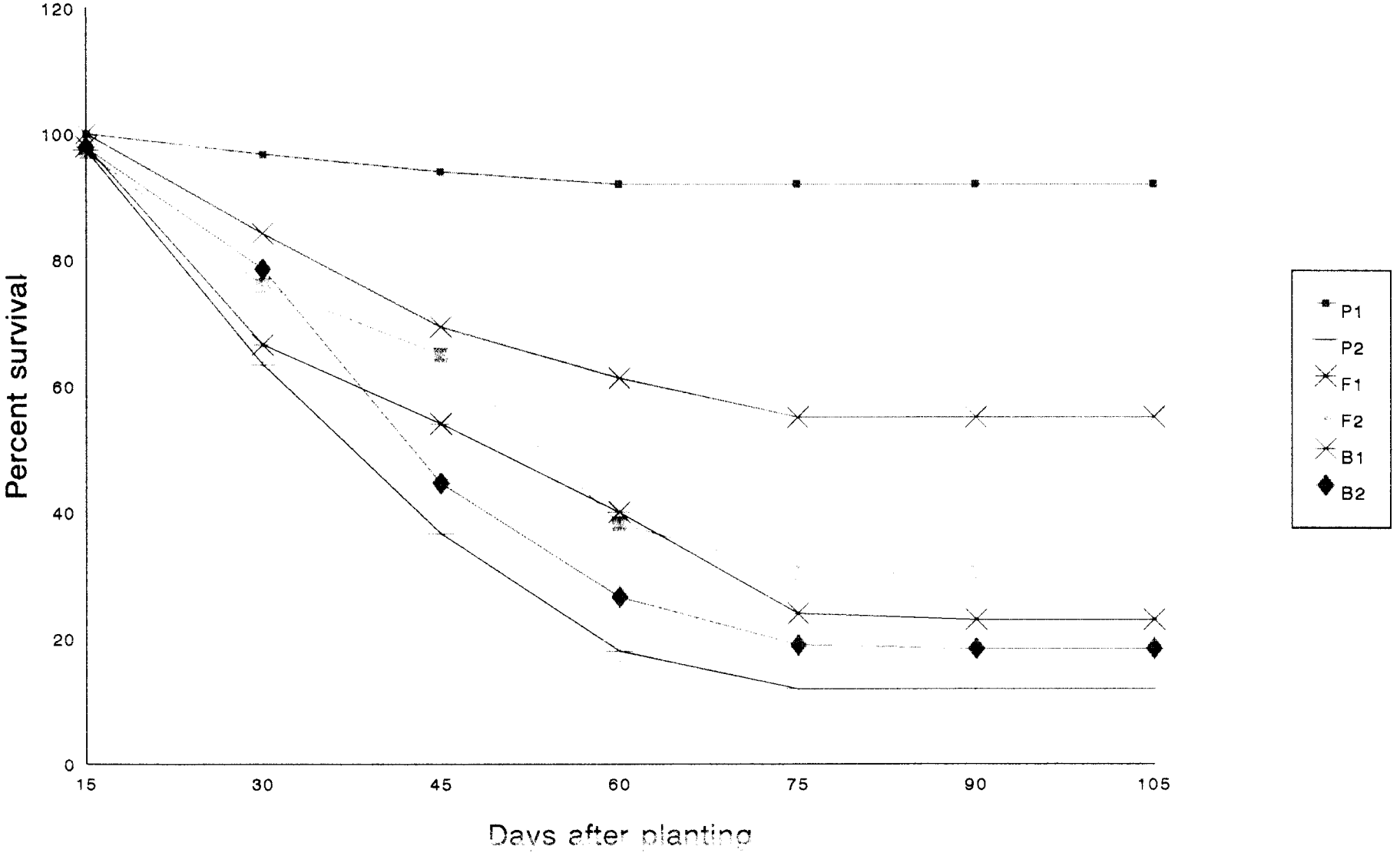
Table 2. Incidence of bacterial wilt in chilli at different growth stages

Generations	Wilt incidence (%)						
	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI	CD
P_1	3.20	2.80	2.00	0.00	0.00	0.00	2.12*
P_2	34.00	26.80	18.60	6.00	0.00	0.00	5.93**
F_1	31.40	12.60	14.00	16.00	0.80	0.00	9.79**
F_2	21.20	11.00	26.80	7.80	0.00	0.00	6.84**
B_1	15.80	14.80	8.20	6.20	0.00	0.00	5.28**
B_2	19.20	34.00	18.00	7.60	0.60	0.00	8.20**
CD	7.47**	7.97**	5.93**	8.41**	NS		

** Significant at 1%
* Significant at 5%
NS - Nonsignificant

Stage I 15 to 30th day after transplanting
Stage II 30 to 45th day after transplanting
Stage III 45 to 60th day after transplanting
Stage IV 60 to 75th day after transplanting
Stage V 75 to 90th day after transplanting
Stage VI 90th to 105 day after transplanting

Fig.1 Percent survival against bacterial wilt at various stages of crop growth in Ujwala (P1) Pusa Jwala (P2) and their F1,F2 and BCs in bacterial wilt sick field



4.2.2 Inheritance of resistance to bacterial wilt

Mendelian basis of resistance

In segregating and nonsegregating generations, the counts of wilted and survived plants were taken (Table 3). The results showed that resistance to bacterial wilt in Ujwala is inherited by a single incompletely dominant gene.

The parent Ujwala was highly resistant but the penetrance to resistance was not complete (92%) and in the susceptible parent the penetrance to susceptibility was also not complete (88%). There was a survival of 23.3 per cent of plants in F_1. From the background performance of heterozygotes in F_1, the expected ratio of wilt resistant plants to susceptible plants in F_2 should be 173:327. The actual ratio observed was 152:348 which fitted the expectations well ($X^2 = 3.72$, $0.10 > P > 0.05$). The segregation pattern in the back cross populations further supported the F_2 data. The monogenic incompletely dominant inheritance of wilt resistance was confirmed.

In the B_1 generation, involving resistant parent, 275 plants were resistant and 225 susceptible (expected ratio 288:212) showing a good fit to the test cross ratio 1:1 ($X^2 = 1.85$, $0.20 > P > 0.10$). In the back cross with the susceptible parent, the expected and observed ratio of plants resistant and that susceptible was 88:412 and 92:408 respectively ($X^2 = 0.05$, $0.90 > P > 0.80$) which showed a good fit indicating the action of single incompletely dominant gene for wilt resistance.

Table 3. Inheritance of resistance to bacterial wilt in chilli

Generations	No. of plants			Expected ratio assuming partial penetrance	X^2	P
	Total	Resistant	Susceptible			
P_1 (Ujwala)	150	138	12			
P_2 (Pusa Jwala)	150	18	132			
F_1 (P_1 x P_2)	150	35	115			
F_2	500	152 (173)	348 (327)	1.38:2.62	3.72	0.10-0.05
B_1 (F_1 x P_1)	500	275 (288)	225 (212)	0.58:0.42	1.85	0.20-0.10
B_2 (F_1 x P_2)	500	92 (88)	408 (412)	0.18:0.82	0.05	0.90-0.80

Figures in parenthesis are expected number assuming partial penetrance of resistance

Scaling tests and generation mean analysis

The six generations P_1, P_2, F_1, F_2, B_1 and B_2 with varying levels of resistance to bacterial wilt were used for the test (Table 4).

Absence of epistasis was detected by scaling tests (Tables 5a and b). The estimates of three parameters along with their standard error are presented in Table 5a. The result showed that all the parameters were not significant. The three parameter model was found to be adequate to explain the generation means which indicated that the resistance was not governed by epistatic gene action.

Since the calculated $X^2_{(3)}$ was not significant at 5 per cent level, the model was found adequate and is a good fit to explain the mode of inheritance. The expected and observed generation means are presented in Table 5b. The expected values are very close to the observed values.

Degree of dominance obtained was 76 per cent from the following formula

$$h/d = \frac{29.89}{39.33} = 0.76$$

4.3 Biochemical basis of bacterial wilt resistance

4.3.1 Estimation of total phenols and OD phenol

Analysis of variance showed significant difference in total phenol content in the roots of the resistant variety Ujwala and susceptible variety Pusa Jwala. In OD phenol content, significant difference was noticed in roots and shoots.

Table 4. Level of resistance in parents and its progenies to bacterial wilt in chilli (%)

Replications	P_1	P_2	F_1	F_2	B_1	B_2
R_1	93.00	10.00	20.00	21.00	50.00	17.00
R_2	93.00	7.00	23.00	31.00	56.00	18.00
R_3	93.00	13.00	13.00	31.00	51.00	19.00
R_4	87.00	13.00	27.00	28.00	56.00	16.00
R_5	94.00	17.00	33.00	41.00	62.00	22.00
Total	460.00	60.00	116.00	152.00	275.00	92.00
Mean	92.00	12.00	23.20	30.40	55.00	18.40

Table 5a. Estimates of the three parameters along with their standard error in scaling tests

Parameter	Estimate	SE of estimate (+)
m	52.09	1.0
d	39.33	0.94
h	-29.89	2.47

Table 5b. Testing the goodness of fit of the model

Generation	Observed mean (O)	Expected mean (E)
P_1	92.00	91.42
P_2	12.00	12.76
F_1	23.20	22.20
F_2	30.40	37.15
B_1	55.00	56.81
B_2	18.40	17.48

$X^2(3) = 6.40$

Total phenols

Contents of total phenol varied with stages of growth in all parts of the plant except leaves (Table 6). Maximum phenol content was observed at 45 days after sowing in stem and whole plant in Ujwala and all parts except roots in Pusa Jwala. The roots and leaves of Ujwala and roots of Pusa Jwala had maximum phenol content at 60th day.

The total phenol content of bacterial wilt resistant and susceptible genotypes differed significantly only in roots at 45th and 60th days after sowing (Table 6 and Fig.2a & b). In Ujwala the total phenol content of roots was 1.174 mg/g and 1.180 mg/g at 45th day and 60th day respectively where as in Pusa Jwala it was only 1.123 mg/g and 1.137 mg/g respectively.

OD phenol

There was significant difference in OD phenol in both Ujwala and Pusa Jwala at different growth stages in all parts except leaves in Pusa Jwala (Table 7). Maximum OD phenol content was at 45th day after sowing in both genotypes in all parts except roots of Ujwala, where it was maximum at 60th day.

Among the resistant and susceptible genotypes, significant difference was noticed in roots and shoots (Table 7 and Fig. 3a & b). The OD phenol content of Ujwala was higher than that of Pusa Jwala at 30th day in all the plant parts at different stages except in leaves. In Ujwala, the OD phenol content of roots was 0.096 mg/g and 0.099 mg/g at 45th and 60th day respectively where as in Pusa Jwala, it was only 0.083 mg/g and 0.054 mg/g.

Effect of artificial inoculation on total phenols and OD phenol contents

Artificial inoculation of the pathogen resulted in a significant increase in total phenols in both resistant (Ujwala) and susceptible (Pusa Jwala) genotypes of chilli (Table 8 and Fig. 4). Before inoculation total phenol content of whole plants were 2.649 mg/g and 2.610 mg/g in Ujwala and Pusa Jwala respectively. After inoculation, it increased to 4.267 mg/g (61.08%) and 4.272 mg/g (63.68%) respectively. In roots, the increase was 73.14 per cent in Ujwala and 111.70 per cent in Pusa Jwala. In shoots and leaves the increase was 60.36 per cent and 85.04 per cent in Ujwala and 77.43 per cent and 97.94 per cent in Pusa Jwala.

Significant difference was observed between inoculated and uninoculated seedlings for OD phenol content in all the plant parts except leaves in Pusa Jwala (Table 9 and Fig. 5). Before inoculation, the OD phenol content of whole plant was 0.246 mg/g and 0.243 mg/g in Ujwala and Pusa Jwala respectively which was increased to 0.398 mg/g (61.79%) and 0.325 mg/g (33.74%) respectively after inoculation. In roots, shoots and leaves the increase was 146.46 per cent, 76.33 per cent and 53.24 per cent respectively in Ujwala and 133.33 per cent, 67.69 per cent and 29.94 per cent in pusa Jwala respectively.

Total phenol content of plants after inoculation did not vary significantly among resistant and susceptible genotypes in any of the plant parts. However, the OD phenol content between varieties varied significantly in all the plant parts (Table 10). In general the OD phenol content was higher in the resistant variety. In Ujwala it was 93.65 per cent higher in roots than that in Pusa Jwala.

Table 6. Total phenol content in bacterial wilt resistant and susceptible genotypes (mg/g of fresh tissue)

Growth stage	Root			Stem			Leaf			Whole plant		
	Ujwala	Pusa Jwala	t value	Ujwala	Pusa Jwala	t value	Ujwala	Pusa Jwala	t value	Ujwala	Pusa Jwala	t value
30th day	0.853	0.758	NS	1.586	1.614	NS	3.083	2.850	NS	2.622	2.667	NS
45th day	1.174	1.123	3.312**	1.873	1.748	NS	3.265	3.148	NS	2.795	2.768	NS
60th day	1.180	1.137	2.689**	1.708	1.684	NS	3.343	2.861	NS	2.649	2.610	NS
75th day	1.164	1.110	NS	1.532	1.442	NS	3.228	3.152	NS	2.013	1.981	NS
CD	0.089**	0.089**	-	0.118**	0.177**	-	NS	NS	-	0.325**	0.354**	-

** Significant at 1%
NS - Nonsignificant

Table 7. OD phenol content in bacterial wilt resistant and susceptible genotypes (mg/g of fresh tissue)

Growth stages	Root			Stem			Leaf			Whole plant		
	Ujwala	Pusa Jwala	t value	Ujwala	Pusa Jwala	t value	Ujwala	Pusa Jwala	t value	Ujwala	Pusa Jwala	t value
30th day	0.066	0.061	NS	0.112	0.103	NS	0.319	0.327	NS	0.263	0.224	NS
45th day	0.096	0.083	NS	0.178	0.136	2.944**	0.470	0.413	NS	0.311	0.256	NS
60th day	0.099	0.054	2.669**	0.169	0.130	2.906**	0.355	0.344	NS	0.246	0.243	NS
75th day	0.096	0.072	2.605**	0.152	0.137	2.652**	0.299	0.294	NS	0.192	0.180	NS
CD	0.030**	0.030**	-	0.030**	0.030**	-	0.089**	NS	-	0.059**	0.059**	-

**Significant at 1%
NS - Nonsignificant

Table 8. Total phenol content of inoculated and uninoculated bacterial wilt resistant and susceptible genotypes (mg/g fresh tissue)

	Ujwala				Pusa Jwala			
	Root	Stem	Leaf	Whole plant	Root	Stem	Leaf	Whole plant
Uninoculated	1.180	1.708	3.343	2.649	1.137	1.684	2.861	2.610
Inoculated	2.043 (73.14)	2.739 (60.36)	6.186 (85.04)	4.267 (61.08)	2.408 (111.70)	2.988 (77.43)	5.663 (97.94)	4.272 (63.68)
t value	8.567**	9.492**	3.587*	7.063**	7.179**	6.434**	7.454**	4.637**

Table 9. OD phenol content of inoculated and uninoculated bacterial wilt resistant and susceptible genotypes (mg/g fresh tissue)

	Ujwala				Pusa Jwala			
	Root	Stem	Leaf	Whole plant	Root	Stem	Leaf	Whole plant
Uninoculated	0.099	0.169	0.355	0.246	0.054	0.130	0.344	0.243
Inoculated	0.244 (146.46)	0.298 (76.33)	0.544 (53.24)	0.398 (61.79)	0.126 (133.33)	0.218 (67.69)	0.447 (29.94)	0.325 (33.74)
t value	14.041**	6.070**	4.138**	6.852**	9.727**	5.936**	NS	2.574**

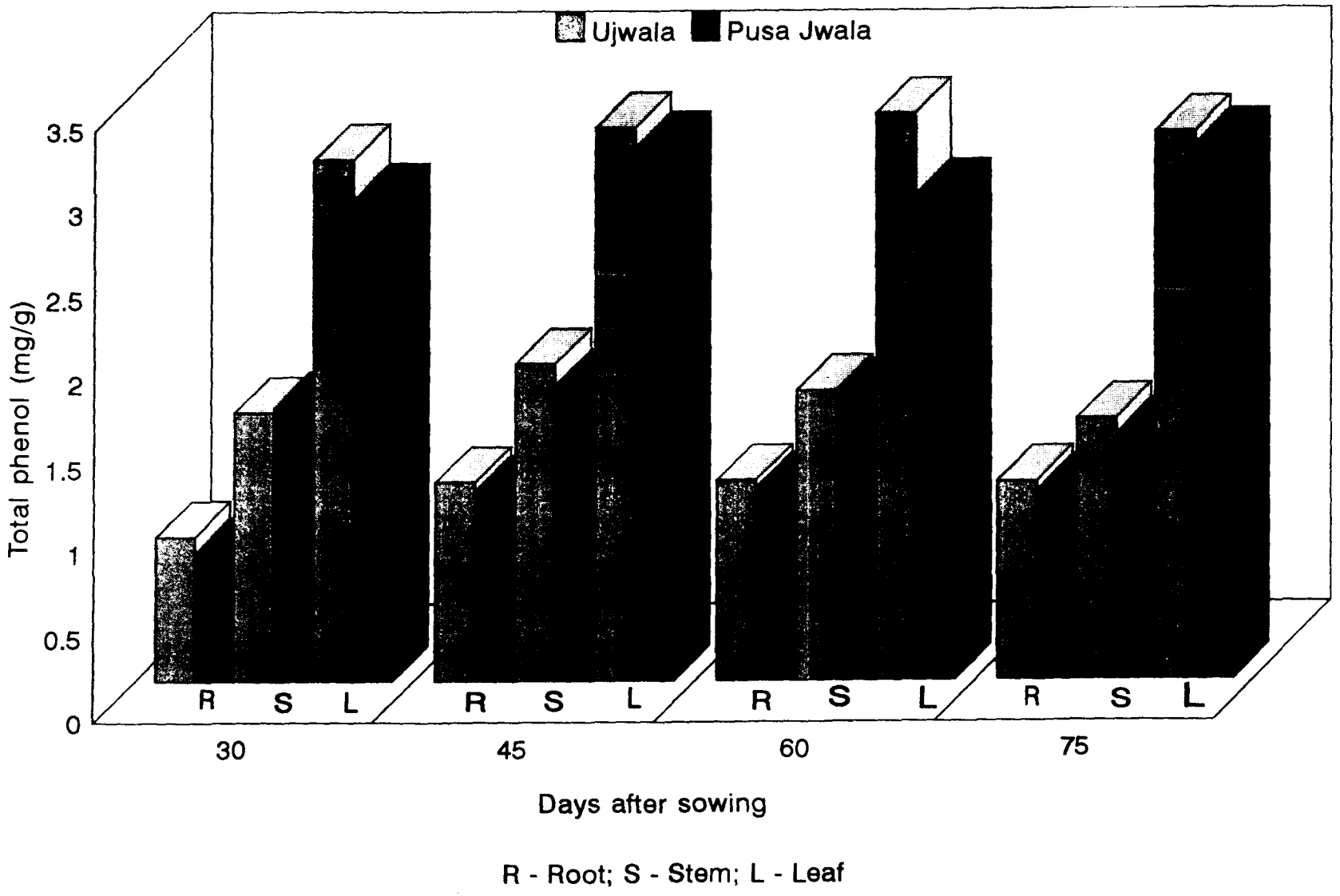
**Significant at 1%
NS - Nonsignificant
Figures in parenthesis are per cent increase over uninoculated

Table 10. Total phenol content and OD phenol content of wilt resistant and susceptible genotypes after inoculation (mg/g fresh tissue)

	Total phenol				OD Phenol			
	Root	Stem	Leaf	Whole plant	Root	Stem	Leaf	Whole plant
Ujwala	2.043	2.739	6.186	4.267	0.244	0.298	0.544	0.398
Pusa Jwala	2.408	2.988	5.663	4.272	0.126	0.218	0.447	0.325
t value	NS	NS	NS	NS	4.289**	3.196**	2.283**	2.305**

**Significant at 1%
NS - Nonsignificant

Fig.2b. Total phenol content in bacterial wilt resistant and susceptible genotypes



R - Root; S - Stem; L - Leaf

Fig.3a OD phenol content in bacterial wilt resistant and susceptible genotypes

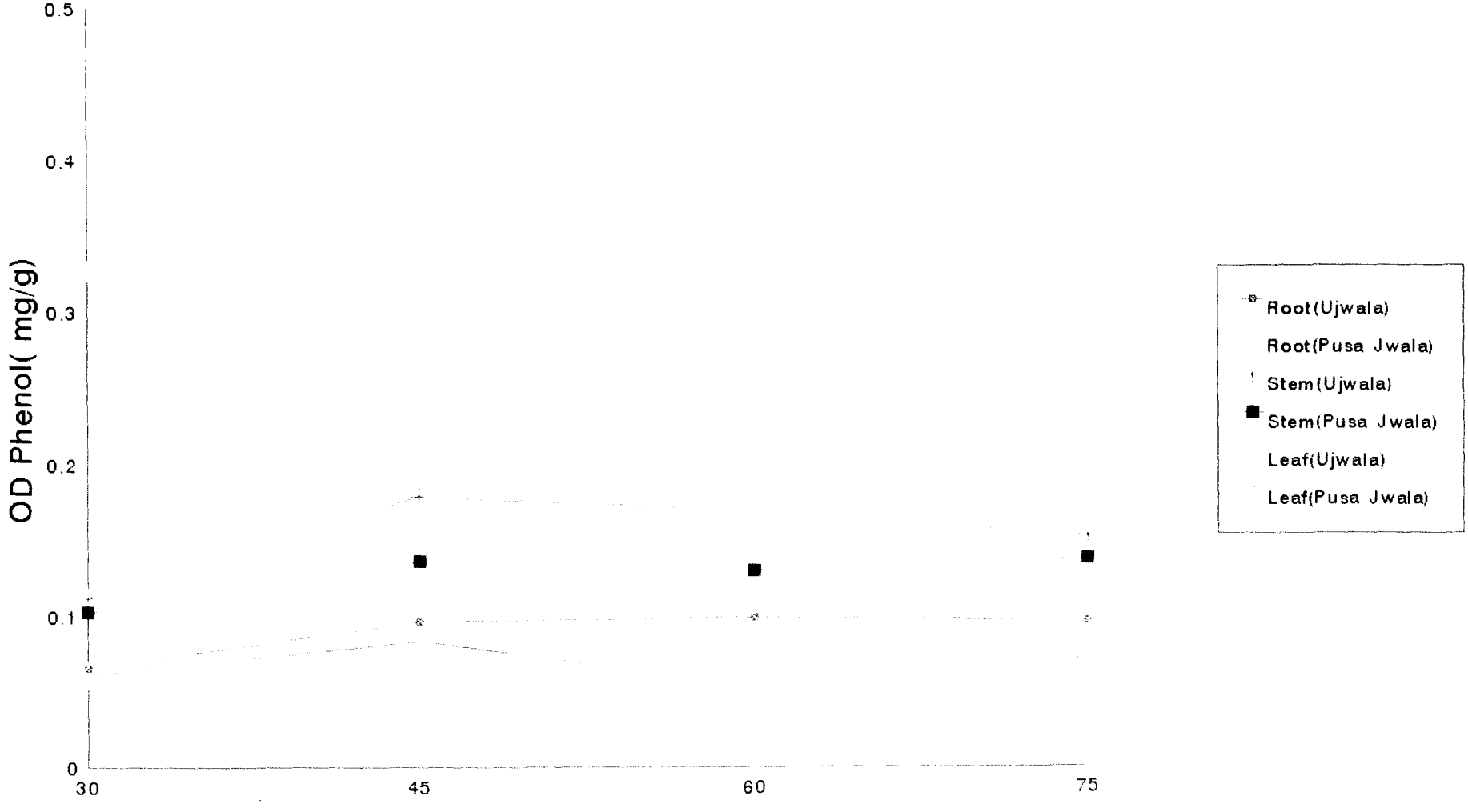
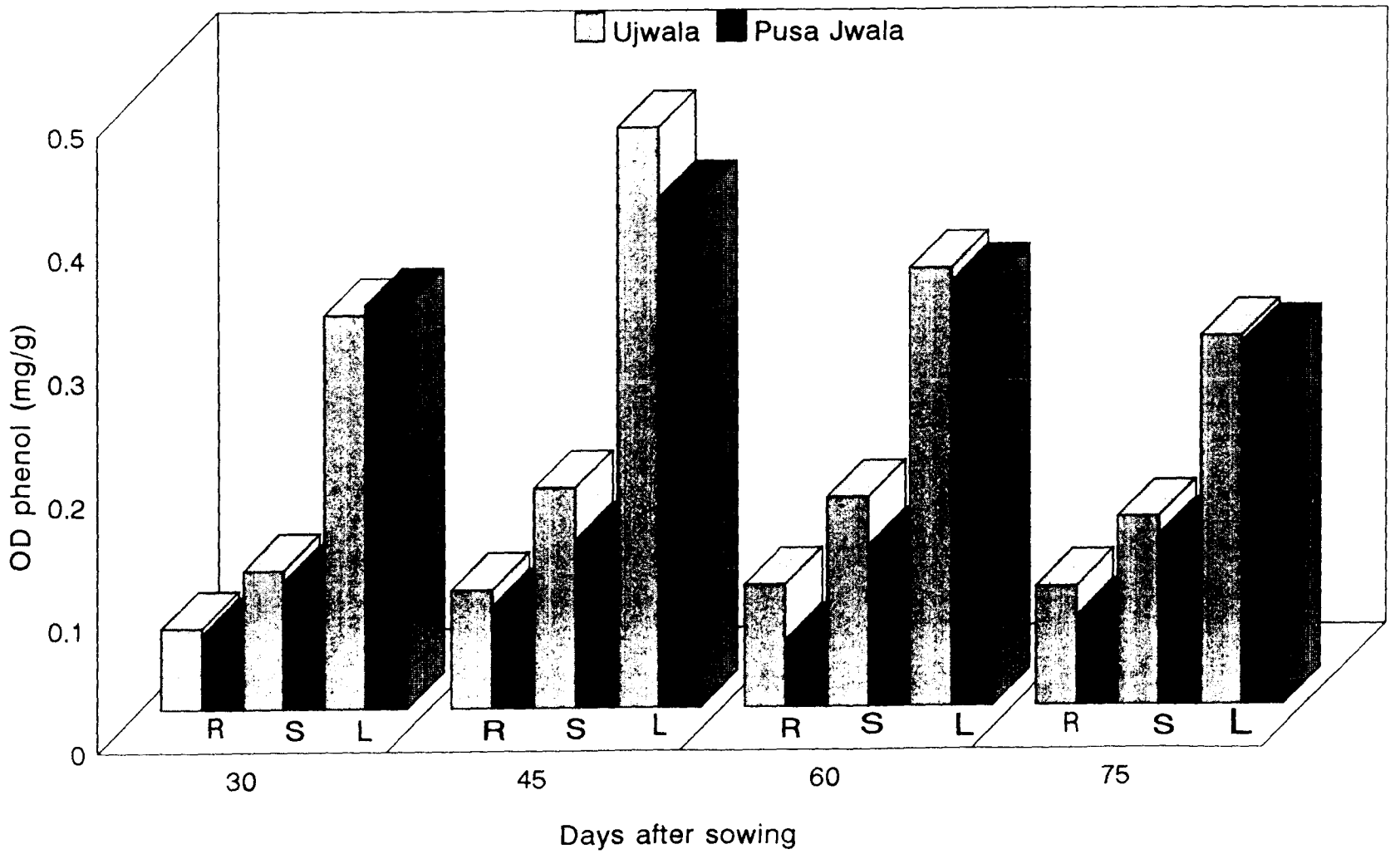


Fig.3b. OD phenol content in bacterial wilt resistant and susceptible genotypes



R - Root; S - Stem; L - Leaf

Fig.4. Total phenol content of uninoculated and inoculated wilt resistant and susceptible genotypes

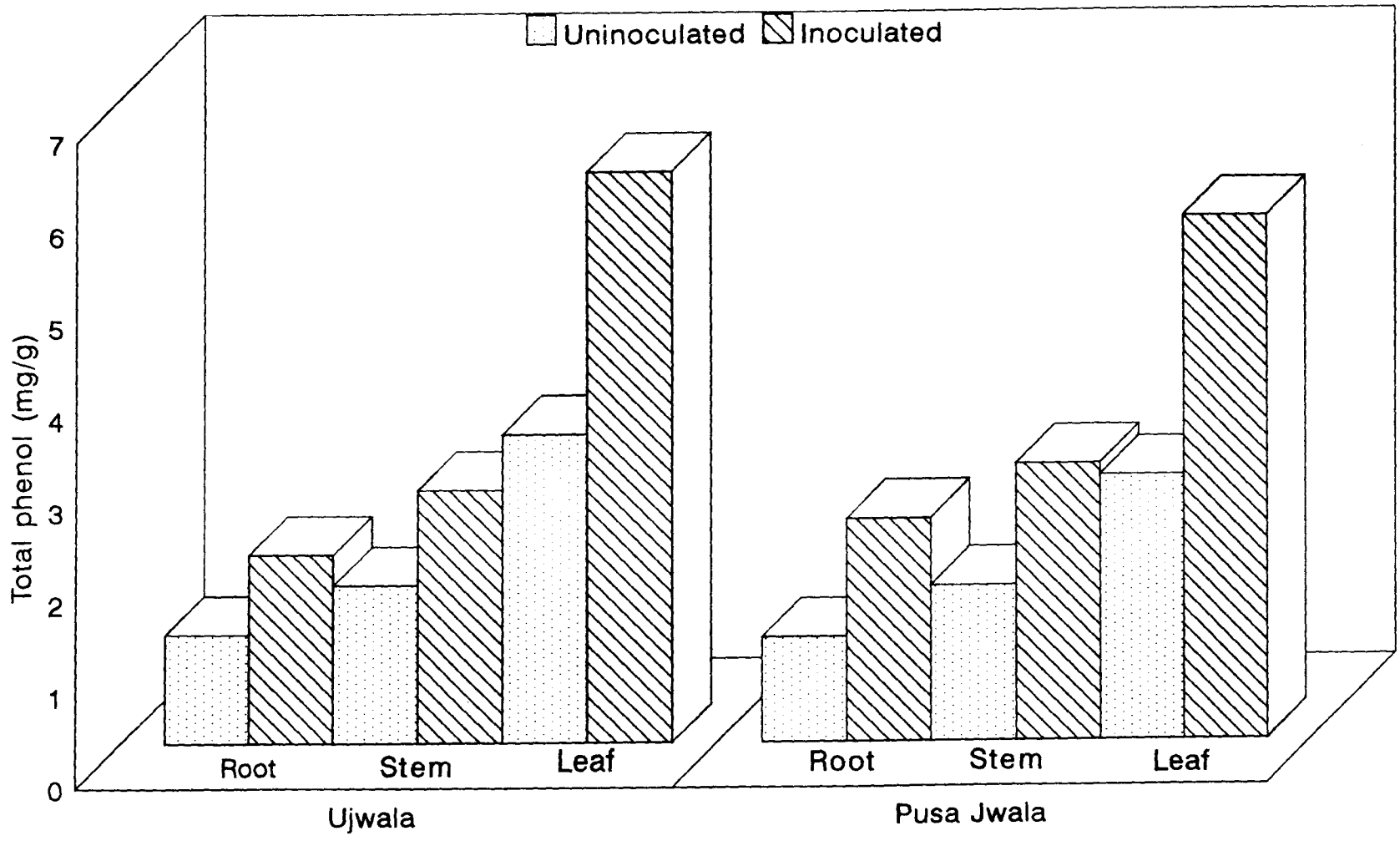
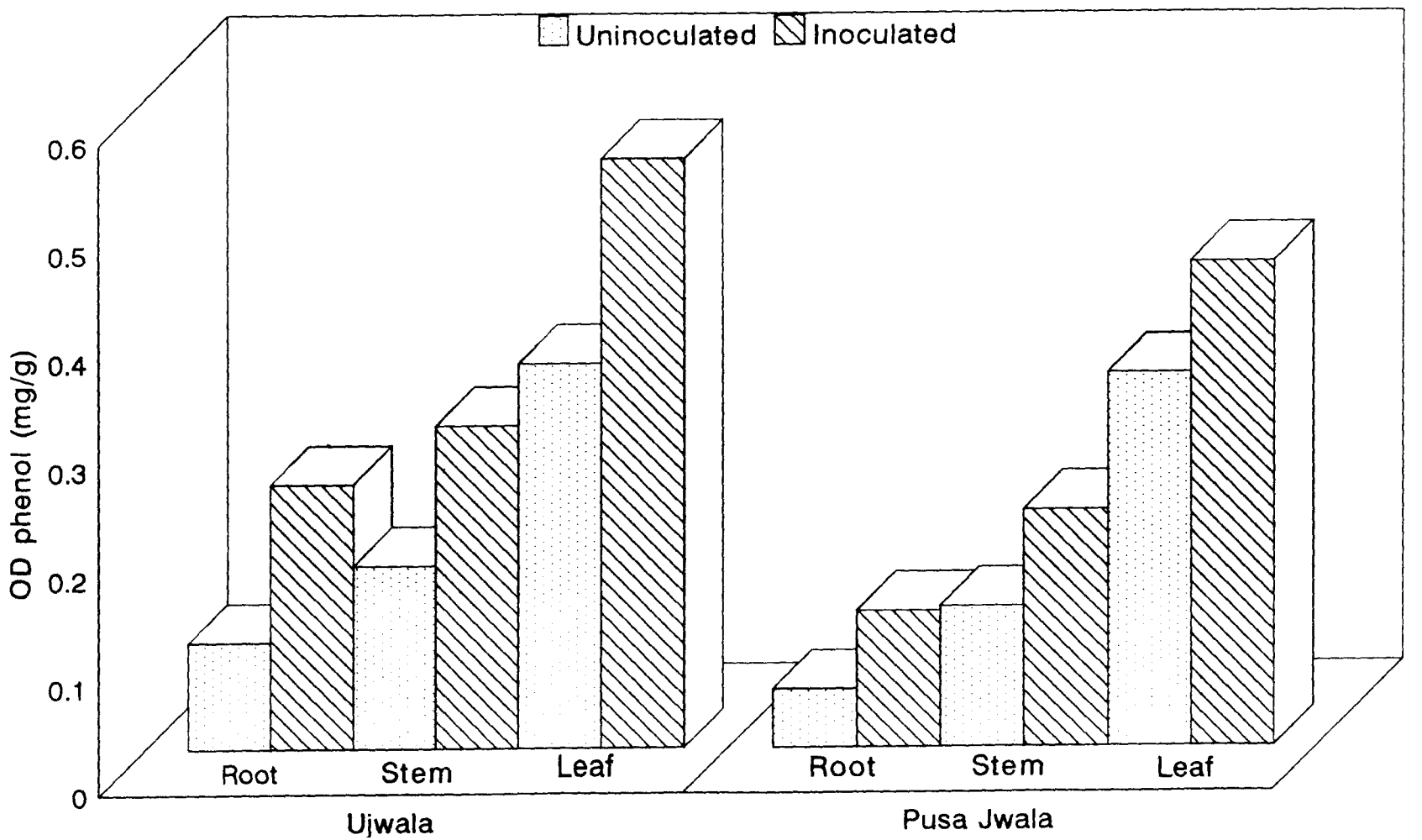


Fig.5. OD phenol content of uninoculated and inoculated wilt resistant and susceptible genotypes



4.3.2 Protein content

The protein content of bacterial wilt resistant and susceptible genotypes differed significantly only in roots and stems (Table 11). The resistant genotype Ujwala had a higher protein content at 30th day and 60th day after sowing in all plant parts except leaves. At 45th day only roots showed enhanced protein content while at 75th day the protein content was higher in stem and whole plant when compared to Pusa Jwala.

The protein content of the resistant and susceptible varieties increased progressively with increase in plant age up to 60 days after sowing (Table 11 and Fig. 6a & b). After 60th day, there was reduction in protein content. Significant variation was observed in protein content at different growth stages of plant. Maximum protein content (35.667 mg/g) was observed in leaves of Ujwala at 60th day followed by leaves of Pusa Jwala (35.333 mg/g). Roots at 30th day had the lowest protein content (13.583 mg/g and 14.833 mg/g) in Pusa Jwala and Ujwala.

Inoculation of chilli plants resulted in a progressive increase in protein content in both resistant and susceptible genotypes (Table 12 and Fig. 7). In Ujwala significant differences were noticed in all plant parts while in Pusa Jwala only the stem and whole plant showed significant differences. In Ujwala, the inoculation resulted in 9.58-12.08 per cent increase in protein where as only 3.07-7.61 per cent increase was observed in Pusa Jwala. The increase in protein content in Pusa Jwala was low when compared with Ujwala. The highest protein content was seen in leaf (39.083 and 36.417 mg/g in Ujwala and Pusa Jwala respectively) while roots recorded the lowest protein content (24.750 mg/g in Ujwala and 21.583 mg/g in Pusa Jwala).

Table 11. Protein content in bacterial wilt resistant and susceptible genotypes (mg/g fresh tissue)

Growth stages	Root			Stem			Leaf			Whole plant		
	Ujwala	Pusa Jwala	t value	Ujwala	Pusa Jwala	t value	Ujwala	Pusa Jwala	t value	Ujwala	Pusa Jwala	t value
30th day	14.833	13.583	3.212**	18.583	16.500	4.580**	22.833	22.750	NS	22.583	20.083	7.408**
45th day	18.500	16.333	4.540**	22.250	21.833	NS	32.417	32.000	NS	28.417	28.083	NS
60th day	22.083	20.417	3.147*	24.250	23.000	2.274*	35.667	35.333	NS	32.083	30.917	NS
75th day	17.750	17.333	NS	22.500	21.917	2.568*	34.833	34.583	NS	27.083	25.583	3.834*
CD	1.151**	0.974**	-	1.180**	0.679**	-	1.829**	1.328**	-	1.092**	0.915**	-

* Significant at 5% level
** Significant at 1% level
NS - Nonsignificant

Table 12. Protein content of inoculated and uninoculated bacterial wilt resistant and susceptible genotypes (mg/g fresh tissue)

	Root			Stem			Leaf			Whole plant		
	Ujwala	Pusa Jwala	t value	Ujwala	Pusa Jwala	t value	Ujwala	Pusa Jwala	t value	Ujwala	Pusa Jwala	t value
Unino-culated	22.083	20.417	3.147*	24.250	23.000	2.274*	35.667	35.333	NS	32.083	30.917	NS
Inoculated	24.750 (12.08)	21.583 (5.71)	4.394** -	26.750 (10.31)	24.750 (7.61)	2.349** -	39.083 (9.58)	36.417 (3.07)	3.500** -	35.167 (9.61)	33.083 (7.01)	3.222** -
t value	3.700**	NS	-	3.417**	3.416**	-	3.740**	NS	-	3.916**	4.218**	-

* Significant at 5% level
** Significant at 1% level
NS - Nonsignificant
Figures in parenthesis are per cent increase over uninoculated

Fig.6a Protein content in bacterial wilt resistant and susceptible genotypes

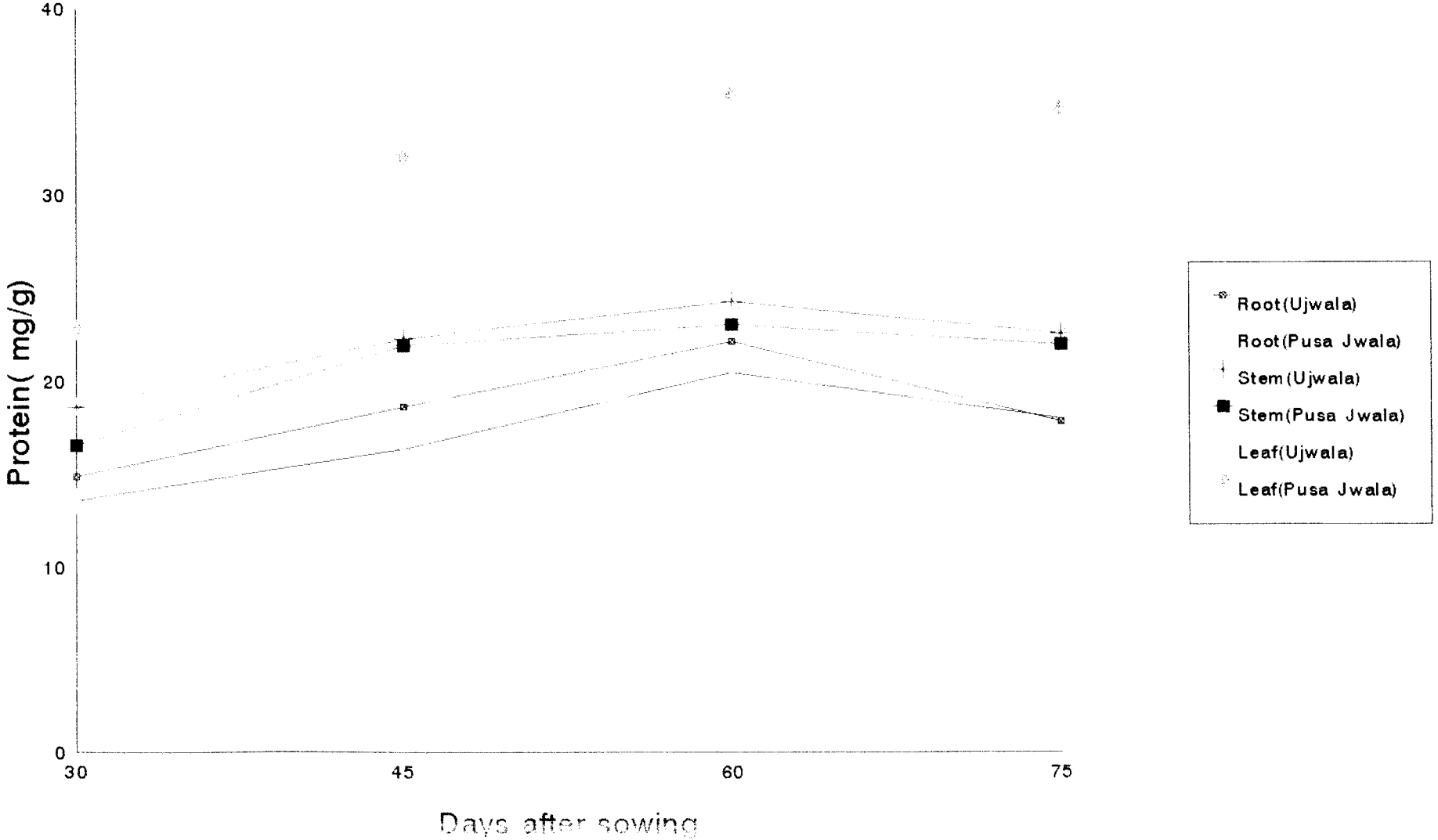
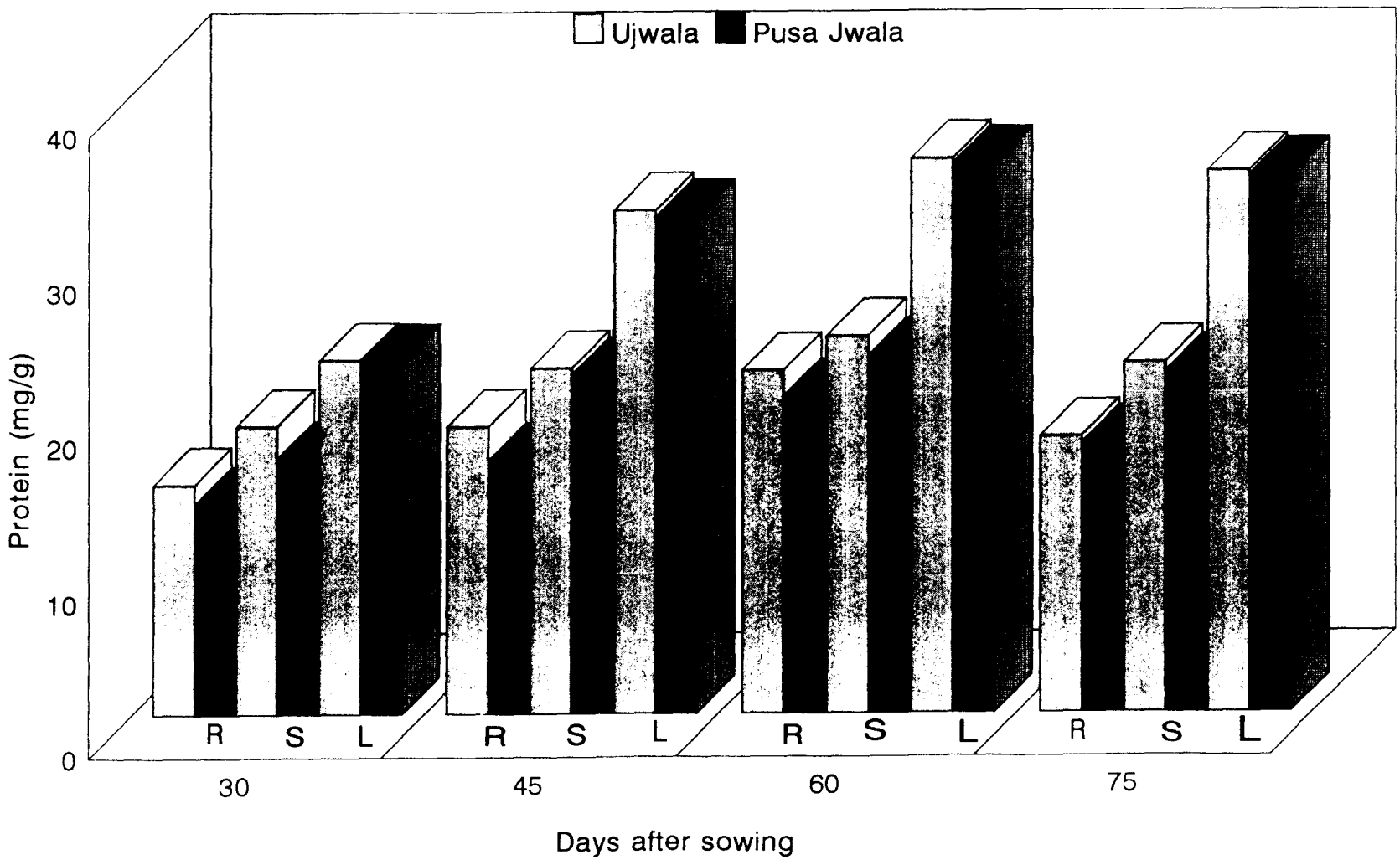
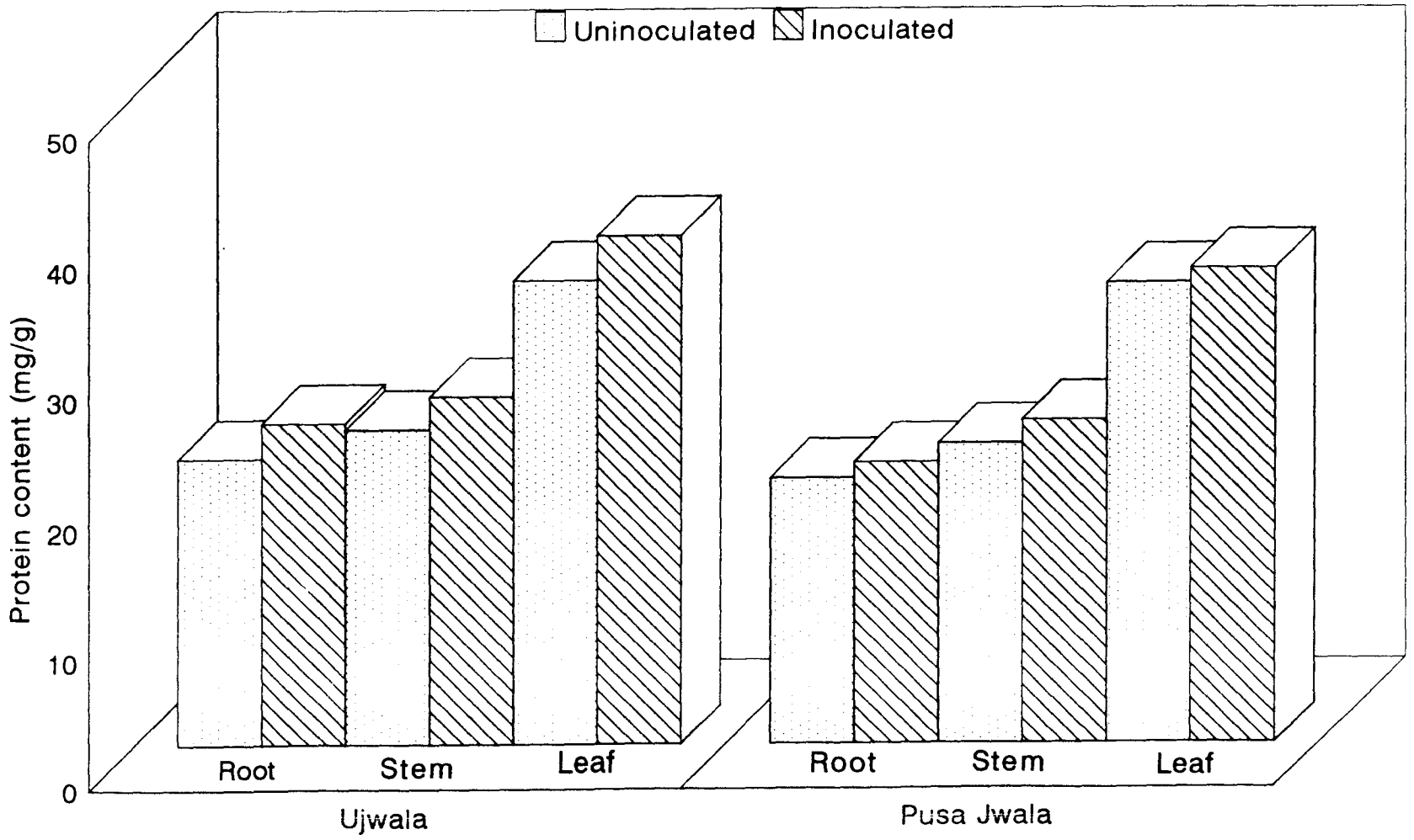


Fig.6b. Protein content in bacterial wilt resistant and susceptible genotypes



R - Root; S - Stem; L - Leaf

Fig.7. Protein content of uninoculated and inoculated wilt resistant and susceptible genotypes



4.3.3 Enzyme activities

Peroxidase

The peroxidase activity of bacterial wilt resistant genotype (Ujwala) was significantly higher than that of the susceptible variety (Pusa Jwala) in all the plant parts at various growth stages (Table 13 and Fig. 8a & b). The enzyme activity significantly varied with different growth stages. Maximum activity was observed at 45th and 60th day after sowing. The figure 8 showed a progressive increase in peroxidase activity with age up to 60 days and a slow decrease there after. At 60th day the peroxidase activity of roots of Ujwala was 184.952 units/litre as against 104.995 units/litre in Pusa Jwala. In the shoot, the enzyme activity of both Ujwala and Pusa Jwala was 163.198 units/litre and 103.277 units/litre respectively. In the leaves it was 193.367 units/litre and 99.957 units/litre in Ujwala and Pusa Jwala respectively.

Artificial inoculation resulted in a significant enhancement of peroxidase activity only in the resistant genotype Ujwala, while in the susceptible genotype a small increase was observed (Table 14 and Fig. 9). In the roots of Ujwala, the peroxidase activity increased from 184.952 to 326.527 units/litre (76.55%) where as in Pusa Jwala it was from 104.995 to 138.388 units/litre (31.80%). In shoots and leaves, the increase was 91.94 per cent and 46.37 per cent in Ujwala where as in Pusa Jwala it was only 25.94 per cent and 16.41 per cent. The enzyme activity was stimulated to greater levels in Ujwala than in Pusa Jwala in all plant parts after inoculation.

Polyphenol oxidase

Absorbance at 495 nm for a period of five minutes at an interval of one minute was recorded. The absorbance value of each minute was plotted and linear phase of the curve was obtained. A steady increase of activity was observed up to second minute in all samples and thereafter the increase was slow (Fig. 10).

It is evident from the Tables (Table 15a, b, c and d) that the polyphenol oxidase activity was higher in the first minute and then decreased gradually in all plant parts at different growth stages. Maximum polyphenol oxidase activity was observed in the roots where as, the activity was minimum in the leaves of both genotypes.

Polyphenol oxidase activity was higher in resistant variety in all the plant parts at various growth stages (Table 16 and Fig. 11a & b). A progressive increase in polyphenol oxidase activity was observed with increase in age up to 60 days after sowing and slowly decreased thereafter. Maximum enzyme activity was observed at 60th day in all the plant parts of both genotypes (Fig. 11). At 60th day, the roots of Ujwala and Pusa Jwala recorded an enzyme activity of 0.103 and 0.096 respectively. In the shoots, it was 0.097 and 0.085 and in leaves 0.037 and 0.028 respectively. The whole plants recorded an activity of 0.077 and 0.048 in Ujwala and Pusa Jwala respectively.

Specific activity of both resistant and susceptible variety is also in agreement with the activity curve and enzyme activity (Table 17). Higher values were obtained for roots and the resistant variety Ujwala recorded higher specific activity than that of Pusa Jwala in almost all parts at various growth stages. In other

cases Ujwala and Pusa Jwala recorded activity at same rate. The specific activity was also higher in the first minute and afterwards decreased gradually in all plant parts (Table 18).

On inoculation, polyphenol oxidase activity was similar to that of peroxidase in chilli genotypes. In both genotypes, the inoculation enhanced the polyphenol oxidase activity. However, in resistant genotype, the enzyme activity increased to greater levels in all plant parts than in susceptible genotype, Pusa Jwala (Table 19).

At 2nd minute the increase was 84 per cent and 64 per cent in roots of Ujwala and Pusa Jwala respectively. In the stems, leaves and whole plant Ujwala recorded an increase of 67 per cent, 84 per cent and 75 per cent, where as in Pusa Jwala, the increase was only 38 per cent, 43 per cent and 46 per cent respectively (Fig. 12).

The specific activity also showed the same trend. The inoculated resistant plants revealed dramatic increase in specific activity over healthy uninoculated plants at all samples (Table 20). Maximum acceleration of polyphenol oxidase specific activity was recorded in roots in the first minute. In the fifth minute, difference between the inoculated and uninoculated plants was not significant.

4.3.4 **Protein pattern by electrophoresis**

Extracts from root and leaf of the resistant variety 'Ujwala' and the susceptible variety 'Pusa Jwala' were used for polyacrylamide gel electrophoresis. In roots, resolution of band was feeble but in leaves it gave fairly good result (Plate 4).

Table 13. Peroxidase activity in bacterial wilt resistant and susceptible genotypes (units/litre of enzyme extract)

Growth stages	Root			Stem			Leaf			Whole plant		
	Ujwala	Pusa Jwala	t value	Ujwala	Pusa Jwala	t value	Ujwala	Pusa Jwala	t value	Ujwala	Pusa Jwala	t value
30th day	99.967	46.670	6.653**	121.738	53.343	11.680**	186.633	84.978	18.685**	111.683	82.977	2.415*
45th day	188.395	60.017	14.746**	160.147	61.687	9.423**	188.395	93.300	4.644**	125.100	81.648	3.575**
60th day	184.952	104.995	6.791**	163.198	103.277	3.280**	193.367	99.957	5.186**	174.968	115.032	4.061**
75th day	123.433	45.002	8.611**	100.00	29.997	7.234**	116.675	28.328	6.563**	110.007	26.663	11.623**
CD	21.546**	17.966**	-	26.400**	11.889**	-	24.869**	38.203**	-	27.760**	21.063**	-

* Significant at 5% level
** Significant at 1% level

Table 14. Peroxidase activity of inoculated and uninoculated bacterial wilt resistant and susceptible genotypes (units/litre of enzyme extract)

	Root			Stem			Leaf			Whole plant		
	Ujwala	Pusa Jwala	t value	Ujwala	Pusa Jwala	t value	Ujwala	Pusa Jwala	t value	Ujwala	Pusa Jwala	t value
Unino-culated	184.952	104.995	6.791**	163.198	103.277	3.280**	193.367	99.957	5.186**	174.968	115.032	4.061**
Inoculated	326.527 (76.55)	138.388 (31.80)	8.571**	313.247 (91.94)	130.015 (25.94)	8.988**	283.025 (46.37)	116.667 (16.41)	9.808**	266.490 (52.31)	131.588 (14.39)	9.275**
t value	8.389**	NS		10.709**	NS		9.173**	NS	-	5.387**	NS	-

**Significant at 1% level
NS - Nonsignificant
Figures in parenthesis are per cent increase over uninoculated

Table 15a. Polyphenol oxidase activity (OD value) in roots of bacterial wilt resistant and susceptible genotypes for 1 to 5 minutes

Growth stages	Time (minutes)											
	1		2		3		4		5		Total	
	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala
30th day	0.061	0.053	0.059	0.048	0.043	0.041	0.033	0.032	0.016	0.012	0.212	0.196
45th day	0.104	0.089	0.096	0.065	0.053	0.048	0.038	0.040	0.019	0.023	0.310	0.265
60th day	0.133	0.117	0.103	0.096	0.061	0.060	0.040	0.047	0.020	0.020	0.357	0.340
75th day	0.105	0.094	0.085	0.082	0.055	0.054	0.036	0.029	0.022	0.020	0.303	0.279

Table 15b. Polyphenol oxidase activity (OD value) in stems of bacterial wilt resistant and susceptible genotypes for 1 to 5 minutes

Growth stages	Time (minutes)											
	1		2		3		4		5		Total	
	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala
30th day	0.051	0.047	0.054	0.048	0.048	0.038	0.026	0.027	0.016	0.011	0.189	0.178
45th day	0.099	0.070	0.081	0.060	0.045	0.042	0.036	0.033	0.020	0.014	0.281	0.219
60th day	0.120	0.097	0.097	0.085	0.045	0.057	0.036	0.034	0.012	0.012	0.310	0.285
75th day	0.093	0.079	0.079	0.078	0.042	0.036	0.028	0.039	0.020	0.020	0.262	0.242

Table 15c. Polyphenol oxidase activity (OD value) in leaves of bacterial wilt resistant and susceptible genotypes for 1 to 5 minutes

Growth stages	Time (minutes)											
	1		2		3		4		5		Total	
	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala
30th day	0.027	0.023	0.022	0.019	0.020	0.016	0.018	0.013	0.012	0.009	0.099	0.080
45th day	0.035	0.034	0.021	0.023	0.033	0.022	0.014	0.018	0.008	0.009	0.112	0.106
60th day	0.060	0.039	0.037	0.028	0.027	0.022	0.010	0.017	0.009	0.011	0.143	0.117
75th day	0.039	0.032	0.033	0.031	0.029	0.022	0.026	0.022	0.014	0.017	0.141	0.124

Table 15d. Polyphenol oxidase activity (OD value) in whole plant of bacterial wilt resistant and susceptible genotypes for 1 to 5 minutes

Growth stages	Time (minutes)											
	1		2		3		4		5		Total	
	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala
30th day	0.053	0.042	0.044	0.040	0.039	0.036	0.028	0.030	0.018	0.016	0.182	0.164
45th day	0.066	0.064	0.066	0.050	0.042	0.032	0.028	0.020	0.019	0.013	0.221	0.179
60th day	0.114	0.089	0.077	0.048	0.026	0.024	0.014	0.012	0.01	0.008	0.241	0.181
75th day	0.086	0.054	0.060	0.049	0.046	0.039	0.035	0.033	0.015	0.019	0.242	0.194

Table 16. Polyphenol oxidase enzyme activity (OD value) at 2nd minute in wilt resistant and susceptible genotypes

Growth stages	Root		Stem		Leaf		Whole plant	
	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala
30th day	0.059	0.048	0.054	0.048	0.022	0.019	0.044	0.040
45th day	0.096	0.065	0.081	0.060	0.024	0.023	0.066	0.050
60th day	0.103	0.096	0.097	0.085	0.037	0.028	0.077	0.048
75th day	0.085	0.082	0.079	0.078	0.033	0.031	0.060	0.049

Table 17. Polyphenol oxidase - specific activity at 2nd minute in wilt resistant and suscepitible genotypes

Growth stages	Root		Stem		Leaf		Whole plant	
	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala
30th day	0.020	0.018	0.016	0.013	0.005	0.004	0.010	0.010
45th day	0.026	0.020	0.018	0.014	0.003	0.003	0.012	0.009
60th day	0.023	0.023	0.020	0.018	0.005	0.004	0.012	0.008
75th day	0.024	0.024	0.018	0.018	0.005	0.004	0.011	0.010

Table 18. Polyphenol oxidase - specific activity in wilt resistant and susceptible genotypes at 60th day after sowing

Parts	Time (minutes)									
	1		2		3		4		5	
	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala	Ujwala	Pusa Jwala
Root	0.030	0.029	0.023	0.023	0.014	0.014	0.009	0.012	0.005	0.005
Stem	0.025	0.021	0.020	0.018	0.009	0.012	0.007	0.007	0.002	0.002
Leaf	0.008	0.006	0.005	0.004	0.004	0.003	0.001	0.002	0.001	0.001
Whole plant	0.018	0.014	0.012	0.008	0.004	0.004	0.002	0.002	0.002	0.001

Table 19. Polyphenol oxidase enzyme activity (OD value) of inoculated and uninoculated bacterial wilt resistant and susceptible genotypes for 1 to 5 minutes

	Time (minutes)											
	1		2		3		4		5		Total	
	UI	I	UI	I	UI	I	UI	I	UI	I	UI	I
Root												
Ujwala	0.133	0.264 (98.49)	0.103	0.190 (84.47)	0.061	0.018	0.040	0.062	0.020	0.027	0.357	0.671
Pusa Jwala	0.117	0.184 (57.26)	0.096	0.158 (64.58)	0.060	0.096	0.047	0.040	0.020	0.014	0.340	0.492
Stem												
Ujwala	0.120	0.251 (109.17)	0.097	0.162 (67.01)	0.045	0.102	0.036	0.050	0.012	0.024	0.310	0.589
Pusa Jwala	0.097	0.137 (41.24)	0.085	0.117 (37.65)	0.057	0.084	0.034	0.048	0.012	0.024	0.285	0.410
Leaf												
Ujwala	0.060	0.102 (70.00)	0.037	0.068 (83.78)	0.027	0.056	0.010	0.038	0.009	0.023	0.143	0.287
Pusa Jwala	0.039	0.065 (66.67)	0.028	0.040 (42.86)	0.022	0.032	0.017	0.025	0.011	0.016	0.117	0.178
Whole plant												
Ujwala	0.114	0.180 (57.89)	0.077	0.135 (75.32)	0.026	0.046	0.014	0.035	0.010	0.022	0.241	0.418
Pusa Jwala	0.089	0.122 (37.08)	0.048	0.070 (45.83)	0.024	0.060	0.012	0.027	0.008	0.018	0.181	0.297

(Figures in parenthesis are per cent increase over uninoculated
UI - Uninoculated
I - Inoculated

Table 20. Polyphenol oxidase - specific activity of inoculated and uninoculated bacterial wilt resistant and susceptible genotypes for 1 to 5 minutes

	Time (minutes)									
	1		2		3		4		5	
	UI	I	UI	I	UI	I	UI	I	UI	I
Root										
Ujwala	0.030	0.053	0.023	0.038	0.014	0.026	0.009	0.013	0.005	0.005
Pusa Jwala	0.029	0.043	0.023	0.037	0.014	0.022	0.012	0.009	0.005	0.003
Stem										
Ujwala	0.025	0.047	0.020	0.030	0.009	0.019	0.007	0.009	0.002	0.004
Pusa Jwala	0.021	0.028	0.018	0.024	0.012	0.017	0.007	0.009	0.002	0.004
Leaf										
Ujwala	0.008	0.013	0.005	0.009	0.004	0.007	0.002	0.005	0.001	0.003
Pusa Jwala	0.006	0.009	0.004	0.005	0.003	0.004	0.002	0.003	0.001	0.002
Whole plant										
Ujwala	0.018	0.026	0.012	0.019	0.004	0.009	0.002	0.005	0.002	0.003
Pusa Jwala	0.014	0.018	0.008	0.011	0.004	0.007	0.002	0.004	0.001	0.003

UI - Uninoculated; I - Inoculated

Fig.8a Peroxidase activity in wilt resistant and susceptible genotypes

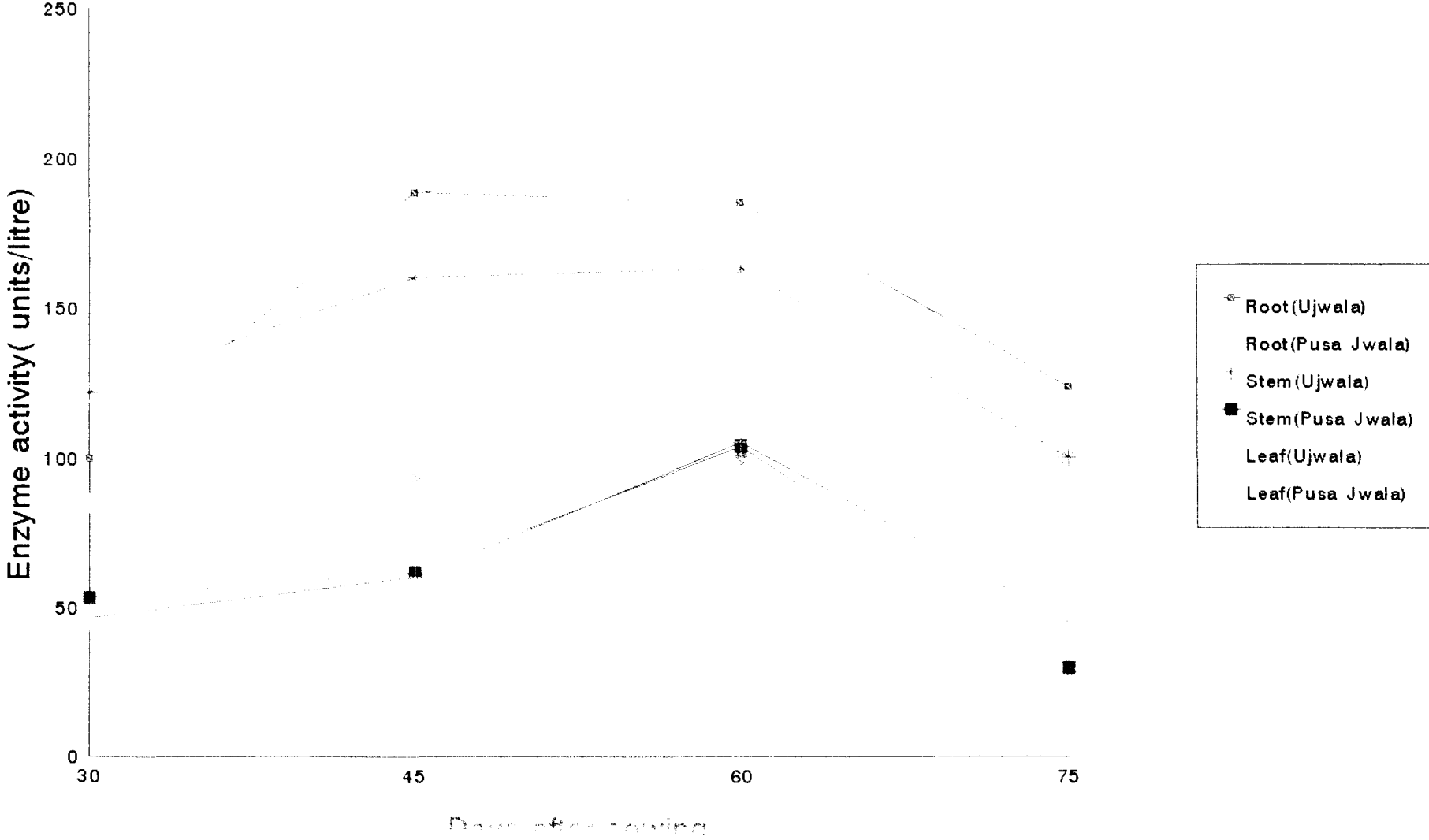
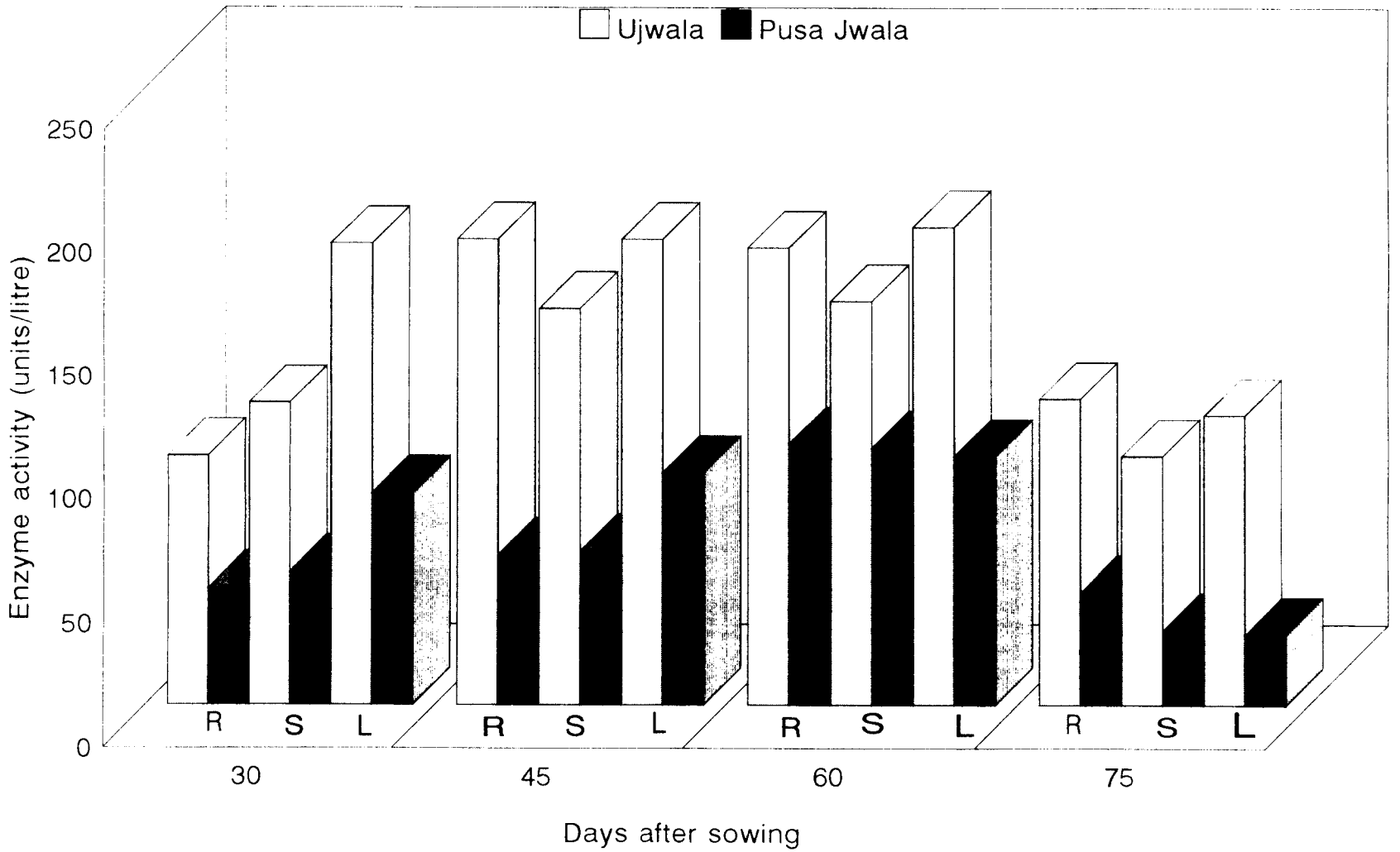


Fig.8b. Peroxidase activity in wilt resistant and susceptible genotypes



R - Root; S - Stem; L - Leaf

Fig.9. Peroxidase activity of uninoculated and inoculated wilt resistant and susceptible genotypes

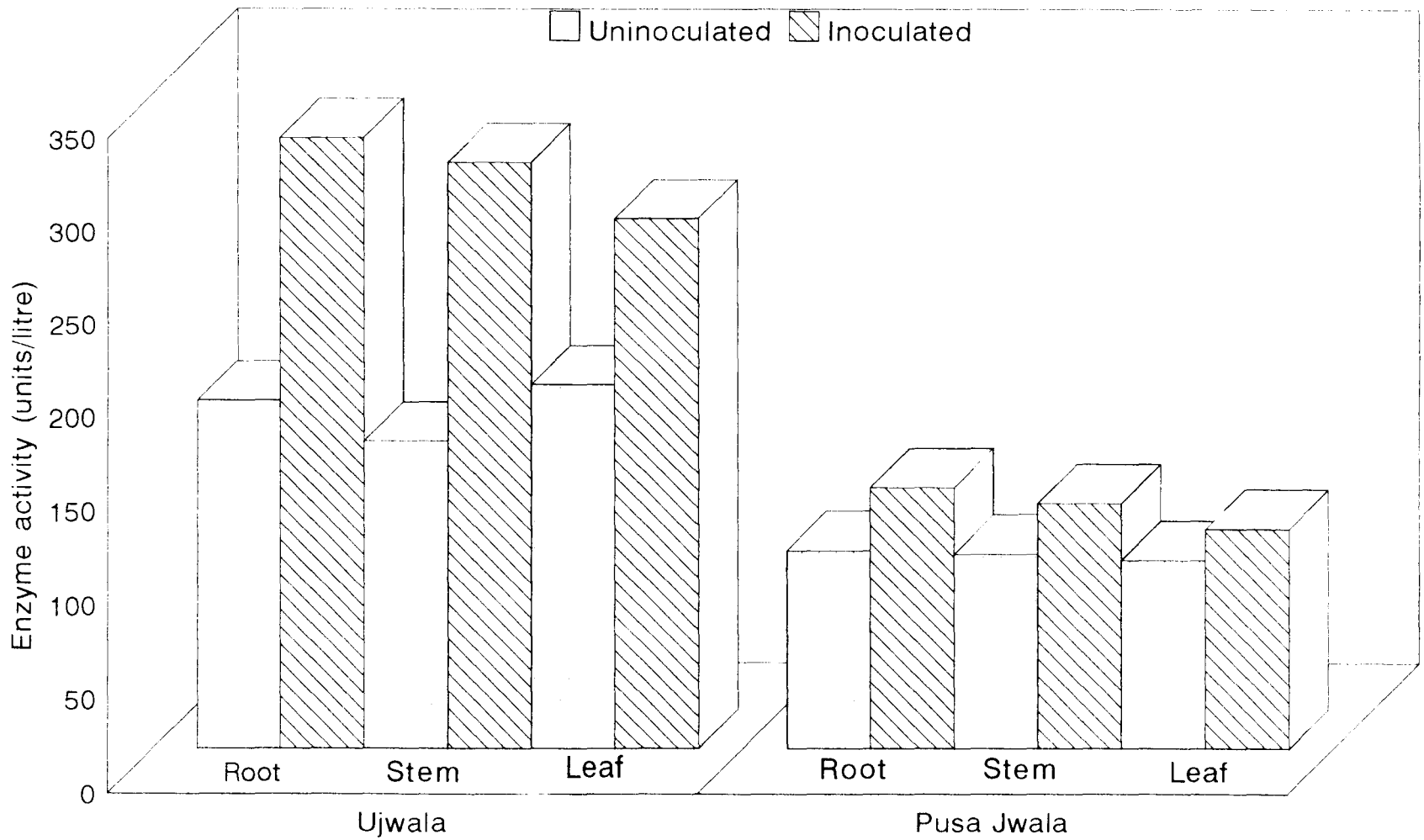


Fig.10 Progressive curve of polyphenol oxidase activity at 60th day after sowing

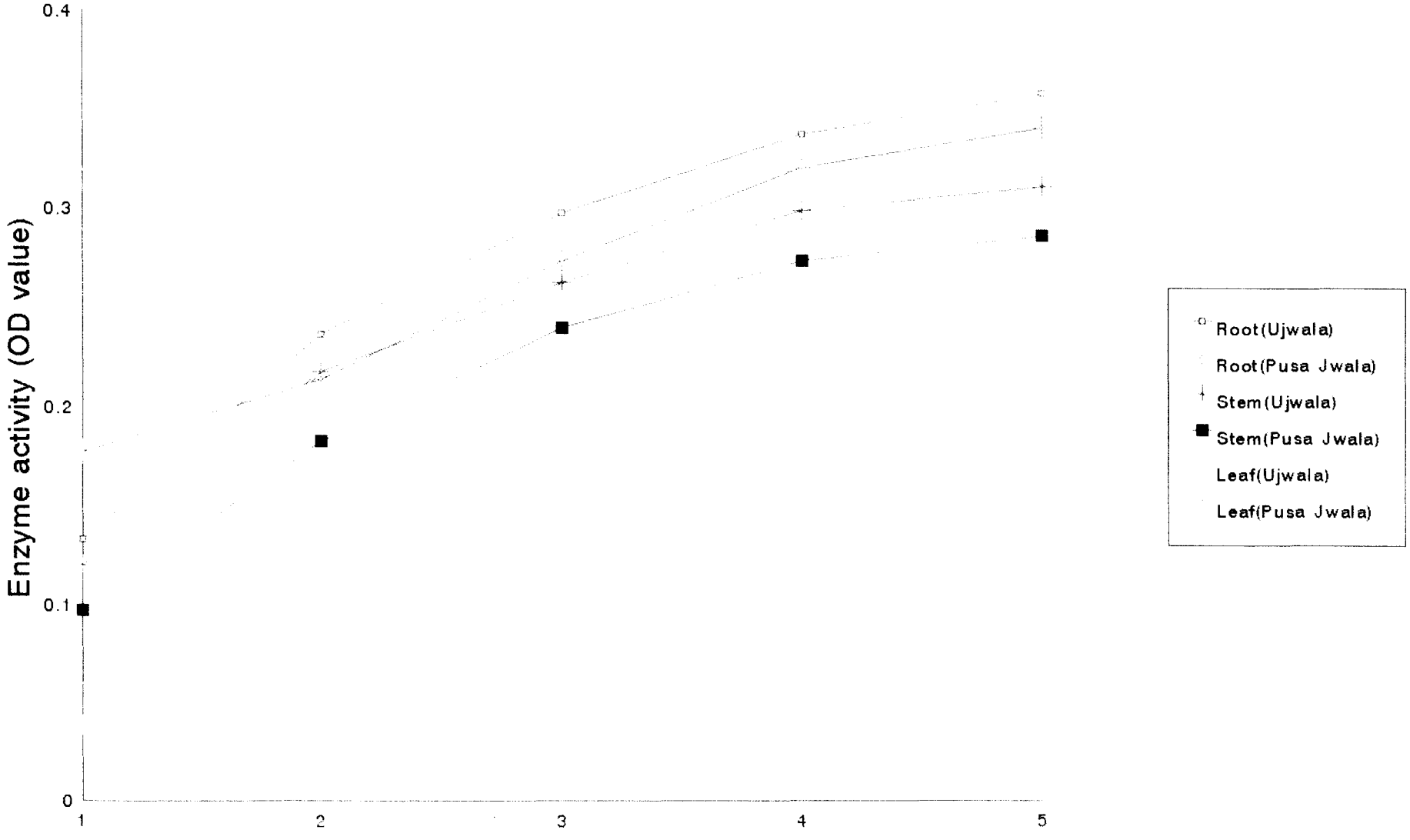


Fig.11a Polyphenol oxidase activity in wilt resistant and susceptible genotypes

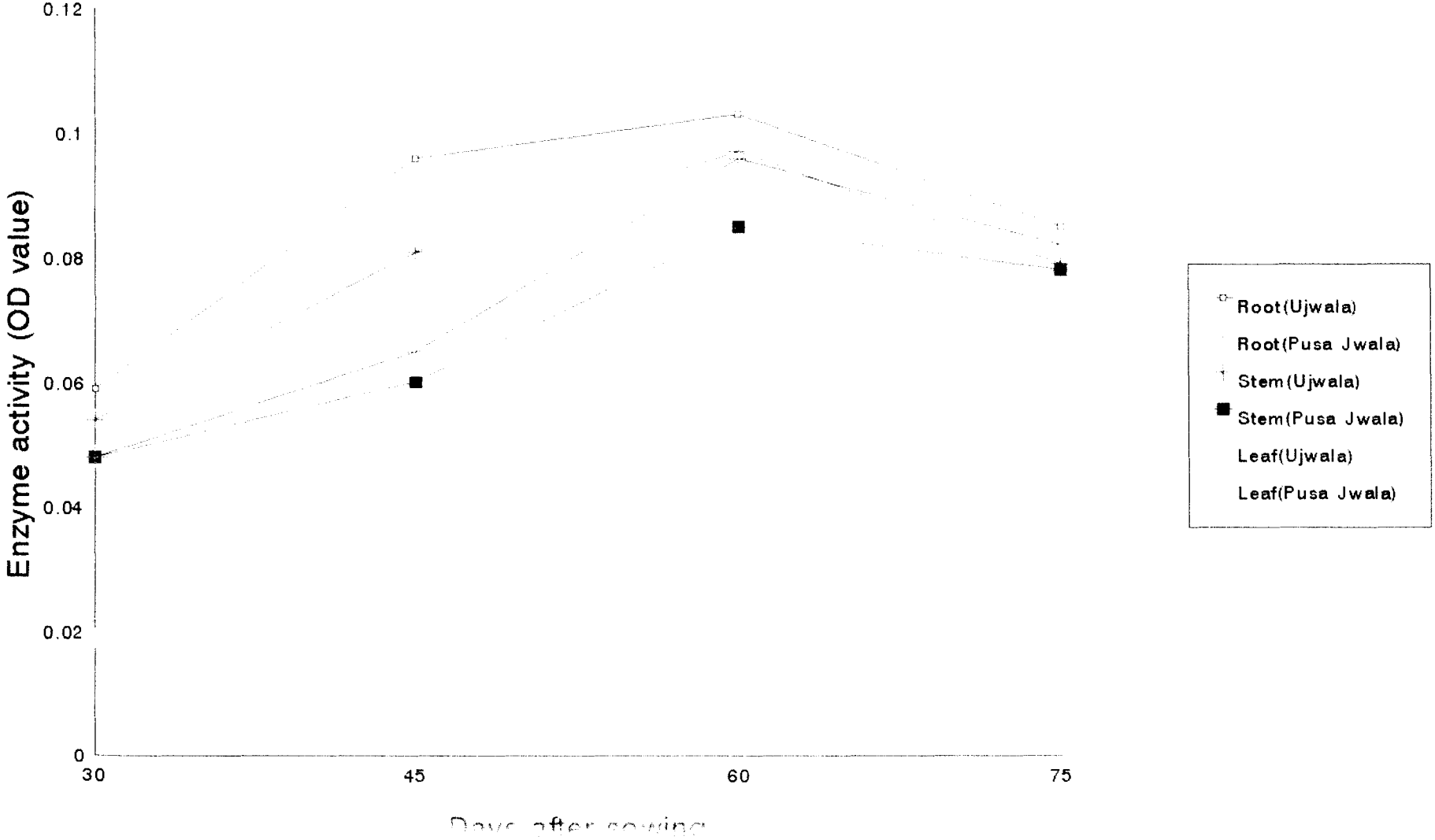
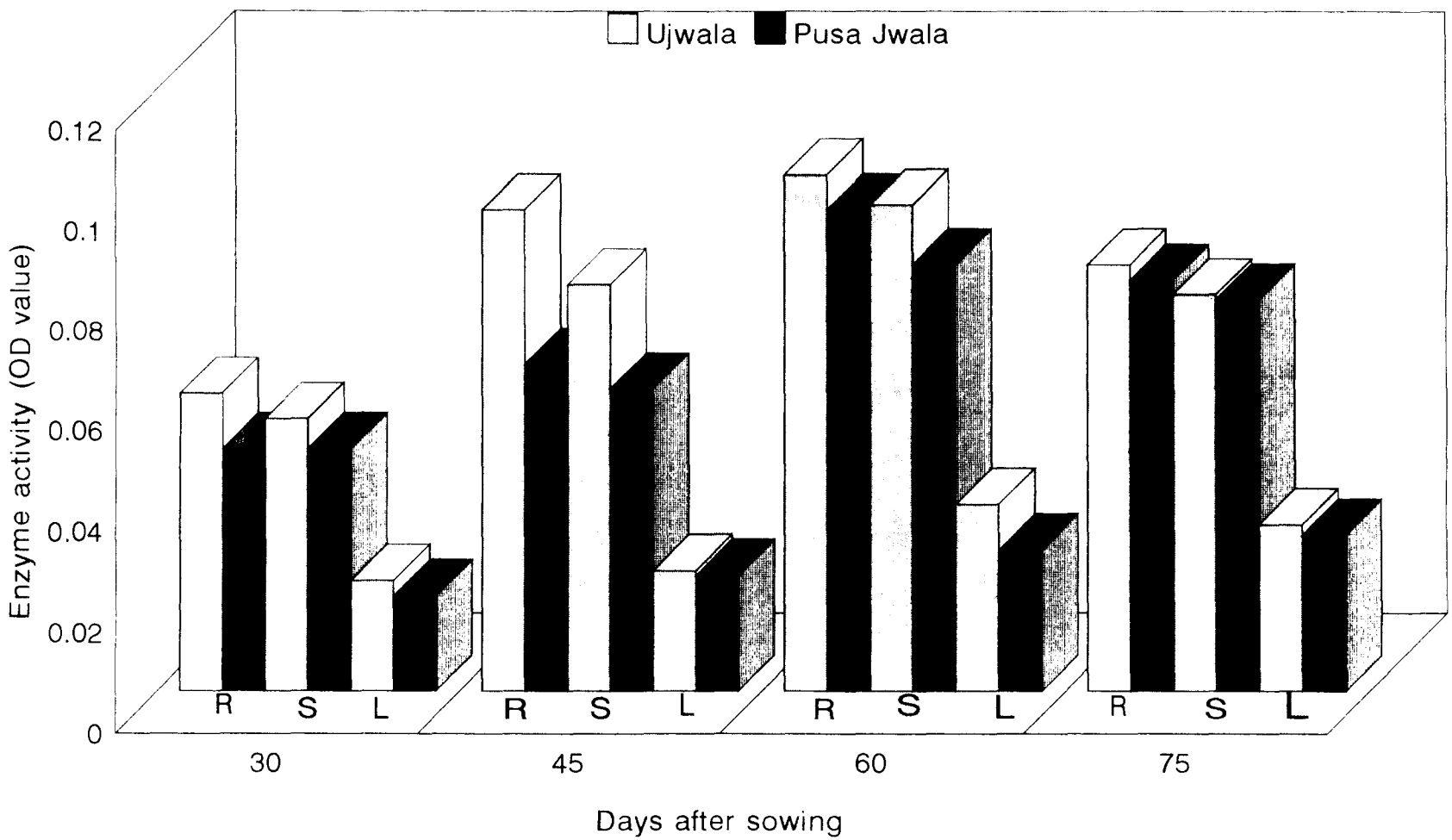
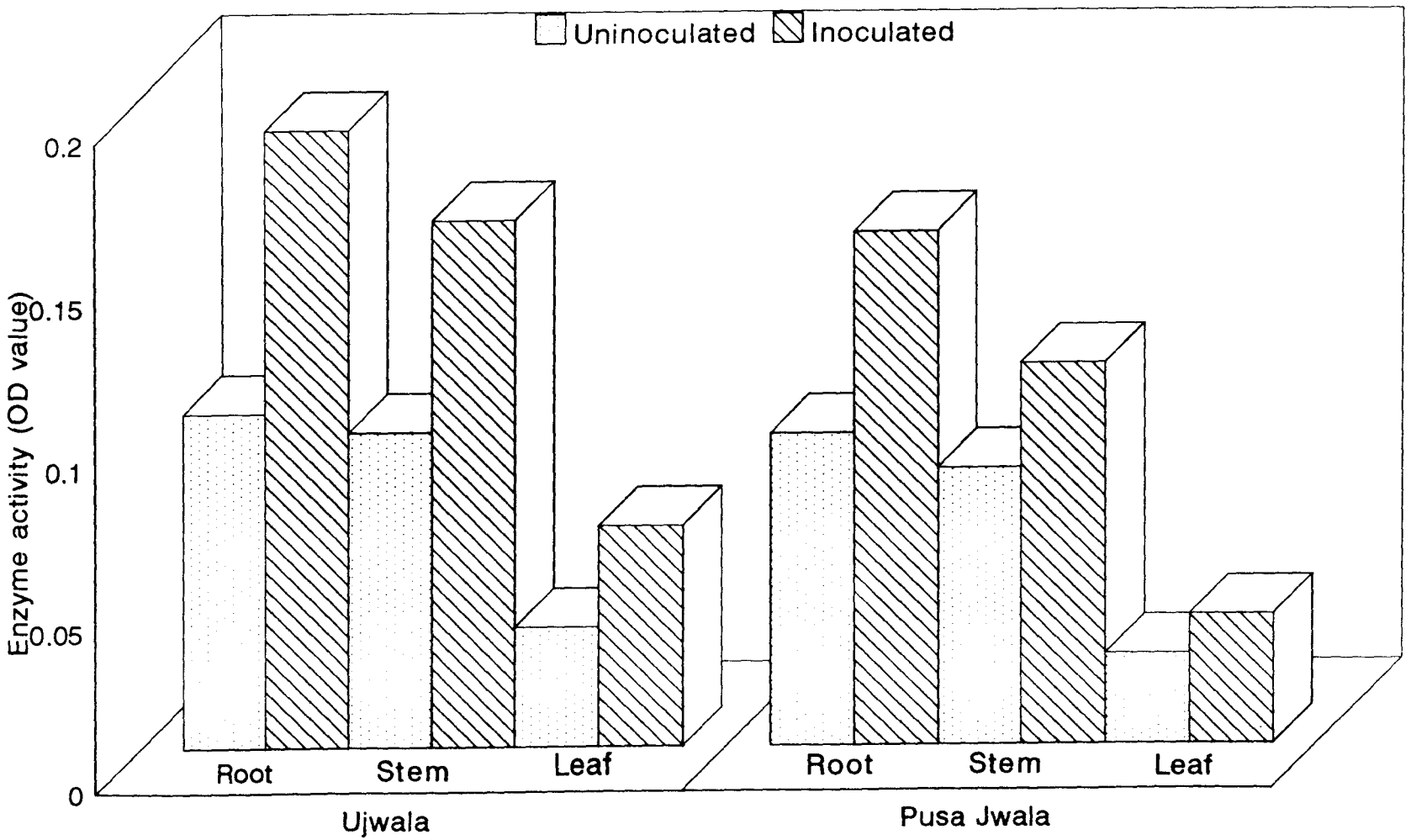


Fig.11b. Polyphenol oxidase activity in bacterial wilt resistant and susceptible genotypes



R - Root; S - Stem; L - Leaf

Fig.12. Polyphenol oxidase activity of uninoculated and inoculated wilt resistant and susceptible genotypes



The two genotypes possessed similar bands with identical electrophoretic mobility. The resistant and susceptible varieties showed no difference in the number of bands and the Rm (Relative mobility) value before inoculation. In roots, there were three bands (Pr 1, Pr 2 and Pr 3). The Rm values of these bands were 0.038, 0.146 and 0.246 respectively. In leaves there were five bands (Pr 1, Pr 2, Pr 3, Pr 4 and Pr 5) and the Rm values were given in Table 21.

The banding pattern were feeble in the inoculated roots of both resistant and susceptible varieties. The leaves of the resistant variety (Ujwala) showed no difference in the electrophorograms on inoculation, while the susceptible variety (Pusa Jwala) exhibited one more band (Pr 6) and the mobility of that protein was slow (Rm 0.015) (Fig. 13).

4.3.5 **Isoenzyme analysis**

Peroxidase

Sample of 30 days and 60 days old seedlings of uninoculated and 60 days old inoculated seedling from Ujwala and Pusa Jwala were taken for this study. Root and leaf extracts were used. Clear and thick bands were obtained for the roots where as leaves exhibited only feeble bands (Plate 5) under the same condition.

In roots, peroxidase activity increased with advancement of plant growth. Sixty days old seedlings showed better resolution of bands than 30 days old seedlings. The resistant variety Ujwala showed only two bands (PRX 2 and PRX 3) in roots of uninoculated (30 days and 60 days old seedling) and inoculated (60 days old seedlings) while three bands (PRX 1, PRX 2 and PRX 3) were exhibited by the susceptible variety Pusa Jwala. The Rm values of these bands were 0.144, 0.20 and

0.256 respectively. Inoculation had no effect in the banding pattern of both resistant and susceptible genotypes, ie., before and after inoculation there was no difference in the banding pattern.

In the case of leaves, number of bands increased with advancement of plant growth. At 30th day both resistant and susceptible varieties showed only one feeble band (PRX 3) of Rm value 0.144. At 60th day, though the resistant variety exhibited only one band (PRX 3), the susceptible variety had two thick bands (PRX 2 and PRX 3) of Rm values 0.144 and 0.200 respectively. Inoculated plants exhibited a similar trend. There was no difference between inoculated and uninoculated seedlings of both genotypes for peroxidase isoenzyme banding pattern (Plate 5 and Fig. 14).

Esterase

The roots and leaves of 15 days and 60 days old seedlings of uninoculated and 60 days old inoculated seedlings were used to identify the esterase pattern. Phosphate buffer of pH 9.5 was used for extracting the sample. Difference in the pattern of esterase banding was obtained for resistant and susceptible genotypes (Plate 6). Leaves showed clear bands compared to root tissues. No difference was observed in the banding pattern with increase in plant age. Fifteen and 60 days old seedlings showed similar types of bands.

Three medium thick bands (EST 1, EST 2 and EST 3) were observed in all samples of the resistant variety (Ujwala), whereas only one feeble band (EST 3) was observed in all samples of the susceptible variety (Pusa Jwala). The Rm values were 0.083, 0.167 and 0.367 respectively (Fig. 15). EST 3 was common for all

Table 21. Banding pattern of protein gel electrophoresis of bacterial wilt resistant and susceptible genotypes

	Root				Leaves			
	Ujwala		Pusa Jwala		Ujwala		Pusa Jwala	
	Unino-culated	Inocu-lated	Unino-culated	Inocu-lated	Unio-culated	Inocu-lated	Unio-culated	Inocu-lated
No. of bands	3	3	3	3	5	5	5	6
Rm values	0.246	0.246	0.246	0.246	0.338	0.338	0.338	0.338
	0.146	0.146	0.146	0.146	0.246	0.246	0.246	0.246
	0.038	0.038	0.038	0.038	0.184	0.184	0.184	0.184
					0.123	0.123	0.123	0.123
					0.046	0.046	0.046	0.046
								0.015

Plate 4. Protein electrophorogram of the root and leaf of bacterial wilt resistant and susceptible varieties

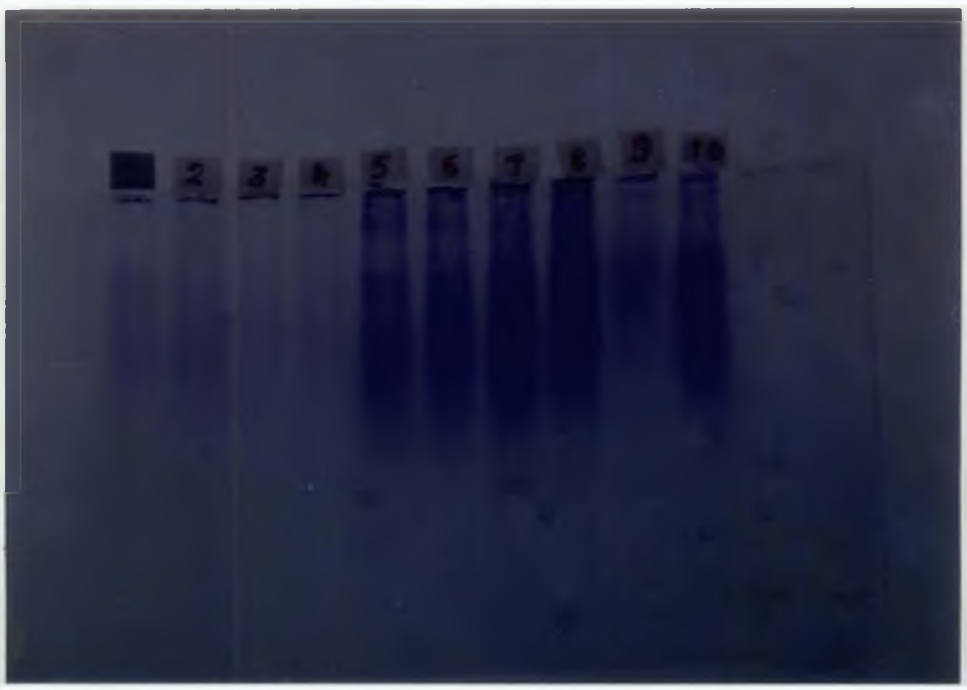
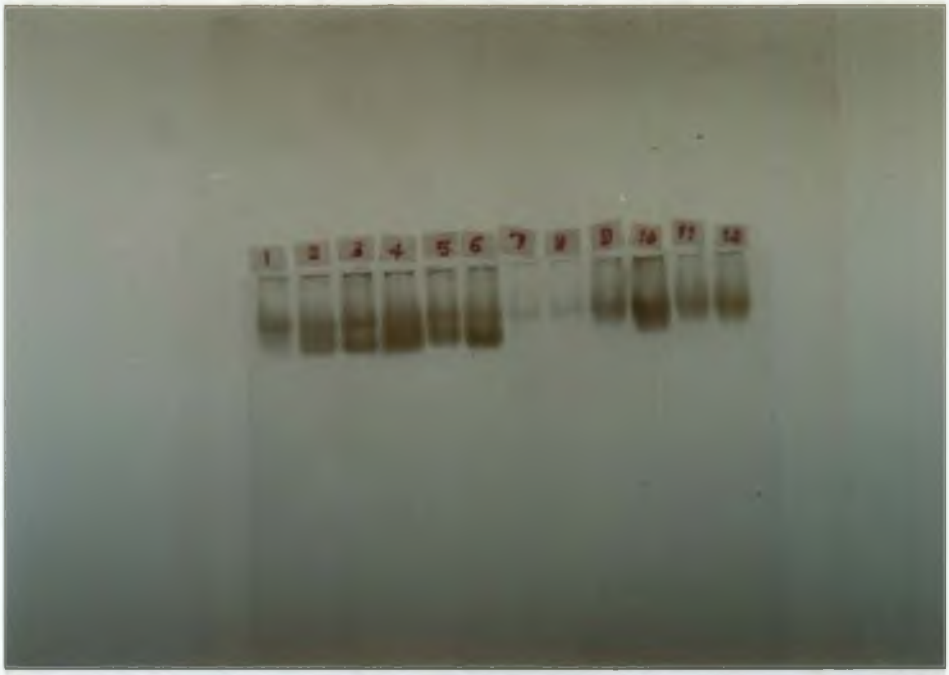


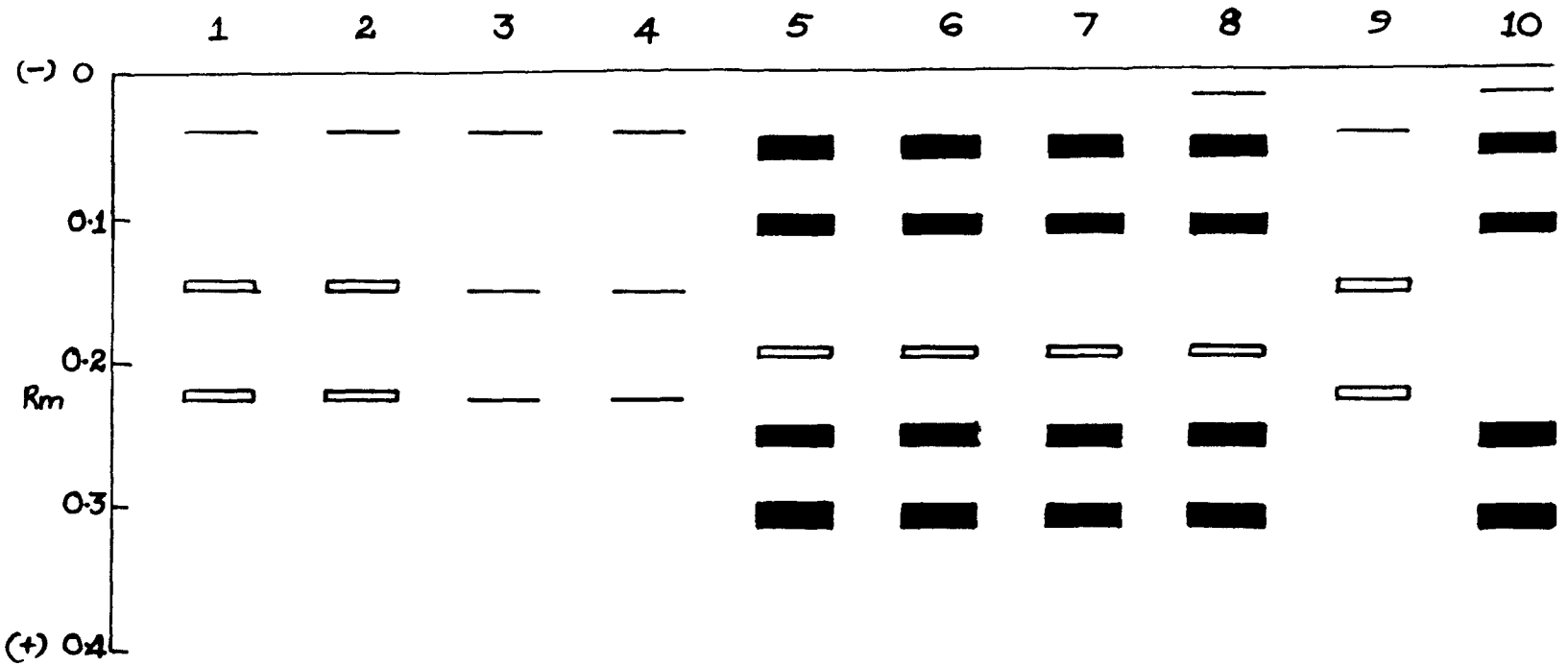
Plate 5. Peroxidase electrophorogram of the root and leaf of bacterial wilt resistant and susceptible varieties

Plate 6. Esterase electrophorogram of the leaf of bacterial wilt resistant and susceptible varieties


1 2 3 4 5 6 7 8 9 10 11 12


1 2 3 4 5 6

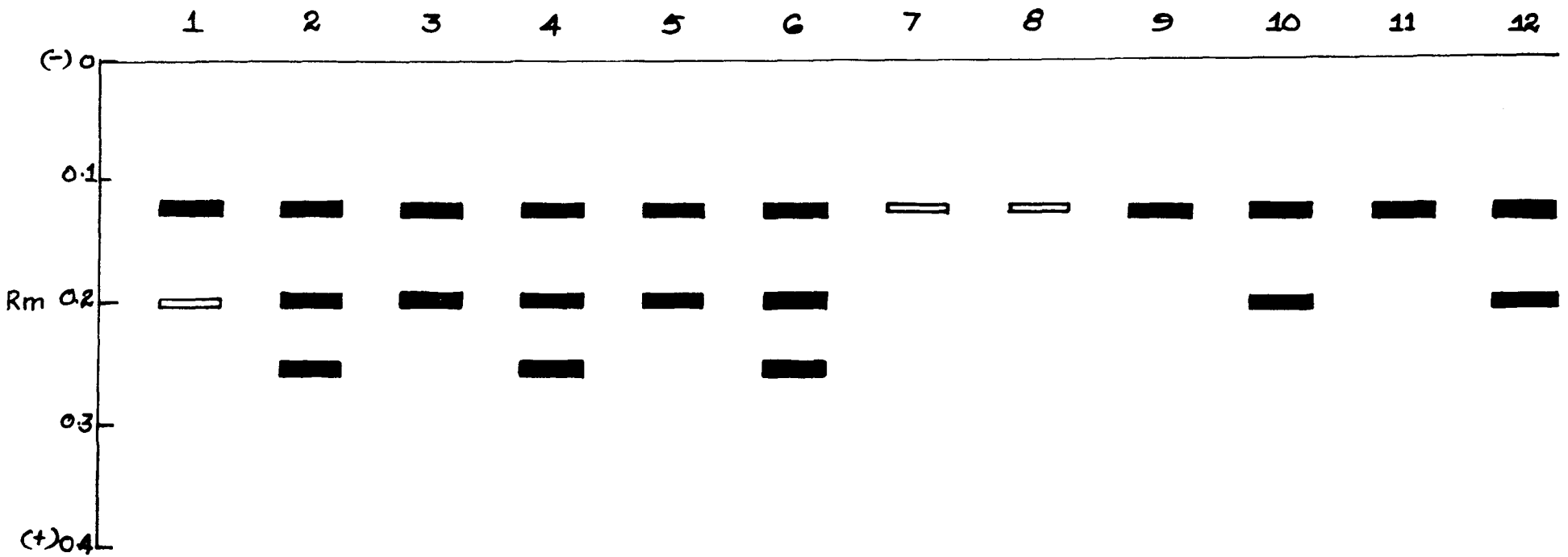
Fig.13. Protein electrophorogram of resistant and susceptible varieties



1. Ujwala root - uninoculated
2. Pusa Jwala root - uninoculated
3. Ujwala root - inoculated
4. Pusa Jwala root - inoculated
5. Ujwala leaf - uninoculated
6. Pusa Jwala leaf - uninoculated
7. Ujwala leaf - inoculated
8. Pusa Jwala leaf - inoculated
9. Pusa Jwala root - wilt sick field
10. Pusa Jwala leaf - wilt sick field

feeble
intermediate
thick

Fig.14. Peroxidase electrophorogram of resistant and susceptible varieties



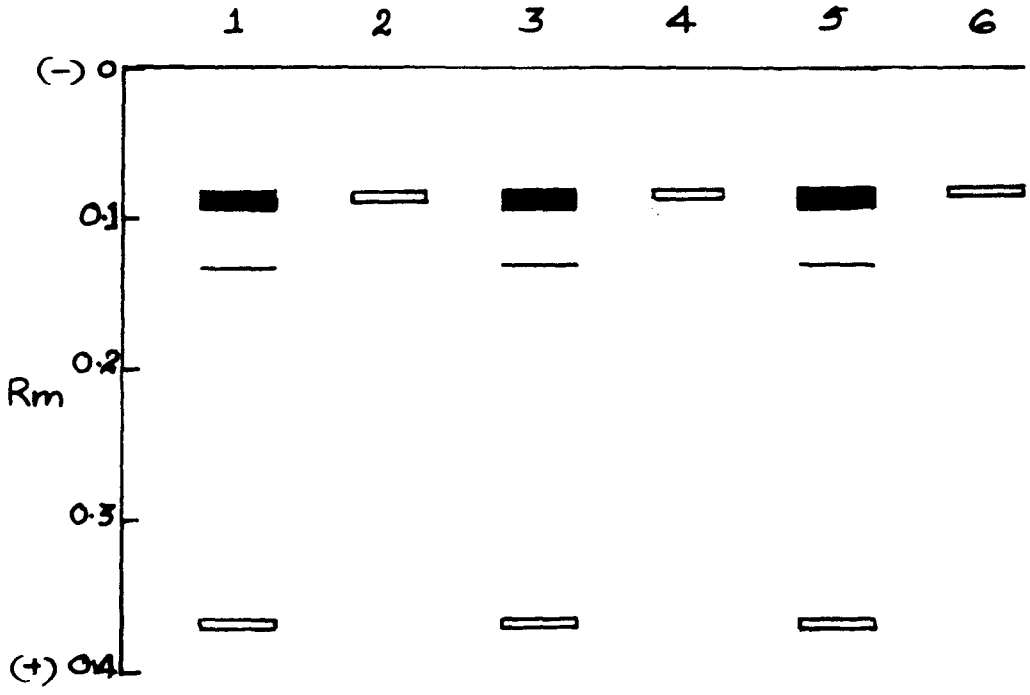
1. Ujwala root (30 days) - uninoculated
2. Pusa Jwala root (30 days) - uninoculated
3. Ujwala root (60 days) - uninoculated
4. Pusa Jwala root (60 days) - uninoculated
5. Ujwala root (60 days) - inoculated
6. Pusa Jwala root (60 days) - inoculated
7. Ujwala leaf (30 days) - uninoculated
8. Pusa Jwala leaf (30 days) - uninoculated
9. Ujwala leaf (60 days) - uninoculated
10. Pusa Jwala leaf (60 days) - uninoculated
11. Ujwala leaf (60 days) - inoculated
12. Pusa Jwala leaf (60 days) - inoculated

feeble

intermediate

thick

Fig.15. Esterase electrophorogram of resistant and susceptible varieties



1. Ujwala leaf (15 days) - uninoculated
2. Pusa Jwala leaf (15 days) - uninoculated
3. Ujwala leaf (60 days) - uninoculated
4. Pusa Jwala leaf (60 days) - inoculated
5. Ujwala leaf (60 days) - inoculated
6. Pusa Jwala leaf (60 days) - inoculated

feeble
intermediate
thick

samples. The additional bands, EST 1 and EST 2 were fast moving (0.167 and 0.367) and they have significance in resistance.

Inoculation had no effect in the esterase electrophorogram of Ujwala and Pusa Jwala.

4.4 Anatomical basis of resistance to bacterial wilt

Transverse sections of stem and tap root of the resistant variety Ujwala and susceptible variety Pusa Jwala were observed. Histological observations revealed that there was significant difference between the two genotypes for the cell arrangement in the stem and root.

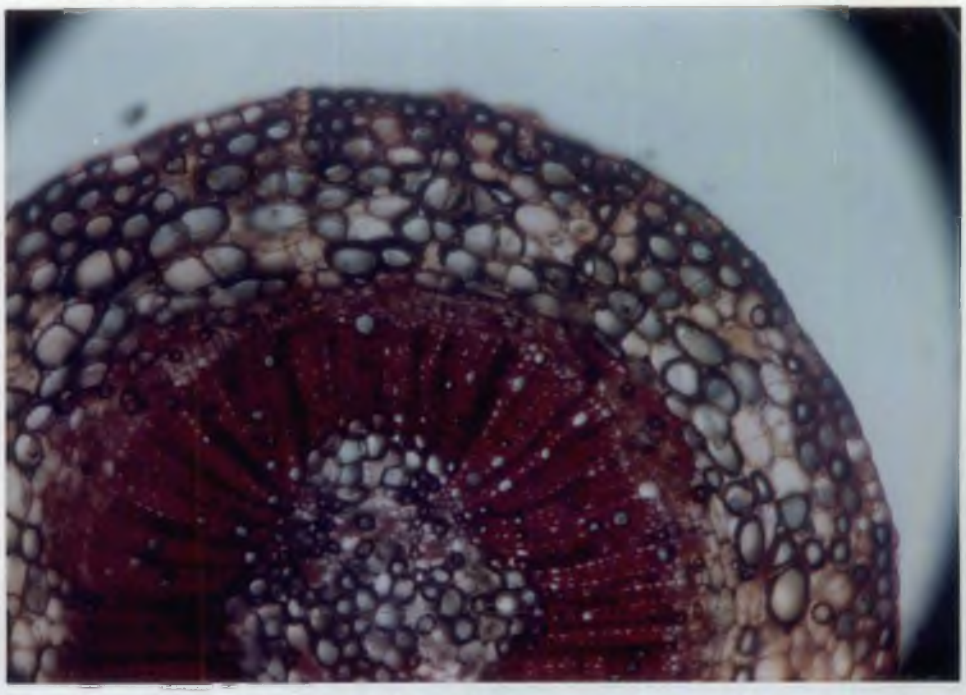
4.4.1 Histology of stem

The wilt resistant varieties had a well developed secondary xylem in contrast to susceptible variety (Plate 7 and 8). The secondary xylem was mostly tracheids and vessels while in susceptible ones the secondoary xylem consisted of less of tracheiry elements and vessels. The proportion of xylem parenchyma was higher in them. In resistant stem, there was more of secondary xylem which formed a solid cylinder (Plate 7a). In the susceptible stem, secondary xylem appeared like a wavy band (Plate 8a). More of secondary xylem was noticed at the region where primary xylem was there (centrifugal). There was slow development of secondary xylem in the interfasicular region.

Cortical cells of the susceptible stem were more succulent and loosely packed with a lot of intercellular spaces (Plate 9 and 10).

Plate 7a. T.S. of stem of bacterial wilt resistant variety (x 300)

b. Magnified view of a portion of secondary xylem of the stem of resistant variety (x 1250)



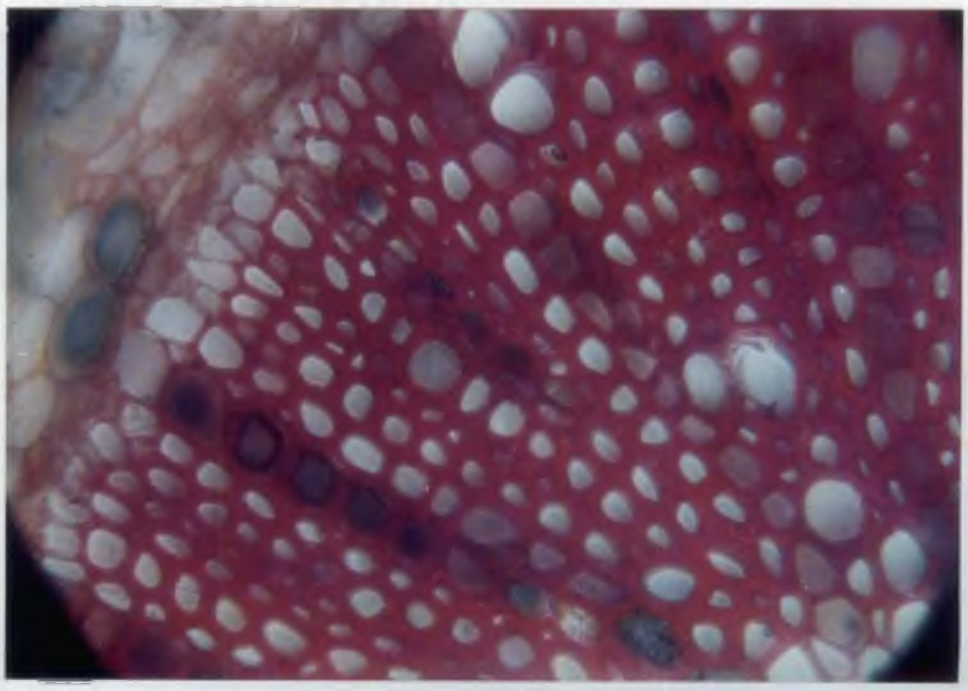
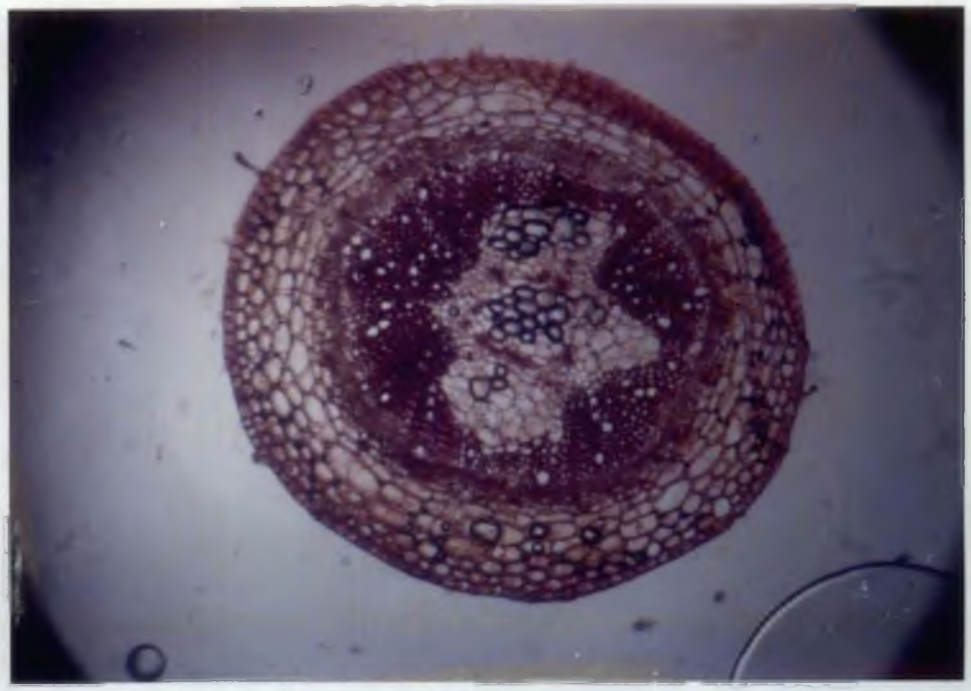


Plate 8a. T.S. of stem of bacterial wilt susceptible variety (x 150)

b. Magnified view of a portion of T.S. of stem of susceptible variety (x 300)



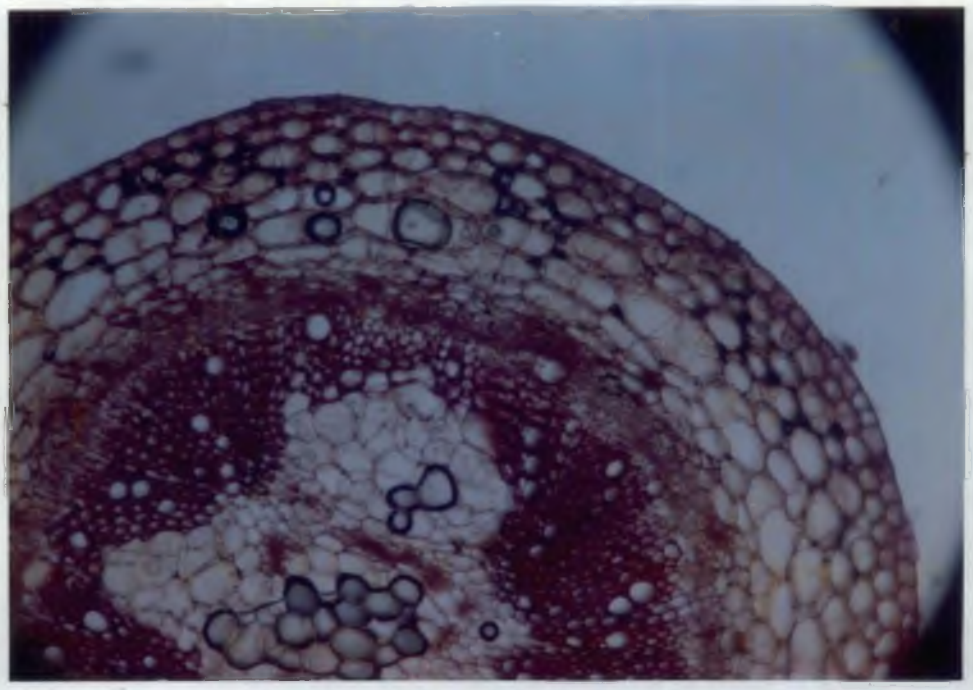
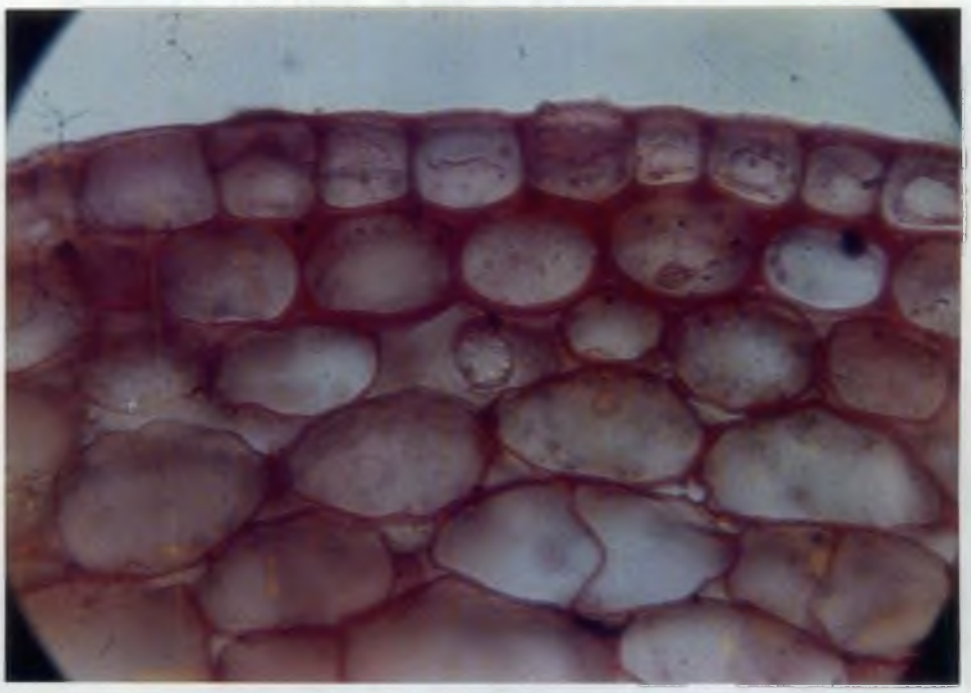


Plate 9. Magnified view of epidermis and outer cortex of stem of bacterial wilt resistant variety (x 1250)

Plate 10. Magnified view of epidermis and outer cortex of stem of bacterial wilt susceptible variety (x 1250)



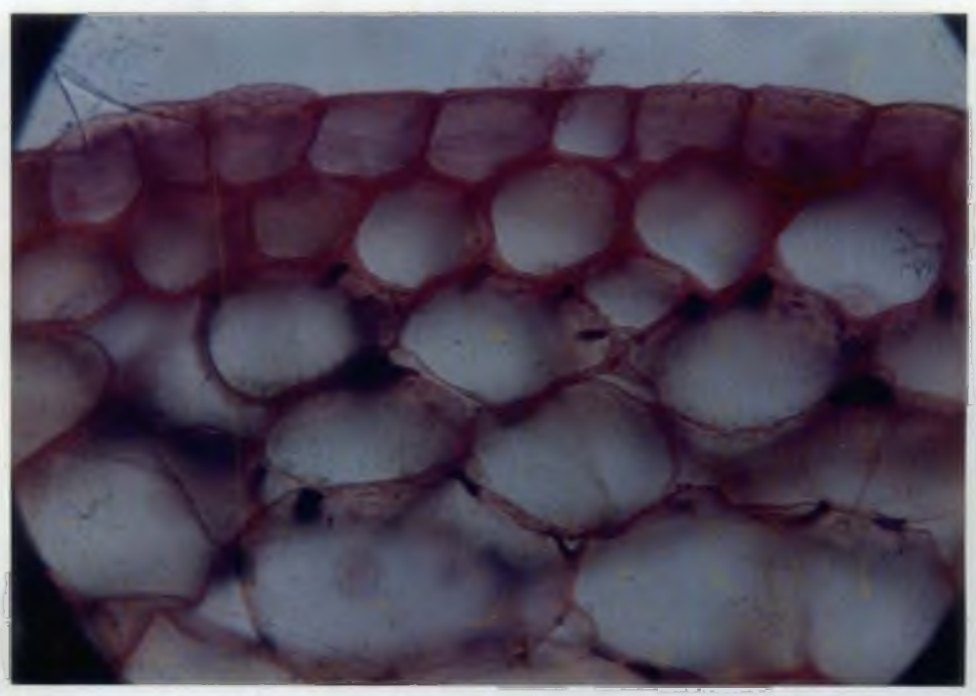
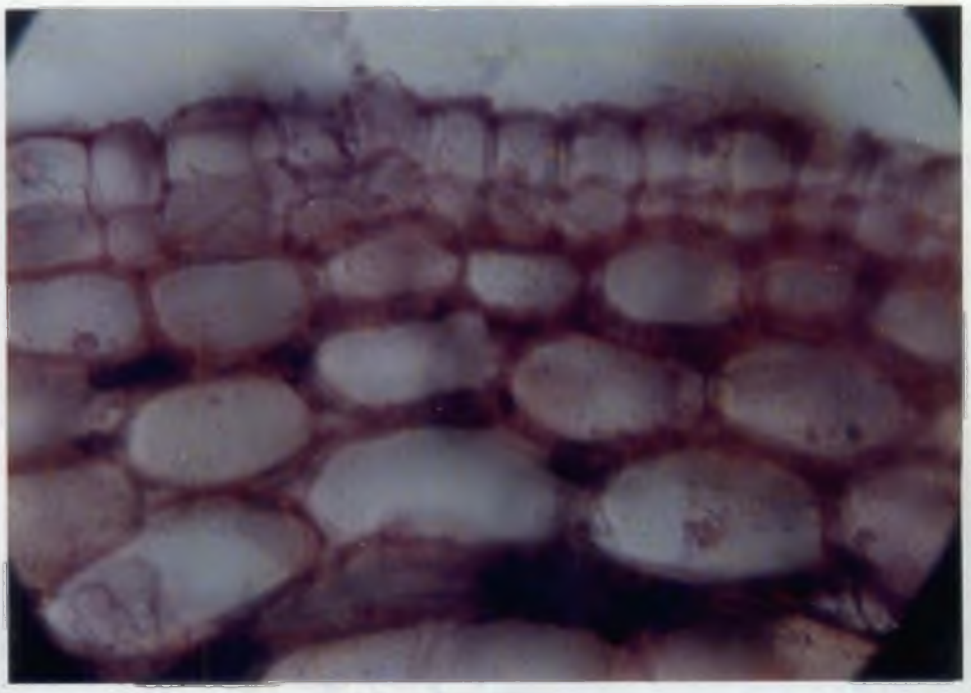


Plate 11. Magnified view of piliferous layer and outer cortex of root of bacterial wilt resistant variety (x 1250)

Plate 12. Magnified view of piliferous layer and outer cortex of root of bacterial wilt susceptible variety (x 1250)



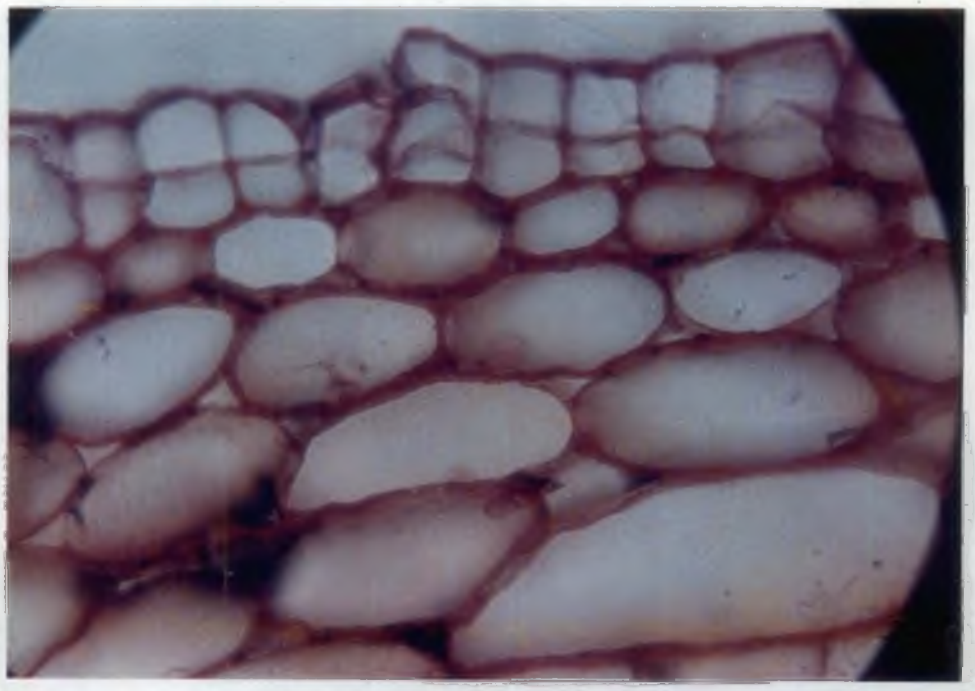
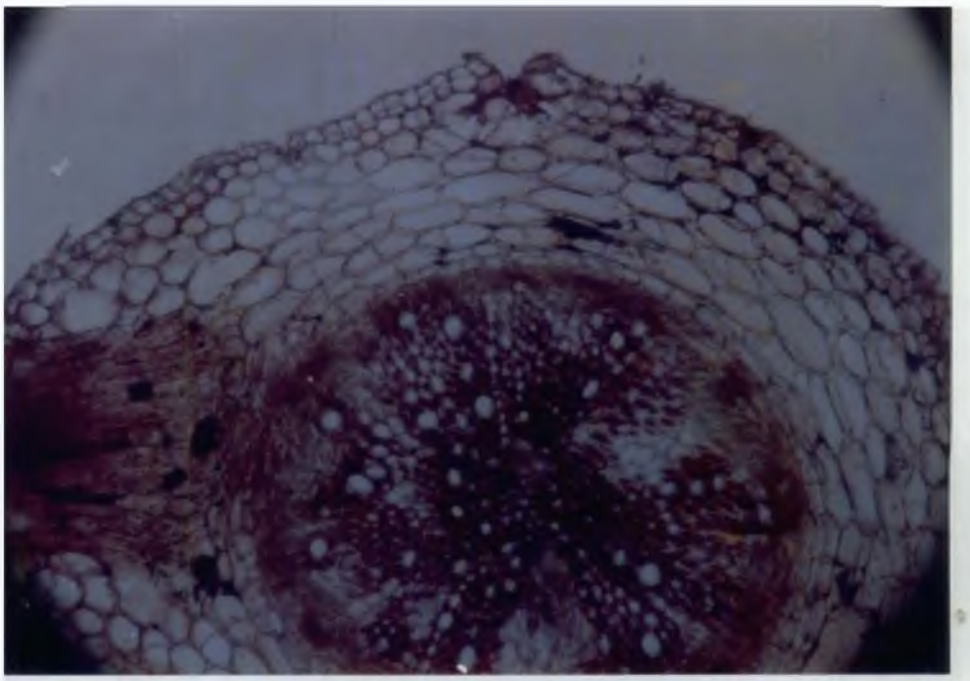


Plate 13. Portion of T.S. of root of bacterial wilt susceptible variety

a) Showing tangential division of the piliferous layer and damage caused to the integrity of the piliferous layer at certain regions (x 300)

b) Magnified view of the damage brought about in the piliferous layer (x 1250)



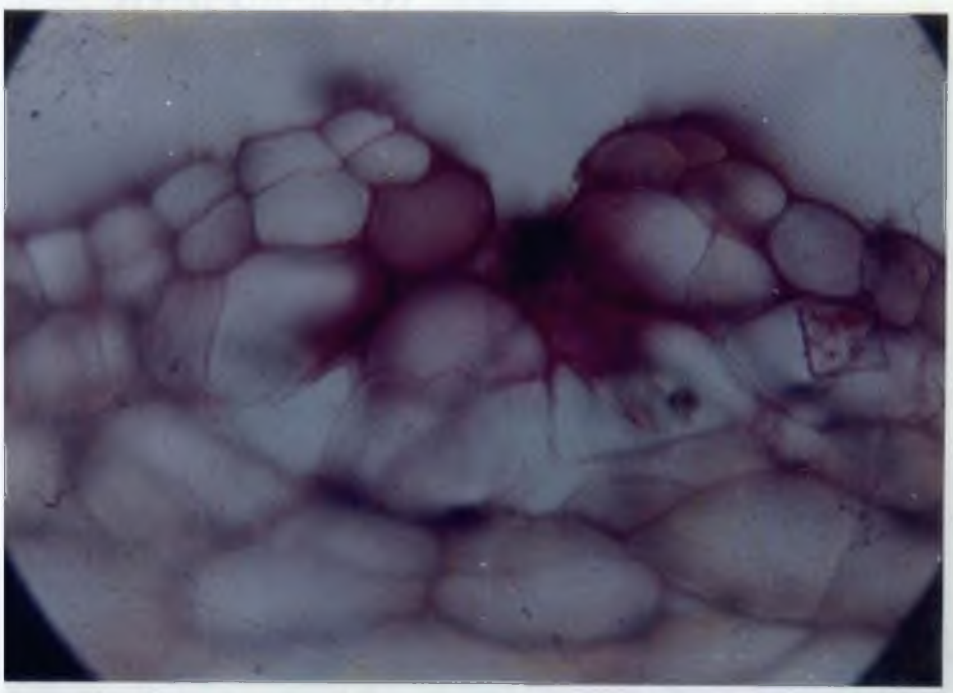
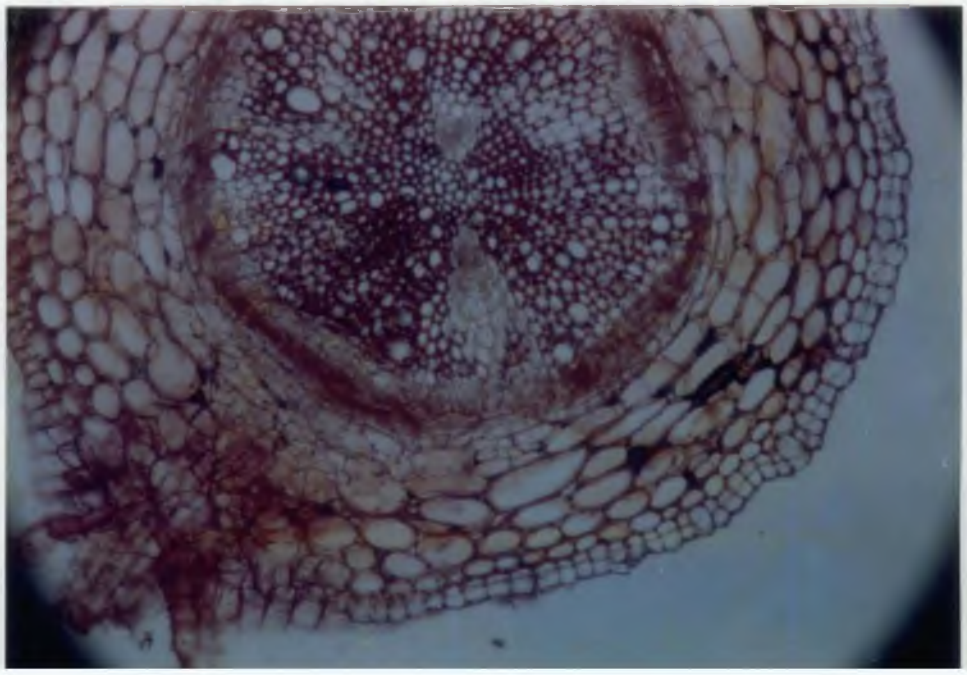


Plate 14. T.S. of root of bacterial wilt resistant variety having more secondary xylem (x 300)

Plate 15. T.S. of root of bacterial wilt susceptible variety (x 300)



4.4.2 Histology of root

In the roots of resistant genotype, epidermal cells as well as cortical cells were more compact with less intercellular space. On the other hand the epidermal cells in susceptible variety were loosely packed, thin walled and tangentially divided (Plates 11 and 12). Subsequent suberization and destruction of cuticle in the piliferous layer was observed (Plate 13a & b). The cortical cells were more succulent and fleshy with more of intercellular space in susceptible root.

Amount of secondary xylem of same age group was more in resistant root (Plates 14 and 15).

4.5 Radiotracer studies on growth and infection of *Pseudomonas solanacearum*

The pattern of infection of *Pseudomonas solanacearum* (Smith) Smith, its entry, movement and growth inside the host tissues and multiplication rate and colonisation were studied by radiotracer techniques using ^{32}P and ^{35}S.

4.5.1 Tolerance and growth of bacterium to radioactivity

The growth response of *Pseudomonas solanacearum* at different levels of radioactivity was carried out using ^{32}P and ^{35}S.

Growth of bacteria under ^{32}P

Measurement of turbidity

There was significant difference in the growth and tolerance of bacteria under different concentrations of ^{32}P (Table 22). Radioactivity of 111 kBq

(3 μci)/5 ml and 222 kBq (6 μci)/5 ml enhanced the growth of bacteria (OD 0.443 and 0.455). There was reduction in growth of the bacteria at 444 kBq/5 ml, 777 kBq/5 ml and 1110 kBq/5 ml.

Serial dilution plate technique

There was significant difference in the number of virulent colonies obtained in different treatments (Table 23). The range was from 180 x 10^4 in T_6 to 580 x 10^4 in T_3. The maximum colony counts were obtained in 222 kBq/5 ml and 111 kBq/5 ml (Plate 16a). Though virulent colonies were obtained from 444 kBq/5 ml and 777 kBq/5 ml, their number was less when compared to control.

In both the above studies 222 kBq/5 ml was found to be the most favourable concentration for the growth of *P. solanacearum* and hence for further plant inoculation experiment concentration of 222 kBq/5 ml (44.4 kBq/ml) was chosen.

Growth of bacteria under ^{35}S

Measurement of turbidity

Measurement of turbidity showed significant difference in the growth and tolerance of bacteria under different concentrations of ^{35}S (Table 24). An increase in the population of the bacterium and tolerance to radioactivity were observed at higher concentrations.

Serial dilution plate technique

There was significant difference in the number of virulent colonies obtained in different treatments and it was increasing as the

concentration of radioactivity increased (Table 25). The range was from 284 x 10^4 in T_1 to 645 x 10^4 in T_{10}. Though the number of virulent colonies were increasing as the concentration increases, upto 3700 kBq (100 μci)/5 ml, the virulent colonies had characteristic pin point pink centre (Plate 16b). But from 4440 to 5550 kBq/5 ml, pink centre disappeared but good slimy colonies were noticed. The virulence of such nonsignificant slimy colonies could not be tested, but they produced good growth on subculturing.

For further plant inoculation experiment, concentration of 3700 kBq/5 ml (740 kBq/ml) was chosen.

4.5.2 Distribution pattern of ^{32}P and ^{35}S tagged *P. solanacearum* in resistant and susceptible varieties

After the prescribed period of absorption, the aerial plant parts were detached and kept for drying. Re-isolation attempts were positive and very good growth of the bacterium was observed in PCA slants and thus verified the recovery of the bacteria.

Tagging with ^{32}P

Autoradiography

The radioautograph revealed no appreciable difference in rate or extent of distribution of ^{32}P tagged bacteria in resistant and susceptible varieties (Plates 17 and 18). The accumulation of tagged bacteria in both resistant and susceptible plants was more in the stem.

Radioassay

Radioassay showed differences among the resistant and susceptible genotypes for accumulation of radiolabelled bacteria in different plant parts.

The radiolabelled bacterial counts of bacterial wilt resistant variety Ujwala was less than that of the susceptible variety Pusa Jwala in both stem and leaves (Table 26). The highest radioactivity was observed in the stem of Pusa Jwala (49,594 cpm g^{-1}). In Ujwala it was only 17,259 cpm g^{-1}. In the case of leaf also there was difference among resistant (2,291 cpm g^{-1}) and susceptible (6,644 cpm g^{-1}) varieties. The accumulation of ^{32}P labelled bacteria in both varieties was more in stem portions than in leaves.

The absorption time given also showed marked difference in the accumulation of bacteria. In susceptible Pusa Jwala, after one hour of absorption the radiolabelled bacteria amounted to 3933 cpm g^{-1} and 1831 cpm g^{-1} in stem and leaves respectively while in the resistant variety Ujwala, it was only 1347 cpm g^{-1} and 168 cpm g^{-1} respectively.

Tagging with ^{35}S

Autoradiography

Autoradiography showed that radioactive bacteria were accumulated mainly at the nodal regions of the stem. The labelled bacteria were more in susceptible than in resistant variety. The photographs (Plates 19 and 20) revealed bacterial accumulation in all the leaves of the susceptible variety, whereas such accumulation was more in older leaves in the case of the resistant variety.

Table 22. Tolerance and growth of *P. solanacearum* to ^{32}P - measurement of turbidity

Treatment	Concentration (kBq/5 ml)	Absorbance (OD)
T_1	0	0.406
T_2	111	0.443
T_3	222	0.455
T_4	444	0.363
T_5	777	0.336
T_6	1110	0.259
CD		0.031**

Table 23. Tolerance and growth of *P. solanacearum* to ^{32}P - serial dilution plate technique

Treatment	Concentration (kBq/5 ml)	Colony count
T_1	0	400,0000 (6.602)
T_2	111	530,0000 (6.724)
T_3	222	580,0000 (6.762)
T_4	444	361,6666 (6.557)
T_5	777	326,3333 (6.511)
T_6	1110	180,0000 (6.252)
CD		0.092**

**Significant at 1%
Data in parenthesis indicate transformed values

Table 24. Tolerance and growth of *P. solanacearum* to ^{35}S - Measurement of turbidity

Treatment	Concentration (kBq/5 ml)	Absorbance OD
T_1	0	0.203
T_2	370	0.340
T_3	740	0.377
T_4	1480	0.408
T_5	2220	0.438
T_6	2960	0.488
T_7	3700	0.553
T_8	4440	0.552
T_9	5180	0.682
T_{10}	5550	0.692
CD		0.089**

** Significant at 1%

Table 25. Tolerance and growth of *P. solanacearum* to ^{35}S - serial dilution plate technique

Treatment	Concentration (kBq/5 ml)	Colony count (ml^{-1})
T_1	0	284,0000 (6.450)
T_2	370	430,0000 (6.633)
T_3	740	494,3333 (6.694)
T_4	1480	495,0000 (6.694)
T_5	2220	456,6666 (6.659)
T_6	2960	507,0000 (6.704)
T_7	3700	554,0000 (6.743)
T_8	4440	542,6666 (6.734)
T_9	5180	596,6666 (6.775)
T_{10}	5550	645,3333 (6.807)
CD		0.059**

** Significant at 1%
Data in parenthesis indicate transformed value (log)

Table 26. Distribution pattern of ^{32}P tagged *P. solanacearum* in resistant and susceptible genotypes

Variety	Time interval (hr)	Plant parts digested	Radioactive counts (cpm g^{-1})
Ujwala	1	Stem	1,347
,,	1	leaf	168
,,	24	Stem	17,259
,,	24	Leaf	2,291
Pusa Jwala	1	Stem	3,933
,,	1	Leaf	1,831
,,	24	Stem	49,594
,,	24	Leaf	6,644

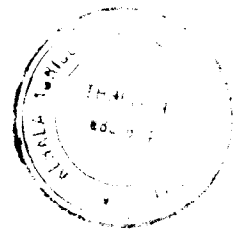


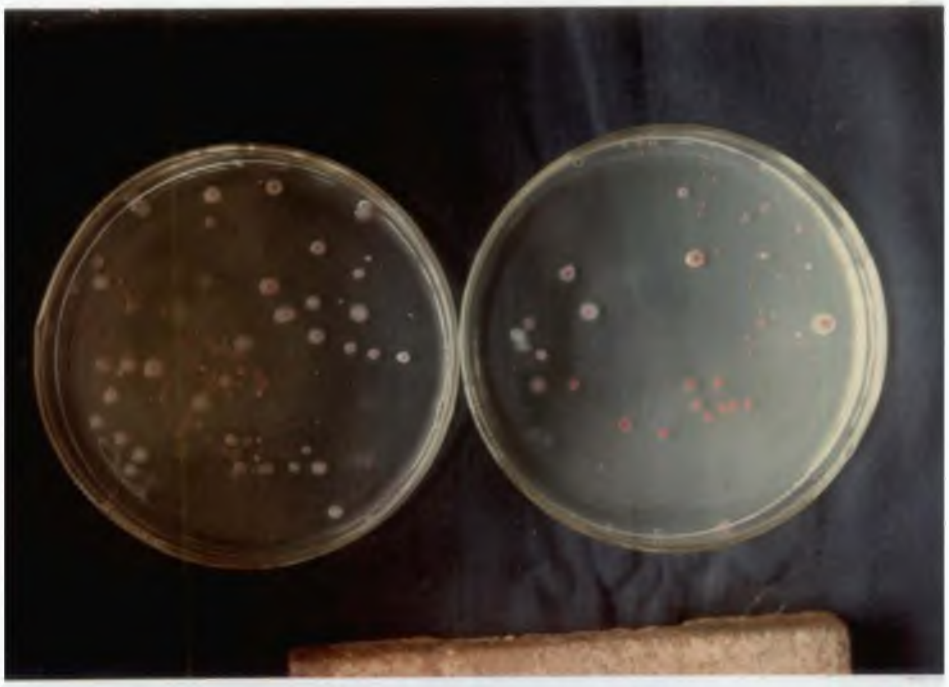
Table 27. Distribution pattern of ^{35}S tagged *P. solanacearum* in resistant and susceptible genotypes

Variety	Time interval (hr)	Plant parts digested	Radioactive counts (cpm g^{-1})
Ujwala	1	Nodal regions	0
,,	1	Other stem parts	0
,,	1	Leaves	0
,,	24	Nodal regions	41,443
,,	24	Other stem parts	32,768
,,	24	Leaves	6,136
Pusa Jwala	1	Nodal regions	15,821
,,	1	Other stem parts	4,978
,,	1	Leaves	0
,,	24	Nodal regions	70,220
,,	24	Other stem parts	53,003
,,	24	Leaves	33,828

Plate 16. Serial dilution plate technique

a) ^{32}P labelled bacteria - 444 kBq/5 ml (left) and control (right)

b) ^{35}S labelled bacteria - 3700 kBq/5 ml (left) and 5550 kBq/5 ml (right)



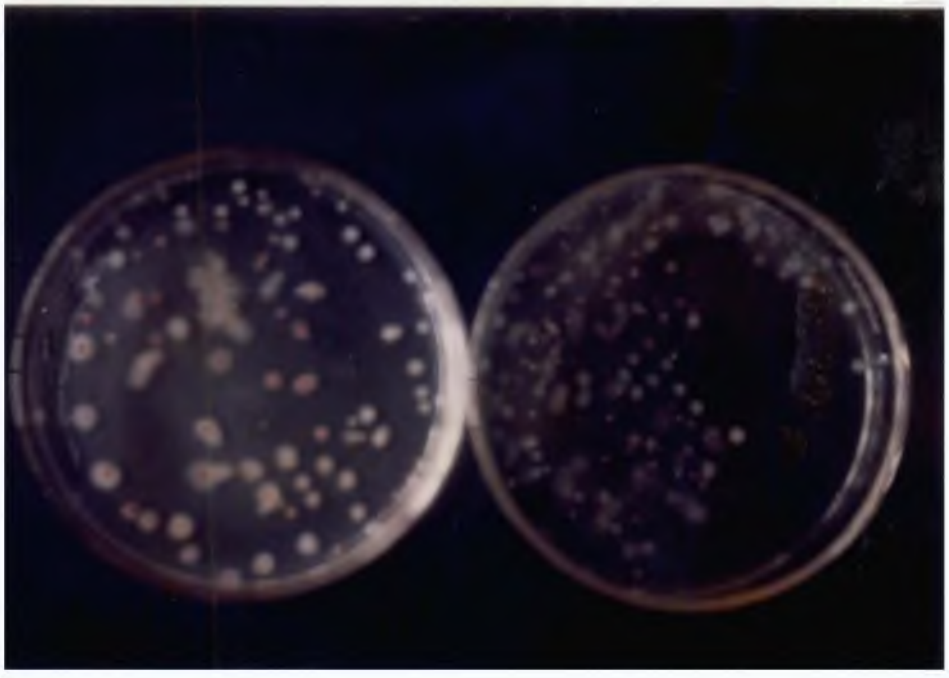


Plate 17. Distribution pattern of ^{32}P tagged *P. solanacearum* in bacterial wilt resistant variety

a) Seedling inoculated with ^{32}P tagged bacteria

b) Radioautograph showing the distribution pattern of ^{32}P tagged bacteria

Table 18. Distribution pattern of ^{32}P tagged *P. solanacearum* in bacterial wilt susceptible variety

a) Seedling inoculated with ^{32}P tagged bacteria

b) Radioautograph showing the distribution pattern of ^{32}P tagged bacteria

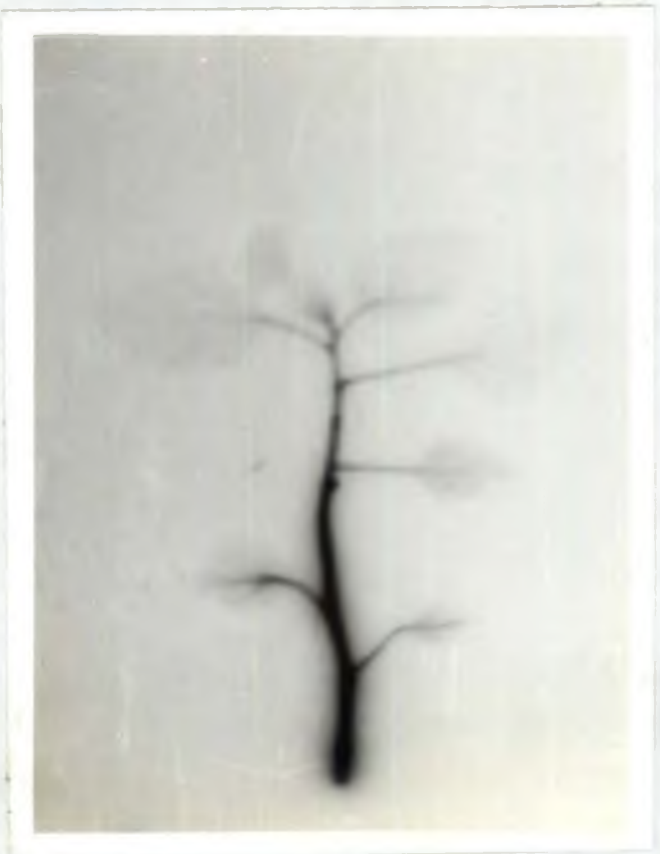


Plate 19. Distribution pattern of ^{35}S tagged *P. solanacearum* in bacterial wilt resistant variety

a) Seedling inoculated with ^{35}S tagged bacteria

b) Radioautograph showing the distribution pattern of ^{35}S tagged bacteria

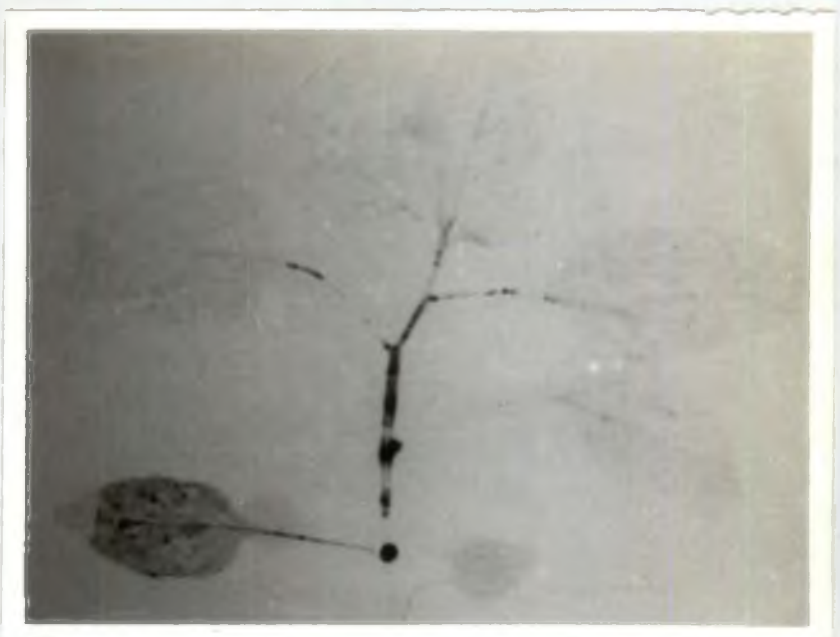


Plate 20. Distribution pattern of ^{35}S tagged *P. solanacearum* in bacterial wilt susceptible variety

a) Seedling inoculated with ^{35}S tagged bacteria

b) Radioautograph showing the distribution pattern of ^{35}S tagged bacteria

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Radioassay

Radioassay showed that the resistant and susceptible varieties were significantly different in accumulation of radioactive bacteria in various plant parts (Table 27). The radioactive counts of bacterial wilt resistant Ujwala were less than that of Pusa Jwala in all the plant parts. The highest radio-activity was observed in nodal stem parts of Pusa Jwala (70,220 cpm g^{-1}) followed by other stem parts (53,003 cpm g^{-1}) while in resistant variety Ujwala it was only 41,443 cpm g^{-1} and 32,768 cpm g^{-1} respectively in nodal region and other stem parts. The radioactive bacteria in the leaves was 33,828 cpm g^{-1} in Pusa Jwala and only 6,136 cpm g^{-1} in Ujwala.

Radioassay after one hour of inoculation indicated significant differences among resistant and susceptible genotypes. In Ujwala, the tagged bacterium could not enter the stem and leaves after one hour while in Pusa Jwala, the nodal regions showed radio active counts of 15,821 cpm g^{-1} followed by 4,978 cpm g^{-1} in other stem parts and the tagged bacterium could not enter the leaves at all.

4.6 Development of near isogenic line possessing field resistance to bacterial wilt

The biometric characters of parents of the near isogenic line are described in Table 28 (Plates 21 and 22). The resistant plants of BC_2 generation (Plate 23) resulted from cross of BC_1 and Pusa Jwala were selfed to produce BC_2F_2 (Plate 24). Bacterial wilt resistant plants having characteristics of Pusa Jwala were selected and are presented in Table 29. They were used as the initial material for producing near isogenic line of Pusa Jwala having resistance to bacterial wilt. They

were further advanced to BC_2F_3 (Plate 25) and BC_2F_4 (Plate 26) and bacterial wilt resistant plants having characteristics of Pusa Jwala were selected and presented in Tables 30 and 31.

Survival percentage of different generations of crosses are given in Table 32. The different generations were classified as suggested by Mew and Ho (1976). Ujwala was resistant with a survival of 92 per cent and Pusa Jwala was susceptible with susceptibility of 88 per cent. In F_1, BC_1 and BC_2, the survival of plants were 23.20 per cent, 20.77 per cent and 16 per cent respectively. In the BC_2F_2, survival per cent was 28.00 which was further enhanced to 46 per cent in BC_2F_3 and 61 per cent in BC_2F_4.

Percentage of resistant plants having characteristics of Pusa Jwala from the segregating population of BC_2F_2 onwards are given in Table 33. In BC_2F_2, 21.43 per cent resistant plants had characteristics of Pusa Jwala which was further enhanced to 32.60 per cent in BC_2F_3 and 54.10 per cent in BC_2F_4.

The characteristics of Pusa Jwala and its near isogenic line (BC_2F_4) having 61 per cent survival against bacterial wilt is given in Table 34. The selected plants in BC_2F_4 generation recorded an average plant height of 49.32 cm with a fruit length of 10.38 cm. Both Pusa Jwala and its near isogenic line had pendulous, solitary and light green coloured fruits. The near isogenic line recorded a higher yield (222.48 g/plant) compared to Pusa Jwala (204.20 g/plant).

Table 28. Performance of bacterial wilt resistant and susceptible genotypes of chilli

Number	Characters	Ujwala	Pusa Jwala
I	Vegetative characters		
	1. Plant height (cm)	71.70	47.30
	2. Plant spread (cm)	126.50	147.40
	3. Primary branches/plant	10.10	7.20
	4. Petiole length (cm)	3.5	2.51
	5. Leaf length (cm)	8.21	6.01
	6. Leaf width (cm)	4.02	2.56
II	Earliness		
	7. Days to flower	79.80	66.66
	8. Days to green fruit harvesting	107.65	95.34
	9. Days to fruit ripening	119.24	104.25
III	Productive characters		
	10. Pedicel length	3.30	2.75
	11. Fruit length (cm)	6.00	10.48
	12. Fruit perimeter (cm)	3.04	3.14
	13. Clustered/solitary	Clustered	Solitary
	14. Fruits/plant	101.05	100.01
	15. Average fruit weight (g)	2.31	2.63
	16. Green fruit yield/plant (g)	208.80	290.50
	17. Dry fruit yield/plant (g)	68.25	71.37
	18. Fruit orientation	Erect	Pendulous
	19. Fruit colour at mature stage	Dark green	Light green
	20. Seeds/fruit	69.60	40.15
	21. Bacterial wilt incidence (%)	8.00	88.00

Table 29. Morphological characteristics of BC_2F_2 generations

Plant No.	Plant height (cm)	Score	Fruit orientation	Score	Clustered/ solitary	Score	Fruit length (cm)	Score	Fruit colour at maturity	Score
1	2	4	5	6	7	8	9	10	11	12
P_1	62.50	0	Pendulous	1	Solitary	1	10.00	1	Light green	1
P_2*	50.00	1	Pendulous	1	Solatiry	1	9.35	1	Light green	1
P_3	58.00	0	Pendulous	1	Solatiry	1	6.00	0	Light green	1
P_4	58.20	0	Pendulous	1	Solitary	1	8.25	1	Light green	1
P_5*	50.45	1	Pendulous	1	Solitary	1	9.25	1	Light green	1
P_6	50.50	1	Pendulous	1	Solitary	1	9.25	1	Dark green	0
P_7	59.00	0	Erect	0	Solitary	1	5.65	0	Dark green	0
P_8*	49.50	1	Pendulous	1	Solitary	1	9.05	1	Light green	1
P_9	60.00	0	Pendulous	1	Solitary	1	9.00	1	Light green	1
P_{10}	62.00	0	Pendulous	1	Solitary	1	6.25	0	Light green	1
P_{11}	43.00	1	Pendulous	1	Clustered	0	4.95	0	Dark green	0
P_{12}	62.00	0	Erect	0	Clustered	0	5.20	0	Dark green	0
P_{13}	50.20	1	Pendulous	1	Solitary	1	10.20	1	Dark green	0
P_{14}	51.75	1	Erect	0	Solitary	1	5.20	0	Light green	1
P_{15}	70.65	0	Erect	0	Solitary	1	4.35	0	Dark green	0
P_{16}	62.50	1	Erect	0	Solitary	1	9.85	1	Light green	1
P_{17}*	50.50	1	Pendulous	1	Solitary	1	9.25	1	Light green	1
P_{18}	66.20	1	Erect	0	Clustered	0	5.95	0	Dark green	0
P_{19}*	50.25	1	Pendulous	1	Solitary	1	9.50	1	Light green	1

Contd.

Table 29. Continued

1	2	3	4	5	6	7	8	9	10	11
P_{20}	55.00	0	Pendulous	1	Solitary	1	9.00	1	Light green	1
P_{21}*	51.00	1	Pendulous	1	Solitary	1	10.65	1	Lighg green	1
P_{22}	63.00	0	Erect	0	Solitary	1	8.56	1	Dark green	0
P_{23}	40.00	1	Erect	0	Clustered	0	6.96	0	Dark green	0
P_{24}	49.50	1	Pendulous	1	Solitary	1	8.65	1	Dark green	0
P_{25}	65.50	0	Erect	0	Clustered	0	5.65	0	Dark green	0
P_{26}	50.00	1	Pendulous	1	Solitary	1	4.50	0	Light green	1
P_{27}	65.00	0	Pendulous	1	Solitary	1	7.40	0	Light green	1
P_{28}	60.90	0	Erect	0	Clustered	0	5.40	0	Dark green	0

* Plant with Pusa Jwala characteristics
Score for Pusa Jwala characteristics

1. Plant height Score

< 52 cm	1
> 52 cm	0

2. Fruit orientation

Pendulous fruits	1
Erect fruits	0

3. Clustered/solitary fruits

Solitary	1
Clustered	0

4. Fruit length Score

> 8 cm	1
< 8 cm	0

5. Fruit colour at maturity

Light green fruits	1
Dark green fruits	0

Table 30. Morphological characteristics of BC_2F_3 generations

Plant No.	Plant height (cm)	Score	Fruit orientation	Score	Clustered/ solitary	Score	Fruit length (cm)	Score	Fruit colour at maturity	Score
1	2	3	4	5	6	7	8	9	10	11
P_1*	50.00	1	Pendulous	1	Solitary	1	10.65	1	Light green	1
P_2	65.00	0	Pendulous	1	Solitary	1	9.65	1	Light green	1
P_3	46.00	1	Erect	0	Solitary	1	5.35	0	Dark green	0
P_4	58.00	0	Erect	0	Solitary	1	4.90	0	Dark green	0
P_5	52.50	1	Erect	0	Clustered	0	5.50	0	Dark green	0
P_6	62.00	0	Pendulous	1	Solitary	1	9.25	1	Light green	1
P_7	65.00	0	Pendulous	1	Solitary	1	8.35	1	Light green	1
P_8	70.00	0	Pendulous	1	Clustered	0	7.36	0	Dark green	0
P_9*	48.00	1	Pendulous	1	Solitary	1	9.25	1	Light green	1
P_{10}*	45.00	1	Pendulous	1	Solitary	1	8.50	1	Light green	1
P_{11}	51.40	1	Pendulous	1	Solitary	1	9.55	1	Dark green	0
P_{12}	72.00	0	Pendulous	1	Solitary	1	10.25	1	Light green	1
P_{13}	49.00	1	Pendulous	1	Solitary	1	10.00	1	Dark green	0
P_{14}*	48.25	1	Pendulous	1	Solitary	1	9.50	1	Light green	1
P_{15}	53.40	1	Erect	0	Clustered	0	5.00	0	Dark green	0
P_{16}	54.50	1	Erect	0	Clustered	0	7.25	0	Dark green	0
P_{17}*	50.25	1	Pendulous	1	Solitary	1	10.50	1	Light green	1
P_{18}*	47.30	1	Pendulous	1	Solitary	1	8.75	1	Light green	1
P_{19}	72.00	0	Erect	0	Solitary	1	8.25	1	Dark green	0
P_{20}	70.40	0	Erect	0	Solitary	1	6.50	0	Dark green	0

Contd.

Table 30. Continued

1	2	3	4	5	6	7	8	9	10	11
P_{21}	68.40	0	Pendulous	1	Solitary	1	10.25	1	Light green	1
P_{22}	62.50	0	Pendulous	1	Solitary	1	8.25	1	Light green	1
P_{23}	58.20	0	Pendulous	1	Solitary	1	10.25	1	Light green	1
P_{24}	55.50	0	Pendulous	1	Solitary	1	9.85	1	Light green	1
P_{25}	50.25	1	Erect	0	Solitary	1	7.10	0	Dark green	0
P_{26}*	47.50	1	Pendulous	1	Solitary	1	10.20	1	Light green	1
P_{27}*	48.00	1	Pendulous	1	Solitary	1	9.10	1	Light green	1
P_{28}	45.00	1	Pendulous	1	Solitary	1	5.25	0	Dark green	0
P_{29}	52.00	1	Pendulous	1	Solitary	1	10.50	1	Light green	1
P_{30}	50.00	1	Erect	0	Solitary	1	7.20	0	Light green	1
P_{31}*	48.00	1	Pendulous	1	Solitary	1	9.80	1	Light green	1
P_{32}	38.00	1	Pendulous	1	Clustered	0	7.50	0	Dark green	0
P_{33}	42.50	1	Pendulous	1	Solitary	1	9.50	1	Dark green	0
P_{34}	72.00	0	Pendulous	1	Solitary	1	10.50	1	Light green	1
P_{35}	65.00	0	Erect	0	Solitary	1	5.35	0	Light green	1
P_{36}	62.00	0	Pendulous	1	Solitary	1	7.35	0	Dark green	0
P_{37}	64.25	0	Erect	0	Clustered	0	6.50	0	Dark green	0
P_{38}*	41.50	1	Pendulous	1	Solitary	1	8.55	1	Light green	1
p_{39}*	49.50	1	Pendulous	1	Solitary	1	10.50	1	Light green	1
P_{40}	50.00	1	Pendulous	1	Solitary	1	7.25	0	Light green	1
P_{41}*	50.50	1	Pendulous	1	Solitary	1	10.25	1	Light green	1
P_{42}	62.00	0	Pendulous	1	Solitary	1	9.80	1	Light green	1

Contd.

Table 30. Continued

1	2	3	4	5	6	7	8	9	10	11
P_{43}*	50.00	1	Pendulous	1	Solitary	1	9.20	1	Light green	1
P_{44}*	49.25	1	Pendulous	1	Solitary	1	10.00	1	Light green	1
P_{45}	62.50	0	Erect	0	Clustered	0	6.85	0	Dark green	0
P_{46}*	48.50	1	Pendulous	1	Solitary	1	9.80	1	Light green	1

* Plant with Pusa Jwala characteristics
Score for Pusa Jwala characteristics - same as in Table 29

Table 31. Morphological characteristics of BC_2F_4 generations

Plant No.	Plant height (cm)	Score	Fruit orientation	Score	Clustered/ solitary	Score	Fruit length (cm)	Score	Fruit colour at maturity	Score
1	2	3	4	5	6	7	8	9	10	11
P_1	55.65	0	Pendulous	1	Solitary	1	9.50	1	Light green	1
P_2*	42.50	1	Pendulous	1	Solitary	1	10.50	1	Light green	1
P_3*	50.45	1	Pendulous	1	Solitary	1	9.50	1	Light green	1
P_4*	50.50	1	Pendulous	1	Solitary	1	8.80	1	Light green	1
P_5	68.25	0	Pendulous	1	Solitary	1	5.50	0	Light green	1
P_6	72.50	0	Erect	0	Solitary	1	4.50	0	Dark green	0
P_7	50.25	1	Erect	0	Clustered	0	8.20	1	Light green	1
P_8*	46.50	1	Pendulous	1	Solitary	1	8.75	1	Light green	1
P_9	53.50	1	Pendulous	1	Solitary	1	5.85	0	Light green	1
P_{10}*	50.75	1	Pendulous	1	Solitary	1	10.30	1	Light green	1
P_{11}	59.22	0	Pendulous	1	Solitary	1	7.25	0	Light green	1
P_{12}	65.70	0	Erect	0	Solitary	1	6.75	0	Dark green	0
P_{13}	72.58	0	Pendulous	1	Solitary	1	9.25	1	Light green	1
P_{14}	75.20	0	Pendulous	1	Solitary	1	9.80	1	Light green	1
P_{15}*	50.25	1	Pendulous	1	Solitary	1	10.50	1	Light green	1
P_{16}*	50.50	1	Pendulous	1	Solitary	1	10.00	1	Light green	1
P_{17}*	48.25	1	Pendulous	1	Solitary	1	8.50	1	Light green	1
P_{18}*	44.50	1	Pendulous	1	Solitary	1	8.50	1	Light green	1
P_{19}*	49.50	1	Pendulous	1	Solitary	1	9.80	1	Light green	1
P_{20}	59.25	0	Erect	0	Solitary	1	9.25	1	Light green	1

Contd.

Table 31. Continued

1	2	3	4	5	6	7	8	9	10	11
P_{21}	65.25	0	Erect	0	Clustered	0	5.50	0	Dark green	1
P_{22}	64.50	0	Erect	0	Solitary	1	9.50	1	Light green	1
P_{23}	67.50	0	Pendulous	1	Solitary	1	7.00	0	Light green	1
P_{24}	60.50	0	Pendulous	1	Solitary	1	10.15	1	Light green	1
P_{25}*	48.50	1	Pendulous	1	Solitary	1	9.20	1	Light green	1
P_{26}*	50.00	1	Pendulous	1	Solitary	1	10.50	1	Light green	1
P_{27}*	49.00	1	Pendulous	1	Solitary	1	9.80	1	Light green	1
P_{28}*	50.00	1	Pendulous	1	Solitary	1	10.85	1	Light green	1
P_{29}	68.00	0	Pendulous	1	Solitary	1	8.25	1	Light green	1
P_{30}	72.00	0	Pendulous	1	Solitary	1	5.45	0	Light green	1
P_{31}	70.50	0	Erect	0	Solitary	1	8.20	1	Dark green	0
P_{32}*	48.25	1	Pendulous	1	Solitary	1	10.00	1	Light green	1
P_{33}*	50.00	1	Pendulous	1	Solitary	1	10.25	1	Light green	1
P_{34}	55.25	0	Pendulous	1	Solitary	1	9.25	1	Light green	1
P_{35}	58.50	0	Erect	0	Solitary	1	8.75	1	Light green	1
P_{36}*	49.20	1	Pendulous	1	Solitary	1	9.85	1	Light green	1
P_{37}*	35.00	1	Pendulous	1	Solitary	1	8.25	1	Light green	1
P_{38}*	47.00	1	Pendulous	1	Solitary	1	9.50	1	Light green	1
P_{39}*	50.40	1	Pendulous	1	Solitary	1	10.80	1	Light green	1
P_{40}	58.00	0	Pendulous	1	Solitary	1	8.50	1	Light green	1
P_{41}*	50.20	1	Pendulous	1	Solitary	1	10.35	1	Light green	1
P_{42}*	49.25	1	Pendulous	1	Solitary	1	10.50	1	Light green	1

Contd.

Table 31. Continued

1	2	3	4	5	6	7	8	9	10	11
P_{43}*	48.75	1	Pendulous	1	Solitary	1	10.40	1	Light green	1
P_{44}*	50.85	1	Pendulous	1	Solitary	1	10.30	1	Light green	1
P_{45}*	50.25	1	Pendulous	1	Solitary	1	8.25	1	Light green	1
P_{46}	53.50	1	Erect	0	Solitary	1	9.25	1	Dark green	0
P_{47}*	52.75	1	Pendulous	1	Solitary	1	10.00	1	Light green	1
P_{48}	54.25	1	Erect	1	Solitary	1	6.50	0	Dark green	1
P_{49}*	50.75	1	Pendulous	1	Solitary	1	10.00	1	Light green	1
P_{50}*	51.50	1	Pendulous	1	Solitary	1	9.90	1	Light green	1
P_{51}	50.80	1	Erect	0	Clustered	0	6.50	0	Light green	1
P_{52}*	50.00	1	Pendulous	1	Solitary	1	10.50	1	Light green	1
P_{53}*	50.50	1	Pendulous	1	Solitary	1	10.75	1	Light green	1
P_{54}	72.00	0	Erect	0	Solitary	1	9.75	1	Light green	1
P_{55}	68.00	0	Pendulous	1	Solitary	1	4.50	0	Light green	1
P_{56}*	51.00	1	Pendulous	1	Solitary	1	8.75	1	Light green	1
P_{57}*	50.20	1	Pendulous	1	Solitary	1	10.75	1	Light green	1
P_{58}	56.00	0	Pendulous	1	Solitary	1	5.45	0	Light green	1
P_{59}	58.00	0	Pendulous	1	Solitary	1	7.75	0	Light green	1
P_{60}	59.60	0	Erect	0	Solitary	1	6.25	0	Light green	1
P_{61}*	44.75	1	Pendulous	1	Solitary	1	10.50	1	Light green	1

* Plant with Pusa Jwala characteristics
Score for Pusa Jwala characteristics - same as in Table 29

Table 32. Survival (%) of Ujwala, Pusa Jwala and their different generations against bacterial wilt

Generations	Total no. of plants	Resistant	Susceptible	Survival (%)	Score
Ujwala (P_1)	150	138	12	92.00	R
Pusa Jwala (P_2)	150	18	132	12.00	S
F_1	150	35	115	23.20	S
BC_1	130	27	103	20.77	S
BC_2	125	20	105	16.00	S
BC_2F_2	100	28	72	28.00	S
BC_2F_3	100	46	54	46.00	MS
BC_2F_4	100	61	39	61.00	MR

Score for bacterial wilt resistance

R - Resistant (>80% survival)
MR - Moderately Resistant (60-80% survival)
MS - Moderately Susceptible (40-60% survival)
S - Susceptible (<40% survival)

Table 33. Percentage of resistant plants having Pusa Jwala characteristics in advanced generations

Generations	Number of resistant plants	Number of plants having Pusa Jwala characteristics	Percentage of plants having Pusa Jwala characteristics
BC_2F_2	28.00	6.00	21.43
BC_2F_3	46.00	15.00	32.60
BC_2F_4	61.00	33.00	54.10

Table 34. Characteristics of Pusa Jwala and near isogenic line of Pusa Jwala (BC_2F_4)

Number	Characters	Pusa Jwala	Near isogenic line of Pusa Jwala (BC_2F_4)
I	Vegetative characters		
	1. Plant height (cm)	48.30	49.52
	2. Plant spread (cm)	136.54	142.32
	3. Primary branches/plant	7.00	7.50
	4. Petiole length (cm)	2.50	2.61
	5. Leaf length (cm)	5.82	6.06
	6. Leaf width (cm)	2.54	2.60
II	Earlines		
	7. Days to flower	65.60	62.20
	8. Days to green fruit harvesting	95.25	90.20
	9. Days to fruit ripening	104.25	101.35
III	Productive characters		
	10. Pedicel length (cm)	2.95	3.08
	11. Fruit length (cm)	10.25	10.38
	12. Fruit perimeter (cm)	3.14	3.15
	13. Clustered or solitary	Solitary	Solitary
	14. Fruits/plant	99.02	105.32
	15. Average fruit weight	2.23	2.36
	16. Green fruit yield/plant (g)	204.20	222.48
	17. Dry fruit yield/plant (g)	65.25	65.94
	18. Fruit orientation	Pendulous	Pendulous
	19. Fruit colour at maturity	Light green	Light green
	20. Seeds/fruit	39.62	44.35
	21. Bacterial wilt incidence (%)	86.98	61.00

Parents used in the breeding programme

Plate 21. Ujwala (CA 219)

Plate 22. Pusa Jwala (Jwala)


CA-219


JWALA

Plate 23. Field view of BC_2 generation

Plate 24. Field view of BC_2F_2 generation


BC_2


BC_2F_2

Plate 25. Field view of BC_2F_3 generation

Plate 26. A near isogenic line of Pusa Jwala (BC_2F_4 generation)


BC_2F_3

Discussion

DISCUSSION

Bacterial wilt caused by *Pseudomonas solanacearum* is a major disease of chilli in the warm humid tropics. The warm humid weather and acidic soil conditions in Kerala accelerate disease incidence due to high population dynamics of the wilt pathogen. Management of the pathogen by chemical means has not been effective or economically feasible. This necessitates a need based study to circumvent the disease. Development of resistant varieties seems to be more ideal and viable in the present circumstance.

A thorough knowledge on the sources and genetics of wilt resistance and biochemical and anatomical factors influencing this malady is essential to throw light on the reasons and to bring out possible solutions. At present such information are rather scanty in chilli. Evolving bacterial wilt resistant line(s) in chilli with wider adaptability and desirable plant type is also contemplated in the present study. The results of the present investigations are discussed here.

5.1 Isolation and establishment of pure culture(s) of the bacterium *Pseudomonas solanacearum*

The bacterial isolates of *Pseudomonas solanacearum* used in the present study were collected from infected chilli plants and their pathogenicity proved. Out of the different methods of artificial inoculation like stem puncture, root dip and soil drenching, root dip method was the most ideal and was used throughout the present investigation. The bacterial isolates were maintained under standard conditions for the above studies.

5.2 Genetic basis of bacterial wilt resistance

Mode of gene action governing wilt resistance is of immense use in the choice of appropriate breeding method(s) for incorporating resistance either from a cultivated or from wild species into commercially popular varieties. Attempts on exploration of source(s) of resistance to bacterial wilt in chilli are very limited. Though it is a major problem in chilli cultivation, systematic attempt on breeding for resistance to wilt was initiated only in 1983 in Kerala Agricultural University. Resistance to bacterial wilt in Manjari and Ujwala has been reported already (Gopalakrishnan and Peter, 1991).

The progenies of Ujwala and Pusa Jwala were developed and progressed for six generations. The survival of plants at different stages i.e. upto 75 days after transplanting were highly significant. There was no significant difference for the survival of plants after 75 days. The difference on the stages of incidence of the disease as observed in the present study was reported in other crops. Tikoo *et al.* (1983) and Nirmaladevi (1987) reported that the mortality of susceptible lines reached maximum by 80th day in tomato. The survival of plants in the present study also reached a plateau by 75th day confirming earlier results in tomato. The bacterial multiplication and resultant blocking of xylem vessels would have been over or reduced by 75th day. Hence it could be suggested that selection of plants for resistance to wilt could be done on a later stage from a sick field. This would enable the breeder to select resistant plants and to plan his back cross breeding programme without waiting for the end of the crop. Such a system ensures availability of pollen for crossing within the same generation.

Inheritance of bacterial wilt resistance was studied using resistant variety Ujwala and the susceptible Pusa Jwala by developing six generations of P_1, P_2, F_1, F_2, B_1 and B_2. Performance of the six generations showed that the resistance in Ujwala is monogenically inherited and is incompletely dominant with a penetrance of 92 per cent. A survival of 23.3 per cent plants in F_1 indicated that the gene action was incompletely dominant. The single partially dominant gene action for wilt resistance makes it rather easy to manipulate this trait in breeding programme. The finding of Rajan (1985) that bacterial wilt resistance mechanism in tomato variety 'Sakthi' was monogenic and incompletely dominant supports the present results.

Attempts to test the epistatic effect showed that the joint scaling test was insignificant. It revealed that survival rate (resistance) was not governed by epistatic gene action indicating the suitability of simple additive dominance model to explain the level of wilt resistance. If epistatic effects were present, inheritance would not have been monogenic. The three parameter model was adequate to explain the generation means. The three parameter model was a good fit to the generation means evidenced by the close agreement of expected and observed generation means. This close fit of the model along with the agreement of mendalian ratios for single factor segregation points to the theory of monogenic inheritance.

5.3 Biochemical basis of bacterial wilt resistance

The defence mechanisms developed by plants in response to injury and/or infection are dyr :mic which culminate in the sealing off the injury or the invading parasite. The interaction between plant and pathogen is the result of co-evolution, governed by the rule of natural selection. It is to the host's advantage to evolve mechanisms to inhibit pathogen growth (resistance mechanism) and to the

pathogen's advantage to evolve mechanisms which overcome these defences (pathogenicity factors). It is then the host's advantage to develop an improved defence and so *ad infinitum* (Collinge *et al.*, 1996).

The resistant varieties achieve the resistance response through several defence mechanisms. An important aspect in understanding the host-parasite relationship is the study of biochemical changes occurring in host plants due to pathogenic infection. In this context, a comparative study was carried out on the changes in the levels of phenols, OD phenols, proteins, enzymes and isozymes of both resistant and susceptible cultivars of chilli invaded by *Pseudomonas solanacearum*.

5.3.1 **Total phenols and OD phenol**

The total phenol content and OD phenol content of bacterial wilt resistant genotype (Ujwala) showed an increasing trend compared to susceptible genotype (Pusa Jwala) in all the plant parts at different stages of growth studied. However, in total phenol the difference was significant only in roots at 45th and 60th days after sowing. The OD phenol content was significantly superior in the roots and shoots of the resistant variety. Maximum phenol content and OD phenol content were observed at 45th to 60th days after sowing.

Higher contents of phenols and OD phenols in resistant plants suggest the role of phenols in imparting resistance to bacterial wilt. Protective role of phenolics against disease incidence was reported by Vidhyasekharan (1990), which supports the present findings. OD phenols are important in disease resistance reactions. They are easily oxidised by polyphenol oxidase and the resulting quinones are highly

reactive and toxic to pathogens (Mahadevan, 1966). The findings of Rajan (1985) that the resistant line of tomato (LE 79) had lower total phenol and higher OD phenols before and after inoculation of *P. solanacearum* is in agreement with the present results.

In the present study, artificial inoculation increased the total phenol content and OD phenol content significantly in both resistant and susceptible genotypes of chilli. The total phenol content did not differ significantly among resistant and susceptible genotypes while OD phenol content varied significantly. Eventhough the inhibition of enzymes take place in the inoculated plants, the reason for higher content in resistant variety could be attributed to higher percentage of phenolic production in resistant varieties than in susceptible varieties. This increased level of OD phenol formed as a result of inoculation in resistant lines plays an important role in wilt resistance. Fluctations in the content of chemicals with age implies that the degree of resistance also varies with age as reported by many workers (Winstead and Kelman, 1952; Bell, 1981; Coyne and Schuster, 1983). Association of wilt resistance and higher OD phenol content obtained in the present study is also in line with the studies of Rajan (1985) in tomato and Vidhyasekharan (1990) in rice, wheat, tomato and grape vine.

5.3.2 **Protein content**

The protein content of bacterial wilt resistant and susceptible genotypes differed significantly only in roots and stems. The resistant genotype Ujwala had a higher protein content at 30th and 60th day after sowing in all plant parts except leaves. The protein content increased progressively with age up to 60 days after sowing. The higher levels of protein imparts indirect defence mechanisms in plant

and the earlier reports indicate that normal level of protein in plants may not be involved in disease resistance (Gabriel and Ellingboe, 1982). Inoculation caused a progressive increase in protein content in both resistant and susceptible genotypes, but resistant one responded by accumulation of more protein as compared to the susceptible one. The higher protein content indicates higher enzyme concentration in the plants for defence mechanisms. As a result, Ujwala responded to inoculation by increasing the protein content. Enhanced protein synthesis appears to be a universal phenomenon in incompatible host-pathogen (resistant) interaction (De Wit and Baker, 1980; Ahmed *et al.*, 1994; Jiang *et al.*, 1994). *De novo* synthesis of new proteins has also been reported by Tani and Yamamoto (1979) and Yamamoto and Tani (1982). The synthesized protein may not be inhibitory to the pathogen. They may activate the synthesis of defence chemicals such as phenolics, lignins, phytoalexins etc. and hence they are involved only in the early recognition process as reported by De Wit and Bakker (1980).

5.3.3 **Enzyme activities**

Peroxidase

Peroxidase is an important enzyme in many plant systems which has been correlated with disease resistance. Moreover, the enzyme itself has been reported to be toxic to microorganisms (Pegg and Young, 1982). The results indicate that resistance is associated with higher levels of peroxidase activity in the host tissue. The peroxidase activity of bacterial wilt resistant genotype was higher than that of the susceptible one in all the plant parts at all stages of the growth studied. Significant differences were observed in peroxidase activities between the resistant and susceptible genotypes before and after inoculation. In resistant genotype,

peroxidase activity increased by 76.55 per cent in roots and 91.94 per cent in shoots after inoculation. In the susceptible genotype, the increase was only 31.80 per cent and 25.94 per cent respectively. The higher activity of peroxidase in resistant plants suggests the role of enzymes in imparting resistance to bacterial wilt. Peroxidase uses the native auxin as a substrate and destroys the same. The native auxin synthesised in shoot and root tissues of resistant genotype would have been destroyed to a significant extent resulting in the prevention of the formation of more thin walled linear parenchymatous cells. On the other hand the lower peroxidase activity in susceptible genotype would have helped in the utilization of auxin for the formation of thin walled parenchymatous cells in the outer layer which would have eventually made the genotype more prone to pathogen entry. These findings are important in terms of resistance which may be associated with increased levels of peroxidase activity before and after inoculation. Similar results observed in many varieties of french bean, tobacco, tomato and groundnut showing resistance to fungi, bacteria and viruses support the present results (Rudolph and Stahmann, 1964; Lovrekovich *et al.*, 1968; Retig, 1974; Shan and Tan, 1994). The less peroxidase activity in the susceptible variety resulted lower defence mechanism. The peroxidase may also help in the synthesis of lignin. Thus the enhanced peroxidase activity may induce resistance by helping to generate hydrogen peroxidase as well as by increasing the concentration of free radicals and their polymerization products such as lignin. The relation of peroxidase activity in host plant to resistance has also been reported by Retig (1974); Duan *et al.* (1994) and Kang and He (1994).

In the present study, peroxidase activity was found increasing progressively up to 60 days after sowing, maximum activity being observed between 45 and 60 days. The lower activity after 60 days may be due to production of

metabolites inhibitory to the enzyme within the plant. Hence transplanting age is more important for wilt resistance. It is also important to see that the early transplanting is not advisable because of low activity of peroxidase for both resistant and susceptible varieties.

Polyphenol oxidase

The activity of polyphenol oxidase is directly influenced by the genetic make up of genotypes. The increased activity of this enzyme results in accumulation of potentially bactericidal quinonic substances and tannins possessing antibiotic properties and hence has been assigned a role in disease resistance (Obukuwicz and Kennedy, 1981). In the present study this enzyme was analysed in fresh samples of root, stem, leaves and whole plant at an interval of one minute at room temperature for a period of five minutes. The increasing absorbance value per minute was plotted and progressive curves were prepared. Polyphenol oxidase activity was higher in the first minute and then decreased gradually in all plant parts at different growth stages. The decrease in the activity could be due to product inhibition in course of plant growth.

A steady increase in activity was observed up to second minute and there after, the increase was slow. *In vitro* activity of polyphenol oxidase is progressive and degraded the substrate in a specific manner in resistant and susceptible varieties. Activity per minute in resistant and susceptible varieties indicated that the higher the rate of activity, the better is the resistance.

The present result revealed that polyphenol oxidase activity is higher in roots and the lowest in leaves. It is important in the metabolic process of plant

growth and development. The findings of Duan *et al.* (1994) that roots are the most appropriate part for the study of resistance mechanism in bacterial wilt of groundnut, is in line with the present results.

Ujwala, the resistant variety showed higher polyphenol oxidase activity in all the plant parts at various growth stages. A comparison of this enzyme activity in root, stem, leaf and whole plants of both resistant and susceptible genotypes revealed that the higher the polyphenol oxidase activity, better is the resistance. These findings have significance in terms of resistance because polyphenol oxidase can catalyse the oxidative polymerization of phenolic substance into quinones and tannins. It is well known that quinones are toxic to bacteria and the tannins possess antibiotic properties and hence phenolics and tannin formations may be a consistant feature of resistance reaction (Obukowicz and Kennedy, 1981). Phenolics are degraded to quinones and are translocated to the plant parts where the infection is possible. It is a time dependent process where genetic control is important.

Activity trend of polyphenol oxidase is comparable with that of peroxidase activity. A progressive increase in polyphenol oxidase activity was observed with increase in age up to 60 days after sowing and decreased slowly there after.

The enzyme activity and protein content were observed for each sample. The specific activity of both resistant and susceptible variety is also in agreement with the activity curve where higher values are obtained for roots at room temperature. The protein content is higher in the leaves, where the polyphenol oxidase activity is less, which implies that the infection is through roots where the plant has a defence mechanism by producing quinone like products in the root.

The changes induced by the pathogen in the polyphenol oxidase activity in resistant and susceptible plants indicated different types of responses to infection. Increased polyphenol oxidase activity was observed in both resistant and susceptible genotypes after infection. There was only a small rise of polyphenol oxidase activity in susceptible variety in contrast to resistant variety. In the first minute, the increase was 98 per cent in the resistant genotype while in the susceptible, it was only 57 per cent. Polyphenol oxidase activity in the shoot showed an increase of 109 per cent and 41 per cent in the resistant and susceptible genotypes respectively. These findings are in agreement with the reports of Maine and Kelman (1961), Obukowicz and Kennedy (1981) and Duan *et al.* (1994) in bacterial wilt diseases of tobacco and groundnut where they stressed the role of this enzyme in disease resistance. The enhanceed activity of polyphenol oxidase enzyme in resistant genotypes following inoculation indicates that the resistant plants are endowed with greater potentiality to activate polyphenol more rapidly so that quinone and tannin production may be increased and thus the invasion of pathogen could be effectively controlled.

5.3.4 **Protein pattern by electrophoresis**

Gel electrophoresis of protein has become a standard and powerful research tool for cost effective methods of genetic investigation in plants. This method reveals electrophoretic variation of the different molecular forms of proteins observed as bands of colour in a slab of gel. The shape and size of a protein molecule relative to the size of pores of the electrophoretic matrix influences its migration. Side chains of the aminoacids are responsible for the movement of proteins through a gel during electrophoresis. An analysis of protein structure using

electrophoresis is an analysis of a gene. The pattern of protein bands is an expression of the particular protein system assayed and its mode of inheritance. From this the electrophoretic phenotype can be interpreted in genetic terms. The factors that determine the number of bands observed on a gel are the quaternary structure of the protein products (sub units).

In the present study, the resistant and susceptible varieties showed no differences in the number of protein bands and their Rm values in both roots and leaves before inoculation. These findings are in agreement with Gabriel and Ellingboe (1982) who suggested that existing proteins might not be involved in disease resistance.

Banding pattern of wilted plants showed an exception to the number of bands in leaves. In leaves number of bands were increased to six ie., they had an additional band (Table 21). A shift in protein pattern could have been a triggering mechanism for synthesis of specific biochemicals such as abscisic acid. This dynamic change in the protein pattern may be an adaptation or metabolic disorder supporting the wilted condition of the plant after an infection. Host pathogen interaction and incompatibility are involved in the expression of protein number in wilted leaf.

5.3.5 **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, genetic or other bio-chemical methods.

The pattern of enzyme bands is an expression of the particular enzyme system assayed and its mode of inheritance. Many enzymes are coded by more than a single gene, ie. more than one isozyme is present. Additional bands or shifts in migration may arise from post-translational modification of enzymes. In isozyme electrophorogram, expression of substrate-enzyme interaction followed with staining is taking place whereas in protein pattern, it is an expression of staining of protein in a particular condition without any protein-substrate interaction. The protein number obtained in electrophoresis may or may not have expressed as particular isozyme like peroxidase and esterase.

In the present study, isozyme pattern of peroxidase and esterase enzymes were studied in both resistant and susceptible chilli genotypes. Among the different plant parts studied, clear bands were obtained in the roots for peroxidase enzymes and in leaves for esterase enzymes. These are in agreement with the findings of Wang and Dehua (1987) and Indira (1994) in chilli Sujatha (1987) in *Cucumis* spp. and Sebastian (1995) in pepper.

Peroxidase

The resistant and susceptible varieties exhibited significant differences in peroxidase electrophorogram. Three bands were found in the root extracts of Pusa Jwala compared to two in Ujwala. In the case of leaf extracts Ujwala showed only one band where as Pusa Jwala had two bands. Ujwala was lacking the fastest moving PRX 1 of Pusa Jwala in both root (0.256) and leaves (0.200) in the peroxidase system. This is in agreement with Bashan *et al.* (1987) in tomato against Fusarium wilt and Linde and Rhodes (1988) in cucumber against anthracnose resistance.

Banding pattern was not influenced by inoculation in both resistant and susceptible genotypes. Polyacrylamide electrophoresis showed no difference in peroxidase isozymes as a result of the various host pathogen interaction (Jennings *et al.*, 1969). Similar results were also reported by Yi *et al.* (1994) with *P. solanacearum* in tobacco.

Three band expression of peroxidase in susceptible genotype showed low activity in the *in vitro* studies. Two bands were obtained in resistant genotype and the peroxidase activity of resistant genotype was substantially high which implies that the protein subunits have an influence in activity and resistance. The above results indicated that the increase of subunits in the plant decreased the activity of peroxidase enzyme.

Esterase

The resistant and susceptible varieties exhibited significant differences for esterase electrophorogram. Leaves showed clear bands compared to root tissues. Three bands were found in the resistant variety compared to one in the susceptible variety. The findings of Xia *et al.* (1994) where cotton plants resistant to *Verticillium dahliae* had higher levels of esterase isozyme than the susceptible ones, supports the present results. The resistant variety Ujwala possessed additional two fast moving bands EST 1 (Rm 0.367) and EST 2 (Rm 0.167) which indicated the involvement of esterase enzyme in resistance mechanism. This could be due to the synthesis of cytokinin in roots and translocation to leaves. Resistant genotype should posses the better ability for synthesis, protection and translocation of cytokinin from roots to leaves so that wilting of leaves is prevented. Three bands were obtained for Ujwala and one band for Pusa Jwala. In the case of peroxidase,

banding pattern is just the reverse which again indicate the independent functioning of esterase and peroxidase.

Inoculation did not influence the banding pattern of both resistant and susceptible genotypes. Same banding pattern of inoculated and uninoculated plants and different banding patterns in resistant and susceptible genotypes showed the specificity of the isozymes which may be used as a marker for screening different genotypes. Comparative evaluation of protein and isozyme are given in Table 35.

5.4 Anatomical basis of bacterial wilt resistance

Transverse sections of stem and root from resistant and susceptible genotypes were examined critically to study the arrangement of vascular bundles, epidermis, cortex, endodermis and thickness of cuticle.

Anatomical studies revealed distinct differences in cell arrangement among resistant and susceptible genotypes. Resistant genotype (Ujwala) had a well developed secondary xylem, formed as a solid cylinder. Secondary xylem is a hard tissue and one of the functions of secondary xylem is to give mechanical resistance. It is difficult for bacteria to pierce the hard and thick walled cylindrical secondary xylem. In the wilt susceptible variety (Pusa Jwala), cylinder was wavy and in the interfascicular region, slow development of secondary xylem occured. This becomes a vulnerable spot for the entry of bacteria.

The primary function of epidermis and cortex is the protection of internal tissues. Cortical cells were more succulent and loosely packed in susceptible stem with a lot of intercellular spaces which easily facilitate the free entry and movement of the pathogen.

Table 35. Comparative evaluation of protein and isozyme by electrophoresis

		Number of bands			
		Protein electrophoresis	Peroxidase	Esterase	Remarks
Uninoculated					
					Pr
Root	Ujwala	3	2	3	root - feeble bands leaf - thick and clear bands
	Pusa Jwala	3	3	1	
					PRX
Leaf	Ujwala	5	1	3	root - thick and clear
	Pusa Jwala	5	2	1	leaf - Ujwala feeble Pusa Jwala thick and clear
Inoculated					
					EST
					root - feeble
Root	Ujwala	3	2	3	leaf - Ujwala-medium thick and clear
	Pusa Jwala	3	3	1	leaf - Pusa Jwala feeble
Leaf	Ujwala	5	1	3	
	Pusa Jwala	6	2	1	

The vascular infection of wilt pathogen *Pseudomonas solanacearum* was already reported by many workers (Walker, 1952; Kolwal, 1978; Saqueira, 1993; Grimault and Prior, 1994 b,c and Vasse *et al.*, 1995). The pathogen first enters into the intercellular space of cortex and then to the xylem vessels. Grimault *et al.* (1994) suggested that resistance to bacterial wilt in tomato was mainly attributed to an induced and non specific physical barrier.

In the piliferous layer, cells were more compact and thick walled in resistant roots whereas the outer layer of cells were loosely packed, thin walled and tangentially divided in susceptible ones. This may probably be due to the availability of native auxin which was destroyed at lesser rate by the lower peroxidase activity in the susceptible variety. Because of this loose arrangement, thin walled nature and the tangential division of cells and subsequent suberization and destruction of cuticle, the piliferous layer looses its integrity as a protective covering (Plate 13a and b).

The lesser degree of secondary xylem development, the loose arrangement of piliferous layer and the cortical cells and the succumbant nature of the cells of the susceptible ones make them most vulnerable to the attack of the pathogen. Lignin may act as a physical barrier for the development of pathogen and the highly lignified cells prevent the colonization of the pathogen in host cells (Henderson and Friend, 1979 and Hammerschmidt and Kuc, 1982). Grimault and Prior (1994a) reported existence of a host defence mechanism for resistance to bacterial wilt.

Thus it appears that wilt resistance in genotypes can be attributed to higher degree of development of secondary xylem which forms a thick and solid cylinder making it quite formidable obstrucle for the pathogen. The thick cuticle

and the compact nature of the cortex of the resistant genotypes add to their ability to resist the attack of the pathogen.

The above mentioned histological specialities existing in the resistant genotypes seems to protect the plants physically from the onslaught of the pathogen. This can be one of the reasons for the wilt resistance in resistant genotypes.

5.5 Radiotracer techniques to study the growth and infection of *Pseudomonas solanacearum* in chilli

In the present study, the mode of entry, movement, growth and multiplication, colonisation and infection of *Pseudomonas solanacearum* in resistant and susceptible varieties of chilli were conducted by radiotracer techniques using ^{32}P and ^{35}S.

Before radioisotopes were used in studying the actual distribution of bacteria within the host, it was considered necessary to determine the radioactive effect due to the use of radioactivity on the bacterium and to find out optimum concentrations of the two radionuclides below which there was no adverse effect on the growth of the bacterium.

Growth responses of *P. solanacearum* to different concentrations of radioactive ^{32}P and ^{35}S in PCA broth medium were compared. Radioactivity levels of 44.4 kBq/ml (1.2 μCi/ml) of ^{32}P and 740 kBq/ml (20 μCi/ml) of ^{35}S were not found to affect the growth of bacteria. These radioactivity levels were found to enhance the growth of the bacterium. Similar results were reported by Warren (1951) who observed that radioactivity of 0.37-1.00 μCi/ml of ^{32}P increased growth

of the bacteria *Erwinia stewartii* and the higher doses of radiation decreased the growth of the bacteria in corn plants.

In the present study radioactivity of 111 to 222 kBq/5 ml of ^{32}P increased the bacterial growth. Concentrations above 222 kBq/5 ml decreased the growth of bacteria significantly. In the case of ^{35}S labelling the growth of bacteria was found to increase with the increasing concentrations of radioactivity. But in concentration above 3700 kBq/5 ml, the pink centering of the bacterial colony was absent.

Results obtained from the measurement of turbidity and the serial dilution plate technique also confirmed the above results. The concentrations of 222 kBq/5 ml for ^{32}P and 3700 kBq/5 ml for ^{35}S were found to be the ideal radio-activity dosages for growth and multiplication of the bacterium, *P. solanacearum* for such plant pathological studies.

Based on this preliminary study, the movement of *P. solanacearum* within the host tissues (both resistant and susceptible varieties) was studied using ^{32}P and ^{35}S labelled bacteria. The radioactivity of ^{32}P and ^{35}S were taken as evidence for the presence of bacteria in a particular tissue. The autoradiographs revealed that the bacteria had entered the plant and moved to the various tissues. The presence of radioactivity arising from ^{32}P or ^{35}S in stem, leaf and growing points is indicative of this. Similar results were reported by Warren (1951) wherein the distribution or rate of movement of bacteria *Erwinia stewartii* within susceptible or resistant varieties showed no significant difference in the autoradiographs. This indicated that even in resistant varieties, the bacteria were widely distributed and resistance does not depend only upon the localization of bacteria for infection.

The radioassay indicated that the resistant and susceptible varieties differed in the accumulation of radiolabelled bacteria in different plant parts. In the resistant variety Ujwala, after one hour of ^{32}P labelled bacterial inoculation, the stem portion recorded counts of 1347 cpm g^{-1} and leaves 168 cpm g^{-1} only while in the susceptible variety Pusa Jwala, it was 3933 cpm g^{-1} and 1831 cpm g^{-1} in stem and leaves. In the case of ^{35}S labelled bacterial inoculation, the radioactive counts were totally absent in Ujwala in both stem and leaves, while in the Pusa Jwala, nodal regions and other parts of stem recorded counts of 15,821 cpm g^{-1} and 4,978 cpm g^{-1} respectively and could not detect any counts from leaves.

The radio labelled bacterial counts were higher in Pusa Jwala in all the plant parts (stem and leaves) (49,594 and 6,644 cpm g^{-1}) after 24 hours of inoculation when compared to Ujwala, the resistant variety (17,259 and 2,291 cpm g^{-1}). The absorbed ^{32}P and ^{35}S were mainly accumulated in the stem portion than in the leaves in both the resistant and susceptible varieties. The radio activity counts were more in susceptible variety than in the resistant varieties in both ^{32}P, ^{35}S labelled plants at both the inoculation periods. The difference is also visible in the autoradiograph of the plants inoculated with ^{35}S labelled bacteria. This indicated that the resistant variety Ujwala offered some amount of resistance to bacterial entry, mobility and colonization.

The results of the autoradiography confirm presence of the labelled bacteria in the plant parts (stem and leaves) of both the varieties. In the autoradiography, the ^{35}S labelled bacteria were more in susceptible variety than in resistant ones. However, there was no appreciable difference betweeen the resistant and the susceptible varieties when inoculated with ^{32}P labelled bacteria. But, it was possible

to differentiate the amount of bacterial growth in the resistant and susceptible varieties from the radioassay.

The study highlighted the possibilities of tracing the bacterial entry, movement and colonisation of the host plant. The difference between resistant and susceptible varieties in the amounts of colonization is also evident from the studies. Therefore it is clear that there is a defence mechanism in the resistant host plant which prevent the bacteria to enter and multiply in the resistant variety. More studies are, however, required to elucidate the mechanism of resistance operating in the resistant variety Ujwala against the bacterial wilt pathogen, *P. solanacearum*.

The present studies with radiotracer techniques using ^{32}P and ^{35}S labelled bacteria, *P. solanacearum* to understand its entry, movement, growth, colonisation and infection of the host plant chilli, have given encouraging results and appears to be a potential tool in such plant pathological studies.

5.6 Development of near isogenic lines for resistance to bacterial wilt

Pusa Jwala is a high yielding variety of chilli and is highly acceptable for both green and dry chilli purposes. But it cannot be grown in Kerala because of its susceptibility to bacterial wilt. In the present investigation, an attempt was made to produce a near isogenic line of Pusa Jwala with resistance to bacterial wilt. Pusa Jwala was crossed with a bacterial wilt resistant line Ujwala and the resultant generations F_1, BC_1, BC_2, BC_2F_2, BC_2F_3 and BC_2F_4 were grown in a bacterial wilt sick field for testing the incidence of bacterial wilt. Confirmation of resistance was made by spot planting with Pusa Jwala. From BC_2F_2 generation onwards selection was made for plants with characteristics of Pusa Jwala.

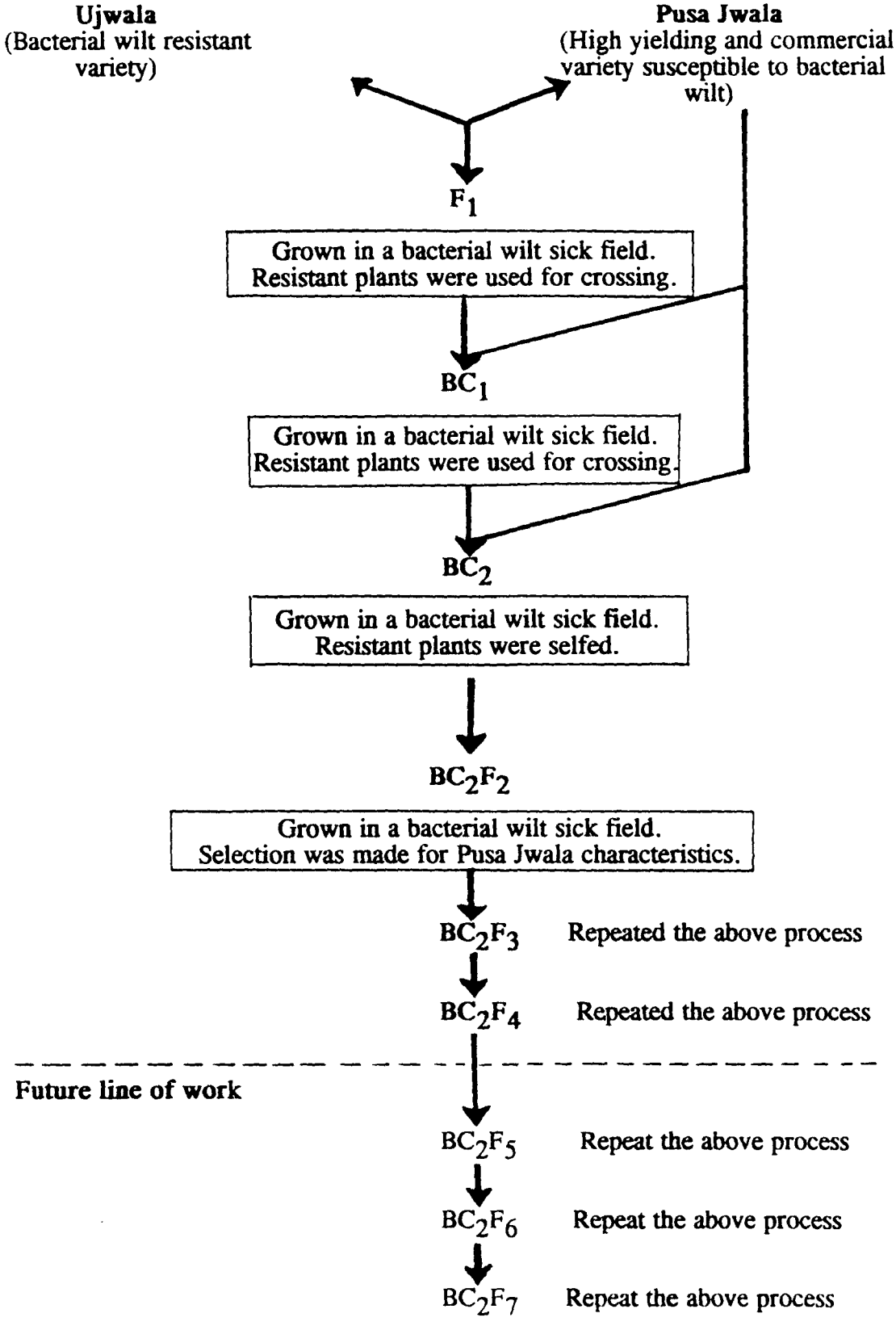
Pusa Jwala recorded a survival per cent of only 12. In the F_1, BC_1 and BC_2 generation, survival per cent were 22.20, 20.77 and 16.00 respectively. From BC_2F_2 onwards, there was an increase in the survival per cent and in BC_2F_4 generation, a near isogenic line of Pusa Jwala with 61 per cent survival was obtained. This increase in the number of resistant plants is achieved by eliminating possible escapes by the technique of spot planting, thus concentrating the favourable gene for resistance. This mode of accentuation of resistance by spot planting technique was as reported by Sadhankumar (1995) in tomato.

From the BC_2F_2 generation onwards, the resistant plants were scored for characters of Pusa Jwala. The percentage of resistant plants having characteristics of Pusa Jwala was increased from BC_2F_2 (21.43%) to BC_2F_4 (54.10%) generation.

By advancing these plants to BC_2F_7, uniformity can be obtained and an isogenic line of Pusa Jwala resistant to bacterial wilt can be obtained. Thus it could be possible to develop a genotype with green and dry chilli qualities possessing resistance to bacterial wilt.

Near isogenic lines are useful in advanced genetic and biochemical studies to explain the mechanism of resistance in a genetic background as reported by Geetha (1989) in brinjal and Mozzetti *et al.* (1995) in chilli. The plants with resistance to bacterial wilt and characteristics of Pusa Jwala would be a break through in chilli breeding. Such plant types will revolutionise the cultivation of chilli in tropics and subtropics in general and Kerala in particular. A schematic representation of the development of this near isogenic line is given below:

SCHEMATIC REPRESENTATION OF BREEDING TECHNOLOGY



By BC_2F_7 generation, isogenic line of Pusa Jwala can be obtained

Summary

SUMMARY

Bacterial wilt, caused by *Pseudomonas solanacearum*, has become a serious threat to chilli cultivation particularly in warm humid areas. Development of resistant varieties is not only economically feasible but environmentally safe to manage this malady. For this knowledge on the source(s) of resistance, mode of inheritance and mechanism of host plant resistance is important pre-requisite. Hence the present investigations on "Genetic and biochemical bases of resistance to bacterial wilt in chilli" was undertaken in the Department of Olericulture, College of Horticulture, Vellanikkara, Trichur during 1992-1996.

The experiments consisted of isolation and establishment of pure culture(s) of *Pseudomonas solanacearum*; genetic, biochemical and anatomical bases of bacterial wilt resistance; radiotracer studies on growth and infection of *P. solanacearum* and development of near isogenic line(s) for resistance to bacterial wilt. The results of the above experiments are summarised here.

The bacterial wilt pathogen *Pseudomonas solanacearum* was isolated from infected chilli plants and its pathogenicity established. The bacterial isolate produced circular, smooth, greyish white, fluidal and slimy colonies with pin point pink centre in TZC medium. Root dip method was ideal for better results out of the different methods of artificial inoculation.

Studies on genetic basis of resistance using the resistant (Ujwala) and susceptible (Pusa Jwala) parents and the progenies of F_1, F_2, B_1 and B_2 generations of their cross revealed that the inheritance of resistance to bacterial wilt was monogenic and incompletely dominant.

Investigation on biochemical basis of resistance revealed that the resistant variety Ujwala exhibited significant increase in total phenol content in roots. The maximum total phenol content in both varieties was recorded in 45 and 60 days old plants. Artificial inoculation resulted in a significant increase in both resistant and susceptible genotypes with higher level in susceptible variety (Pusa Jwala).

In Ujwala significant increase of OD phenol content was noticed in roots and shoots and the maximum content was in 45-60 days old plants. On artificial inoculation, there was an increased content in both varieties but resistant variety Ujwala had significantly higher content.

Roots of Ujwala showed a higher protein content at 30th, 45th and 60th days after sowing and in shoots at 30th, 60th and 75th days as compared to Pusa Jwala. Artificial inoculation resulted in significant increase of protein content in all plant parts in Ujwala while in Pusa Jwala only stem and whole plant showed similar increase.

Resistant genotype, Ujwala showed significantly higher peroxidase activity than the susceptible variety before and after inoculation. In Ujwala, the increase of peroxidase activity was 76.55 per cent and 91.94 per cent in roots and shoots after inoculation where as in Pusa Jwala the increase was only 31.80 per cent and 25.94 per cent respectively.

Polyphenol oxidase activity was higher in resistant variety in all the plant parts at various growth stages except in stems at 30th day of sowing. Maximum enzyme activity was observed on 60th day in both the genotypes. Artificial

inoculation enhanced the polyphenol oxidase activity in both genotypes, but to a greater extent in resistant genotype.

Electrophoretic studies showed that resolution of protein bands were feeble in roots, but fairly good results were observed in leaves. The two genotypes possessed similar bands with identical electrophoretic mobility. On inoculation, the susceptible variety exhibited an additional band in the leaves. Isozyme studies with peroxidase and esterase showed that the roots showed clear bands of peroxidase enzyme while clear bands of esterase enzyme were visible only in leaves of both the genotypes. The number of bands of peroxidase in roots was two in Ujwala while it was three in Pusa Jwala. In leaves, Ujwala had only one band while it was two in Pusa Jwala. In the case of esterase enzyme, there were three medium thick bands in Ujwala whereas Pusa Jwala had only one feeble band. Inoculation had no effect in the banding pattern for both enzymes.

The anatomical studies divulged that, in the resistant variety the higher degree of secondary xylem development coupled with thick and compact arrangement of piliferous layers and cortical cells acted as a physical barrier preventing the entry of the pathogen.

The radiotracer studies on growth and infection of *Pseudomonas solanacearum* showed that the ideal doses of radioactivity of ^{32}P and ^{35}S were 44.4 kBq (1.2 μci)/ml and 740 kBq (20 μci)/ml respectively. An increase in growth of bacteria was observed as the level of radioactivity increased. In the autoradiograph there was no appreciable difference between the resistant and the susceptible varieties of chilli when inoculated with ^{32}P labelled bacteria. However the ^{35}S labelled bacteria were more in susceptible plants than in resistant ones. The labelled

bacteria accumulated more in the stem than in leaves. The radioassay of the inoculated resistant and susceptible varieties of chilli revealed that the radioactivity counts were more in susceptible variety than in the resistant variety labelled with ^{32}P and ^{35}S at one as well as 24 hours of inoculation.

Near isogenic line of Pusa Jwala was developed by crossing Ujwala (92% resistance) with Pusa Jwala (88% susceptibility). Bacterial wilt resistant plants having characteristics of Pusa Jwala were selected from BC_2F_2 generations onwards and advanced to BC_2F_4. In F_1, the survival per cent was 23.20 which was further enhanced to 61 per cent in BC_2F_4. In BC_2F_2, 21.43 per cent resistant plants were having characteristics of Pusa Jwala which was enhanced to 54.01 per cent in BC_2F_4. The near isogenic line resulted from BC_2F_4 selected plants had pendulous, solitary, light green fruits (Fruit length -10.38 cm) resembling Pusa Jwala. The genotype recorded an yield of 222.48 g/plant against 204.20 g/plant in Pusa Jwala. This genotype with resistance to bacterial wilt and characteristics of Pusa Jwala was realised from this investigation.

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* Originals not seen

GENETIC AND BIOCHEMICAL BASES OF RESISTANCE TO BACTERIAL WILT IN CHILLI

By

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ABSTRACT OF A THESIS

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ABSTRACT

Investigation on "Genetic and biochemical bases of resistance to bacterial wilt in chilli" was carried out in the Department of Olericulture, College of Horticulture, Vellanikkara during 1992-96 to study the inheritance and mechanisms of bacterial wilt resistance and to develop a near isogenic line of Pusa Jwala with resistance to bacterial wilt.

Pseudomonas solanacearum, the bacterial wilt pathogen was isolated from infected chilli plants and its pathogenicity was established. The resistance to this disease showed a monogenic and incompletely dominant type of inheritance.

Studies on biochemical basis of resistance revealed that total phenol, OD phenol and protein content and enzyme activities had a positive association with bacterial wilt resistance. Ujwala, the resistant variety showed high protein content and had higher activities of peroxidase and polyphenol oxidase enzyme in all plant parts. Higher total phenol was found only in roots and OD phenol in roots and stems of Ujwala. All these biochemical constituents recorded an increasing trend on inoculation in both resistant and susceptible varieties. The wilt resistant variety had higher OD phenol and protein contents on inoculation whereas the susceptible variety was higher in total phenol content. Both peroxidase and polyphenol oxidase activity were higher in resistant than in susceptible variety. The higher amount of OD phenol and their oxidation products like quinones formed by increased peroxidase and polyphenol oxidase were ascribed for resistance in Ujwala.

In the protein gel electrophoresis, the resistant and susceptible genotypes possessed similar bands with identical electrophoretic mobility. The isozyme patterns

of peroxidase and esterase were studied. Roots for peroxidase and leaves for esterase showed clear bands. The resistant and susceptible varieties were different in banding pattern before inoculation. Inoculation did not evince any change in the banding pattern for peroxidase and esterase enzymes.

The resistant variety had a well developed secondary xylem with thick and compact piliferous layers and cortical cells compared to the susceptible variety.

In the radiotracer studies, radioactivity counts were more in susceptible than in resistant variety after inoculation in both ^{32}P and ^{35}S labelled plants. In the autoradiography, the ^{35}S labelled bacteria were more in susceptible plants than in resistant ones. However there was no appreciable difference between resistant and susceptible plants when ^{32}P tagged bacteria were inoculated.

A near isogenic line of Pusa Jwala, moderately resistant to bacterial wilt, was developed by crossing Ujwala with Pusa Jwala and advancing the F_1s to BC_2F_4 generation.