STUDY OF GENETICS AND SOME FACTORS ASSOCIATED WITH LEAF CURL VIRUS IN *Lycopersicons*

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CERTIFICATE

This is to certify that the thesis entitled STUDY OF GENETICS AND SOME FACTORS ASSOCIATED WITH LEAF CURL VIRUS IN LYCOPERSICONS submitted by Shri S.H. Jalikop, in partial fulfilment of the requirement of degree of DOCTOR OF PHILOSOPHY in Genetics and Plant Breeding to the University of Agricultural Sciences, Bangalore, is a record of bonafide research work carried out by Shri S.H. Jalikop under my guidance and supervision and that no part of the thesis has, been submitted for the award of any degree, diploma, associateship, fellowship or other similar titles.

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INTRODUCTION

INTRODUCTION

In India the cultivation and production of tomato (Lycopersicon esculentum Mill.), one of the largely consumed vegetables, is threatened by the leaf curl virus. The virus inflicts serious damage, often resulting in farmers drawing blank from their tomato field due to total crop loss. In fact Saikia and Muniyappa (1989a) reported cent per cent infection and fruit yield losses exceeding 90 per cent. According to Banerjee and Kalloo (1987a) several farmers have discontinued tomato cultivation during summer in South India and autumn in North India when the disease incidence will be at its peak following steep rise in the vector population. At such times, tomatoes become scarce and prices shoot up by many - folds. Disease intensity is increasing during the remaining period of the year too. Presence of many alternate hosts for the vector as well as virus ensures ample inoculum in nature, making swift spread of disease unavoidable. Thus Indian tomato industry is in desperate need of tomato leaf curl virus tolerant varieties to stabilize tomato production.

Tomato leaf curl virus (TLCV), a geminivirus transmitted by the tobacco whitefly (Bemisia tabaci Genn.) was first reported in India by Vasudeva and Sam Raj in 1948. Affected plants are severely stunted, leaflet size markedly reduced and curled showing severe interveinal chlorosis and branches assuming erect position. Fruit set is greatly affected and infected young plants almost fail to produce any marketable yield.

Tomato yellow leaf curl virus (TYLCV) another geminivirus vectored by _B.tabaci_ Genn., is devasting disease in the Middle East (Pilowsky and Cohen, 1990). TYLCV symptoms are akin to those produced by TLCV.

Although numerous F_1 hybrids, improved and local varieties represent tomato cultivation in India, none is known to possess resistance/tolerance for TLCV. Leaf curl virus control and tomato production are very much linked as the disease has an important bearing on plant yield. Effective disease control technology helps in furthering the area by making its cultivation possible all round the year.

For the control of leaf curl virus nothing analogous to fungicide for the field use is yet known. However many indirect approaches like checking the vector by using insecticides, oils, antibiotics and protection of the nursery by nylon net covering have been suggested,These measures may reduce the infection to varying levels, providing a facile defense against the virus. Besides, application of large amount of vector killing pesticides is not only uneconomical to the farmers but also has serious environmental consequences. Clearly, none would be as effective as the use of resistant cultivars.

Host plant resistance is arguably the most important disease control strategy which is environmentally sound with low running costs. As the virus solely banks upon the whitefly for its spread, use of vector resistant cultivars will help break virus-vector-host transmission cycle. Therefore, breeding tomato cultivars possessing built-in resistance either for virus or vector or both is an appropriate approach for the control of leaf curl virus.

A pre-requisite for achieving the above objective is the identification of source of resistance. A few workers based on techniques unlikely to be fool-proof, reported resistance for TLCV in accessions of certain wild relatives of cultivated tomato (Muniyappa et al., 1991a and Banerjee and Kalloo, 1987a). Occurrence of high levels of resistance to whitefly in L.hirsutum augurs well for the use of this species as a source of vector resistance. In general the wild species of genus Lycopersicon are well documented as rich reservoirs of several useful genes and todate out of resistance located for 30 diseases 16 have been introgressed into commercial tomatoes. But, a comprehensive effort to incorporate TLCV and B.tabaci resistance from the wild types into the edible tomatoes has been slow and lagging, hence work in this direction is called for.

In order to realize more efficiently the resistance source, information needs to be secured on the genetics of resistance in the donors, the breeding methods to be used, the size of the population to be grown, and the breeding strategy to be adopted. More often, resistance is a function of a number of mechanisms in concert, each contributing somewhat to overall defense. Hence, complete realization of resistance either for the virus or vector involves, apart from identification of resistance source, the understanding of the underlying genetical, physical and biochemical mechanisms. Further, by identification of isozyme markers it would be possible to cut short the lengthy process of conventional backcross scheme applied in such gene transfer.

Till date our knowledge on the above aspects of TLCV resistance has been incomplete and inadequate to evolve suitable breeding strategy. Hence, a study confining to the following objectives was carried out.

1. Identification and confirmation of TLCV resistance source through fool-proof tests.
2. Interspecific hybridization and evaluation of hybrids.
3. Understanding the genetics of TLCV resistance and other possible mechanisms.
4. Trichomes and their role in vector resistance.
5. Isoperoxidase activity in TLCV resistant lines.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

2.1 EPIDEMIOLOGY OF TOMATO LEAF CURL VIRUS

2.1.1 Tomato leaf curl virus disease

Among the several diseases of tomato crop in India, the one caused by tomato leaf curl virus (TLCV) is the most serious. The occurrence of tobacco leaf curl virus (Tob LCV) was observed by Pruthi and Samuel in 1939 and subsequently the devastating nature of this virus on tomato was reported by Vasudeva and Sam Raj in 1948. Presently, the disease has become a national problem, and needs preferential research consideration.

Apart from India TLCV was also reported from Egypt (Nour Eldin et al., 1969), Sudan (Yassin and Nour 1965), Somalia (Castellani et al., 1981), Philippines (Retuerma et al., 1971) and Thailand (Thanapase et al., 1983).

Tomato yellow leaf curl virus (TYLCV) closely resembling TLCV symptoms has been reported to be a serious problem in Middle East and Tunisia (Ioannou 1987; Pilowsky and Cohen, 1990 ; Al-Musa, 1982 ; Cohen and Nitzany, 1966). It can cause loss upto 100 per cent in summer and autumn plantings (Pilowsky and Cohen, 1990).

2.1.2 Symptomatology

The disease affected plants have been described by Vasudeva and Sam Raj (1948), Sastry and Singh (1973), Saklani and Mathai (1977), Raychaudhuri and Nariani

(1977), Seetharam Reddy (1978), Capoor (1981), Muniyappa (1980), Muniyappa and Veeresh (1984). Such plants exhibited vein clearing, reduction in leaf size and stunted growth (Fig.1). The reduction in leaf size was more pronounced in the successive leaves accompanied by shortening of the internodes resulting in curling and crowding of leaves. The leaflets were deformed and their margins curling inward or outward. The leaflets showed a tendency to become stiff and crinkled with their tips coiled or twisted in the form of cork - screw. The younger leaves were pale in colour with light green and dark green areas. Puckering of the leaflets was a characteristic symptom and the plants had a greater tendency to produce stunted lateral branches imparting a bushy appearence. The plants infected in young age seldom attained height of not more than 25 to 37 cm. The disease induced non-fruitfulness due to deformed floral structure. The diseased plant usually developed purple patches especially on the older leaves.

Leaf curl infected leaves had less chlorophyll, xanthophyll and carotene and lower photosynthetic rate but had high respiration rate with increased catalase and peroxidase activities. More protein, pectin and ascorbic acid and less sugar and phenolics were recorded in the fruits (Sasikumaran et al., 1979). Ultrastructure changes in phloem tissue of tomato leaves with symptoms of leaf curl disease was recorded by

Saikia and Muniyappa (1989b). Virus - like particles were found in nuclei of phloem parenchyma cells and in the cytoplast of mature sieve elements. Hypertrophy of nucleus and nucleoplasmic accumulation of dark staining granules were also recorded.

2.1.3 Tomato leaf curl virus

Immunosorbent electron microscopy studies of extracts of TLCV infected tomato leaves revealed the presence of geminate virus particles (Robinson _et al._, 1987 and Harrison _et al._, 1991). Geminiviruses are characterized in having a genome of cirucular single stranded DNA contained in geminate particles that typically measure about 30nm x 20nm (Muniyappa _et al._, 1991b). Several strains of TLCV have been identified based on the host reaction. Three strains by Singh and Lal (1964) ; five by Seetharam Reddy (1978) and "enation leaf curl virus of tomato" by Nariani (1968) were reported. Muniyappa _et al._ (1991b) studied TLCV isolates from nine different locations of Karnataka and found slight differences in epitome profiles.

2.1.4 Host range of TLCV

Many crop plants and weeds form source of this virus. Pruthi and Samuel (1939), Muniyappa and Veeresh (1984) recorded TLCV on a few host plants. While Saikia and Muniyappa (1989a) demonstrated that _B.tabaci_ can transmit this virus to 23 host plants.

2.1.5 Vector

Bemisia tabaci Genn. (tobacco or potato whitefly) is implicated as the sole vector responsible for TLCV transmission (Vasudeva and Sam Raj, 1948 ; Varma, 1959 ; Ramkrishnan *et al*., 1964 ; Singh and Lal, 1964, Butter and Rataul, 1973 ; Seetharam Reddy and Yaraguntaiah, 1981 ; Yassin and Nour, 1965 ; Retuerma *et al*., 1971 and Castellani *et al*., 1981)

Whiteflies are tiny insects with piercing and sucking type of mouth parts belonging to family Aleyrodidae and order Homoptera.

2.1.6 Vector biology

Vector biology, behaviour, host range, population assessment and virus vector relationships are aspects of concern to a breeder engaged in vector/insect resistance breeding. A number of workers have studied the biology of *B.tabaci* (Pruthi and Samuel, 1942; Gameel, 1977 ; Gerling *et al*., 1982, Butler *et al*., 1983 and Musuna, 1983).

Mound (1983) observed that an adult female may lay upto 200 eggs and the life cycle of *Bemisia tabaci*, from egg to adult under normal conditions in tropics, is often completed in less then 3 weeks. The eggs laid on the undersurface of leaves, hatch (in about 48h) to produce minute, actively crawling larvae. This first instar larvae soon settles to feed, and the subsequent be taken advantage to take counts of adult whiteflies on individual plants to find the genotypic differences for whitefly preference.

2.1.9 Virus-vector disease relationship

The knowledge of this is useful in developing appropriate screening tests for resistance. TLCV is transmitted in a persistant manner, but is not transvorial. Three per cent transmission by B.tabaci was observed by Butter and Rataul (1977), with 31 min acquisition threashold. They also recorded with inocultion access of 24 h on tomato plants, the transmission rose to 24 per cent. Starving the vector for one hour pre-acquistion or one hour pre-inoculation resulted in 36 and 40% transmission of TLCV respectively as compared with 20% for non-starved whiteflies. Larvae also can acquire and transmit the virus. According to Seetharama Reddy and Yaraguntaiah (1981) minimum acquistion and inoculation feeding periods was 30min each.

A strong positive correlation was recorded ($r = +0.970$; $P = 0.01$) between the percentage of incidence of TLCV disease and the vector population density by Saikia and Muniyappa (1989a).

2.1.10 Disease intensity and crop loss

Disease intensity is influenced by the vector population dynamics which in turn is a funciton of temperature, humidity and the rainfall. Sastry et al., 1978 demonstrated that tomato planted in Karnataka during December to May experience low rainfall, humidity and high temperature which favoured the build up of population of whitefly resulting in increased incidence of leaf curl. The studies of Saikia and Muniyappa (1989a) in the same location also revealed that in sequential sowings, 90-100% of plants were infected in plots sown between February and the end of May. They also noted that during July to November the low incidence of TLCV was due to fall in whitefly population brought about by low temperature.

The stage of the plant at which the infection occurs has an important bearing on disease severity and subsequent yield loss. In plants infected within 20 days after planting the loss was 92.3 % ; infection after 35 and 50 days resulted in 74% and 28.9 % loss respectively. According to Saikia (1985) the plants which are affected 2, 4 and 6 weeks after planting were severely stunted and yield loss was 94.94, 90.02 and 78.00 per cent respectively. However, when the plants were infected 10 weeks after planting the crop loss was low (10.18 %).

2.1.11 Disease management

A number of workers observed that application of various insecticides partially reduced the leaf curl in tomato by controlling the vector population (Varma, 1959;

Vasudeva,1959 ; Sastry and Singh,1971 ; Singh *et al*., 1973 ; Thirumalachar *et al*., 1973 ; Sastry *et al*., 1976; Rataul and Butter, 1975, 1976 ; Yassin, 1975 ; Mote, 1976; Saklani and Mathai, 1978 ; Kisha, 1981, Yassin *et al*., 1982 and Saikia and Muniyappa, 1989a). The residual effect of these chemicals and the possibility of whiteflies developing resistance to them (Cohen *et al*., 1974) make the chemical control a stop-gap arrangement. Nylon net covering of nursery seedlings combined with 2-3 sprays of monocrotophos after transplanting (Saikia and Muniyappa, 1989a) was suggested to keep the disease incidence under check. Intercropping with *Crotalaria juncea* (Sastry *et al*., 1977), *Cajanus cajan* (Yassin *et al*., 1982) that attract the vector are other measures to mitigate the damage of TLCV. However, it appears that since difficulties in managing *B.tabaci* are enormous (Maramorosch, 1975), the most feasible approach is to breed resistant varieties for whiteflies.

2.2 SOURCE OF TLCV RESISTANCE

So far no tomato cultivar with resistance to TLCV has been reported (Banerjee and Kalloo 1987a; Muniyappa *et al*., 1991a). When resistance is not available within the crop species, breeder is constrained to look beyond. Hence, several workers resorted to examine related species of *L.esculentum*, for source of TLCV resistance. Before reviewing this aspect, a brief description of Lycopersicon genus is dealt with.

2.2.1 The genus Lycopersicon

Lycopersicon is relatively small genus having nine species (Warnock, 1988), within the extremely large and diverse family Solanaceae. Earlier taxonomic treatments meted out to this genus (Muller, 1940 ; Luckwill, 1943) have become inadequate as number of species and races collected from South America have increased. Rick(1976) divided the genus into those species which can be easily crossed with the commercial tomato (esculentum - complex) and those which cannot (Peruvianum - complex). The species (2n=24) under these 2 groups are listed below :

Species forming the 'esculeutum-complex' : Lycopersicon esculeutum Mill ; L. pimpinellifolium (Jusl.) Mill. ; L. parviflorum Rick, Kes., Fob. Sr Holle; L.cheesmanii Riley ; L.chmielewskii Rick,Kes., Fob.& Holle ; L.hirsutum Humb and Bonpl. ; L.pennellii (Correll) D' Arcy.

Species forming the peruvianum - complex : L.chilense Dun. ; L.peruvianum (L.) Mill.

Both cultivated (L.esculentum) and wild species embrace an unusually vast amount of genetic variation (Rick, 1987). So immense is the genetic potential that despite continuing best efforts by several researchers, as yet a tiny fraction is realized. Presently, both sexual hybridization and contemporary tools of molecular biology provide an opportunity for exploiting the vast reservoir of traits in Lycopersicon.

A number of useful traits that have been detected in wild species of Lycopersicon includes : resistance to several diseases and pests, stress tolerance (tolerance to high and low temperatures, drought, water logging), fruit shape, colour, firmness, flavour, B-carotene, vitamin C, male sterility and incompatibility, and jointless pedicel. (Rick, 1987). Wild species can also add significantly to the cause of variation in many useful quantitative traits by means of transgressive and other novel variation in the segregating progenies of interspecific crosses (Rick, 1982).

2.2.2 Screening procedures

The success of disease resistance breeding solely hinges on the reliable evaluation tests applied. It is essential to employ most scrupulous test/tests of resistance, while dealing with evasive disease like TLCV. Many workers have based their evaluation for TLCV/TYLCV reaction on visual symptoms of the disease either by observing under field conditions (Yassin, 1985) or after vector inoculation (Pilowsky and Cohen, 1974 and 1990 ; Kalloo and Banerjee, 1990) or both (Nariani and Vasudeva, 1963 ; Hayati and Varma, 1984 ; Banerjee and Kalloo, 1987a and 1987b ; Muniyappa et al., 1991a). Hassan *et al.,* (1984a) employed vector inoculation, graft transmission and progency tests for screening TLCV resistant genotypes. Som and Choudhury (1976) examined for resistance source by growing plants in summer, using *B.tabaci* and graft inoculation.

To assess the resistance of a given genotype, symptom severity grades, designated with numerical values 0-4, have been given on the basis of visual inspection (Som and Choudhury, 1976 ; Banerjee and Kalloo, 1987a and Kalloo and Banerjee, 1990). Likewise four grades were adopted by Hassan *et al.,* 1984a and Muniyappa *et al.,* 1991a). Reaction of test genotypes were scored by Yassin (1985) and Pilowsky and Cohen (1990) as diseased and healthy. Banerjee and Kalloo (1987a) assigned arbitary response value to each of the grades, and quantified the disease severity by working out coefficient of infection which was obtained by multiplying the % disease (% plants infected) by 'response value'.

2.2.3 Resistance source

Vasudeva and Sam Raj (1948) screened more than sixty varieties of tomato and reported all of them to be susceptible to TLCV. Subsequently, Nariani and Vasudeva (1963) tested 98 varieties of tamato and *Lycopersicon* species including lines of *L.pimpinellifolium*, *L.hirsutum* and *L.peruvianum* but did not find resistant genotype. Varma *et al.* (1980) and Hayati and Varma

(1984) showed that *L.glandulosum* (EC 66003) was resistant to TLCV. Joshi and Chaudhury (1981) studied TLCV reaction in 166 cultivars, 28 lines of allied species of the genus *Lycopersicon* and two species of *Solanum*. Among them three viz., P_{13} (B2247), XXXII - 345-AA-Silvestra and 65537 (51.498) exhibited a fair level of tolerance.

Banerjee and Kalloo (1987a) screened 122 varieties, lines and wild accessions of *Lycopersicon*, and recorded that *L.hirsutum* F. *typicum* (A 1904) and *L.peruvianum* possessed resistance to TLCV and observed no disease symptoms in *L.pimpinellifolium* (A 1921) till 90 days of age. Muniyappa *et al.* (1991a) reported that two accessions of *L.hirsutum* (PI 390658 and PI 390659) and two of *L.peruvianum* (PI 127830 and PI 127831) showed resistance to TLCV based on field and laboratory evaluation.

2.3 INTERSPECIFIC HYBRIDIZATION

Since resistance source is located in species other than the cultivated, interspecific hybridization, an unconventional means of gene transfer, gains importance here.

L.esculentum can be crossed with eight other tomato species with varying degrees of success, and the resultant hybrids are sufficiently fertile to yield required progenies (Rick 1982). *L.pimpinellifolium* crosses most freely with cultivated species producing fertile progenies (Rick and Butler, 1956). Rick (1979a) updated the status of interspecific crossability relations in *Lycopersicon* species.

L.hirsutum shows unilateral incompatibility with cultivated tomato. Normal seeds and hybrid plants can easily be obtained using *L.esculentum* as female, but the reverse of this cross does not result in fruit-set (McGuire and Rick, 1954). A Similar limitation also applies to the F_1 hybrids in that they are successfully mated to *L.esculentum* only as pollen and to the wild parent only as female. However, a form of *L.hirsutum* f *glabratum*, has been reported by Chmielewski, (1966) to exhibit reciprocal compatibility with cultivated species. Some deviation in normal segregation, F_1 hybrid suffering often from premature senescence after a short period of vigorous growth are reported (Sawant, 1956 and Chmielewski, 1966) in *L.esculentum* x *L.hirsutum* crosses. However, this has not unduly impeded the transferring of potentially valuable genes from the wild species into the cultivar.

It is possible to cross *L.peruvianum* with *L.esculentum* but at the expense of great effort. In hybridizing *L.peruvianum* with *L.esculentum* , fruits are obtained only when the latter is used as pistillate parent, and even then early embryo breakdown occurs, which can, however, be circumvented by embryo culture and other techniques (Smith, 1944 ; Alexander, 1956 ; Choudhury, 1955 and Barbano and Topoleski, 1984). Some post-fertilization barrier exists in the first backcross of the hybrid to L.esculentum (Choudhury, 1959). The primary reason for the endosperm and consequent embryo degeneration is little understood, but it could be due to growth regulator imbalance (Barbano and Topoleski, 1984). Choudhury (1959) proposed the failure of the primary endosperm nucleus in fertilization in L.esculentum x L.peruvianum cross, as the cause of postfertilization failure. Barbano and Topoleski, (1984) confirmed this, and reported that endosperm of the hybrid ovules disintegrated totally in 10 days after pollination and observed total embryo breakdown in 24 days. Earlier, Choudhury (1959) and Smith (1944) observed that the embryo begins to disintegrate 30-40 days after pollination.

Rick (1983) reported that a race of L.peruvianum gave a small proportion of viable seeds in crosses with L.esculentum. Pilowsky and Cohen, (1990) used mixture of pollen to overcome interspecific hybridization barrier.

2.4 GENETICS OF TLCV RESISTANCE

Complete and effective realization of available genetic sources lies in better understanding of genetic basis of resistance.

2.4.1 The genetics of virus resistance

Fraser (1990) tabulated the information available on the genetics of resistance for 87 viruses, to gain an overall insight on the relative frequency of different types of genetic controls operating for virus resistance. He noted that among the examples considered, 38 showed single dominant gene control (i.e., where the F_1 displayed the same or more or less similar resistance reaction to the homozygous resistant parent) ; 13 incomplete/partial dominance which is gene-dosage dependent (i.e., the F_1 was intermediate between susceptible and homozygous resistant parent) ; 18 exhibited control by apparently recessive genes (F_1 developed slower or less severe symptoms than the susceptible parent) ; and 5 oligogenic control. The remaining 13 in addition to monogenic control showed possible presence of modifiers having mostly indirect or nonspecific influence such as an effect through growth rate.

The above record of over-whelming simplicity of virus resistance in genetic terms may reflect the fact that the sample studied by Fraser (1990) was composed of cultivares which were in the hands of plant breeders, farmers, who intended to maximize other characters, as well as virus resistance. But the genetics of resistance in wild species might be more complex, and has not been examined so far (Fraser 1986). In tomato there have been attempts to breed for oligogenic (vertical) resistance, by transferring of individual resistance genes from several related species (Fraser, 1987).

2.4.2 The genetics of TLCV resistnace

Som and Choudhury (1976) reported that the resistance for TLCV in L.pimpinellifolium was incompletely dominant and governed by polygenes. While screening for virus resistance these workers relied upon natural occurrence of the disease and visual ratings. However, Banerjee and Kalloo (1987 a) tested the reaction for local (Hissar), strain of TLCV using viruliferious whitefly and found that the resistance of L.pimpinellifolium (A 1921) was due to a single incompletely dominant gene. They designated this gene as 'TLC'. Same workers (1987b) studied the inheritance of resistance to TLCV in the progenies derived from L.esculentum (5 cultivars) and L.hirsutum f.glabratum (line B 6013) and showed that the resistance derived from this species is based on 2 epistatic genes, one from the wild and the other from the crop species, resulting in a 13 : 3 segregation in the F_2 generation.

The work carried out in Oman by Yassin (1985) indicated that the L.pimpinellifolium (LA 1582) carried a dominant factor for TLCV resistance. But in Israel the TYLCV tolerance of LA 121 of the same species was controlled by a single incompletely dominant gene (Pilowsky and Cohen, 1974). Hassan et al. (1984a) observed in Egypt that the resistance for TYLCV derieved from *L.hirsutum* (LA 386) was dominant and controlled by more than one gene, while the inheritance studies of Pilowsky and Cohen (1990) indicated that tolerance to TYLCV of *L.peruvianum* was conferred by five recessive genetic factors. As yet no study directed towards working out relative virus concentration in the plant in relation to TLCV symptom severity is reported. Such a study on threshhold limits may be more appropriate especially when incomplete gene dominance or recessive resistance is involved.

2.5 TRICHOMES AND THEIR SIGNIFICANCE IN VECTOR PREFERENCE

Trichomes are unicellular or pluricellular outgrowths from the epidermis of leaves, shoots and roots (Uphof and Hummel, 1962) and occur in diverse forms and structures. Some plants such as tomato, have several types of morphologically and chemically distinct trichomes or hairs (Beckman *et al.*, 1972).

2.5.1 Types of trichome in *Lycopersicon*

Luckwill (1943) identified seven distinct trichome types that occur on leaves and stems of plants in the genus *Lycopersicon* and applied it as a taxonomic criterion for differentiating species. Roman numerals were assigned to identify each type. Type II, III and V are non-glansular and Type I, IV, VI and VII are glandular (Fig. 17). Of these, only Type VI and VII have multicellular tips. In L.esculentum Type I, III, V and VI trichomes are abundant, Type VII are sparse, while Type II and IV are absent. In L.hirsutum, Type I, IV and VI are abundant, Type III are sparse and Type II and V are absent.

2.5.2 Trichomes and insect resistance

Often, resistance in many plant species to arthropods has been correlated with the relative hairiness of the leaves. Pubescence as a passive resistance factor interferes with insect oviposition, attachment to the plant, feeding and ingestion. The mechanical effects of the trichomes depend on their density, erectness, length and shape. Sometimes the toxins of glandular trichomes may kill insects on contact or act as repellents. In some plants, stickly exudates glue the insect legs, impede locomotion or the toxic constituents may make tissue impalatable or toxic.

2.5.2.1 Trichomes as a source of chemical resistance

The trichomes in addition to physical forms of resistance exhibit biochemical defence mechanism. For example the glandular trichomes of Nicotiana sp. carry chemicals poisonous to aphids (Thurston and Webster, 1962). The symptoms of toxicity resemble those of nicotin poisoning-leg paralysis, loss of equilibrium and death.

One of the more exciting recent discoveries is that *L.hirsutum* f. *glabratum* contains the natural insecticide 2-tridecanone (Williams *et al.*, 1980) produced by Type VI trichomes. This wild tomato contains about 72 times as much of this toxin as cultivars. Demock and Kennedy (1983) observed that this compound was present only on the leaflet surfaces, but absent in the leaflet interior. Kennedy *et al.* (1987) observed this was acutely toxic to a number of insect species. When the toxin was removed by swabbing with ethanol from the leaf surfaces of *L.hirsutum* f. glabratum PI. 134417, they noticed loss of resistance to tomato fruit worm larvae, *Heliothis zea*.

Subsequent research into mechanism of *H.zea* resistance has identified other potential biochemical contributors to resistance (Kennedy *et al.*, 1981). 2-Undecanone (2-U), a second methyl ketone associated with Type VI trichomes of PI 134417, also adversly affects *H.zea*, but is less toxic and is less abundant in trichome tips (1.1 µg/tip VS 6.3 µg/tip for 2-T). Although 2-U as 2 - T both are potential factors in resistance of PI134417 to *H.zea*, they cannot account for all the resistance, since most larvae die after having fed for several days. Research has also demonstrated potential roles of α-tomatine, phenols and proteinase inhibitors as factors contributing for *H.zea*. resistance (Kennedy *et al.*, 1987).

2.5.2.2 Effects of trichomes on oviposition

Data on ovipositional preference is available mostly with lepidopterous species. Non-hairy cotton genotypes are less favourable than pubescent ones for oviposition by *Heliothis zea* or whiteflies (Stadelbacher and Scales, 1973). This is because glabrous types provide a poor foothold for oviposition. Pubesence thus at times may be beneficial for the insects. The wild species of *Solanum* have dense glandular trichomes. Gibson (1971) observed that when the delicate cell walls of these glands were ruptured by contact with the aphids, a clear water soluble liquid oozed out and changed upon exposure to atmospheric oxygen into an insoluble black substance that harden around the aphid legs and immobilize them quickly resulting in their death.

Resistance in several varieties of tomato to oviposition by carmine spider mite seems to be due to the presence of glandular hairs (Stoner *et al.*, 1968). Resistance to two spotted and carmine spider mite was identified in accessions of *L.hirsutum* (Gentile *et al.*, 1968). Stoner (1970) could select tomato plants in F_2-population, which were resistant to the common spider mite by visually selecting plants with greater concentration of gladular hairs on the leaves. On such leaves the females oviposited fewer eggs than on plants with few hairs.

There is some evidence to show that the structure of pupal case of B.tabaci is influenced by the trichomes or leaf hairs. Mound (1962) demonstrated that pupal cases on hairy leaves of different hosts developed long dorsal setae, whereas pupal cases on glabrous leaves had short setae. This phenomenon is apparently directly induced by the presence of leaf hairs and circular rugosity, but it is not known how whitefly percives the hair. Estop (1969) showed that these dorsal setae develop after the final moult from third to fourth instar, unlike other insects in which setae develop before moulting. Another adaptation of B.tabaci was shown in tobacco by Mound (1983) wherein before ovipositioning the female deposited wax to cover the glandular hairs.

2.5.3 Trichomes of L.hirsutum

Among different types of trichomes of tomato, considerable effort has been expended in studying Type VI, V and IV trichomes.

Rodriguez et al. (1972) surveyed 32 accessions of Lycopersicon species and found that L.hirsutum P.I. 251303 was most resistant to spider mites. They determined that the mode of resistance was not simply non-preference because many mites died when placed on L.hirsutum leaflets. They also noted that abaxial surfaces of younger leaves were more resistant than older leaves, and that young leaves and abaxial surfaces possessed greater densities of Type VI trichomes. Based on these observations they concluded that a toxic substance was more abundant in younger leaves and on abaxial surfaces. Aina _et al._, 1972) further characterized the resistance of P.I.251303 and demonstrated that mites placed on leaflets were either killed or repelled. Application of trichome exudates to mites resulted in mortality (Aina _et al._, 1972). Thus, suggested the exudate was acting as both a toxicant and repellent.

Type VI glandular trichomes of _L.hirsutum_ were more toxic to spidermites than those of _L.esculentum_, variation in density of Type IV trichomes accounted for most of the observed variation in mite resistance (Snyder and Carter, 1984, Carter and Snyder, 1985 and 1986). Good and Snyder (1988) reported that Type IV trichome density is mechanistically and causally related with host plant resistance of _L.hirsutum_ to mites.

2.5.4 Inheritance of trichomes

Information available on inheritance of trichome appears to be inadequate. Based on a study of crosses between _L.esculentum_ and _L.pennellii_, Lemke and Mutschler (1984) presented data suggesting that the presence of Type IV trichomes is controlled by 2 unlinked genes, but that Type IV density is inherited quantitatively with a rather high broad sense heritability. In contrast, their data for Type VI trichomes revealed a quantitative inheritance with low to moderate broad sense heritability, indicating rather large environmental influence. This finding is supported by the results of Fery and Kennedy (1987) for crosses between *L.esculentum* and *L.hirsutum f.glabratum*. While environmental effects on trichome density appear to be stronger for Type IV than Type VI trichomes, the densities of both are influenced by day length and this can influence the expression of arthropod resistance (Kennedy *et al*., 1981).

Results of various workers reported above indicate that the nature of the pest resistance available within the *Lycopersicon* gene pool is not fully defined. The data indicate that the trichomes and compounds may be involved in the host plant resistance displayed in the *Lycopersicon* genus.

2.6 ISOZYME STUDIES

The term isozyme or isoenzyme refers to different molecular forms of enzyme with the same substrate specificity (Market and Moller, 1959). Isozymes are excellent, reasonably reliable and easily detectable markers which are tissue and species specific.

2.6.1 Isozymes and disease resistance

Studies on changes in expression of isozymes during viral infection helps to look for enzymes which may be involved in plant defence against viral infection as well as enzymes expressed during cell damage (Mc Millan, 1983).

The rapidity of the response of peroxidase to the infection of plants by pathogens, together with many reports of positive correlations between their activity and disease resistance (Hislop and Stahmann, 1971), has led to the hypothesis that peroxidases may be an integral part of the host plants defence mechanism (Burdon and Marshall, 1983 and Fernandez and Jouve, 1990). However the physiological role of plant peroxidase is poorly understood (Fernandez and Jouve, 1990).

Many authors have reported the synthesis of additional isoperoxidases and increase in the activity of the enzyme following infection. Two new peroxidase isozymes were observed by Farkas and Stahmann (1966) in zymograms of *Phaseolus vulgaris* affected by southern bean mosaic virus. Extra bands were observed in highly resistant cultivar of wheat by Yang *et al.* (1984) following infection with powdery mildew fungus. Chahal *et al.* (1986) observed one prominant band in ergot resistant lines of pearl millet. But, Jin and Shen (1985) observed one extra band in powdery mildew susceptible cultivars of barley. However, no change in isozyme pattern was observed in tomato (Retig, 1974) and rice (Ye and Liu, 1984) affected respectively by *Fusarium* and blast.

Johnson and Lee (1978) ascribed increase in the intensity of a particular band to quantitative increase in the level of isoperoxidase. Andryushchenko and Dubinets (1975) observed higher enzyme activity in smut resistant panicum varieties than in susceptible varieties. Matasuyama and Kozaka (1981) showed that in an incompatible combination of rice cultivar and blast fugus, the rapid increase in peroxidase activity in rice leaves occured within 24 hours after inoculation, while a little or no increase was observed in a compatible case in early stages of infection. Similar results were recorded by Chung and Chung (1982) in rice for blast fungus, Fric (1984) in barley for powdery mildew, Yang et al. (1984) in wheat for powdery mildew, Arora and Wagle (1985) in wheat for loose smut.

Seevers and Daly (1970) were able to demonstrate that the increase in peroxidase activity associated with resistance to stem rust in wheat was a consequence, rather than a determinant of resistance. It has been argued that the increase in the activity of peroxidase, among others, is due partly to increased respiratory and metabolic activity associated with diseased plant tissue (Sako and Stahmann, 1972).

2.6.2 Isoperoxidases in tomato

In tomato 41 isozymic genes, corresponding to 15 unique enzymatic reactions, have been characterized. Several of these genes have been mapped on chromosomes

(Tanksley and Bernatzky, 1987). Such information is used for a variety of purposes including tagging genes of economic importance (Medina Filho, 1980) and detecting introgression of genes and chromosomes from wild species (Rick *et al.*, 1986).

Seven peroxidase loci have been genetically delineated in tomato, three in anodal field, three in cathodal and one with activity in both zones and alleles have been reported for most of these loci (Rick and Fobes, 1976). Activity for all seven can be found in crude extracts of roots. Prx-1,2 and 3 are assayable in shoot tissue (Tanksley and Rick, 1980b). Though each tissue displayed its characterstic set of isozymes, the zymogram patterns were relatively indifferent to age of the plant (2 weeks seedlings to flowering) and, to a considerable extent, to cultural conditions (Rick *et al.*, 1974 and Suurs *et al.*, 1989).

2.6.3 Role of isozymes in introgression of genes from wild species

Backcrossing has been widely used in plant breeding, particularly to introgress disease resistance from alien genotypes (Simmonds, 1979). The amount of time which must be invested makes backcrossing a costly technique for the plant breeder. Tanksley and Rick (1980b) suggested exploiting difference in isozymic alleles between the donor and recurrent parent for reducing the number of backcross generations. Tanksley et al. (1981) demonstrated this by crossing L.esculentum and L.pennellii (Solanum pennellii). The backcross seedlings were screened electrophoretically engaging 12 allozymic loci. Comparison of morphological observations and isozymic data showed that the latter had significantly higher predictive value for recurrent parent phenotype. In the backcross, selection would be made for plants with profiles most like recurrent parents.

Subsequently, Tanksley and Bernatzky (1987) reported that the molecular marker map of tomato prepared on restriction fragment length polymorphism (RFLP) analysis play an important role in detecting the introgression of genes from wild species. These workers argued that the progress in completing map of tomato chromosomes using isozome markers has been handicapped for want of number of good histochemical stains required for detecting enzyme activity in gels. Presently a relatively detailed RFLP map is available for tomato (Dixon and Harrison 1990).

In tomato, linkage has been established between isozyme marker loci and a number of genes or blocks of genes affecting several morphological and other characters (Tanksley et al., 1982). For example, an acid phosphatase allozyme which is tightly linked to a gene for nematode resistance has been successfully engaged to screen genotypes resistant to this parasite (Medina - Filho, 1980).

MATERIAL AND METHODS

III MATERIAL AND METHODS

The material for the present investigation on tomato leaf curl virus (TLCV) which comprised Lycopersicon germplasm were received from the Plant Virology Laboratory, University of Agricultural Sciences, Bangalore. The study was carried out at the Main Research Station, University of Agricultural Sciences, Hebbal, Bangalore over a period of 3 years (1988 to 1990). Field experiments were carried out during summer (January to May) when the maximum temperature is around 35°C.

A total of 42 Lycopersicon germplasm accessions (Table 1), most of which were drawn from over 1200 tomato cultivars, breeding lines and accessions of Lycopersicon species that had indicated field tolerance/resitance earlier (Muniyappa et al, 1991b) formed material for the present study.

It is to note that the material used in the present investigation for the sake of brevity is regarded as under: 1.Arka Saurabh as A. Saurabh; 2. Arka Vikas as A.Vikas; 3. L.pimpinellifolium as L.pim.; 4. L.hirsutum (PI390658 and PI390659) as L.hir.'58 and L.hir. '59 respectively;5. L.peruvianum(PI 127830) as L.per.

Accessions A.Saurabh and A.Vikas represented the cultivated species (L.esc.). A. Saurabh is an improvement over a breeding line V685 from Canada. The plants are semi-determinate in growth habit and produces medium sized fruits (average fruit weight 70 g) suitable for fresh market as well as processing into juice and ketchup. This cultivar, on an average yields 35 tons per ha. A.Vikas is a selection from the variety Tip-Top. Like A.Saurabh it is also semi-determinate and produces fruits weighing on an average 80-85 g suited for fresh market. It gives upto 40 tons fruits per ha.

The studies carried out in the present investigation included five experiments: location of resistance source ; interspecific hybridization and evaluation of hybrids ; genetics of TLCV resistance; trichome types and density and their significance in vector preference;and role of isoperoxideses in TLCV resistance.

3.1 EXPERIMENT I : LOCATION OF TLCV RESISTANCE

Non - availability of fool - proof screening procedures in testing for TLCV resistance necessiated the standardization and subsequent use of such tests for confirmation of resistance. Following approaches were adopted while screening of Bangalore strain (Muniyappa *et al*.,1991b) of TLCV.

3.1.1 Preliminary screening.

A preliminary screening was carried out in the field by growing at least 10 plants each of 42 tomato lines in 1988 summer. Summer time-frame was preferred to other periods of the year as the dense vector(*B*.*tabaci*)

Reaction such as the intensity of TLCV symptoms and age of the plant at which the first TLCV symptoms appeared i.e. number of days required for first TLCV symptoms to show up were recorded. The enzyme linked immunosorbent assay for TLCV of the parents was carried out in leaf samples of A.Saurabh,A.Vikas, L.pim. and L.hir. ('58 and '59).

3.1.2.1 Enzyme linked immunosorbent assay (ELISA)

As mentioned above, ELISA was used to detect the presence of virus and its relative intensity in the leaf tissue. This sensitive immunological technique was orginally developed for human and animal virus diseases. In this test, antigen (virus), antiserum and antigen specific enzyme are successively adsorbed to a special microplate. When the enzyme substrate is added, a colour develops, the intensity of which is measured by a microplate reader.

Direct antibodies sandwitch-enzyme linked immunosorbent assay (DAS-ELISA) was employed with a slight modification of the technique described by Clark and Adams (1977). African cassava mosaic virus (ACMV) antibodies were used to detect the antigen TLCV.

Procedure : Top 2-3 leaves from the fresh growth were extracted in Tris-EDTA pH 8 containing 2% PVP and 0.05% Tween-20 (10 ml/g) and filtred through muslin cloth.

ELISA plates were coated with ACMV-r-globulin (1 μg m^{-1}) in coating buffer by adding 100 μl/well. The margin row of each side of the plate was left blank. The plates covered with lid were incubated at room temperature for 3 h and washed with PBS-T. Three quick washes and 3 washes at 3 min interval were done. While washing, care was taken to avoid spilling of washing buffer in the neighbouring wells. After the sixth wash,plates were dried by using a tissue paper pad. Crude leaf extracts (test antigen) in extraction buffer,were added to wells at the rate 100 µl/ml.Plates were incubated overnight at 4^{O}C. After the incubation the plates were washed with PBS-T 3quick and 3 at 3min intervals. Wells were blocked with 5% dried milk (200 µl/well) in PBS-T PVP and incubated for 30min at room temperature. Later the excess milk was removed by inverting the plates, but not washed. African cassava mosaic virus polyclonal conjugate (with alkaline phosphate) diluted in the ratio 1:1000 in PBS-T-PVP-OA was added at the rate of 100 µl/well. Plates covered with lids were incubated for 3h at room temperature. Then washed with PBS-T as above. The substrate freshly prepered by dissolving P-nitrophenyl phosphate (0.6mg/ml) in substrate buffer was added at the rate of 150 µl/well and covered with aluminum foil to avoid auto-degradition of P-nitrophenyl phoshate. Absorbance was recorded after 1h and then overnight at 405 nm in BioTeck microplate reader (EL 309).

3.1.2.2 Monoclonal ELISA

Triple antibody sandwitch ELISA (TAS-ELISA) was used as described by Muniyappa *et al.* (1991b), plate washing, sample extraction and incubation were performed as described in polyclonal ELISA. After washing the plates to remove excess sample, they were blocked for 30 to 60 min at room temperature with 200/ul per well of PBS-T-PVP plus 5% fat free milk. The supernatant third of a monoclonal antibody (2-H12) to Indian cassava mosaic virus (kindly supplied by B.D. Harrison, SCRI, Scotland) was used at a dilution of 1/3 in conjugate buffer and incubated at room temperature for 3h. With another series of washes, rabbit anti-mouse alkaline phosphatase conjugate (Sigma) was added at 1/1000 dilution in conjugate buffer and incubated at room temperature for 3h. After washing, P-nitrophenyl phosphate at 0.6 mg per ml was added as described above. Absarbance values were read at 405 nm after 1h room temperature and after overnight incubation at 4°C using a Bio-Tek Microplate Auto Reader (EL-309).

3.1.2.3 Interpretation of ELISA readings

The ELISA results were interpreted mainly in relation to values for healthy leaf assays. The healthy leaf tissue was obtained from plants grown in insect proof glass house. Such samples giving over and above two times ELISA values for healthy were considered as positive indication of presence of high levels of antigen (virus);1.25 times or less as negative ;1.26 to 1.5 as very weak;1.51 to 1.75 as weak; and above 1.75 times as weak to positive.

Composition of buffers and solutions used in ELISA.

1. Coating buffer,pH 9.6 for one liter

Na2 Co3	1.59g
NaHCO3	2.39g

2. Phosphate buffer saline(PBS),pH 7.4 (for one liter)

NaCl	8.00g
Na2HPO4-2H2O	1.44g
KH2PO4	0.20g
KCl	0.20g

3.PBS-Tween-20(PBS-T)

PBS + 0.5 % Tween-20

4.PBS-Polvinyal Pyrrolidone (PVP)
PBS-T+2%PVP

5.PBS-T-PVP-Ovalbumin(OA)

PBS-T-PVP+0.2%OA

6.#Extraction bufferpH 8.00

Tris	0.05M
EDTA	0.005M

2%PVP and 0.05% Tween-20 were added.

7.#Substrate buffer,pH 9.8

10% diethanolomine
#pH adjusted with 6N HCl.

Dried milk

Sagar skimmed milk powder was defattened by steeping in diethylether overnight at 4°to 8°C and then dried at room temperature.

3.1.3 Vector inoculation

In order to ensure the validity of results obtained under field conditions vector inoculation was resorted to. The whitefly vector (*B.tabaci*) was employed for transferring the local TLCV isolate from the infected *L.esc.*(A.Saurabh or A.Vikas) to the genotypes under test excepting L.per. Fifteen to twenty vectors that had undergone 24h acquistion time were released for 24h inoculation on 20(+/-4) days old seedlings. Susequently the seedlings were observed at least for 50 days for symptom development. Procedural details of vector inoculation are as follows.

Collection of vectors: The whitefly culture was maintained on cotton(*Gossypium hirsutum* cv.Varalaxmi) in an exclusive glasshouse. They were collected by using an aspirator after gently turning the leaves upwards. Aspirator consisted of a glass tube (25cm length and 0.5cm diameter) fitted into a transparent plastic tube,both separated by a piece of muslin cloth that enabled to gather the flies in the glass tube upon sucking.

Acquisition access: Whiteflies were made viruleferous by allowing an acquistion access period of 24h in a PVC bottle (20x8cm) containing a TLCV affected tomato twig. The bottom position of the bottle was replaced with a muslin cloth which avoided moisture accumulation. The narrow end was closed with a cotton plug after insertnig the twig.The non-viruliferous white flies were released from the aspirator through a side-hole. After the acquisition viruleferous whiteflies were used for inoculation.

Inoculation of seedilings: A small plastic tube (8x2cm) covered at one end with muslin cloth was used to enclose the young seedlings planted in a small cup (Fig.2). About 15-20 viruliferous *B. tabaci* were let into the tube through a side-hole (which was later plugged with cotton) allowing 24h inoculation.

3.1.4 Screening by graft transmission and confirmation for virus by ELISA

Screening by vector inoculation is quite dependable and valid (as it ensures the virus transmission) when none of the test genotypes exhibts vector resistance. But, in this study high level of resistance observed for the vector in *L.hir*. made screening through graft transmission all the more necessary.

The test genotype was wedge grafted on an infected *L.esc.* (A. Saurabh or A. Vikas) rootstock. At the basal end of 6 to 8 cm scion twig a wedge was made by giving smooth cuts on either side by using a sharp knife. An oblique cut was made into the stock, corresponding to wedge of the scion,at an angle of about almost half the way through the stem.The scion was inserted into the stock in such a way as to obtain maximum contact of the cambium layer. Polythene ribbons were used to wrap around the graft junction. Further,the complete scion was enclosed for 3 to 4 days in a polythene cover to avoid desiccation. After the graft establishment the stock was cut off just above the union. At least four fully establised grafts of each entry were obtained for the study.

The test genotype being screened i.e. scion was assyed for TLCV by ELISA not before 28 days of grafting for detecting symptomless virus carriers.

3.1.5 Progeny test

The breeding behaviour of the reaction for TLCV was evaluated by a progeny test. Seeds from plants grown in isolation were used for raising progenies. Randomized,15 plant single line progeny of each of the parent was raised in the field during summer,1990 and TLCV incidence was recorded.

3.2 EXPERIENT II. INTERSPECIFIC HYBRIDIZATION.

As the TLCV resistant source was located exclusively in the wild species, interspecific gene transfer became imperative, and the study of the six interspecific hybrids involving three species was felt relevant in this context.

3.2.1 Selection of parents

Considering aspects such as popularity,local adaptability, yield and fruit quality of the variety, two cultivars of tomato (L.esc.) viz. A. Saurabh, and A. Vikas,were selected as one set of parents. To complement the drawback (TLCV sensitivity) of these two cultivars another set of parents comprising two accessions of

L.hir.('58 and'59) and one each of *L.pim.* and *L. per.* were identified for interspecific hybridization and other studies. Among the three wild species used,*L.pim.* bears red, small fruits and is the lone species that has shown natural introgression with cultivated species. The wild species *L.hir.*and *L. per.* bear tiny green fruits and do not cross freely with *L.esc.*

3.2.2 Hybridization and development of experimental material

Flower buds due to open on the following day were emasculated using forceps, and coverd with a butter paper bag for pollination. The pollen from the freshly dehisced anthers were scooped with a needle and carefully smeared on the stigma. To start with, reciprocal crosses were effected between *L. esc.* cultivars and *L.pim.*,*L.hir.*and *L.per.*accessions. But difficulties posed in fruit set by *L.hir.*,*L.per.* when used as seed parent, led to use them subsequently only as pollen parents. Likewise while backcrossing with wild species the F1 was preferred as female, and for backcrossing with A.Saurabh and A.Vikas the F1 was used as male. Seeds for raising F2 generation was obtained by maintaining potted plants of *L.esc.*X *L.hir.* in isolation as they failed to produce selfed fruits when flower buds were covered with bags. In 1988 F1's were developed and in 1989 F2's ,B1(P1 X F1) and B2 (F1 X P2)along with a fresh lot of F1 were produced.

The cross between L.esc. and L.per. failed to give viable seeds. Hence, the undeveloped minute seeds were dissected after 30 days of pollination and attempts were made to culture them on a sterile medium. Seeds from fully ripe and about to ripe fruits (breaker stage) were also tried. The fruits were surface sterilized using teepol, mercuric chloride (0.1%) as well as sodium hypochlorite (30%) for 15 and 25 min., respectively. The fruits were cut into radial sections. The embryos were excised from the developing seed in a laminar flow hood on the day the fruits harvested. Individual seeds were picked up carefully with a needle and transferred to tubes containing the culture medium . Murashige and Skoog's (MS) basal medium (Murashige and Skoog,1962) was supplemented with insoluble PVP-360, BAP and NAA. The composition of the media are enumerated in Appendix I. The cultures were maintained under constant light provided by white flourescent tubes, at a temperature of approximately 20° C.

3.2.3 Study of interspecific hybrids (Field experiment I)

The interspecific hybrids were studied along with their parents by raising them in a randomised block design with three replications during May, 1989 (field experiment I). Each entry was represented by single row of ten plants spaced 30 cm apart while rows were laid at a distance of 60 cm. The recommended cultural practices were followed. Following parameters on six competitive plants per replication were considered for studying the hybrids.

1. **Plant height** was measured in cm from the ground level to the tip of the plant on 60th day after seeding.
2. **Number of primary lateral branches** was counted on 60th day after seeding and length of three basal most branches were measured in cm.
3. **Leaf length :** third leaf from the top was measured in cm.
4. **Leaf expansion :** one leaf initially measuring 5 cm (± 0.5cm) lengthwise was labelled in each plant and detached 12 days later for measuring the leaf expansion. The area was measured in cm^2 using a LICOR leaf area meter.
5. **Leaf senescence** was the number of days required for initiation of leaf tip drying, from the day the earmarked leaf measured 5 cm (± 0.5cm).
6. **Pollen sterility** was estimated using acetocarmine stainability test. Pollen from the ripe anthers from freshly opened flowers were smeared in 1.5% acetocarmine. The well-filled, deeply stained pollen grains were considered as viable, while poorly stained and partially filled were classified as sterile. Five microscopic fields were examined at random and number of fertile to sterile pollen was recorded and expressed in

percentage, and were subjected to angular transformation before computing least significant difference (LSD).

7. **Fruit set per cent** was worked out by taking count of flowers in five randomly selected inflorescences per plant, and the number of fruit set in the cluster developed from such inflorescence.

8. **Average fruit weight** expressed in grams, was obtained from the weight of 10 random fruits picked from a plant.

9. **Number of seeds** was counted in 10 random fruits in A.Vikas (representing L.esc.) and F_1 hybrids involving this cultivar.

10. **Seed fertility** was recorded by counting the number of seedlings obtained after 3 weeks of sowing 100 seeds of each entry in the nursery beds, not discounting the seedling mortality.

11. **Overall assessments** of the F_1 interspecific hybrids was done taking survival and growth as criteria. The following pattern was followed.

0 : Plant dies prior to flowering and fruit set.

1 : Shoots very brittle, very fast senescence, no or poor fruit set.

2 : Poor growth, senescence of leaves faster than in normal plant and poor fruit set.

3 : Slow growth, leaves roll downwards and normal fruit set.

4 : Growth and fruit set good.

3.3 EXPERIMENT III. GENETICAL STUDIES

As the present research involved interspecific gene transfer, the routine techniques of inheritance studies were applied more discreetly. Although six generations of all the six interspecific F_1s were studied, for reasons furnished on page 126 , L.esc x L.pim. crosses were subjected to six generation mean analysis considering TLCV as a quantitative attribute ; while TLCV resistance in L.hir. was treated as qualitative trait. The data were collected from an experiment designed and laidout as under (field experiment II).

3.3.1 Field studies II

Six generations P_1, P_2, F_1, F_2, B_1 and B_2 of six crosses (A.Saurabh x L.pim.; A.Vikas x L.pim.; A.Saurabh x L.hir.'58; A. Vikas x L.hir.'58; A.Saurabh x L.hir '59 and A.Vikas x L. hir'59) were laid out in a randomized block design with three replications. Two separate, adjacent blocks were assigned for L.esc. x L.pim. and L.esc. x L.hir. generations. The seedlings were transplanted 30 cm apart in 3m rows with 60 cm between the rows. The experimental field was surrounded by plots of horsegram and other tomato varieties that served as a good source of TLCV and B.tabaci. After every 15th line a row of A.Saurabh and A.Vikas was raised which served as both source of inoculum and check.

No insecticide was applied lest it hampered the vector activity. Further effective TLCV evaluation of the material under test was ensured by transplanting in peak summer (March, 1990).

Generations were randomised within crosses. Since the different generations were expected to contain different amounts of genetic variability, larger plots were included for the more genetically variable populations. In each replication parents and F_1's were represented by 2 rows and backcrosses by 3 rows in crosses involving L.pim. and by 2 rows in crosses involving L.hir.(both accessions). Six rows were assigned to F_2 generation derived from L.pim. hybrids while F_2 derived L.hir. ('58 and '59) were provided 2 rows. Because of low seed germination the population size had to be curtailed in case of F_2 and backcrosses of crosses involving L.hir. Observations were recorded on 10 randomly selected plants in each replication in case of non-segregating (P_1,P_2 and F_1) and all in case of segregating (F_2,B_1,P_2) populations. The observations were regarded as follows:

1. **Plant height** was measured as mentioned under section 3.2.3.
2. **Days to flowering** is the plant age in days at which the first flower opened.

3. **Days to TLCV symptom expression** was based on age at which first external disease signs got manifested under field conditions.
4. **TLCV symptom severity** was scored as mentioned under page 34.
5. **Average fruit weight** as noted in section 3.2.3.

3.3.1.1 Statistical analysis

Data on six generations-P_1, P_2, F_1, F_2, B_1 and B_2 of the above five attributes (section 3.3.1) was presented by frequency polygon. However, plant height and average fruit weight were not considered for subsequent analysis as they were drastically influenced by TLCV onslaught.

Analysis of variance as outlined by Snedecor and Cochran (1967) was performed. The replication mean of the competative plants in each of the six generations of A.Saurabh x *L.pim*. and A.Vikas x *L.pim*. was considered for the analysis. The total variance was partitioned into that due to non-segregating generations, (parents and F_1)and segregating generations (F_2 and BC), and the interaction between segregating versus non-segregating generations. The details of the partitioning is furnished in appendix II.

3.3.1.2 Genetic analysis

Genetic analysis were performed in case of A.Saurabh x *L.pim.* and A.Vikas x *L.pim.* crosses seperately for days to first flowering , TLCV symptom expression and symptom severity.

3.3.1.2a The mean and variance for the different quantitative attributes were calculated for each population. The standard error of population mean was worked out as square root of : variance of population/total number of plants.

3.3.1.2b Test of Epistasis : To detect the presence of epistasis ABC scaling tests as outlined by Mather (1949) were performed for a given character. By using the following formulae the values of A,B and C were computed.

$A = 2B_1 - P_1 - F_1$

$B = 2B_2 - P_2 - F_1$

$C = 4F_2 - 2F_1 - P_1 - P_2$

Where, P_1, P_2, F_1, F_2, B_1 and B_2 were the mean values of the respective generations.

The squares of the standard error of different generations provided the variances of means of the corresponding generation and to test the significance of these three quantities, the variance (V) of A,B and C were calculated as :

$V_A = 4VB_1 + VP_1 + VF_1$

$V_B = 4VB_2 + VP_2 + VF_1$

$V_C = 16VF_2 + 4VF_1 + VP_1 + VP_2$

3.3.1.2c Six generation mean analysis : In order to obtain information on the nature of the main gene effect and the non - allelic interaction components governing the traits under study, generation means were analysed by the method proposed by Jinks and Jones (1958) and Hayman (1958). The notations for the variance gene effects (Hayman, 1958) used in herein were means ($\hat{m}$), additive (d), dominance ($\hat{h}$), additive x additive ($\hat{i}$), additive x dominance ($\hat{j}$), dominance x dominance ($\hat{l}$); and were estimated by using following equations.

$m = F_2$

$\hat{d} = B_1 - B_2$

$\hat{h} = F_1 + 2B_1 + 2B_2 - 1/2\ P_1 - 1/2P_2 - 4F_2$

$\hat{i} = 2B_1 + 2B_2 - 4F_2$

$\hat{j} = 2B_1 - 2B_2 - P_1 + P_2$

$\hat{l} = P_1 + P_2 + 2F_1 + 4F_2 - 4B_1 - 4B_2$

The variances (V) of these gene effects were obtained as

$V_m = VF_2$

$V_d = VB_1 + VB_2$

$V_h = VF_1 + 16VF_2 + 1/4\ (VF_1 + VP_2) + 4(VB_1 + VB_2)$

$V_i = 4VB_1 + 4VB_2 + 16VF_2$

$V_j = 4(VB_1 + VB_2) + VP_1 + VP_2$

$V_l = VP_1 + VP_2 + 4VF_1 + 16\ (VF_2 + VB_1 + VB_2)$

The standard error of the estimates was arrived at by taking square root of the respective variances and it was used to test the significance of the estimates by 't' test. The calculated 't' value was compared with 1.96 and 2.58 (5% and 1% significance levels) of the tabulated 't' values.

3.3.1.2d <u>Heritability</u> <u>and</u> <u>genetic</u> <u>advance</u> : Heritability (h^2) and genetic advance (GA) were worked out in accordance with the formulae given by Webber and Murthy (1952) and Johnson <u>et</u> <u>al</u>. (1955).

$$h^2 \text{ (narrow sense)} = \frac{V_A}{V_P}$$

$$h^2 \text{ (broad sense)} = \frac{V_G}{V_P}$$

where, V_A - additive genetic variance [i.e., $2VF_2 - (VB_1 + VB_2)$] ; $V_P \approx$ Phenotypic variance [i.e., VF_2] and V_G = genotypic variance [i.e., $VF_2 - V_E$]. V_E = environmental variance was obtained by $1/3\ (VP_1 + VP_2 + VF_1)$.

$$GA = h^2 \times p \times K$$

where h^2 = heritability ; p = phenotypic standard deviation and K = selection differential which has been shown to take the value of 2.06 at 5% selection intensity.

GA was also expressed as per cent of mean

$$= \frac{GA}{F2} \times 100$$

3.3.2 Glasshouse studies

3.3.2.1 *Latent period* as conceived in this investigation was the least number of days necessary for outward symptoms to develop after inoculation with *B.tabaci*, was studied in parents, F_1's and F_2's of *L.esc* x *L.pim*. and *L.esc* x *L.hir*. A maximum of 40 days for symptom to show up after inoculation was allowed.

3.3.2.2 *Inheritance studies* : A study directed towards understanding the differential reaction of parents, F_1's, F_2's, backcross progenies of *L.esc*. x *L.hir*. crosses for TLCV was carried out under controlled conditions by deploying *B. tabaci* as an agent for transferring the virus. At least eight seedlings in parents and F_1's, and varying number of F_2, B1 and B2 at 2-3 leaf stage were inoculated following procedure detailed on page 38.

While scoring for disease symptoms in glass house, it was visually not possible to classify the young seedlings appropriately into different classes. However they were differentiated into resistant (not showing TLCV symptoms) and susceptible (exhibiting varying levels of symptoms). Data thus collected from the segregating generations were tested for goodness of fit with the assumed genetic ratio by X^2 (Snedecor and Cochran,1967)

3.4 EXPERIMENT IV, TRICHOMES AND THEIR SIGNIFICANCE IN VECTOR PREFERENCE

3.4.1 Trichome types and density

Leaf trichomes (hairs) were categorized according to Luckwill's (1943) classification (Fig.17).

Trichome density was measured 56-70 days after planting. Two leaflets from A.Saurabh, A.Vikas, *L.hir.* '58, *L. hir.* '59, A. Saurabh x *L.hir* '58, A.Vikas x *L.hir* '58, A. Saurabh x *L hir* '59 and A.Vikas x *L. hir.* '59 were removed from the third compound leaf (from the apical meristem) of 5 plants. One of the leaflets provided the adaxial surface sample and the other abaxial sample. Leaflet length were recorded in cm. Type I, IV, V, VI and VII trichomes were counted within a sample area of 1.6 mm^2 at 3 fields of view of a dissecting microscope in interveinal areas on these leaflets. Hemocytometer was used to fix an area of 1.6 mm^2 which was calibrated using stage micrometer. Trichome densities were expressed as the number of different types of trichome per 10 mm^2. In F_1 hybrids type IV and V were counted together, as differentiating them was not possible in such trichomes where the apical gland (in case of type IV) was damaged. Length of each trichome type was measured in mm using ocular micrometer.

3.4.2 Vector preference

In order to understand the relationship between the species, specific trichomes and B. tabaci preference, following data were recorded. B. tabaci density was estimated in L. esc. x L.pim. and L. esc. x L.hir. plots by counting the white flies trapped in the yellow plates containing water. Six trap plates were maintained in each plot from 6 AM to 6 PM on three days at an interval of 10 days during the active phase of the crop.

3.4.2.1 Whitefly preference for genotype was estimated by taking counts of flies underneath upper, middle and lower leaves of plants selected at random. The three leaves were taken as to represent the upper, middle and lower canopy positions of the plant. The observation was taken at an interval of ten days during early hours (before 7AM), by gently turning the leaf. Fifteen plants in non segregating and sixty in segregating generations were checked for the flies. The logarithmic transformed data were analysed for LSD.

A single representative of L. esc., L. hir. and F_1 hybrid was used for studying B. tabaci mortality, egg size and egg laying behaviour.

3.4.2.2 B.tabaci mortality was determined by releasing 35 white flies in bottles containing 3rd and 10th leaf (from apex), where petiole base was immersed in a beaker with water. The set up was kept for 48 h away from bright light. Total white flies died after 48 h and those due to entrapment in the trichomes were counted. Experiment was repeated and the data recorded were expressed in per cent.

3.4.2.3 *Egg size and eggs/groups* were observed by allowing large number of whiteflies in a bottle (as explained above) to remain for 3-4 days. Later the leaves were checked for eggs under dissection microscope, and egg length was measured in mm for 20 eggs at random. Twenty random egg groups were considered for observing the number of eggs in each group. Cotton, the most susceptible natural host of *B. tabaci*, was also included in this study for comparison.

3.4.2.4 *Pupal case counts* on older leaves were carried out in *L. esc.*, *L. hir.* and their all F_1's. Six plants selected at random per replication were used for recording this observation. For pupal count undefined number of leaves were sampled but counts were reduced to 100 cm^2 leaf area.

3.5. EXPERIMENT V. ISOZYME ANALYSIS

Six F_1 hybrids (A.Saurabh x *L.pim.* ; A.Vikas x *L.pim.*; A Saurabh x *L.hir.* '58 ; A.Vikas x *L.hir.* '58, A.Saurabh x *L.hir*, '59 and A.Vikas x *L.hir.* '59) and the five parents (A.Saurabh ; A.Vikas ; *L.pim.* *L.hir.* '58 and *L.hir.* '59) were analysed for anodal peroxidase isozyme by standard vertical polyacrylamide gel electrophoresis. The method suggested by Suurs *et al.*(1989) with minor modificitions was followed. The methodology is given below.

3.5.1 Enzyme extraction

For each sample, 0.5g young, fast growing fresh leaf material was crushed (mortar and pestle) in 0.5ml of icecold 0.05M tris HCl buffer (pH 6.8), containing 20%(v/v) glycerol and 1%(v/v) β-mercaptaethanol. The extracts were subsequently centrifuged at 12000 g for 15min at 4°C, and 200 µl of the supernatant used as the enzyme source, after adding 5 µl of tracking dye solution. Tracking dye was prepared by dissolving 0.5g of bromophenol blue in 10ml water. Fifty µl sample was directly loaded into the wells of polyacrylamide gel. Care was taken to avoid mixing of sample with reservoir buffer, and to avoid cross-contamination of samples.

3.5.2. Electrophoresis

Electrophoresis was carried out in a BioTech vertical gel unit at 4°C and a constant current (35mA) and voltage (100V) were applied. Peroxidase isozymes were separated on 7.5% polyacrylamide gel. A 5X tris-glycine buffer (pH8.3) was used as the electrode (running) buffer. No stacking gel was used. When the tracking dye had migrated to the bottom of gel (after about 2h) electrophoresis was stopped.

3.5.3 Staining

The method of Khan et al.(1981) was used for staining the gels for peroxidase activity. Gels were routinely rinsed in water and placed in 1M acetate buffer (pH 4.7)for 30 min followed by incubacting the gel for 15min in benzidine solution. The benzidine solution was prepared by adding 370mg benzidine, 25ml methanol, 20ml 1M acetate buffer (pH 4.7) and was made to 100ml with distilled water. After 15min, gels were transfered to 2% H_2O_2 solution when immediate banding was observed . After staining the gels were photographed and fixed in 6% glacial acetic acid and zymograms were drawn.

3.5.4 Preparation of stock solutions required in electrophoresis

a) Acrylamide stock solution: Thirty grams of acrylamide, 0.8g NN'methylene bisacrylamide dissolved in distilled water and made upto 100ml. Filtered and stored at 4°C in amber coloured bottle in dark.

b) 1.5 M tris -HCl (pH 8.8) : In about 80ml water 18.15g tris base was dissolved, pH adjusted to 8.8 with 1N HCl and made upto 100ml with distilled water. Stored at 4°C.

c) 0.5M tris-HCl (pH 6.8):Six g tris base dissolved in about 80ml water, pH adjusted to 6.8 using 1N HCl and volume was made to 100ml and stored at 4°C.

d) Ammonium persulphate solution : Ten percent ammonium per sulphate solution was prepared by dissolving 50mg in 500µl distilled water. Fresh solution was used.

e) 5X Electrode buffer (pH 8.3): Tris base (15g)and glycine (72g)were dissolved in distilled water, pH adjusted to 8.3 and volume made to 1000ml with distilled water and stored at 4°C.

3.5.5 Gel preparation

Acrylamide is a synthetic hydrophobic substance that polymerizes in the presence of free radicals and bis-acrylamide to form transperant polyacrylamide gel which exhibits uniform porosity at prescribed acrylamide monomer concentrations. Composition of 7.5% gel which was used in the present analysis is given below.

Components	Quantity
1.5M tris HCl (8.8pH)	7.5 ml.
Distilled water	14.45 ml.
Acrylamide stock solution	6.15 ml.
TEMED	15 μl.
Ammonium persulphate (fresh)	400-600 μl

RESULTS

IV RESULTS

The investigation comprised five studies and the results of which are embodied experiment wise as under.

4.1 EXPERIMENT I. TLCV RESISTANCE SCREENING

4.1.1 Preliminary screening

A total of 42 entries (Table 1), some of which had shown field tolerance/resistance for TLCV in earlier testing, were verified in 1988 summer for their reaction to TLCV. Although entries like 762, V-14, V-27, V-21, V-8 gave relatively larger fruits, but number of fruits that could be harvested from total of five plants/entry was fewer than ten fruits. Possibly these fruits had set prior to TLCV symptoms showing up. The mean disease score in L.esculentum ranged from 1.9 in V-34, 305 and 331 to 3.8 in V-18, 508 and 794. Cultivars A.Saurabh and A.Vikas manifested a mean disease score of 3.6 and produced fruits of reduced size (31 g and 34.5 g respectively). L.pim. showed a very low disease score of 1.7. L.hir. and L.per. showed '0' score indicating their resistance to TLCV infection. But all these wild species produced fruits of small (<2g) size.

4.1.2 Field screening and ELISA for TLCV

Fifteen plants of A. Saurabh, A. Vikas, L. hir. and five plants of L. per. regrown in summer 1989, were observed for the TLCV infection (Table 2). Both in A. Vikas and A. Saurabh the first plant to show symptom was

Table 1. Preliminary screening of *Lycopersicon* lines for TLCV under field conditions

Sl. No.	Lines	Mean score	Average fruit weight (g)
1.	V-2	2.2	16.2
2.	V-3	2.4	19.2*
3.	V-8	2.1	49.9
4.	V-9	2.2	12.0
5.	V-10	2.3	26.1*
6.	V-11	2.0	38.4*
7.	V-13	2.1	41.1*
8.	V-14	2.0	65.0*
9.	V-17	3.0	31.1*
10.	V-18	3.8	30.0*
11.	V-20	2.6	39.2*
12.	V-21	2.0	52.2
13.	V-25	3.4	12.2
14.	V-26	2.2	20.2*
15.	V-27	3.3	60.8*
16.	V-28	3.4	50.1
17.	V-30	3.6	27.1
18.	V-31	3.2	22.2
19.	V-34	1.9	17.2
20.	V-36	2.5	25.2
21.	305	1.9	24.0*
22.	331	1.9	34.3
23.	502	3.6	19.8*
24.	508	3.8	38.7
25.	733	2.4	14.3
26.	734	2.0	16.0
27.	752	1.8	22.6*
28.	762	3.4	89.3
29.	794	3.8	5.4
30.	827	3.3	6.1
31.	856	3.5	6.7
32.	974	3.2	22.4
33.	1094	3.0	4.6
34.	1099	3.3	3.5
35.	1036	3.1	4.8
36.	1617	3.1	10.2
37.	*L. pim.*	1.7	0.96
38.	*L. hir.* ('58)	0.0	1.16
39.	*L. hir.* ('59)	0.0	1.63
40.	*L. per.* ('30)	0.0	1.47*
41.	A. Saurabh	3.6	31.0*
42.	A. Vikas	3.6	34.5

* Mean of less than ten fruits

Table 2. Testing of Lycopersicon species by DAS-ELISA for TLCV.

Sl. No.	Genotypes /species	Reaction to TLCV (symptoms)	Antibody	ELISA readings*			Days for first Symp. expression	Mean days to symptom expection
				Sample	Healthy	Buffer		
1.	L.esc. (A.Saurabh)	Severe	Polyclonal	0.751^{P}	0.183	0.120	60	63.37
2.	L.esc. (A. Vikas)	Severe	Polyclonal	0.982^{P}	0.182	0.120	60	65.75
3.	L.pim.	Moderate	Monoclonal	0.300^{W}	0.180	0.172	72	81.80
4.	L.hir. ('58)	Nil	Monoclonal	0.183^{n}	0.156	0.172	N.S.	N.S
5.	L.hir. ('59)	Nil	Monoclonal	0.191^{n}	0.167	0.172	N.S	N.S
6.	L.per.	Nil	Polyclonal	0.205^{n}	0.181	0.120	N.S.	N.S

* Reaction in ELISA measured as A405 nm after overnight incubation with substrate. Plant sap was diluted 1/10 for testing. Antibody 2-H_{12} was supplied by B.D. Harrison, Scotish Crop Research Institute, Scotland.

N : negative ; P : positive ; W : weak ; N.S : no symptoms.

at the age of 60 days (30 days after transplantation) and the mean number of days for disease appearance was 63.37 in A.Saurabh and 65.75 in A. Vikas. The species L. pim. remained free from symptoms for 72 days and even the mean number of days for disease appearance (81.8) was delayed by about a fortnight than in L.esc. Disease symptoms were more severe in L.esc. (Fig.1) than in L. pim. However, other two species L.hir. and L.per. did not show any disease signs.

The summer grown plants were indexed for the virus (Table 2) by enzyme linked immunosorbant assay (ELISA). The assay was positive for L. esc. (A.Saurabh and A.Vikas); weak for L. pim. and negative for both the accessions of L. hir. and L. per.

4.1.3 Vector inoculation

Reaction of tomato lines used in hybridization to artificial inoculation with TLCV using viruliferous B. tabaci is shown in Table 3. The response of A.Saurabh and A.Vikas was similar. After the inoculation they took on an average 13 and 13.5 days respectively for the symptom to appear and in both the cultivars first symptom was observed 11 days after inoculation. L. pim. showed the earliest signs of disease 23 days following inoculation while the mean worked out to 28 days. TLCV syptoms were intense in L. esc. and moderate in L.pim.

Fig. 1. TLCV infected A. Vikas plants

Fig. 2. Seedling inoculation of TLCV by B.tabaci.

Fig.3. B.tabaci trapped in leaf trichomes of L.hir.

Table 3. Reaction of tomato lines to TLCV upon vector inoculation

Entry	Days for first symptom expression	Mean days for symptom expression	Disease severity
A.Saurabh	11	13.0	severe
A.Vikas	11	13.5	severe
L.pim.	23	28.0	moderate
L.hir. '58	-	-	no symptoms
L.hir. '59	-	-	no symptoms

Number of seedlings in each entry = 20.

No TLCV symptoms were noticed in the two accessions of L.hir. ('58 and '59); but inoculation of the virus itself was questionable as several whiteflies died (Fig.3) during inoculation period - 24h. So graft transmission studies were conducted to confirm the resistance.

4.1.4 Screening by graft transmission and confirmation for virus by ELISA

Graft transmission was accomplished by using the infected L.esc. as stocks and L.pim. and L. hir. as scions for wedge grafting (Fig.4). Moderate TLCV symptoms in L.pim. were observed both in field grown (Table 2) and graft transmitted plants (Table 4) and also alike virus titer (0.3 and 0.292 ELISA values respectively) were recorded under these two conditions.

Fig. 4. Graft inoculation of L.hir. '58 (left) and L.pim (right)

Fig. 5. Inoculated L.esc x L.hir. F_1 - showing TLCV signs.

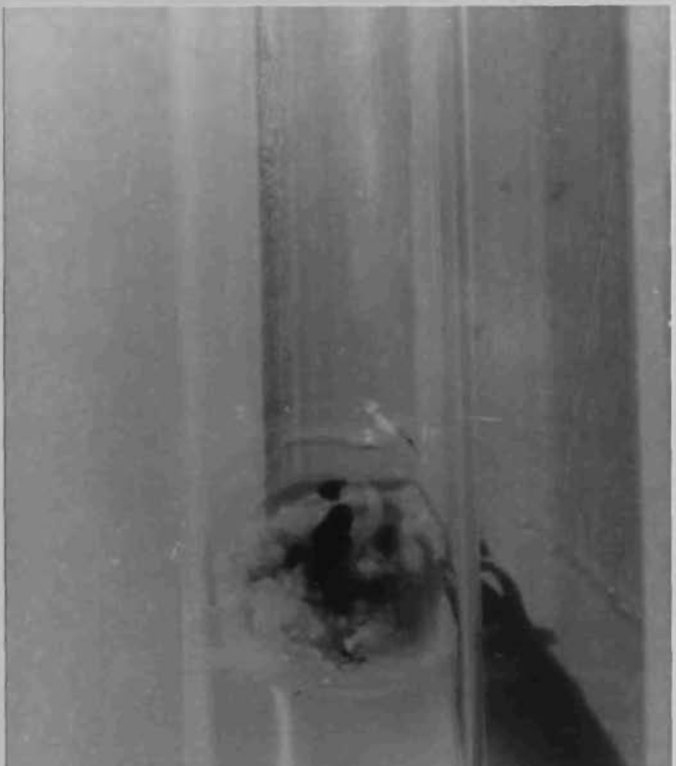


Fig. 6. Callus formation in A.Vikas x L.per. from immature, 35d old F_0 seeds.

Table 4. Graft transmission and ELISA screening of *Lycopersicon* speices for TLCV

Rootstock	Scion	Symptoms	ELISA readings			Remarks
			Inoculated	Healthy	Buffer	
TLCV infected *L. esc.*	*L. pim.*	Moderate	0.292^{W}	0.180	0.172	Mild symptoms, Moderate after 35 days
TLCV infected *L. esc.*	*L. hir.* ('58)	Nil	0.254^{VW}	0.178	0.162	When observed for 60 days no symptoms
TLCV infected *L. esc.*	*L. hir.* ('58)	?	0.270^{VW}	0.178	0.162	Infection suspected, light yellow mosaic
TLCV infected *L. esc.*	*L. hir.* ('59)	Nil	0.190^{N}	0.178	0.162	Observed for 60 days, no TLCV symptoms
TLCV infected *L. esc.*	*L. per.*	Nil	-	-	-	Observed for 66 days, no TLCV symptoms
Confirmation of virus presence in the rootstock and its movement to the scion						
TLCV infected *L. esc.*	*L. esc.*	Severe (rootstock)	1.100^{P}	0.178	0.162	Rootstock sprouts assayed for virus
TLCV infected *L. esc.*	L. esc.	Severe (scion)	0.411^{P}	0.178	0.162	TLCV symptoms after 24 days of grafting
-	L. esc. (Seedling)	Nil (mother plant)	0.184^{N}	0.178	0.162	Mother plant of scion assayed

N : Negative; P - Positive, W - Weak and VW - Very weak

* Reaction in ELISA measured as A 405 nm after overnight incubation with substrate. Plant sap was diluted 1/10 for testing. Monoclonal antibody 2-H_{12} to ICMV was supplied by B.D. Harrison, Scotish Crop Research Institute, Scotland.

Except two grafts none of the four fully established grafts each of L.hir.'58 and '59 and L. per. produced TLCV syptoms when observed for a minimum of 60 days after grafting. The virus assay of L. hir. '58 gave very weak and that of L. hir. '59 negative reaction. Two grafts of L. hir. '58 in which the infection was suspected because of light yellow mosaic on leaves, recorded 0.27 (very weak) ELISA value. L. per. was not assayed for the virus, for want of adequate leaf samples.

Confirmation of virus presence in the stock and its subsequent movement to the scion was done by simultaneous assay of sprouts that appeared now and then on the stock, and the scion tissue. The stock sprouts gave a positive 1.1 ELISA value (Table 4). Four weeks after grafting the leaf samples of both the mother plant and the grafted scion were subjected to ELISA concurrently, the former gave negative (0.184) and the latter positive (0.411) indication of virus.

4.1.5 Progeny test

The reaction of the genotypes for TLCV observed thus far by different screening tests was further examined by progeny test as to know its breeding behaviour. TLCV response of at least 15 progenies of each tomato line was studied by growing them in peak summmer. Confirming the performance recorded earlier, all the progenies of A.Saurabh and A.Vikas consistantly developed sooner or later severe TLCV symptoms and

L.pim. offsprings exhibited mild to moderate symptoms without seriously affecting plant growth and fruiting. Progenies of L. hir. ('58 & '59) L. per. failed to show TLCV signs.

4.2 EXPERIMENT II. INTERSPECIFIC HYBRIDIZATION

4.2.1 Study of interspecific Fl hybrids

The Fl hybrids of L.esc. x L.pim. and L.esc. x L.hir. and their parents were studied for 14 attributes as shown in Table 5. Differences among all the eleven entries were significant for the ten characteres for which the analysis was performed. Although both the cultivars, A.Saurabh and A.Vikas were at par in respect of all the growth attributes (except leaf expansion), but higher pollen fertility, better fruit set (58.33 %) and higher average fruit weight (64.5g) were observed in A.Vikas. In general vegetative growth was better in L.pim. than L.esc., so also the per cent fruit set (83.18) and pollen fertility. But, this wild species bears small fruits (0.88g) having 56.33 seeds on an average.

From the mean values on vegetative growth parameters it may be generalised that L.hir. grew more exuberantly than L.esc. and L.pim., but the leaf growth (expansion) in L.hir. was slower. However, L.hir. leaves showed delayed senescence. Vegetative vigour of L.hir. '58 was more than that of L.hir. '59 especially for

Table 5. Behaviour of interspecific hybrids and their parents for vegetative and reproductive attributes

Attributes	A. Saurabh (P$_1$)	A. Viaks (P$_2$)	L.pim (P$_3$)	L.hir '58 (P$_4$)	L.hir '59 (P$_5$)	P$_1$ x P$_3$	P$_1$ x P$_4$	P$_1$ x P$_5$	P$_2$ x P$_3$	P$_2$ x P$_4$	P$_2$ x P$_5$	C.D. 5%	C.D.01%
Plant height (cm)	54.00	55.00	69.58	90.89	86.50	79.79 (29.13)	47.75 (-34.08)	96.38 (37.19)	87.42 (40.34)	102.50 (40.52)	111.17 (56.99)	17.61	24.02
Laterals (no.)	5.83	5.42	8.92	8.58	6.83	8.42 (14.24)	3.75 (-47.19)	5.58 (-11.84)	9.33 (30.12)	7.83 (11.86)	7.50 (22.54)	2.12	2.89
I Lateral length (cm)	37.13	38.75	63.75	72.17	67.83	57.08 (13.16)	29.83 (-45.41)	68.08 (29.72)	69.95 (36.49)	88.58 (59.72)	71.17 (33.55)	12.06	16.45
II Lateral length (cm)	35.42	36.79	62.67	72.17	64.92	69.50 (41.72)	27.42 (-49.02)	65.83 (31.21)	66.33 (33.38)	81.00 (48.68)	78.00 (53.37)	14.48	19.75
III Lateral length (cm)	25.96	30.92	68.00	86.33	56.33	60.75 (29.31)	31.05 (-44.69)	55.67 (35.32)	65.17 (31.76)	82.00 (39.88)	77.17 (76.91)	14.53	19.82
Leaf length (cm)	26.59	23.09	17.61	20.14	21.39	26.99 (22.12)	15.26 (-34.67)	22.20 (-7.46)	26.05 (28.00)	26.74 (23.74)	26.19 (17.76)	5.21	7.10
Leaf expansion (cm^2)	120.74	86.32	74.03	107.08	91.61	144.68 (48.57)	52.95 (-53.51)	111.96 (4.70)	137.89 (71.99)	125.39 (29.66)	144.97 (62.96)	32.36	44.14
Leaf senescence (d)	19.00	18.50	18.66	23.33	25.80	27.00 (43.38)	15.00 (-29.11)	17.00 (-23.87)	26.00 (39.93)	27.50 (31.45)	24.50 (10.96)	-	-
Pollen sterility (%)	5.26 12.76*	7.08 14.42*	2.69 8.55*	5.56 11.00*	6.28 12.30*	1.89 6.56* (-52.39)	25.05 28.87* (363.03)	18.79 25.32* (225.65)	3.75 9.86* (-23.15)	18.89 24.10* (198.89)	19.63 25.28* (193.86)	5.49	7.26
Fruit set (%)	47.78 43.61*	58.33 49.66*	83.18 65.63*	35.02 36.09*	55.78 48.24*	69.84 56.58* (6.66)	2.91 9.64* (-92.97)	14.07 21.68* (-72.83)	71.26 57.56* (0.72)	45.87 42.49* (-1.73)	33.81 35.39* (-40.75)	6.15*	8.39*
Av. for Wt. (g)	57.26	64.50	0.88	1.14	2.07	16.29 (-43.96)	2.91 (-90.03)	3.72 (-87.46)	11.30 (-65.43)	3.08 (-90.61)	3.99 (-88.01)	1.71	2.33
Seed/fr. (no.)	-	122.0	56.33	71.13	75.80	-	-	-	49.27 (-44.75)	32.20 (-66.66)	24.93 (-74.79)	-	-
Seed germ. (%)	80.00	81.50	60.00	12.12	42.00	79.00 (12.86)	10.00 (-78.27)	9.00 (-85.24)	86.00 (21.98)	15.00 (-67.96)	9.00 (-85.43)	-	-
Overall performance	-	-	-	-	-	4.00	0.89	2.72	4.00	3.78	3.67		

* Angular transformed values.
Figures in parenthesies indicate per cent reduction over mid parent.

number of primary lateral branches which was significantly more (8.58) in the accession '58.

No significant difference in per cent pollen sterility was noted between the two accessions of L.hir., but lowest per cent fruit set (35.02) and seed germination (12.2) among parents was recorded by L.hir. '58. Fruit size was smaller in L.hir. and was significantly not different from L.pim.

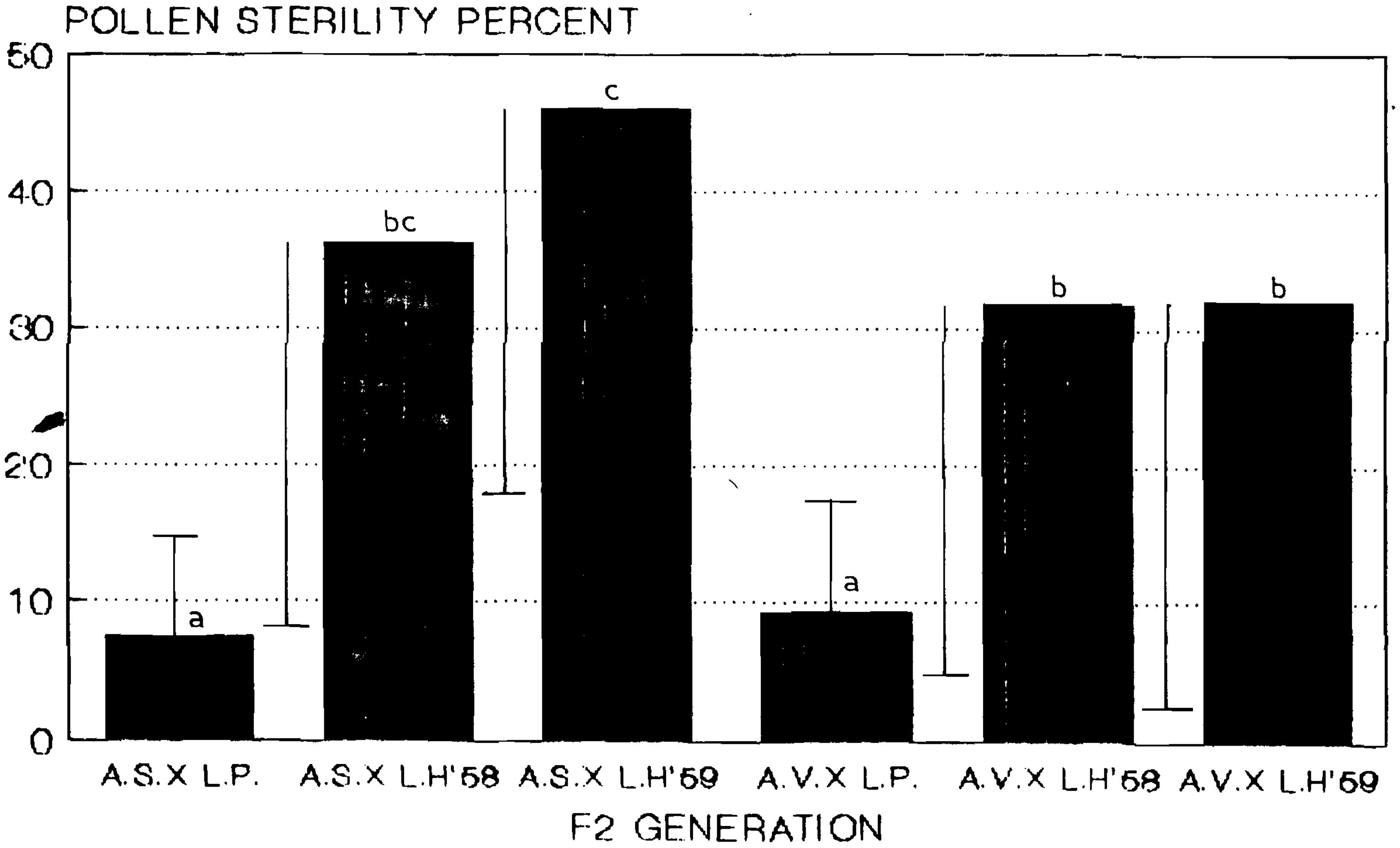
In general the interspecific hybrids between L.esc. x L.pim. was by far better than the L.esc. x L.hir. Both A.Saurabh x L.pim. and A.Vikas x L.pim. were not significantly different for most of the attributes excepting the average fruit weight, the former cross producing bigger (16.29g) fruits. But, among the four Fl hybrids of L.esc. x L.hir., the performance of A.Vikas x L.hir. ('58 and '59) was better than that of A.Saurabh x L.hir. ('58 and '59). All the four crosses of L.esc. x L.hir. recorded pollen sterility per cent that did not differ significantly from one another but per cent fruit set was maximum in A.Vikas x L.hir. '58.

Considering the F_1 behaviour in relation to the respective mid-parent values as yardstick the interspecific F_1's were compared. In case of all the attributes (excepting average fruit weight and number of seeds / fruit) L.esc. x L.pim. surpassed the mid-parent values. While L.esc. x L.hir. crosses were better than mid parent in case of most of the growth parameters, but not in respect of attributes concerning fertility. Failure of A.Saurabh to nick well with *L.hir.* (both the accessions) was notable from high and negative values, especially A.Saurabh x *L.hir.* '58 which exhibited maximum negative effects in respect of plant height, days to leaf senescence, pollen fertility and fruit set per cent. Based on these observations and visual scoring it may, therefore, be concluded that both the accessions of *L.hir.* combined relatively well with A.Vikas, than with A.Saurabh.

4.2.2 Pollen sterility in F2 generation

This was checked in 40 plants at random. A perusal of Table 6 and diagram (Fig.7) shows that the mean pollen sterility was very low in *L.esc.* x *L.pim.* crosses. This parameter measured more in F_2 of *L.esc.* x *L.hir.* crosses (31.71 to 46.04%). A.Saurabh x *L.hir.* crosses recorded maximum mean pollen sterility and were significantly different from other crosses. The earlier finding based on the study of Fl interspecific hybrids that A.Saurabh genome failed to blend well with *L.hir.* is reinforced by higher pollen sterility observed in corresponding F2 of A.Saurabh than A.Vikas. Standard errors associated with *L.esc.* x *L.pim.* was lower than that with *L.esc.* x *L.hir.*

Fig.7 Pollen sterility in F2



A.S. -A. Saurabh; A.V.- A. Vikas; L.P. -*L.pim*; L.H. 58-*L.hir*.58; L.H.59-*L.hir*.'59

Different letters above bars indicatae significant differences between genotypes at P= 0.05; Vertical bars show S.D.

Table 6. Pollen sterility per cent in F_2 generation of L.esc. x L.pim. and L.esc. x L.hir.

Cross	Range		
	Maximum	Minimum	Mean
A.Saurabh x L.pim.	39.13	0.00	7.40
A.Vikas x L.pim.	35.50	0.00	9.31
A.Saurabh x L.hir. ('58)	98.73	1.64	36.21
A.Vikas x L.hir ('58)	84.78	0.98	31.71
A.Saurabh x L.hir. ('59)	91.30	0.00	46.04
A.Vikas x L.hir. ('59)	98.98	3.61	32.06

4.2.3 L.esc. x L.per. crosses

Upon pollinating A.Saurabh and A.Vikas with L.per. fruits were set but no viable seeds were detected when eight fruits in each cross were checked. The seeds when sown failed to germinate. Hence immature seeds dissected from 35 days old and breaker stage fruits, were planted on growth medium as shown in Table 7.

Table 7 makes it clear that the media employed herein were not optimum for satisfactory embryo growth or callus formation for a given age of the seed and cross. For A.Saurabh x L.per. and A.Vikas x L.per. media 32 and 46 respectively were capable of inducing radicle and some amount of calli in 35 days old seeds. Such callus forming A.Vikas x L.per. was pictured as in Fig.6.

Table 7. Culture of undeveloped L.esc. x L.per. seeds on five growth media

Cross	Fruit stage	No. of seeds planted	* Medium code	Remarks
A.Saurabh x L.per.	35d old	58	32	After 49d, one seed produced radicle and another formed callus, but soon dried.
A.Saurabh x L.per.	Breaker stage	60	26	After 51d, 3 seeds bulged, but no further growth.
A.Vikas x L.per.	35d old	55	47	Observed for 90d, no growth.
A.Vikas x L.per.	35d old	39	46	One seed formed the radicle and other two calli, later turned black, dried.
A.Vikas x L.per.	Breaker stage	40	69	After 40d, one seed bulged and dried.

* The compounds and concentration of the media are enumerated in appendix I.

4.3.3 EXPERIMENT III. GENETICAL STUDIES

The experiment laid out in randomised block design during summer for studying inheritance of TLCV resistance, comprised the six populations - P1,P2,F1,F2,B1 and B2 of L.esc. x L.pim. and L.esc. x L.hir. crosses.

4.3.1 *L.esc.* x *L.pim.* crosses

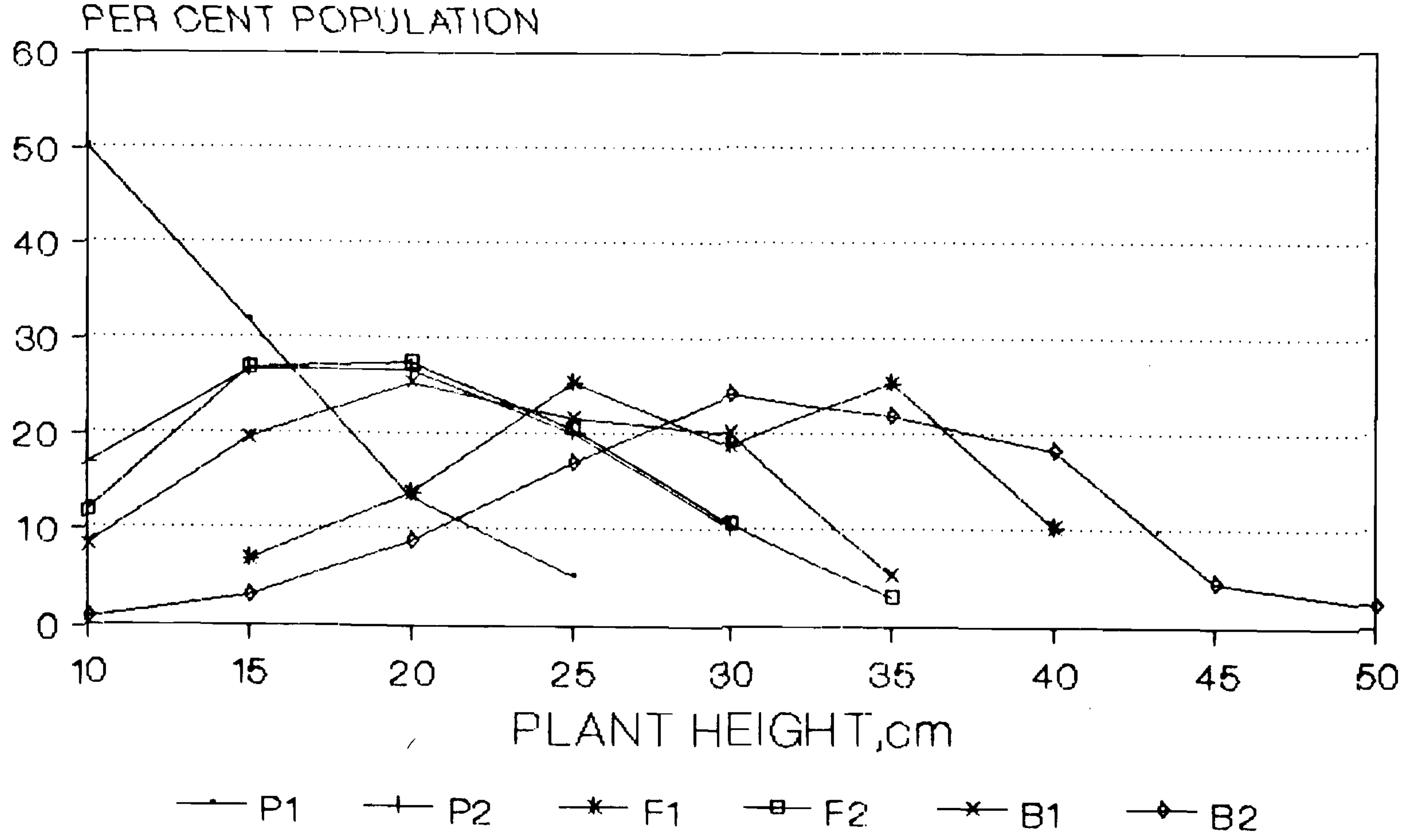
4.3.1.1 Frequency distribution

Frequency distribution in six generations for plant height, days to flower, days to TLCV symptom expression, mean disease score and average fruit weight was worked out and depicted by frequency polygons for *L.esc.* (A.Saurabh and A.Vikas) x *L.pim.*

Plant height : Fifty per cent of the plants in *L.esc.* measured very low height (10cm) while 2 per cent of B2 [(*L.esc.* x *L.pim*) x *L.pim.*] population measured maximum height of 50cm (Fig.8). The variability for this attribute was low in parents and maximum in B2. Major role of environment in the expression of plant height was evident from the larger variation observed in the non-segregating generation - F_1.

Days to flowering (Fig. 9): The variability of the segregating generations (F_2, B_1 and B_2) was markedly greater than that of the non-segregating ones (parents and F_1). *L.pim.* was distinctly early than *L.esc.* Transgressive segregation for early flowering was observed in all the three generations (F_2 B_1 and B_2) but no segregant flowering as late as 74 days appeared. Four per cent of B_2, 3 per cent of B_1 and 0.6 per cent of F_2 populations flowered when 30,34 and 38 days old respectively, indicating feasibility of selecting early types.

FIG.8 PLANT HEIGHT IN SIX GENERATIONS OF TOMATO



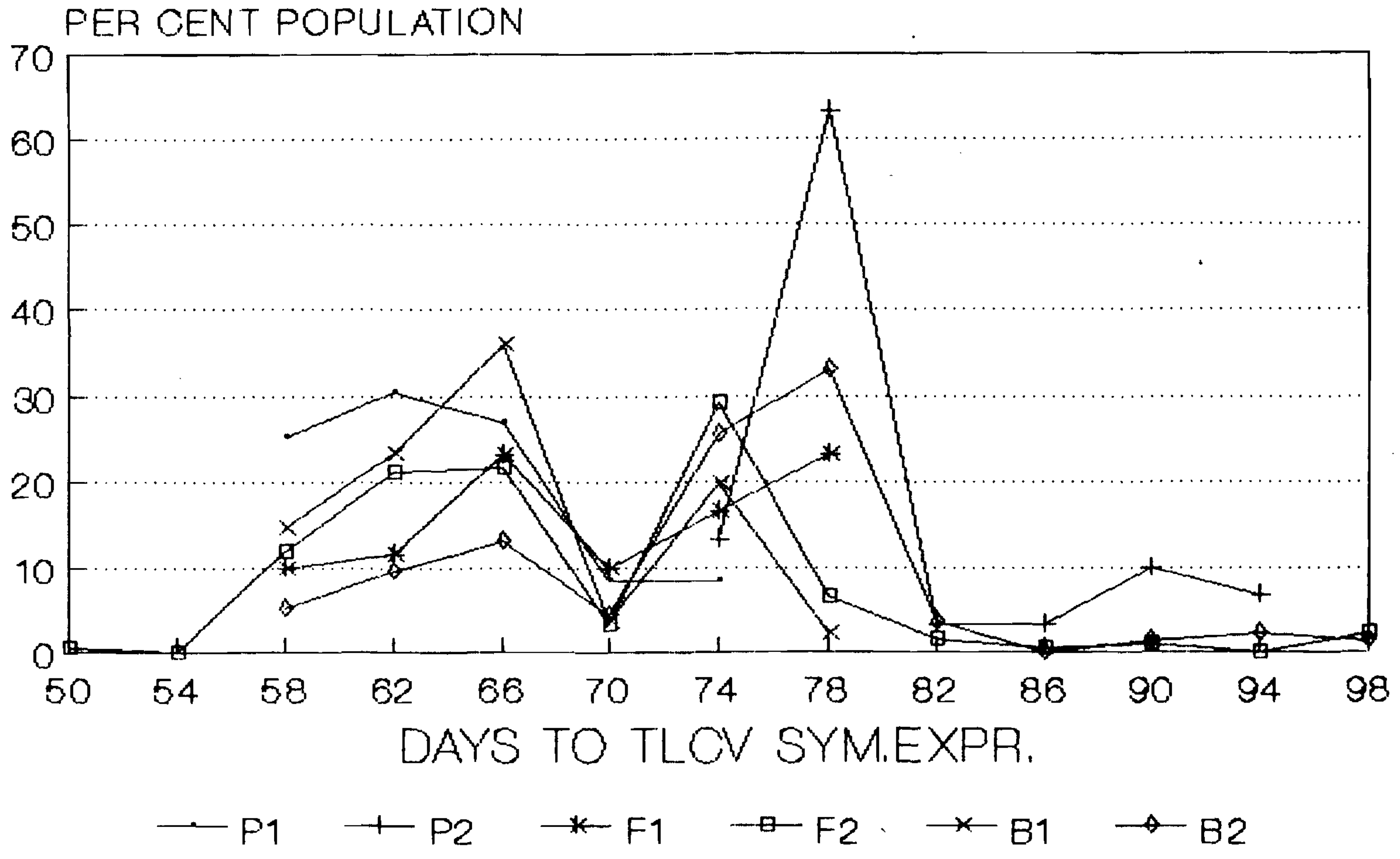
P1 : L.ESC. ; P2 : L.PIM.

B1 : F1 x L.ESC. ;B2 : F1 x L.PIM.

Days to TLCV symptom expression : One fourth of L.esc. population exhibited TLCV symptoms 58 days after seeding and about one third after 62 days (Fig. 10). In 64 per cent of L.pim. TLCV signs appeared when the plants were 78 days old and 7 per cent of the population expressed symptoms when 94 days old. L.pim. was clearely late in producing TLCV symptom as compared to L.esc. F_2 generation followed by B_2 exhibited markedly greater variability than the non-segregating generations (P_1,P_2 and F_1). A shift towards the recurrent parent-early expressor L.esc. in B_1 (F_1 x early expressor) and late expressor - L.pim. in B_2 (F_1 x late expressor) was illustrative of in Fig.10. Transgressive segregants in both the directions, taking as low as 50days (in F_2) and as high as 98 days (in F_2 and B_2) for symptom expression were recorded, indicating that there does exist some potential for improvement of this attribute by selection procedures.

Mean disease score:Low disease score (0 to 2) in L.pim. and high (3,4) in L.esc. was clear from the frequency distribution plotted in Fig.11. In L.esc. 59 per cent recorded highest mean disease score and rest (41%) scored 3, while in L.pim. 56 per cent showed a mean score of 2 and none recorded high score of either 3 or 4. Maximum number of plants (66%) in F_1 registered mean score of 2. Thus, the graphic presentation clearly depicts the distribution curves for mean disease score

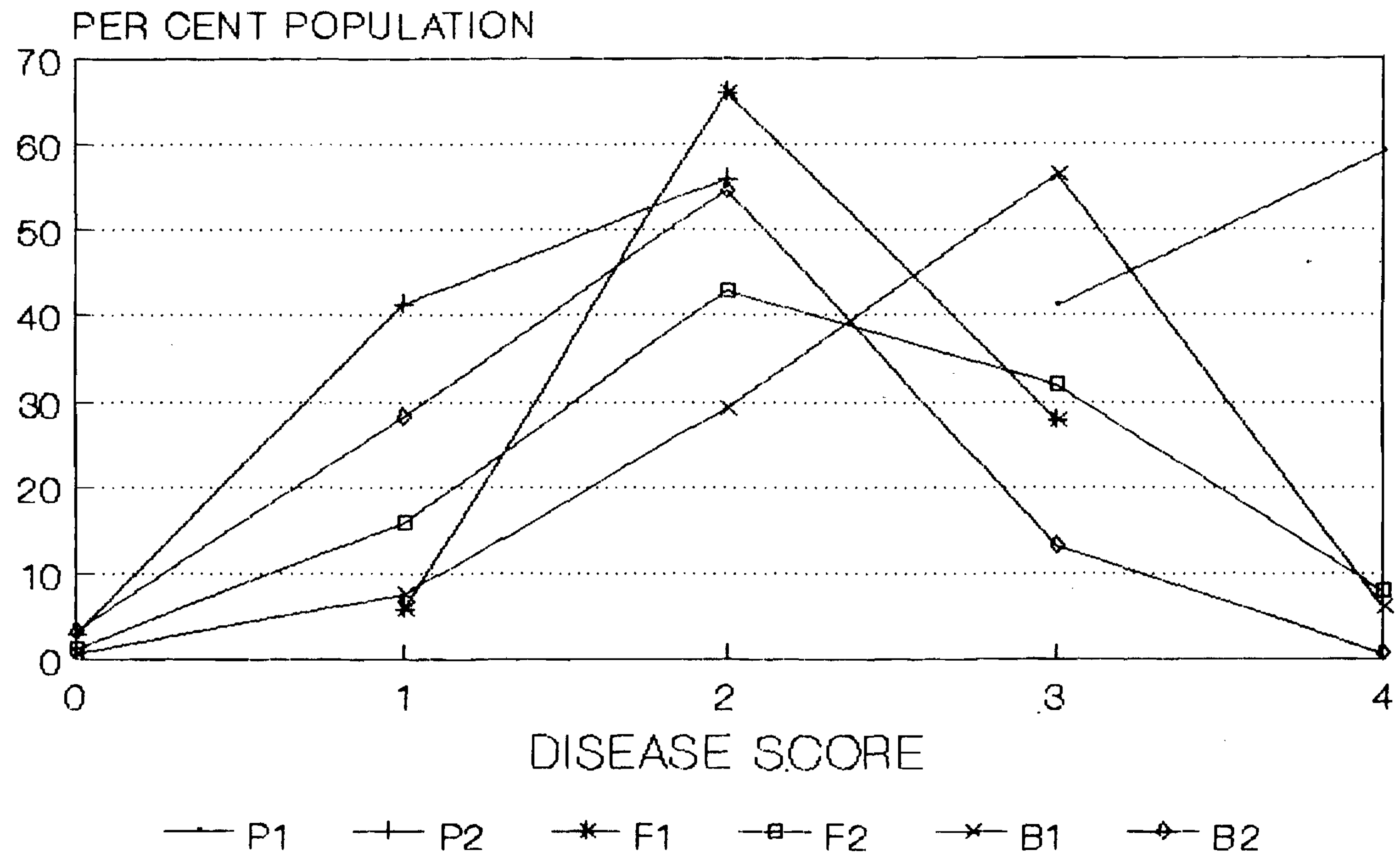
FIG.10 DAYS TO TLCV SYMPTOM EXPRESSION IN SIX GENERATIONS



P1 : L.ESC. ; P2 : L.PIM.

B1 : F1 x L.ESC. ; B2 : F1 x L.PIM.

FIG.11 TLCV DISEASE SCORE IN SIX GENERATIONS OF TOMATO



P1 : L.SEC. ; P2 : L.PIM.

B1 : F1 x L.ESC. ; B2 : F1 x L.PIM.

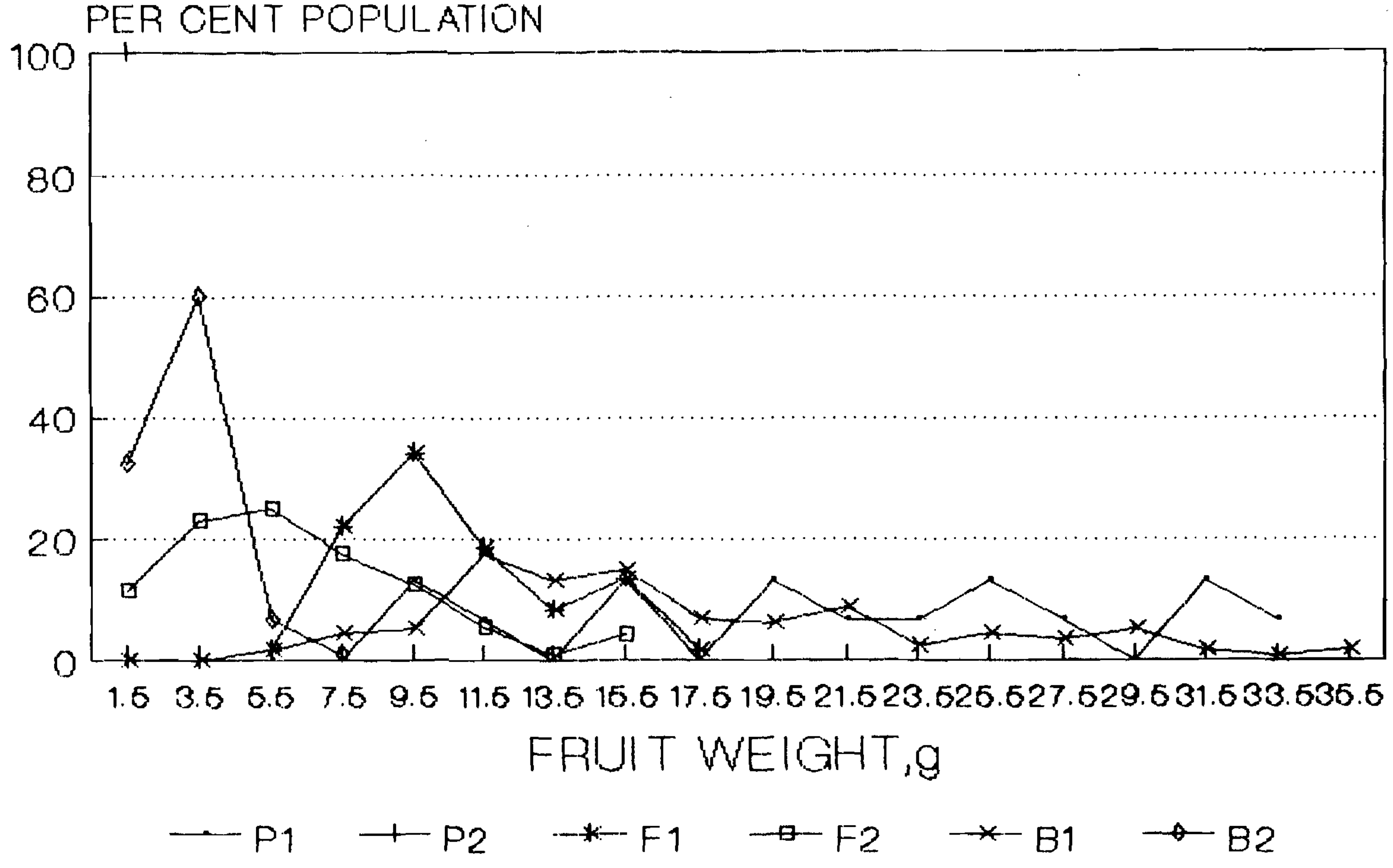
for tolerant species L.pim. to occupy left position and susceptible L.esc. right position while F_1 (susceptible x tolerant) to lay in the middle place (Fig.11). As regards, segregating generations, F_2 distribution showed maximum variability, B_1 (F_1 x susceptible) frequency polygon curve had a slant towards the susceptible parent and B_2 (F_1 x tolerant) towards the tolerant parent.

Average fruit weight : Representative fruit samples of six generation are illustrated in Fig.14 and frequency polygon for this attribute is presented in Fig.12. Average fruit weight varied from 9.5 to 33.5g in L.esc. (under severe TLCV infection) while L.pim. produced fruits weighing on an average between 1 – 2g. The wide variation observed in L.esc. was due to total and severe TLCV infectioon. In F_1 34 per cent of the population had fruit weight of 9.5g. Both F_1 and P_2 had little variability as compared to susceptible parent P_1. Among segregating generations maximum variance was recorded in B_1 (F_1 x susceptible parent) and least in B_2 (F_1 x tolerant parent). Nearly 2 per cent of the B_1 [L.esc. as recurrent parent] gave fruits weighing on an average 35.5g even under TLCV stress.

4.3.1.2 Analysis of variance

For detailed analysis of variance and genetical studies only three attributes viz., days to flowering, days to symptom expression and disease mean score were

FIG.12 MEAN FRUIT WEIGHT IN SIX GENERATIONS OF TOMATO



P1 : L.ESC. ; P2 : L.PIM.

B1 : F1 x L.ESC. ; B2 : F1 x L.PIM.

considered, but not plant height and average fruit weight as they were extremely influenced by TLCV onslaught.

Generation means and mean squares for days to flower, days to TLCV syptom expression and disease score in A.Saurabh x L. pim. and A. Vikas x L. pim. are furnished in Table 8. The analysis of variance revealed highly significant differences for all the three attributes in both the crosses. Further partitioning of the variance showed that the differences among the parents and between the F_1S of two crosses were significant for all the attributes, while the interaction between parents vs F_1's was not significant in case of days to symptom expression.

As regards segregating generations (F_2's and BC's) significant differences were observed for all the traits in backcross populations and days to flower alone in F_2. Likewise for the same trait F_2's vs BC's comparison was significant. However for none of the traits block effects was significant.

4.3.1.3 Population Mean

Days to flower : The TLCV susceptible cultivars A. Saurabh and A. Vikas took on an average 67.4 and 67.8 days to bloom and tolerant species L.pim. flowered earlier by about 14 days, while Fl'S were still early in flowering (Table 8). The lower values of Fl's than the

Table 8. Generation means with standard errors, mean squares (bold face) for different generations in two crosses of L.esc. x L.pim.

Generations	d f	Days to flower	Days to TLCV sympt.expression	TLCV disease score
Replication	2	**0.67**	**0.89**	**0.001**
Entries	10	**142.88****	**82.31****	**1.38****
A Non segr.(NS)	4	**212.93****	**158.53****	**2.41****
(1) Parents	2	**179.68***	**302.43****	**3.98****
(i) A.Saurabh (AS)		67.40 ± 0.90	60.76 ± 0.66	3.71 ± 0.10
(ii) A. Vikas (AV)		67.80 ± 0.54	63.60 ± 1.18	3.48 ± 0.08
(iii) L.pim. (LP)		54.20 ± 0.84	79.40 ± 1.18	1.58 ± 0.10
2 F1's	1	**13.50****	**24.00****	**0.12***
(i) AS X LP		53.10 ± 0.57	67.13 ± 1.22	2.35 ± 0.10
(ii) AV X LP		50.10 ± 0.49	71.13 ± 1.21	2.13 ± 0.08
3 Parents VS F1's	1	**478.86****	**5.27NS**	**1.59****
B Segregating (S)	5	**53.14****	**37.69 ****	**0.49****
(i) F2'S	1	**20.63****	**0.09NS**	**0.03NS**
(i) AS X LP		53.64 ± 0.49	67.69 ± 0.69	2.36 ± 0.06
(ii) AV X LP		57.35 ± 0.92	67.44 ± 1.26	2.23 ± 0.12
(2) BC's	3	**52.06****	**61.71****	**0.79****
(i) AS X (AS X LP)		53.09 ± 0.95	63.39 ± 0.66	2.78 ± 0.08
(ii) (AS X LP)XLP		45.91 ± 0.90	71.46 ± 1.02	2.04 ± 0.07
(iii)AV X(AV X LP)		55.20 ± 0.75	65.98 ± 0.86	2.33 ± 0.09
(iv) (AV X LP) X LP		48.91 ± 0.81	73.03 ± 0.94	1.60 ± 0.9
(3) F2's X BC's	1	**88.88****	**3.21NS**	**0.05NS**
NS VS S	1	**311.44****	**0.50NS**	**1.64****
Error	20	**1.53**	**2.44**	**0.02**
C.D. at 5%		1.74	2.20	0.20
C.D.at 1%		2.55	3.23	0.29

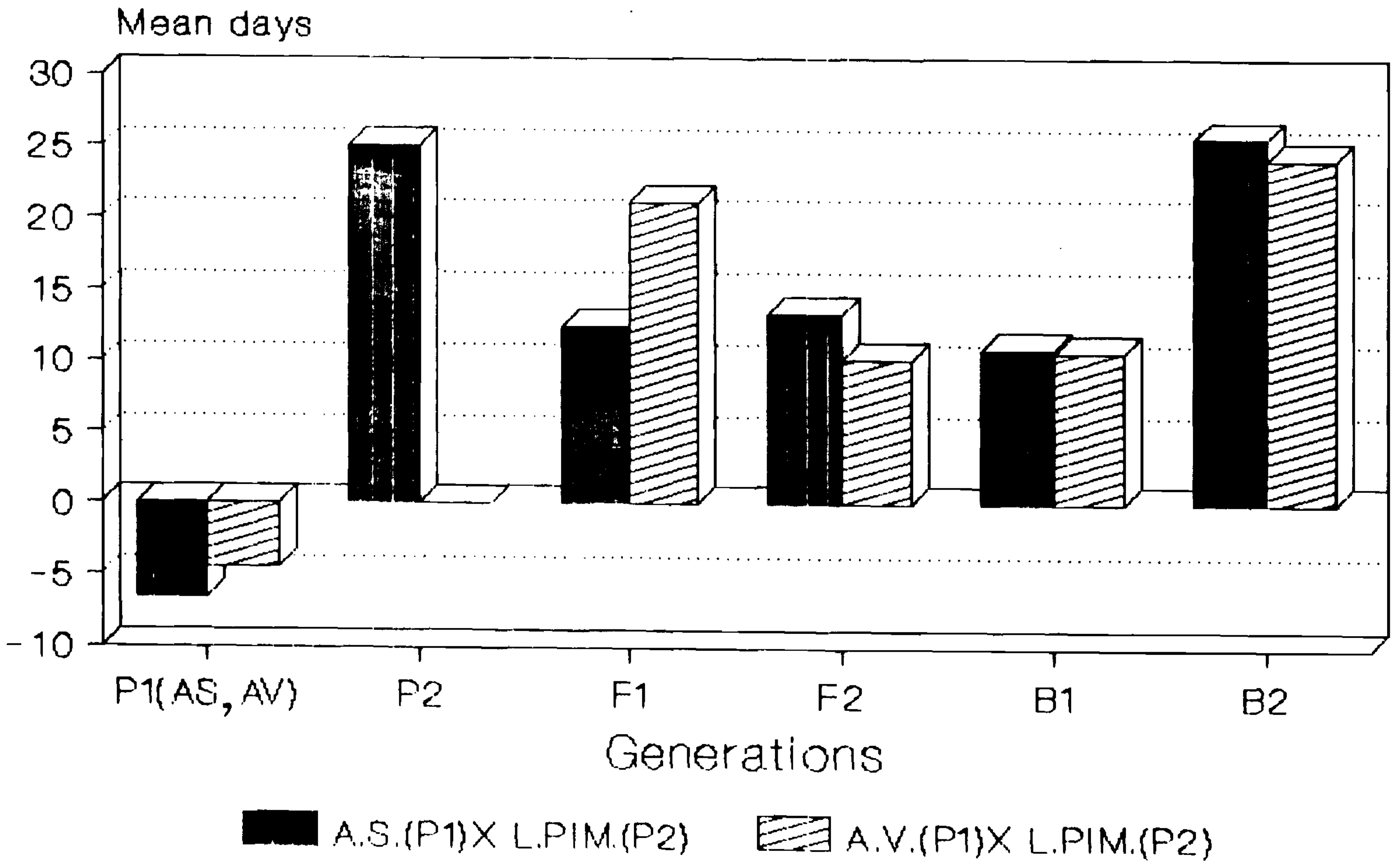
* and ** indicate significant at 5% and 1% levels of probability respectively

corresponding mid parent values indicated the presence of partial dominance for earliness. Higher mean value of F2's (57.35) than the F1's in case of A. Vikas x L. pim. was suggestive of presence of dominant and epistastic components. L. pim. as recurrent parent produced progeny whose mean days to first flower was by far lower than the cultivated varieties, it was as low as 45.91 mean days in case of backcross of (A. Saurabh x L. pim.) X L.pim.

Days to TLCV symptom expression : Number of days at which the TLCV symptom appeared differed significantly in cvs A. Saurabh and A. Vikas. TLCV tolerant L. pim. exhibited the disease symptom at the mean age of 79.4 days which was later than the cultivated types by a fortnight. A. Vikas which expressed TLCV symptom on an average 3 days later than A. Saurabh produced F_1 hybrids that took longer period (71.13 days) than F_1 of A. Saurabh.

Such genotypes showing considerable delay in TLCV symptom appearance after flowering are of much value in escaping from TLCV onslaught. This is depicted in Fig.13 for the six generation populations of both the crosses. It may be seen from the graphic representation that L. pim. and backcross progeny arising from L. pim. as recurrent parent (B2) showed very wide gap (about 25 days) between days to TLCV symptom appearance and days to flowering, while TLVC sensitive A. Saurabh and A.

Fig.13 Mean difference in days to TLCV sympt.expr.and flowering in L.ESC.xL.PIM.



AS : A.SAURABH ; AV : A.VIKAS

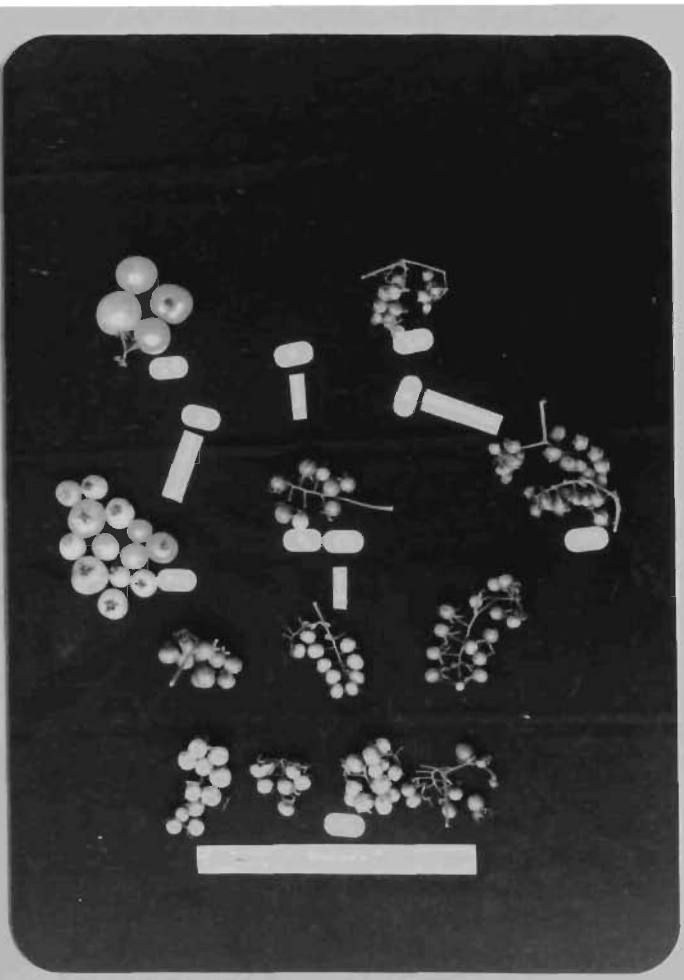


Fig. 14. Representative fruit samples of six generations of L.esc. x L.hir.

Fig. 15. A. Vikas x (A. Vikas x L.pim.) - R_1 34/1/1, good bearing even when infected by TLCV.

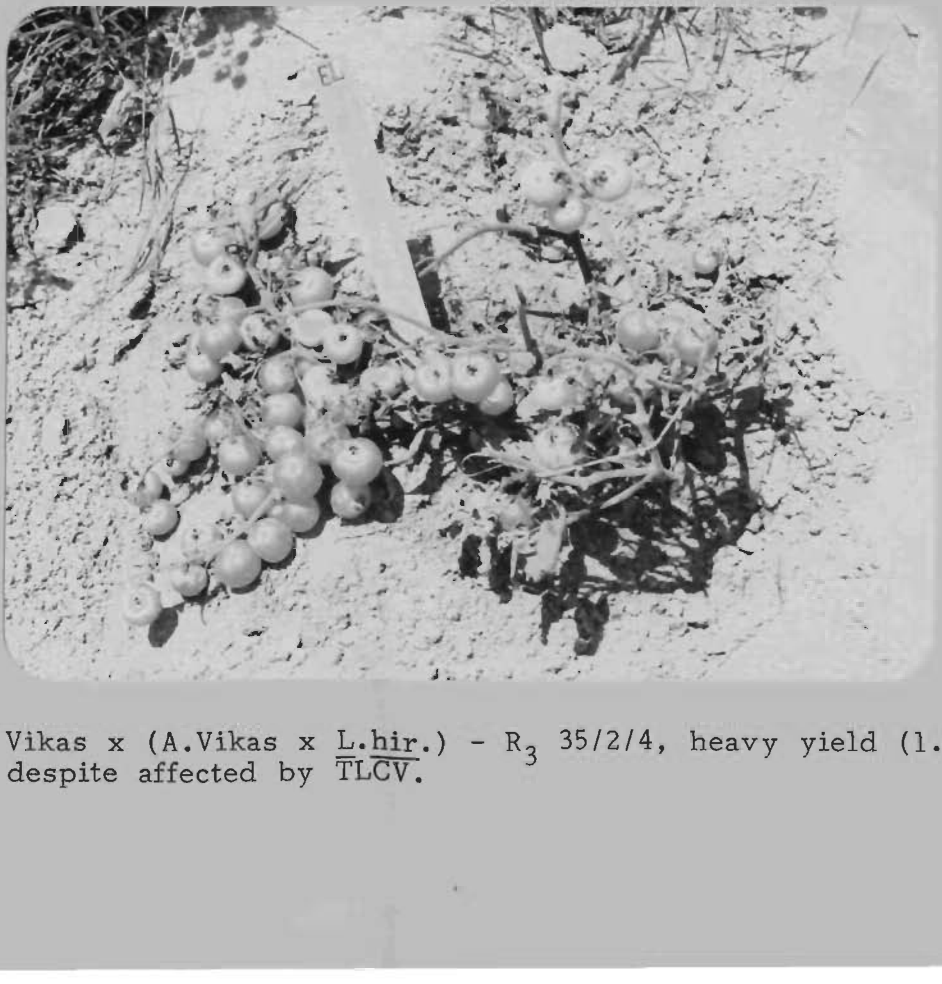


Fig. 16. Vikas x (A.Vikas x L.hir.) - R_3 35/2/4, heavy yield (1.78 kg) despite affected by TLCV.

Vikas gave -6.67 and -4.53 values which meant that TLCV symptom started showing up well before flowering.

TLCV disease score ; Susceptible cvs. A. Saurabh (3.71) followed by A. Vikas (3.48) scored maximum, while *L.pim.* (1.58) scored least. F_1 of A.Saurabh x *L.pim.* had significantly higher (0.05 level) mean score than F_1 of A.Vikas x *L.pim.*, but in F_2 generation the mean scores were at par. In case of B_1, i.e., backcross with A.Saurabh or A.Vikas, the mean TLCV ratings fell well below the mid-parent value and showed clear skewness towards the tolerant species *L.pim.* The mean virus incidence was as low as in donor parent with one dose of backcross with *L.pim.* in case of A. Vikas x *L.pim.*

4.3.1.4 *Gene effects and genetic parameters*

Table 9 & 10 furnish the results of six generation mean analysis including scaling tests and gene effects, apart from heritability and genetic advance estimates for days to flower, days to symptom expression and disease severity score for A. Saurabh X *L.pim.* and A. Vikas X *L. pim.*

Days to flower : Scaling tests A, B and C for this character were highly significant and negative barring test C in case of A. Vikas X *L. pim.* thereby suggesting the presence of epistasis in both the cross combinations. Analysis of genetic components of mean suggested that excepting additive X dominance all the gene effects were

Table 9. Scaling test, gene effects and gene action for three attributs in two <u>L</u>. <u>esc</u>. x <u>L</u>. <u>pim</u>. crosses

Parameter		Days to flowering	Days to TLCV symptom expression	TLCV symptom severity	Days to flowering	Days to TLCV symptom expression	TLCV symptom severity
		A. Saurabh x <u>L.</u> pim.			**A. Vikas x <u>L.</u> pim.**		
Scaling Test	A	-13.84** ± 2.18	0.73 ± 1.92	- 0.47* ± 0..21	- 7.83** ± 1.66	- 2.94 ± 2.38	- 0.95** ± 0.22
	B	-15.51** ± 2.08	- 1.93 ± 2.67	0.15 ± 0.20	- 6.47** ± 1.89	- 4.48 ± 2.53	- 0.24 ± 0.29
	C	-12.98** ± 2.60	- 2.81 ± 3.94	- 0.55 ± 0.35	6.86 ± 3.93	-15.07** ± 5.82	- 0.33 ± 0.53
Gene effects							
Mean	(m)	53.70** ± 0.49	67.10** ± 0.69	2.36** ± 0.06	57.34** ± 0.92	67.55** ± 1.26	2.22** ± 0.12
Additive	($\hat{d}$)	7.43** ± 1.31	- 8.00** ± 1.22	0.75** ± 0.11	6.30** ± 1.10	- 7.13** ± 1.26	0.60** ± 0.16
Dominance	($\hat{h}$)	-24.07** ± 3.40	- 2.91 ± 3.95	- 0.06 ± 0.35	-32.22** ± 4.33	7.28 ± 5.83	- 1.38* ± 0.59
Additive x additive	($\hat{i}$)	-16.37** ± 3.29	1.63 ± 3.69	0.24 ± 0.32	-21.16** ± 4.28	7.65 ± 5.64	- 0.85 ± 0.58
Additive x dominance	($\hat{j}$)	0.83 ± 1.45	1.32 ± 1.39	- 0.31* ± 0.13	- 0.68 ± 1.21	0.77 ± 1.51	0.35* ± 0.17
Dominace x dominace	($\hat{l}$)	45.72** ± 5.87	- 0.46 ± 6.27	- 0.08 ± 0.55	35.46** ± 5.91	- 0.24 ± 7.73	2.04* ± 0.82
Type of epistasis		Duplicate	-	-	Duplicate	-	Duplicate

Significant at 5% (*) and 1% (**) levels, based on corresponding 't' value.

Table 10. Heritability and genetic advance in two crosses of *L.esc.* x *L.pim.*

Parameter	Days to flowering	Days to TLCV sym. expresion	TLCV symptom severity score
A.Saurabh x *L.pim.*			
Heritability (Broad sense)	58.16	33.73	48.95
Heritability (Narrow sense)	-2.62	-0.02	0.88
Genetic advance (GA)	-0.27	-0.003	0.02
GA as % of mean	-0.51	-0.004	0.63
Expected GA in F_3	53.43	67.10	2.37
A.Vikas x *L.pim.*			
Heritability (Broad sense)	87.73	63.32	67.13
Heritability (Narrow sense)	0.61	1.02	-0.05
Genetic Advance (GA)	0.10	0.23	-0.001
GA as % of mean	0.17	0.33	-0.04
Expected GA in F_3	57.45	67.77	2.22

highly significant in both the crosses. Dominance X dominance followed by dominance appeared to play a predominant role in the expression of earliness in both the crosses. The contrasting signs of $\hat{h}$ and $\hat{l}$ effects suggested duplicate type of epistasis in A. Saurabh x L. pim. and A. Vikas x L. pim.

In A.Vikas x L.pim. broad sense heritability was high (87.73), but not the narrow sense (0.61), while the narrow sense heritability (-2.62) and genetic advance (-0.27) were negative in A. Saurabh X L.pim. Expected genetic advance in F_3 was similar in both A. Saurabh x L. pim. (53.43) and A. Vikas x L. pim. (57.45).

Days to TLCV symptom expresion: Non-significance of all the scaling tests in A.Saurabh X L.pim. and two (A and B) in A. Vikas X L. pim. indicated the absence of epistasis for this trait. In both the crosses the mean and additive component of gene effects were of same magnitude and highly significant. None of the inter and intra-allelic components in either crosses appeared to have importance in controling the genetic variability of the attribute in question. No inferences regarding type of gene action was drawn as neither $\hat{h}$ nor $\hat{l}$ estimates was significant.

Both narrow and broadsense heritability were by far greater in A. Vikas X L.pim. than A.Saurabh X L. pim. Value for genetic advance was negative in A.Saurabh x

L.pim., and in A.Vikas x L.pim. it was of low magnitude. This indicated that additive genetic effect did not play an important role in determining expression of this attribute. Expected genetic advance in F_3 was almost same in both the crosses.

TLCV symptom severity : Out of three scales (A, B and C) only one was significant, suggesting the absence of epistasis. Both the crosses expressed preponderance of additive genetic effects in the expression of TLCV symptoms. Other effects operative were additive X dominance in A. Saurabh X L.pim. and dominance, additive X dominance and dominance X dominance in case of A. Vikas X L.pim. But all these interaction effects registered significance only at 5 % level.

Higher heritability (67.13) was recorded in A.Vikas x L.pim. Genetic advance as per cent of mean and expected genetic advance in F_3 were better in A.Saurabh X L. pim. than the other cross.

4.3.2 L. esc. x L. hir. crosses

Six generations of L. esc. x L. hir. crosses although were evaluated, the data were not subjected to detailed analysis because of serious deviations from normal segregation observed following high pollen sterility both in F1 and F2, poor fruit set, reduced seed germination, seedling mortality, slow growth, casuality prior to flowering , late flowering and brittle shoots especially in case of A. Saurabh x L.

hir. For same reasons it was difficult to secure larger population in A.Saurabh x L.hir. However, the means and variances for days to flower, days to symptom expression and disease score alone are furnished in Table 11.

Days to flower: L. hir. took more time to flower (about 85 days) than L. esc. (about 69 days). The flowering in F1's varied from 88.25 mean days in A. Saurabh x L. hir. '58 to 76.35 days in A. Vikas x L. hir. '58, but greater variance was associated with hybrids than with the parents. In F2's the mean number of days for flowering was less than in the corresponding F1's which ranged from 69.91 to 78.67. All the four F2's showed higher variance than F1's. The weak interspecific F1 hybrid, A. Saurabh x L. hir. '59 upon backcorssing with A. Saurabh produced B1 generation which took maximum of 81.41 days to flower, while other B1's came to flowering as early as the early L. esc. types. In the B2 involving backcross to late flowering L. hir., the relatively better performing F1 (A. Vikas x L. hir. '59) exhibited very low mean days (71.64) to flower. The variances associated with B1 and B2 generation were of about the same magnitude.

Days to symptom expression: Among the generations, mean number of days to express TLCV symptoms was lowest in susceptible parents, A. Saurabh (60) and A. Vikas (62.3). L. hir. and some F1 plants failed to get

Table 11. Mean and variance for days to flower and symptom expression disease score in L. esc. x L. hir. crosses

Generations	Days to flowering		Days to symptom to expression		Disease intensity	
	Mean	Variance	Mean	Variance	Mean	Variance
P: P_1	68.00	12.80	60.00	15.20	3.64	0.30
P_2	69.30	10.20	62.30	14.80	3.60	0.32
P_3	85.80	15.36	-	-	0.00	-
P_4	85.00	20.88	-	-	0.00	-
F_1: $P_1 \times P_3$	88.25	39.47	93.00	*	1.51	*
$P_1 \times P_4$	80.14	36.30	97.00	*	1.60	*
$P_2 \times P_3$	76.35	29.47	99.33	80.66	1.67	*
$P_2 \times P_4$	83.45	28.47	85.50	*	1.72	*
F_2: $P_1 \times P_3$	77.27	77.26	79.00	89.30	2.92	0.94
$P_1 \times P_4$	78.67	90.72	87.22	190.49	2.09	0.94
$P_2 \times P_3$	74.95	105.68	81.37	130.64	2.25	0.79
$P_2 \times P_4$	69.91	143.52	79.92	96.63	2.43	1.25
B_1: $P_1 \times (P_1 \times P_3)$	68.02	107.77	70.60	80.12	2.85	0.54
$P_1 \times (P_1 \times P_4)$	81.41	40.01	84.05	220.57	2.62	0.54
$P_2 \times (P_2 \times P_3)$	68.96	83.48	73.92	39.11	2.53	0.57
$P_2 \times (P_2 \times P_4)$	72.70	88.08	72.50	25.74	2.95	0.75
B_2: $(P_1 \times P_3) \times P_3$	76.44	102.53	90.75	297.64	1.88	0.41
$(P_1 \times P_4) \times P_4)$	80.77	44.36	82.33	101.33	2.00	0.67
$(P_2 \times P_3) \times P_3)$	82.87	91.98	80.44	33.78	2.43	1.62
$(P_2 \times P_4) \times P_4)$	71.64	117.38	82.41	120.45	2.40	0.83

P : Parents; P_1 : A. Saurabh; P_2: A. Vikas; P_3: L. hir. 58; P_4: L. hir. 59

* As the sample size was small variance not reported

infected under field conditions. Preference by B.tabaci for F1 hybrids as much as L.esc. and segregating populations may be one reason for lack of uniformity in development of TLCV symptoms by the F1 population under field conditions. The range was narrow in the F2 (79 to 87.22) than in F1 (85.5 to 99.33). Both the F1 and F2 of A.Saurabh xL. hir. '59 took more mean days (97 and 87.22 respectively) to show up symptoms under field conditions. Conversely, F1 and F2 of A. Vikas x L. hir. '59 were quick to react for TLCV. Magnitude of variances (89.3 to 190.49) were larger in all the four F2's. In general B1 showed TLCV symptoms earlier than B2, the former generation had emerged from backcross to the susceptible, early symptom expressing L. esc. Among the crosses of B1, again the late flowering A. Saurabh x (A. Saurabh x L. hir. '59) took more mean days (84.05) to express TLCV, than the rest of the B1's. This particular backcross also showed largest variance (220.57). B2 generation (A. Saurabh x L. hir. '58) x L. hir. '58 requried maximum of 90.75 mean number of days to manifest TLCV, it also had the highest variance (297.64).

Mean disease score: Susceptible parents A. Saurabh (3.64) and A. Vikas (3.60) registered highest mean disease score with minimum variance. However, F_1 did not differ much for mean TLCV incidence which ranged from 1.51 to 1.72. In F2 generation of the four crosses the TLCV was highest in A. Saurabh x L. hir. '58 (2.92) followed by A. Vikas x L. hir. '59, the latter had the highest variance (1.25) also. F2 of A. Saurabh x L. hir. '59 gave lowest score of 2.09. The backcrosses involving (susceptible x resistant) x susceptible had higher score (2.53-2.95) than the corresponding (susceptible x resistant) x resistant crosses (1.88 to 2.40). In B1, the cross A. Vikas x (A. Vikas x L. hir. '59) manifested highest disease score (2.95) and variance (0.75). In B2 (A. Vikas x L. hir. '58) x L. hir. '58 recorded highest mean score and variance of 2.43 and 1.62 respectively.

4.3.3 Indexing segregating population for TLCV

A small fraction of genotypes representing varying levels of disease intensity with good yields and belonging to F_2, B_1 and B_2 were subjected to ELISA. The relevant description of such genotypes and the corresponding ELISA values are presented in Table 12. Almost negative (R_1 23/1/9 and R_1 23/2/1) to positive (R_1 23/2/4) readings in F_2 of A. Saurabh X L.hir. '59 were recorded, while the assay turned out to be clearly negative (0.185) in respect of R_2 22/1/3. One of the selections (R_1 24/1/3) from the F_2 population of A. Vikas X L. pim. showed light yellow islands on leaves but no curling, and was vigorous. It recorded high fruit yield (1.2 kg/plant) and at the same time weak reaction (0.286) for TLCV assay.

Table 12. Polyclonal ELISA and external attributes of segregants sampled from three generations F_2, B_1 and B_2

Generation	Parentage	Individual plant sel.	ELISA Value	Remarks
F_2	AS X LH'58	R_3 22/1/3	0.185^{N}	No leaf curl symptoms, light green leaves resembling L.esc.
F_2	AS X LH'59	R_1 23/1/9	0.205^{N}	Symptoms very mild; not much reduction in vigour; fruiting, flowering normal
F_2	AS X LH'59	R_1 23/2/1	0.206^{N}	Showed leaf curl when 6 weeks old; later recovered
F_2	AS X LH;59	R_1 23/2/4	0.338^{P}	Very severe infection; stunted, poor fruit formation
F_2	AV X LP	R_1 24/1/3	0.286^{W}	Light yellow islands on leaves, but no curling; vigourous and high yield of 1.2kg; average fruit weight 8.2g.
B_1	AS (AS X LH'59)	R_1 33/1/1	0.317^{W-P}	No leaf curl symptoms in field, but poor yield (3 fruits)
B_1	AS (AS X LH'59)	R_1 33/1/1	0.319^{W-P}	Assayed for virus after grafting on infected L.esc.stock, no signs of TLCV
B_1	AS (AS X LH'59)	R_2 33/1/7	0.168^{N}	Exhibited freedom from virus, but was sterile, good flowering but no fruit set
B_1	AS (AS X LH'59)	R_2 33/1/1	0.214^{N}	Mild infection, very good fruiting
B_1	AV (AV X LP)	R_1 34/1/1	0.242^{VW}	Symptoms mostly on the fresh leaves, vigour and yield good (1.38kg); average fruit weight : 28g
B_2	(AS X LH'59)X LH'59	R_1 43/1/3	0.551^{P}	Severe infection, stunted growth, poor yield
B_2	(AS X LH'59)X LH'59	R_1 43/1/4	0.232^{VW}	Slight infection but vigrous
B_2	(AS X LH'59)X LH'59	R_2 43/1/5	0.181^{N}	No signs of leaf curl, vigrous and leaves like resistant parent
B_2	(AV X LP) X LP	R_3 44/2/2	0.291^{W}	Symptoms more pronounced; profuse bearing (1.12kg) with average fruit weight 4.8g

AS - A. Saurabh; AV- A.Vikas; LP - L.pim.; LH'58-L.hir.'58 and LH'59- L.hir.'59; N - Negative;
P - Positive; VW- Very weak, W-P-Weak to positive, W-Weak

Five among the B_1 (suceptible parent X F_1) plants tested for virus, two showed weak to positive reaction, one very weak and the rest two negative. Another segregant (R_2 33/1/1) of A. Saurabh x (A. Saurabh x L. hir. '59) showed no leaf curl signs but the ELISA indicated weak to positive response (0.317). The same plant recorded 0.319 ELISA upon graft transmission, but when it again remained free from TLCV symptoms (observed for 30 days). Thus it appeared to be a symptomless virus carrier. High yield of 1.38 kg with average fruit size of 28 g was observed in another notable segregant (R_1 34/1/1 ; Fig.15) that emerged from a cross involving L. pim. But it showed a reading of 0.242 for ELISA.

Three B_2 plants of (A. Saurabh x L.hir. '59) x L.hir. '59 showing severe to no symptoms were sampled for ELISA. Maximum ELISA reading of 0.551 was recorded in a plant with severe symptoms, while one with mild and no symptoms gave 0.232 and 0.181 values respectively. Inspite of moderately high virus concentration (0.291), (A. Vikas X L.pim.) X L. pim. - R_3 44/2/2, registered good fruit yield (1.12 kg).

4.3.4 Glass house studies

4.3.4.1 Latent period required for TLCV symptom expression

As mentioned earlier (section 4.1.3), after TLCV inoculation by B. tabaci, minimum of 11 days were required for symptoms to show up in A. Saurabh and A. Vikas, while 23 days in *L. pim.*, *L. hir.* accessions failed to develop symptoms when observed for 40 days. In case of F1 of *L. esc.* X *L. pim.* latent period required was 4-5 days more than in *L.esc.* but was about a week earlier than in *L. pim.* (Table 13). Similarly F_1 A. Saurabh X *L.hir.* '58 and A. Vikas X *L.hir.* '58 also needed 3-4 days more minimum incubation time than the female parents, but same parents with *L.hir* '59 took a week more (19 days) to exhibit first signs of TLCV. In the F2 progeny days required for symptom expression varied widely in *L.esc.* X *L. pim.* but no segregant registered as short a period (11 days) as *L. esc.* cultivars. A. Vikas X *L. pim.* produced progeny that delayed TLCV symptom expression by 34 days. In the F2's of four *L.hir.* crosses, minimum days to express TLCV signs had a narrow range of 16-18 days.

4.3.4.2 Inheritance of TLCV resistance in *L. esc.* x *L. hir.* crosses

Variation in symptom intensity in infected young plants in small cups caused ambiguiety in discerning them into appropriate disease grade. Hence seedlings showing signs of TLCV were regarded as susceptible and the rest as resistant. Moderate TLCV symptoms were noticed in all the four hybrids of *L. esc.* x *L. hir.* (Fig. 5). The segregation for TLCV reaction, expected

Table 13. Days required for TLCV symptom expression and frequencies of susceptible and resistant genotypes in F_1, F_2, B_1 and B_2 generations upon vector inoculation

Genoyptes	Days required for TLCV sympton expression	Segreation		X^2/P value
		Suce.	Rest	
F_1: P_1 x P_3	16	13	0	
P_1 x P_4	16	8	0	
P_1 x P_5	19-24	11	0	
P_2 x P_3	15-17	17	0	
P_2 x P_4	15-16	11	0	
P_2 x P_5	19-23	9	0	
F_2: P_1 x P_3	15-29	*	*	
P_1 x P_4	16-30	69	46	
P_1 x P_5	18-28	40	42	
P_2 x P_3	19-34	*	*	
P_2 x P_4	16-26	74	45	
P_2 x P_5	17-25	64	69	
F_2 Total	-	247 253\$	202 196\$	0.280
B_1: P_1x (P_1 x P_4)	-	107	3	(9:7) P value
P_1x (P_1 x P_5)	-	21	1	(0.70-0.50)
P_2x (P_2 x P_4)	-	90	4	
P_2x (P_2 x P_5)	-	71	0	
B_1 Total	-	289	8	
B_2: (P_1x P_4) xP_4	-	13	7	
(P_1x P_5) xP_5	-	6	8	
(P_2x P_4) xP_4	-	15	8	
(P_2x P_5) xP_5	-	25	17	
B_2 Total	-	59	40	

P_1: A.Saurabh; P_2: A.Vikas; P_3:*L.pim.*; P_4:*L.hir*; '58; P_5: *L.hir*;'59

* Resistance in *L. pim*. was treated as a quantitative attribute.

\- Observation on days for TLCV symptom expression was recorded only in F_1 and F_2

\$ Expected ratio

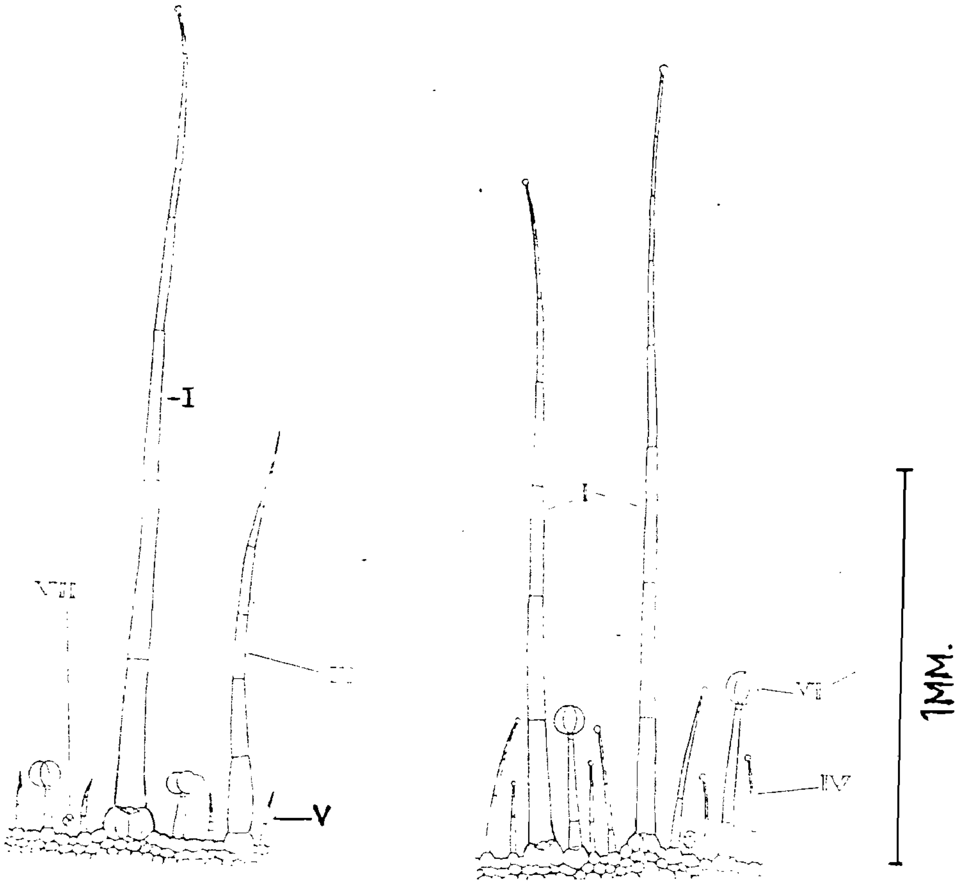
frequencies, their probability values showing goodness of fit of the observed frequencies are presented in Table 13. The segregation in the F_2 generation did not show good fit to any of the known ratios when individual crosses were examined. However, when the four crosses of L. esc. x L. hir. were put together, a good fit for 9:7 (susceptible : resistant) ratio was indicated by X^2 with P value 0.7-0.5. Segregation recorded in B_1 (susceptible parent x F_1) was in support of the complementary gene interaction shown by F_2. However, the eight out of 297 segregants in B_1 generation did not show TLCV symptoms, which possibly may be susceptible escapes. But, the limited B_2 (F_1 x resistant parent) progeny screened failed to uphold the assumed hypothesis based on F_2 and B_1 data.

4.4. EXPERIMENT IV. TRICHOMES AND VECTOR PREFERENCE

4.4.1 Trichome density

Leaf trichome density for L.esc. (A. Saurabh and A.Vikas), L.hir. ('58 and '59) and the four F1 hybrids between L.esc. x L.hir. was estimated. Trichome types I,IV,V, VI and VII (Fig. 17) for both adaxial and abaxial surfaces were counted.

Adaxial surface : Mean trichome density (number/$10mm^2$) and types for adaxial surface for eight genotypes is presented in Table 14. and for those trichome types that showed statistical difference are represented by bar



L.esc. *L.hir.*

Fig. 17. Trichome types of *L.esc.* and *L.hir.*

Table 14. Mean trichome density for 10mm adaxial leaf surface of L.esc., L.hir. and their F_1 hybrids.

Genotype	Mean leaflet length (cm)	Type I	Type IV	Type V	Type VI	Type VII	Total
A.Saurabh	5.16	8.13^{abc}	Absent	135.00	201.25	2.50	346.27^{bc}
A.Vikas	4.88	3.75^{a}	Absent	201.86	222.50	5.00	433.13^{c}
L.esc. (mean)	5.02	5.94	Absent	168.43	211.88	3.75	389.70
L.hir. '58	5.86	57.50^{d}	141.25	Absent	230.63	2.50	431.86^{c}
L.hir. '59	6.18	21.88^{c}	163.75	Absent	178.13	3.13	366.75^{bc}
L.hir. (mean)	6.02	39.69	152.50	Absent	204.38	2.82	399.31
A.Saurabh x L.hir. '58	5.85	15.00^{abc}	56.25	-	96.25	1.25	168.75^{a}
A.Saurabh x L.hir. '59	6.94	21.25^{bc}	165.00	-	183.75	1.25	371.19^{bc}
A.Vikas x L.hir. '58	5.92	14.38^{abc}	145.63	-	170.00	0.63	330.63^{bc}
A.Vikas x L.hir. '59	6.60	6.25^{ab}	85.63	-	171.88	3.13	266.88^{ab}
F_1 hybrids (mean)	6.33	14.22	113.13	-	155.47	1.57	284.36
C.D at 5%	NS	15.57	NS	*	NS	NS	149.29

In F_1 hybrid trichome Type IV and V were counted together.
Means followed by the same letter do not differ at 0.05 level of significance, as determined by LSD.
* 't' value : 0.38

diagram. The two entries of L.esc. and two of L.hir. did not differ significantly within the species (Fig.18). Highest trichome density was observed in A.Vikas ($433.13/10mm^2$) closely followed by L.hir. 58 ($431.86/10mm^2$). Among the F1 hybrids A.Saurabh x L.hir. '59 recorded maximum ($371.19/10mm^2$) mean trichomes per unit area and minimum ($168.75/10mm^2$) was recorded on leaves of A.Saurabh x L.hir. '58. The other two hybrids were at par. Foliage of F_1 hybrids possessed by far lower trichome density ($284.36/10mm^2$) than the parental species.

Among the five types of trichomes, differences were significant for Type I. L.hir'58 registered highest mean Type I (Fig. 19,22) trichomes of 57.5 for 10 mm^2 and was different from L.hir. '59. Mean number of Type I trichomes for $10mm^2$ in L.hir. was highest (39.69) and lowest in L.esc. (5.94), while in F_1 it counted 15.57. Other types of trichomes did not differ significantly among the species and hybrids studied. Type IV trichomes in L.esc. and Type V in L.hir. were absent, while both these types occured together on the foliage of F_1 hybrids (Fig. 25). Type IV trichome density for $10mm^2$ ranged from 56.25 (A.Saurabh x L.hir. '58) to 165 (A.Saurabh x L.hir. '59). Type V trichome (Fig. 23) were more in A.Vikas. Mean density of Type VI trichome was less ($155.47/10mm^2$) in F_1 hybrids (Fig.24) than either of the parental species. Type VII trichome was sparse

FIG.18 TOTAL TRICHOME DENSITY ON ADAXIAL LEAF SURFACE

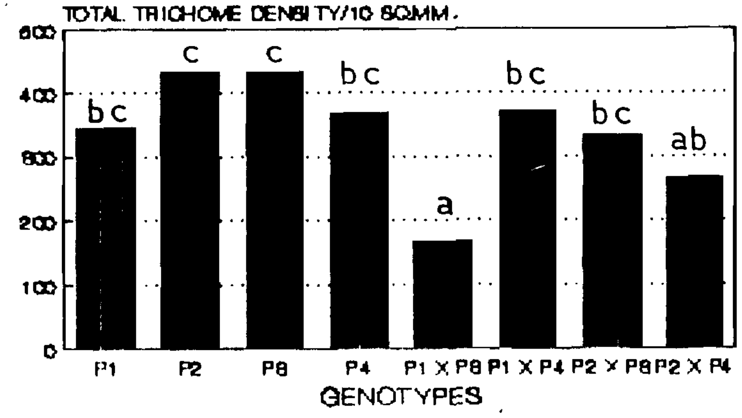
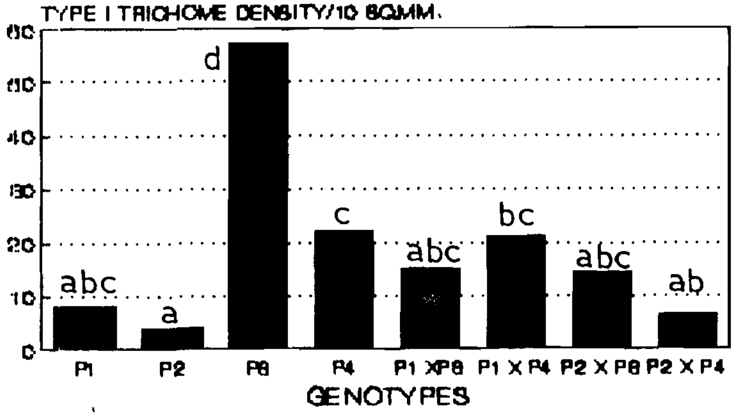


FIG.19 TYPE I TRICHOME DENSITY ON ADAXIAL LEAF SURFACE



NOTES: Fig. 18 and Fig. 19

P1 : A.Saurabh; P2 : A.Vikas; P3 : *L.hir.* '58 P4 : *L.hir.* '59

Genotypes representing the bars with the same letters are not significantly different ($P < 0.05$).

(0.63 to .5/10mm^2) in the species and the hybrids studied.

Abaxial surface : Table 15 provides data on trichome density for abaxial leaf surface. Total trichome density for 10mm^2 of leaf varied from 463.75 to 872.50 in the species and hybrids under study, but statistical analysis did not demonstrate significant difference among them. Maximum total trichome density for 10mm^2 was observed in L.hir. (773.78), followed by L.esc. (649.01), while in hybrids between these two species it measured 635.90.

Out of five types of trichomes, significant difference among the genotypes tested was revealed only for Type VI (Fig. 20) Type VII (Fig. 21). Type VI trichome did not differ within the species or hybrids. L.hir. recorded maximum of 126.88 Type VI trichomes for 10mm^2. In case of all the genotypes checked Type VII trichomes were at par excepting L.hir. '59 which counted highest number of trichome (8.75/10mm^2). Very low number of Type I trichomes were noticed in F_1 hybrids (0.94/10mm^2) and high in L.esc. (5.92/10mm^2). Type IV trichomes varied from 740.63 to 405 for 10mm^2 of abaxial leaf.

4.4.2 Per cent of different trichome types per unit area

Per cent of different types of trichomes is furnished in Table 16 for both the leaf surfaces.

Table 15. Mean trichome density for 10mm^2 abadxial leaf surface of L.esc., L.hir. and their F_1 hybrids.

Genotype	Mean leaf let length (cm)	Type I	Type IV	Type V	Type VI	Type VII	Total
A.Saurabh	5.36	3.73	Absent	562.50	53.75^a	2.50^a	622.46
A.Vikas	5.44	8.10	Absent	592.50	72.50^a	2.50^a	675.56
L.esc. (mean)	5.40	5.92	Absent	577.50	63.13	2.50	649.01
L.hir. '58	6.18	4.38	740.63	Absent	124.38^b	3.13^a	872.50
L.hir. '59	6.80	3.13	533.80	Absent	129.38^b	8.75^b	675.05
L.hir. (mean)	6.49	3.76	637.22	Absent	126.88	5.94	773.78
A.Saurabh x L.hir. '58	5.88	1.88	405.00	-	56.88^a	0.00^a	463.75
A.Saurabh x L.hir. '59	6.94	0.63	657.15	-	87.50^{ab}	0.00^a	746.88
A.Vikas x L.hir. '58	5.92	0.63	682.50	-	72.50^a	1.88^a	757.50
A.Vikas x L.hir. '59	6.60	0.63	488.75	-	83.75^{ab}	2.50^a	575.60
F_1 hybrids (mean)	6.34	0.94	558.35	-	75.16	1.10	635.90
C.D at 5%	NS	NS	NS	NS	50.91	4.87	NS

In F_1 hybrid trichome Type IV and V were counted together.

Means followed by the same letter do not differ at 0.05 level of significance as determined by LSD.

FIG.20 TYPE VI TRICHOME DENSITY ON ABAXIAL LEAF SURFACE

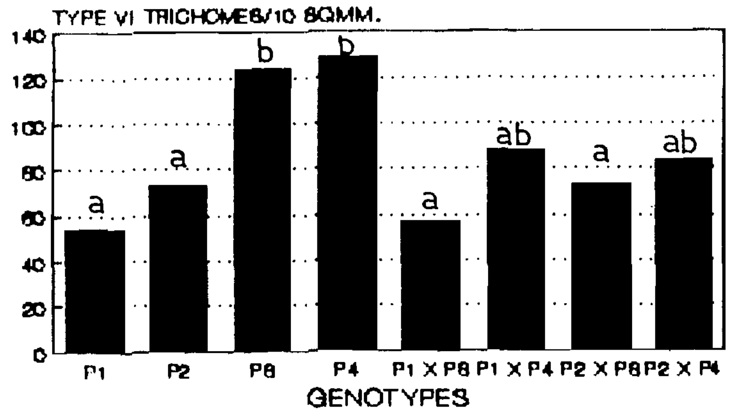
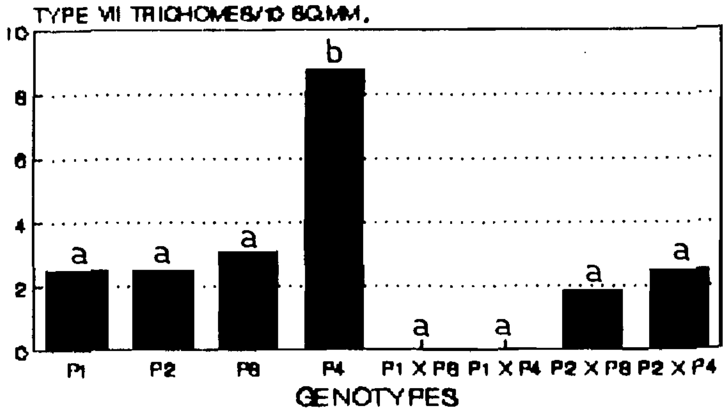


FIG.21 TYPE VII TRICHOME DENSITY ON ABAXIAL LEAF SURFACE



NOTES: Fig. 18 and Fig. 19

P1 : A.Saurabh; P2 : A.Vikas; P3 : L.hir. ' 58 P4 : L.hir '59

The bars with the same letters are not significantly different ($P < 0.05$).

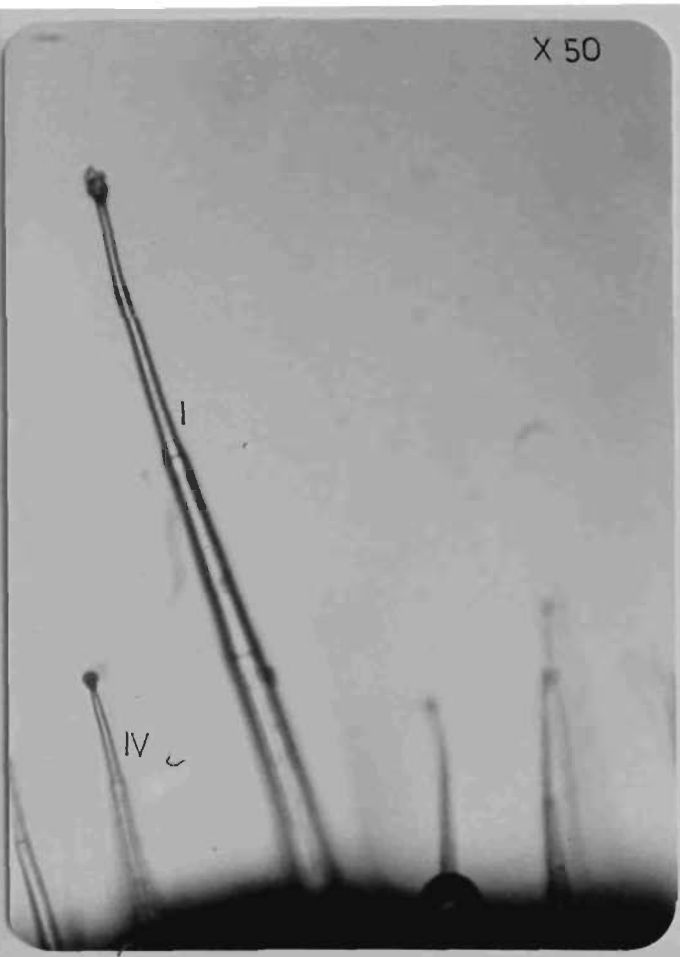


Fig. 22. Type I glandular trichome surrounded by Type IV in L.hir.

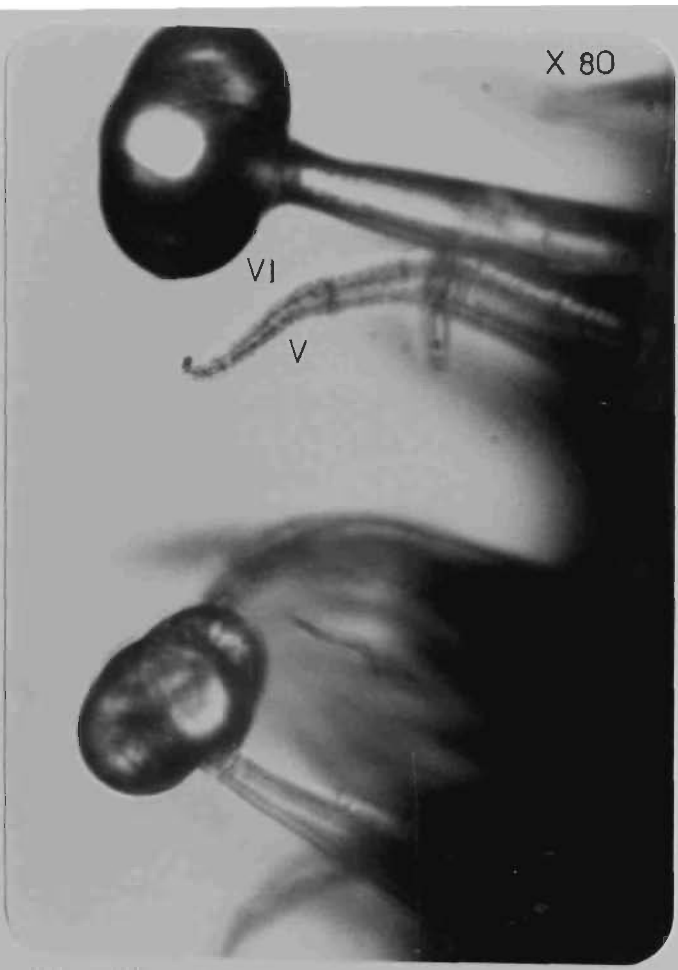


Fig. 23. L.esc. Type VI and Type V trichomes.

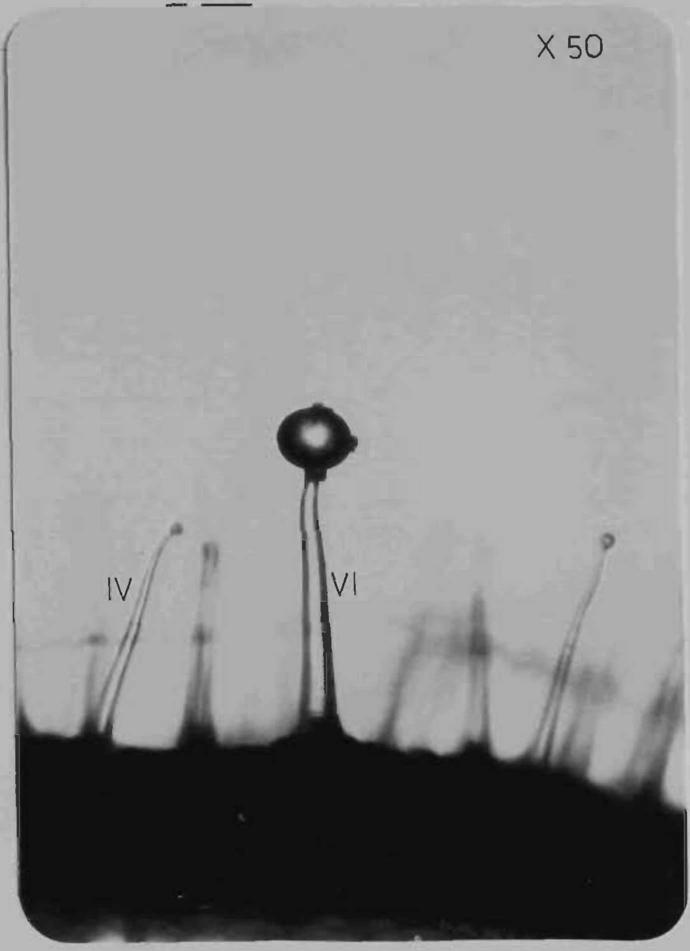


Fig. 24. L.esc. x L.hir. (F_1) Type VI trichomes.

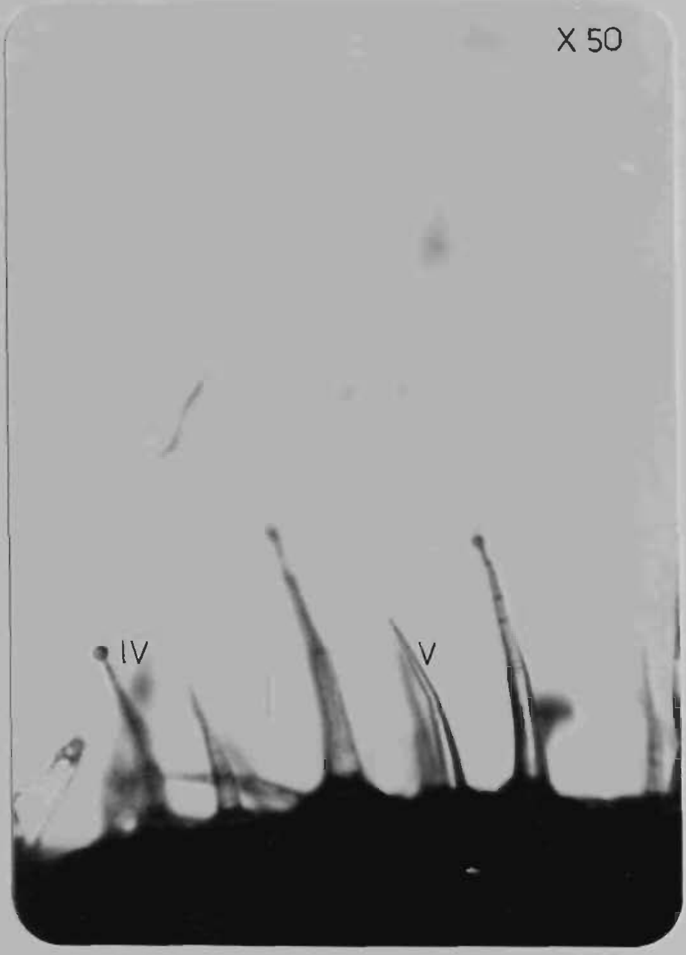


Fig. 25. L.esc. x L.hir. (F_1) Type V, IV trichomes.

Table 16. Per cent of different types of trichomes on leaf surfaces of L.esc., L.hir. and their F_1 hybrids.

	ADAXIAL					ABAXIAL				
Genotype	Type I	Type IV	Type V	Type VI	Type VII	Type I	Type IV	Type V	Type VI	Type VII
A.Saurabh	2.35	Absent	38.99	58.12	0.72	0.60	Absent	90.37	08.64	0.40
A.Vikas	0.87	Absent	46.60	51.37	1.15	1.20	Absent	87.71	10.73	0.37
L.esc. (mean)	1.50	Absent	43.22	54.37	0.96	0.91	Absent	88.98	09.73	0.39
L.hir. '58	13.31	32.70	Absent	53.40	0.58	0.50	84.89	Absent	14.26	0.36
L.hir. '59	5.97	44.65	Absent	48.57	0.85	0.46	79.08	Absent	19.16	1.29
L.hir. (mean)	9.94	38.19	Absent	51.18	0.71	0.49	82.35	Absent	16.40	0.77
A.Saurabh x L.hir. '58	8.89	33.33	-	57.03	0.74	0.41	87.33	-	12.27	0.00
A.Saurabh x L.hir. '59	5.72	44.45	-	49.50	0.34	0.08	87.98	-	11.71	0.00
A.Vikas x L.hir. '58	4.35	44.05	-	51.42	0.19	0.08	90.09	-	9.57	0.25
A.Vikas x L.hir. '59	2.34	32.08	-	64.40	1.17	0.11	84.91	-	14.55	0.43
F_1 hybrids (mean)	5.00	39.78	-	54.67	0.55	0.15	87.80	-	11.81	0.17

In F_1 hybrid trichome Type IV and V were counted together.

4.4.2.1 Adaxial surface : On the leaves of all the genotypes assessed Type VI (Fig. 23) trichome domiated. Density of this glandular trichome Type VI was followed by Type IV (Fig. 24), and a non glandular Type V (43.22%) in L.esc. (Fig.23). Per cent of Type I trichome (Fig.22) was very low in L.esc. (1.5%) and high in L.hir. (9.94%). Type VII trichome were sparse and were less than 1% of the total trichome present in both the species and their hybrids.

4.4.2.2 Abaxial surface : On the abaxial leaf surface Type IV trichomes in L.hir. (82.35%) and F_1 of L.esc. x L.hir. (87.80%) predomiated, while they were absent in L.esc. But in L.esc. preponderence of a non glandular trichome Type V (88.98%) was observed. Type I and VII trichomes on the abaxial surface were very sparse. Trichome density of these two types did not exceed 1 per cent in either of the species and hybrids studied. Trichome Type VI varied from 8.64 per cent (A.Saurabh) to 19.16 per cent (L.hir. '59) in the genotypes studied.

Thus Type I trichomes per cent was more on the adaxial leaf surface than on abaxial. On the adaxial surface trichome Type VI and on the abaxial surface Type V (L.esc.) and Type IV (L.hir. and hybrids) predominated. But proportion of glandular trichome Type VI were by far less on the undersurface of leaves than on the upper. On both the surfaces Type VII trichome was very sparse.

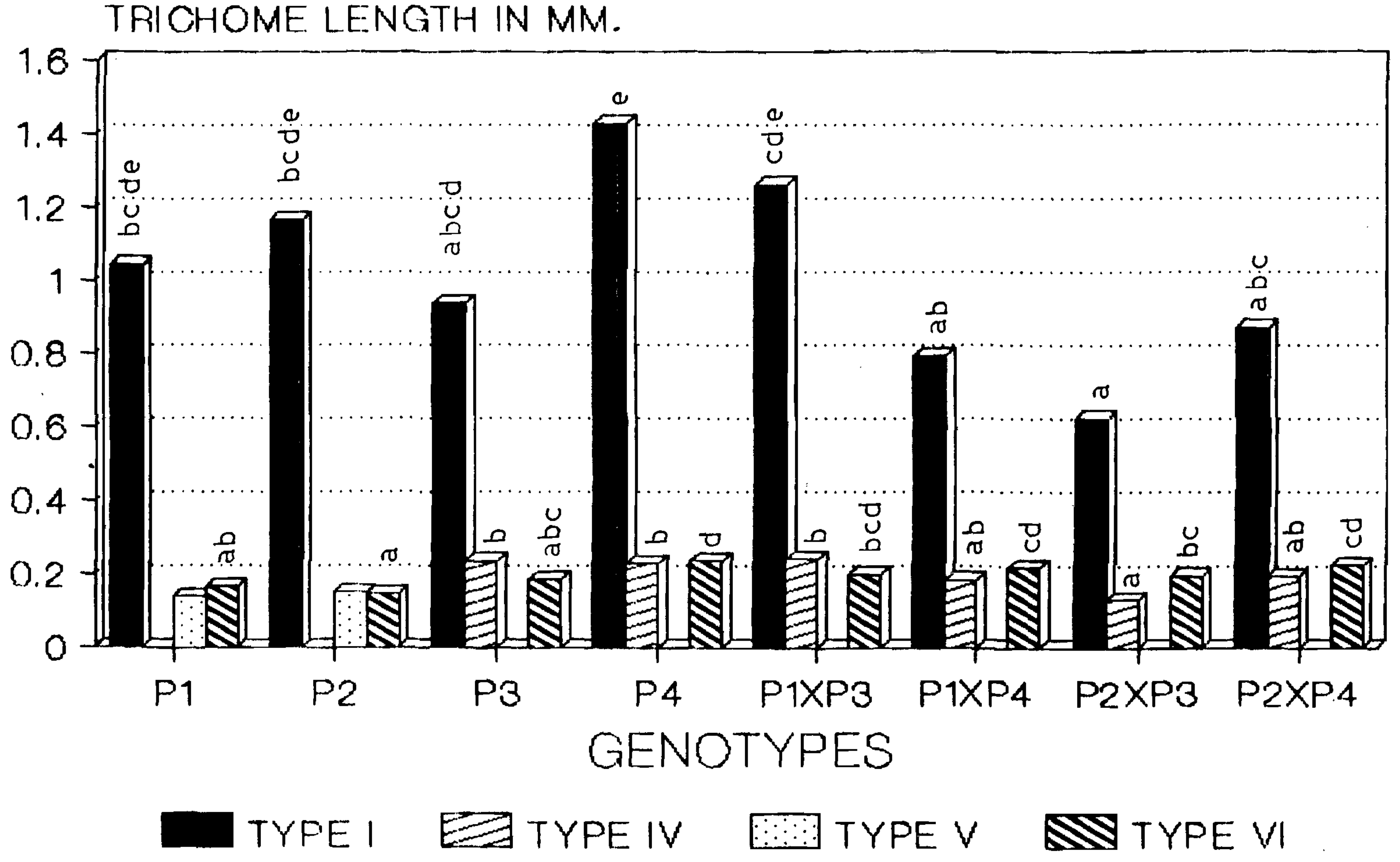
4.4.3 Trichome length

Adaxial trichome length for Type I,IV,V and VI is illustrated by plotting trichome length versus genotypes in Fig. 26. Significant variation in genotypes tested for mean trichome length was observed for Type I,IV and VI. Type I trichomes were the longest among the different types of trichomes observed on the leaf surfaces.

Type I trichomes were significantly longer and at par in L.hir. '59 (1.43 mm.), L.esc. and A.Saurabh x L.hir. '58. Rest of the genotypes excepting A.Saurabh x L.hir. '58 recorded shorter trichomes and the differences among them were not significant. Type IV trichome (noticed only in L.hir.) and in F1 L.esc. x L.hir. hybrids (reckoned along with Type V in the present study) measured 0.13 to 0.24 mm. A non glandular trichome Type V which is observed only in L.esc. measured 0.14mm. in A.Saurabh and 0.15mm. in A.Vikas. Length of Type VI trichome varied from 0.15 (A.Vikas) to 0.24 mm. (L.hir. '59). No significant difference was observed in hybrids for Type VI trichomes.

Length of trichomes on abaxial surface for Type I, IV, V and VI is also presented vide bar diagram (Fig. 27). Differences in mean trichome length were significant only for Type I. Longest trichomes (1.22 mm.) of Type I were observed in A. Saurabh, while in rest of the

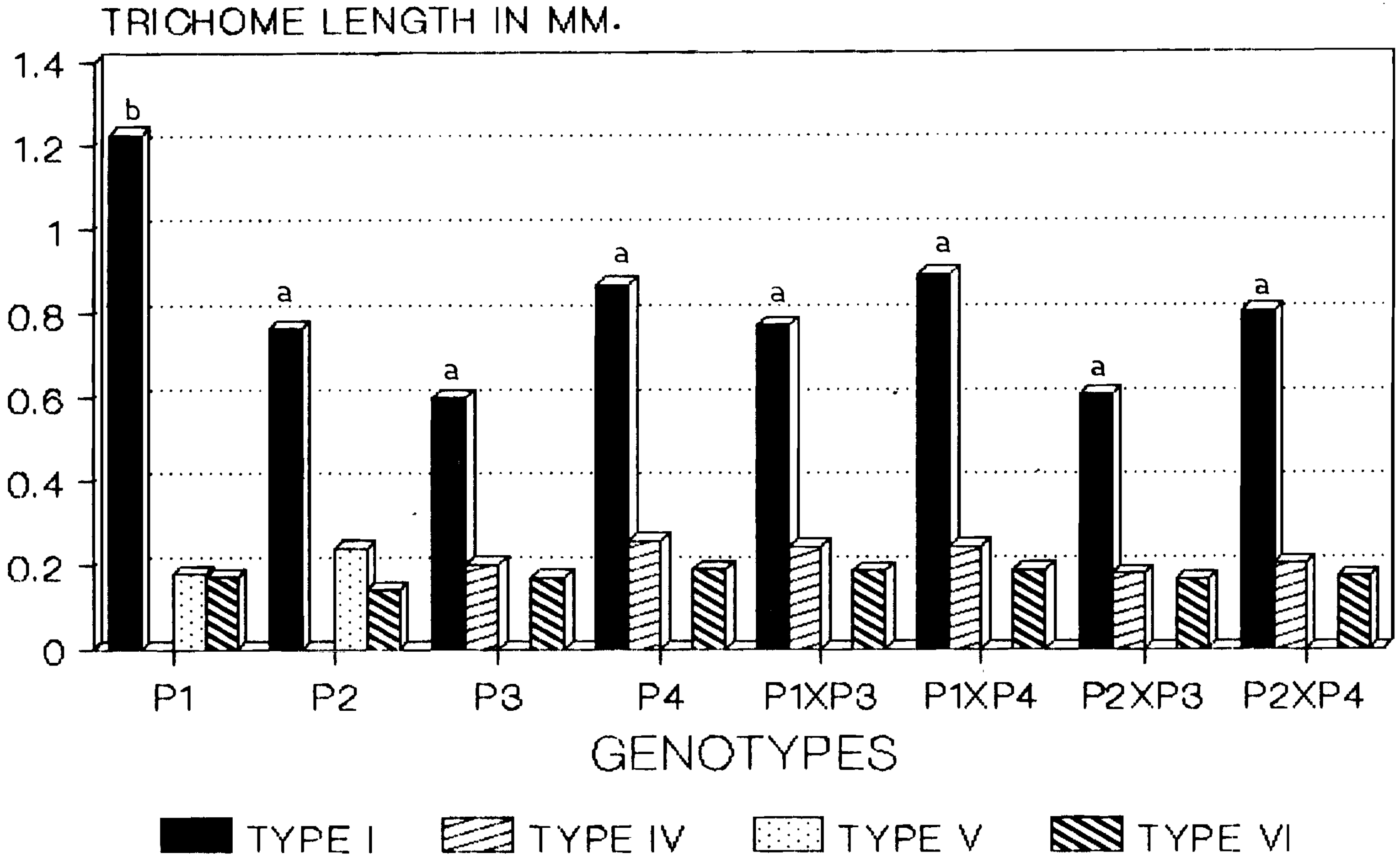
(adaxial surface)



P1-A. Saurabh; P2- A. Vikas; P-3- L. hir. '58;P4- L.hir. '59

Columns labelled with different lower case letters are different at P= 0.05 level of significance.

Fig.27 Trichome length in mm.
(abaxial surface)



P1-A. Saurabh; P2-A. Vikas; P3-*L.hir.*'58; p4- *L. hir.* '59
Columns labelled with different lower case letters are
different at P= 0.05 level of significance.

genotypes which were at par, the length ranged between 0.89 to 0.6 mm. Type IV trichomes were longest in L.hir. '59. Type V trichome length was highest (0.26mm.) in A.Vikas. Type VI mean trichome length which was also not significant, varied from 0.14 mm. in A. Vikas to 0.19mm. in A. Saurabh x L. hir. ('58 and '59) hybrids.

4.4.4 Whitefly preference

Differential preference of the B. tabaci for various tomato genotypes and generations involved in the present research were studied under field conditions.

4.4.4.1 L.esc. x L.pim. and L.esc. x L.hir. plots

The number of whiteflies collected in a day (6AM to 6PM) in the yellow pans with water was used to estimate the activity of the whiteflies in plots planted to L.esc. x L. pim. and L.esc. x L. hir. generations (field experiment II). The data collected from the six pans/plot on three dates (10 days interval) was averaged over dates and depicted graphically (Fig.28). Higher activity of the TLCV vector in case of L.esc. x L. pim. plot was evident from the graph.

4.4.4.2 L.esc. x L.pim. generations

Whiteflies were counted in the early morning (before 7 AM) on three randomly selected leaves representing one each from upper, middle and lower canopies of the plant in the six generations of L. esc.

Fig.28 Whitefly trapped in yellow plate/ plots of L.ESC.XL.PIM.and L.ESC.XL.HIR.

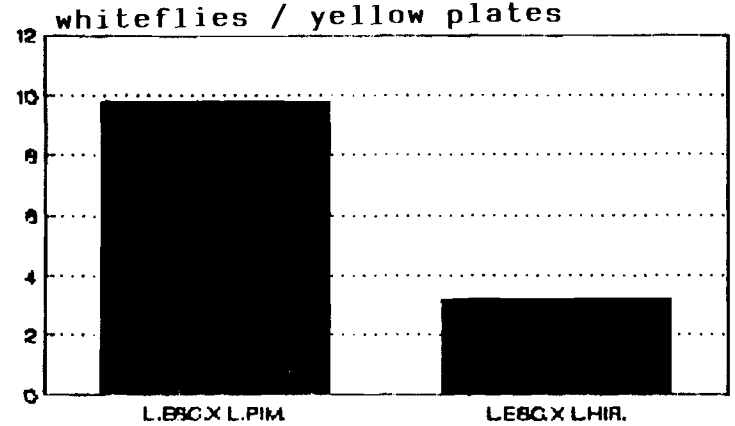
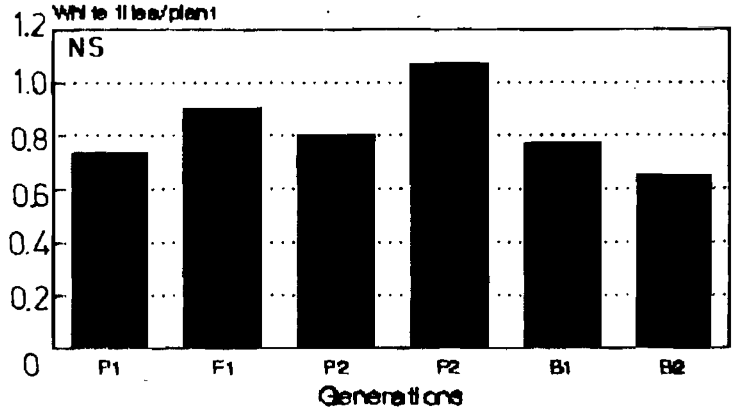
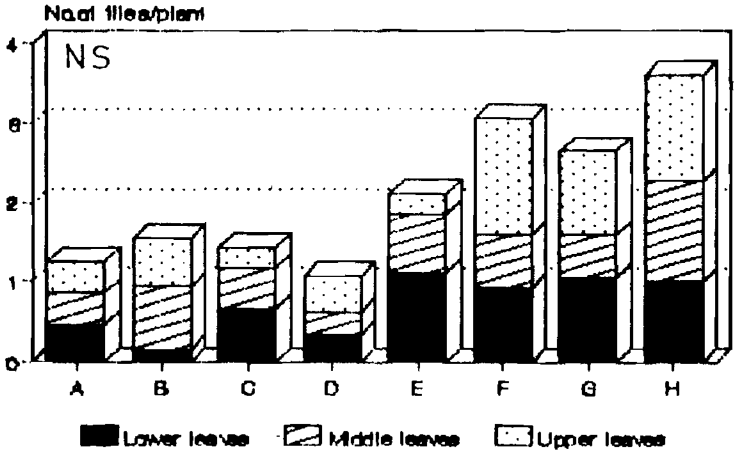


Fig.29 Average white flies /plant in L.ESC X L.PIM. crosses.



P1 -L. esc. (A. Saurabh +A.Vikas);
P2-L. pim. NS-Non significant at
P = 0.05

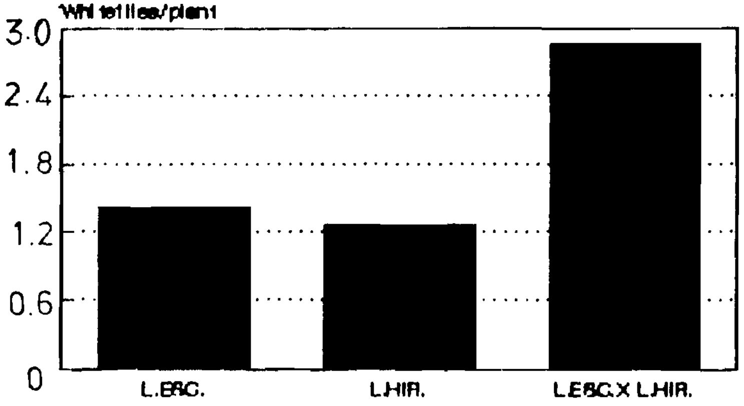
Fig.30 Mean whiteflies/plant in L.ESC X L.HIR. cross



A-A.Saurabh; B-A.Vikas;C-L. hir.'58;
D-L. hir.'59; E-(AxC); F-(AxD);
G-(BxC); H-(BxD).

NS-non Significant at P 0.05

Fig.31 Mean whiteflies/plant averaged over L.ESC.,L.HIR. and L.ESC.XL.HIR.



x L. pim. (field experiment II). The data gathered on three dates at an interval of ten days (Table 17) was averaged over species and was plotted by taking mean whiteflies/plant on 'Y' axis and generations on 'X' axis (Fig. 29). The statistical analysis of the data showed absence of significant differences among six generation for whitefly preference.

Table 17. B.tabaci per plant in six generations of A.Saurabh x L.pim. and A.Vikas x L.pim., averaged over three dates.

Generation	Entry	No. of plants studied	Mean flies/plant	
			entry	generation
P_1	A.Saurabh	15	1.00	0.74
	A.Vikas	15	0.47	
P_2	L.pim.	15	0.80	0.80
F_1	A.Saurabh x L.pim.	15	1.13	0.90
	A.Vikas x L.pim.	15	0.67	
F_2	A.Saurabh x L.pim.	60	1.10	1.07
	A.Vikas x L.pim.	60	1.03	
B_1	A.Saurabh x (A.Saurabh x L.pim.)	60	0.77	0.77
	A.Vikas x (A.Vikas x L.pim.)	60	0.76	
B_2	(A.Saurabh x L.pim.) x L.pim.	60	0.70	0.65
	(A.Vikas x L.pim.) x L.pim.	60	0.60	

Date of recording observation : 12.4.90 ; 22.4.90 and 2.5.90

No significant difference at (P = 0.05) was demonstrated by LSD analysis of logarithamically transformed data.

4.4.4.3 L. esc. x L.hir. crosses

B. tabaci preference was also investigated in L. esc., L.hir. and F1 hybrids (Table 18). The whitefly counts taken as in the above study, on upper, middle and lower leaves on three dates indicated no significant difference for whitefly preference among genotypes

Table 18. Number of B.tabaci per plant under field conditions in L.esc., L.hir. and L.esc. x L.hir. F_1 hybrids, averaged across replications and three dates of observation.

Entry	Canopy position			
	Upper	Middle	Lower	Total
A.Saurabh	0.39	0.39	0.47	1.25
A.Vikas	0.61	0.77	0.17	1.55
L.esc. mean	0.50	0.58	0.32	1.40
L.hir. '58	0.27	0.50	0.67	1.44
L.hir. '59	0.44	0.28	0.35	1.07
L.hir. mean	0.36	0.39	0.51	1.26
A.Saurabh x L.hir. '58	0.25	0.75	1.11	2.11
A.Vikas x L.hir. '58	1.05	0.55	1.05	2.65
A.Saurabh x L.hir. '59	1.46	0.67	0.93	3.06
A.Vikas x L.hir. '59	1.34	1.27	1.00	3.61
L.esc. x L.hir. mean	1.03	0.81	1.02	2.86
Mean	0.73	0.65	0.72	#

\# Differences were not significant at 5% level either for entries or canopy positions as indicated from analysis of logarithmically transformed data.

examined (Fig. 30). However, when averaged over species and the hybrids (Fig. 31), the F1 hybrids recorded higher mean whiteflies/plant (2.86) than parents - L.esc. (1.4) and L.hir. (1.26). The mean whiteflies across three dates and eight entries, on upper, middle and lower canopies was 0.73, 0.65 and 0.72 respectively and showed no significant difference. As this experiment was not carried out in summer the number of whiteflies observed on L.esc. was less than that recorded for the same species in field experiment II.

4.4.5 Whitefly mortality and oviposition

Whitefly mortality, eggs laid in groups of, egg size and pupal cases were studied in L. esc., L. hir. and F_1 hybrid. As the trichome density becomes sparser with leaf expansion, 3rd and 10th leaves (representing dense and sparse respectively) were selected to study the above aspects. However, the 3rd leaf of L. hir. was not used for eggs/group and egg size studies, as the whiteflies failed to survive in this treatment.

4.4.5.1 Mortality of whiteflies : The bar diagram obtained from the data furnished in Table 19 for whitefly mortality for L.esc., L.hir. and F_1 is shown in Fig. 32. Casuality of whiteflies was lowest on 3rd and 10th leaves of L. esc. (5.7 and 20% respectively) and none of the whiteflies were found to be entangled and dead in leaf trichomes. Almost equal number of

Fig.32 Whitefly mortality as influenced by species and leaf position.

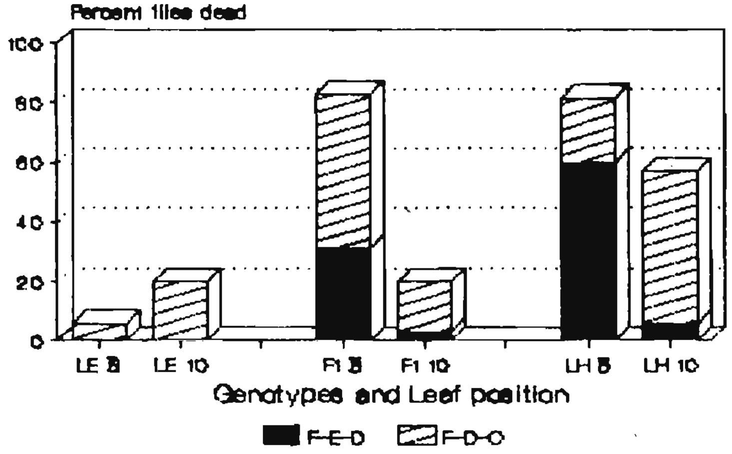


Fig.33 Average number of eggs/group

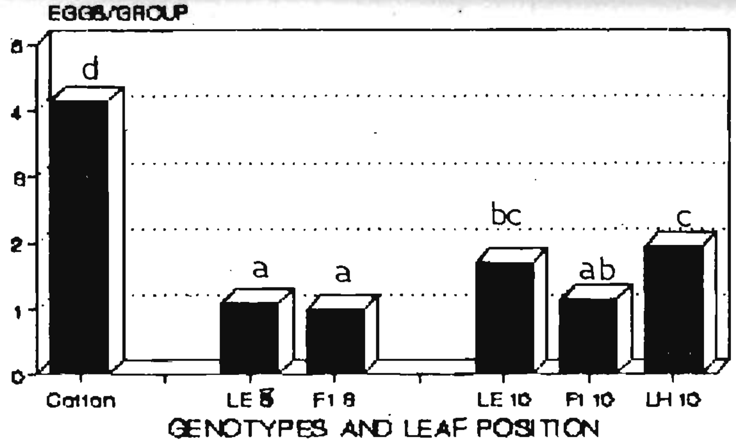


Fig.34 Leaf position and whitefly egg size (mm).

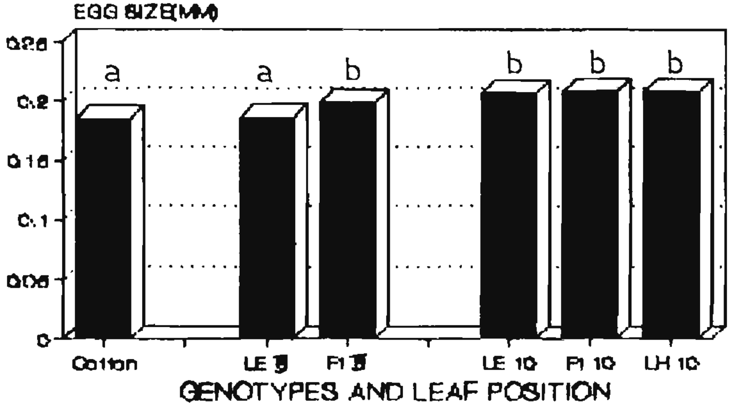
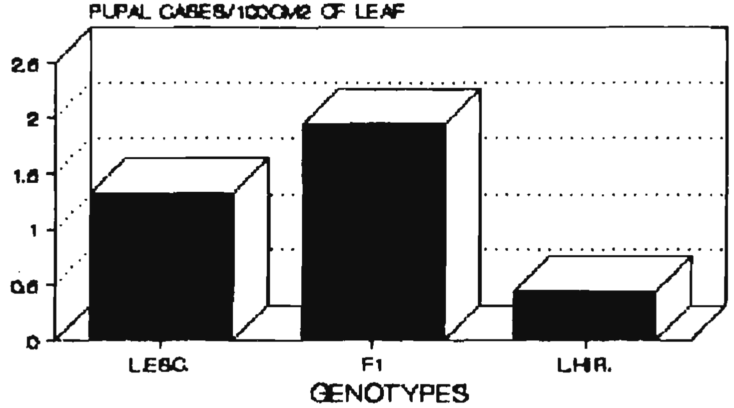


Fig.35 Whitefly pupal cases averaged over L.ESC.,L.HIR.and F1 hybrids.



NOTES: Fig.32 to 34.

LE3- L.esc.(3rd leaf) ; LE10- L.esc.(10th leaf) LH3- L. hir.(3rd leaf); LH10-L.hir. (10th leaf)

F-E-D- flies trapped in leaf trichomes and dead; F-D-O- flies dead otherwise

Columns labelled with different letters are different at P= 0.05

whiteflies died on 3rd leaf of *L.hir.* and *L.esc.* x *L.hir.*, but whiteflies that entangled and dead were far more (60%) in *L. hir.* than *L. esc.* x *L. hir.* (31.4%). While on 10th leaf with sparse trichome density, the total whiteflies dead was 57.1 per cent in *L. hir.* and it was as low as 20 per cent (equal to *L.esc.*, 10th leaf) in case of *L. esc.* x *L. hir.* and the whiteflies caught and dead were also low (2.9%) in the hybrid.

Table 19. Per cent whitefly mortality on *L.esc.* *L.hir.* and F_1 (*L.esc.* x *L.hir.*) leaves in the laboratory

Species	Flies dead (%)			
	Total		Entrapped in trichomes and dead	
	Trichome density*		Trichome density*	
	Sparse	Dense	Sparse	Dense
L.esc.	05.71	20.00	00.00	0.00
F_1 (*L.esc.* x *L.hir.*)	82.86	20.00	31.43	2.86
L.hir.	81.43	51.14	60.00	5.71

Per cent mortality out of a total of 35 whiteflies ; two replicated blocks

* Third and tenth leaves represented the sparse and dense trichome densities respectively.

4.4.5.2 Eggs per group : Mean number of eggs/group (Table 20 and Fig. 33) in cotton was maximum (4.15) and significantly different from *Lycopersicons*. Differences were significant for 3rd and 10th leaf of L. esc. but not for L. esc. x L. hir. for number of eggs/group. Eggs/group were at par in case of 10th leaf of L. esc. (1.7) and L. hir., (1.95), but the latter was significantly higher than 10th leaf of F_1 (1.15). As the whiteflies did not survive on 3rd leaf of L. hir., it was not considered for this study.

4.4.5.3 Whitefly egg size : Table 20 presents length of the eggs laid on L.esc., L.hir. and F_1 leaves and Fig.34

Table 20. Mean eggs/group and egg length of eggs oviposited on leaves of L.esc., L.hir. and L.esc. x L.hir. in the laboratory.

Species	Trichome density■	Eggs/group		Egg length (mm)	
		Mean$^{\#}$	SE	Mean$^{\#}$	SE
L.esc.	Dense	1.10^{a}	± 0.07	0.186^{a}	± 0.005
	Sparse	1.70^{bc}	± 0.06	0.207^{b}	± 0.005
F_1 (L.esc. x L.hir.)	Dense	1.00^{a}	± 0.00	0.199^{b}	± 0.005
	Sparse	1.15^{ab}	± 0.08	0.208^{b}	± 0.004
L.hir.	Dense		\$		\$
	Sparse	1.95^{c}	± 0.34	0.208^{b}	± 0.005
Cotton		4.15^{d}	± 0.71	0.185^{a}	± 0.004

Within each coloumn, means followed by same letter do not differ at the 5% level of significance, as determined by LSD. LSD = 0.58 eggs/group ; LSD = 0.012 mm egg length.

■ Third and tenth leaves represented sparse and dense trichome density respectively.

Mean of 20 observations

$ Large number of flies dead within 24 h of release.

illustrates the same graphically. Egg length (excluding stalk) did not vary between those laid on cotton (0.185mm) and L. esc., 3rd leaf (0.186mm). But eggs on L. esc. (10th leaf), L. hir. (10th leaf), L. esc. x L. hir. (3rd and 10th leaves) were longer and at par.

4.4.5.4 Pupal case count : Whitefly pupal cases were counted on several older leaves in the field and was reduced to 100 cm^2 of leaf in L.esc.xL.hir. crosses. Observations recorded for A. Saurabh, A. Vikas, L. hir. '58 and L.hir. '59 , the four Fl hybrids were averaged over species and hybrids, and graphically presented in Fig. 35. Fl recorded maximum pupal cases (1.95) and L. hir. least (0.45) for $100cm^2$ leaf area.

4.5 EXPERIMENT, V. ISOZYME ANALYSIS

Analysis of isoperoxidase was performed on the young leaf. Leaf samples of four parents and six Fl hybrids were extracted and electrophoresed on polyacrylamide gel stained for isozyme peroxidase. Zymogram and its schematic representation , considering migration distances nearest to 0.05 mm, is shown for seven genotypes including diseased A.Vikas in Fig. 36 and 37 respectively.

A total of 5 bands distinguished were assigned 3 zones as illustrated in Fig.37. Often, isozyme coded by alleles of a single locus band within a specific region of the gel. The number for anodal peroxidase (Prx) loci were assigned, starting at anodal end and moving towards the

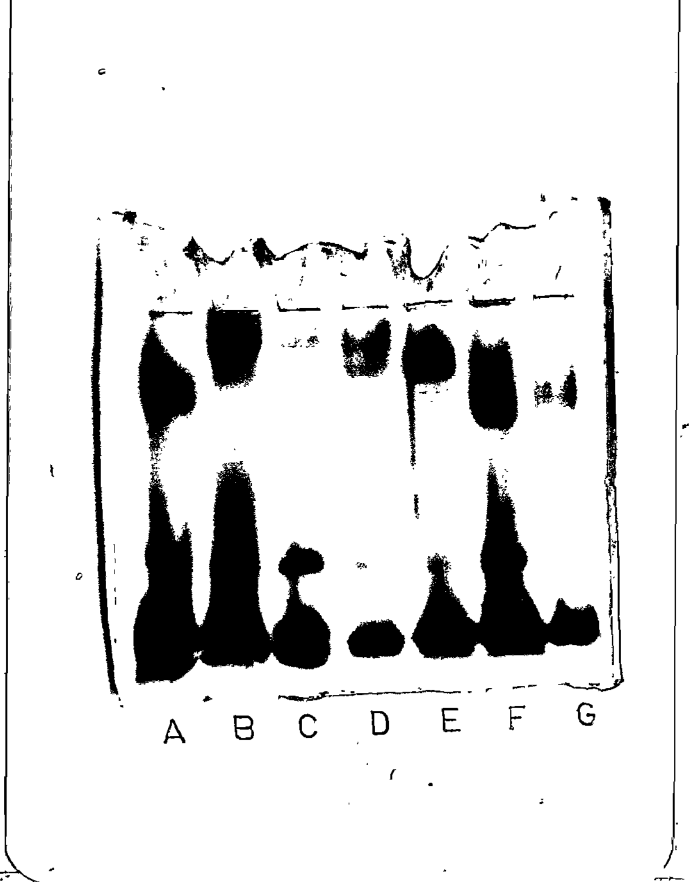


Fig. 36 Polyacrylamide gel zymogram for isoperoxidases of L.esc., L.hir. and F_1 hybrids.

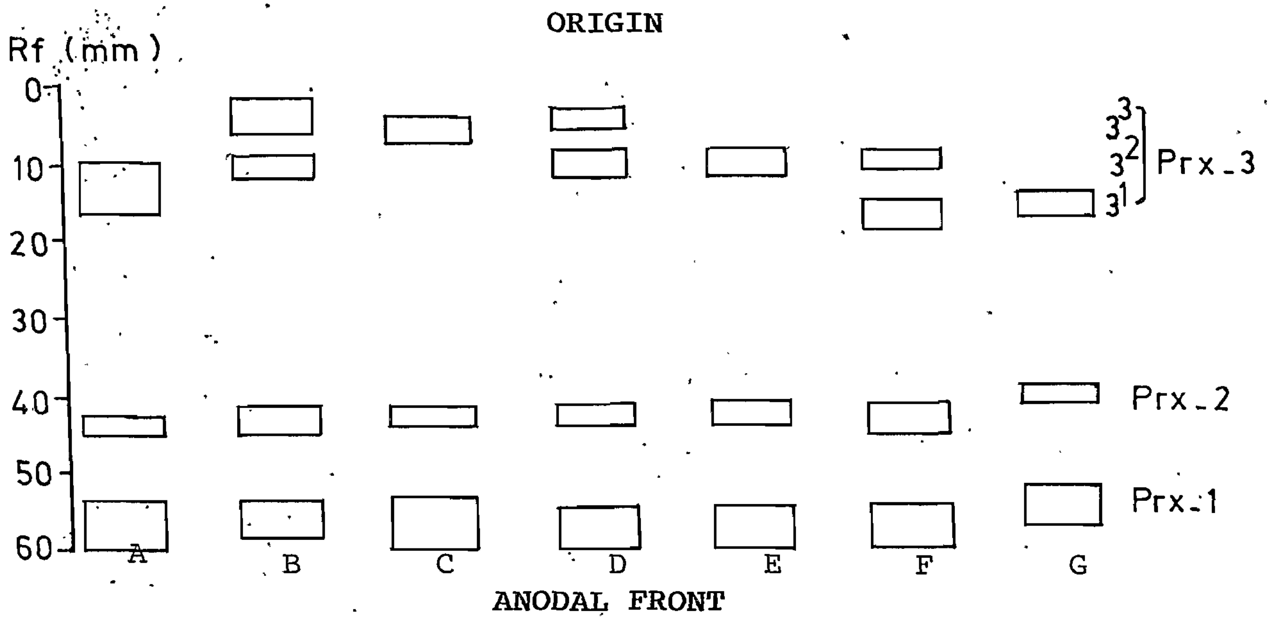


Fig. 37 The schematic illustration of the zymogram shown in the top figure (36).

Legend for Fig. 36 and 37.

A - TLCV affected A.Saurabh; B-A.Vikas X L.hir. '58; C- L.hir. '59 ; D-A.Vikas X L.hir.'59; E-A.Vikas ;F-A.Vikas X L.pim. G-L.pim.

origin and the alleles were distinguished by subscripts in case of polymorphic locus (Prx3).

Three major anodal peroxidase bands,Prxl,Prx2 and Prx3 were resolved for the genotypes examined. Each pair of parents differed from one another at one peroxidase isozyme (Prx3) banding pattern, which allowed identification of hybrids by this method. The hybrid nature of the Fl plants was also seen from Fl zymograms wherein bands found in both the parents were represented. The Fl hybrid A.Vikas x L.pim. (lane F) were characterised by four bands viz., Prxl, Prx2,$Prx3^1$ and $Prx3^2$,the first two being common to both the parents while $Prx3^1$ contributed from L.pim. (lane G) and $Prx3^2$ from A.Vikas (lane E). Likewise,A.Vikas x L.hir. '59 (lane D) also exibhited bands Prxl,Prx2,$Prx3^2$ and $Prx3^3$, the last mentioned band was due to L.hir. '59 (lane C). Similar but not so distinct, banding patterns were also observed in case of A.Saurabh and L.hir. '58. Thus L.esc.,L.pim. and L.hir. were found to possess uniform banding pattern for Prxl and Prx2.

The increased activity of peroxidase in the TLCV infected plants was evident from the dense staining, thick bands recorded in the diseased A.Saurabh (lane A).

DISCUSSION

V DISCUSSION

Without questions, built-in host plant resistance is by far the most important disease control strategy. This is all the more felt in case of virus deseases as nothing parallel to a fungicide is available for field use to check virus. Once a sensitive variety has become infected in the field by virus, there is little that can be done to control. Besides, application of chemicals for the control of vector often has a low efficacy in checking the virus spread.

Host plant resistance for disease is a rule and not an exception. Resistance breeding essentially involves (a) identification of stable resistant source and (b) its successful introgression into commercial variety. The full potential of available resistance can only be realized if the mechanisms are understood at the genetical and biochemcial levels.

Genetically, host susceptibility and resistance on the one hand, and virus pathogenicity and virulence on the other, cannot be considered in isolation. Major aim of any study of genetic mechanisms must be to find out how the host and virus systems interact, or why they may fail to do so. Breeding for resistance genes in the host, and the resulting selection pressure on the pathogen for matching virulence genes, has led to the co-evolution of gene-for-gene interactions. This situation is quite clear with fungal pathogens but with viruses it is not so well known. Similarly vertical and horizontal resistance concepts that describe host pathogen interaction introduced by Vanderplank (1963), are not extensively tested with viruses (Fraser, 1987).

The Tomato. It is the most popular and widely consumed vegetable crop of India covering an area of 83,000 ha with an annual production of 50,000 tonnes (Vishnu, 1990). It was domesticated just in the last century. Tomato is a member of nightshade family Solanaceae, and the genus *Lycopersicon*, which contains nine species of which all except *L. esculentum* are strictly wild, but all are diploids with n = 12 chromosomes. Numerous wild forms of tomato are still found in Andes (Peru), Equador, Bolivia and Gallapagos. The cultivated tomato has self-fertilizing mode of reproduction and has been favoured crop for genetical studies.

Tomato leaf curl virus. Cultivation of tomato, principal vegetable of India, has been endangered by TLCV to the extent that several farmers avoid taking crop especially in summer (January to May) when population of *B.tabaci*, that vectors this geminivirus in a peristant manner becomes dense and at times results in abandoning of whole fields prior to harvest. Several crop plants and weeds serve as a good reservoir of the virus and vector. Curling and twisting of leaves resulting in reduction in leaf size, extreme stunting, bushy appearence and chlorosis, are typical symptoms of the disease caused by TLCV (Sastry and Singh, 1973; Saikia and Muniyappa, 1989a). Apart from great biomass loss, the normal flowering, anthesis, fruit-set will be adversely affected by TLCV. The two important yield components, fruit number and average fruit weight are drastically influenced in TLCV affected plants resulting in sharp decline in plant yields.

Attempts to reduce the disease incidence based on limitation of vector population by chemical control, soil mulching with yellow polythyelene sheets to attract and kill the whileflies, and physical barriers like netting often are ineffective (Saikia and Muniyappa, 1989a). Consequently, host resistance is an attractive option to augment tomato yields. The development and cultivation of resistant varieties offers the most technically feasible, environmentally sound and economical means of disease control. But, development of TLCV resistant cultivars is still awaited despite the disease was reported more than four decades ago by Vasudeva and Sam Raj (1948).

However, progress in this enterprise has understandably been modest, notwithstanding recognition of seriousness of TLCV, benefits of built in resistance and doners for resistance reported. Several technical difficulties in evaluating virus/vector resistance by early and rapid tests, scant understanding of basic mechanisms underlying TLCV resistance, genetic features not formally worked out and limited number of breeders addressing the problem account to some extent for the lag in evolving TLCV resistant cultivars.

Scope of this investigation. The principal concern of the present investigation was to identify a stable tomato leaf curl virus resistant source, initiation of its transfer to the cultivated tomato, to study the genetics of TLCV resistance, role of trichomes in vector resistance and understanding isozyme pattern in resistant/susceptible lines. Research was not focused on several other complementary aspects of TLCV resistance breeding like host-pathogen genetic systems at work, reaction to several TLCV isolates, isozyme tagging with TLCV resistance etc. The study therefore does not pretend to be a complete one from this stand point.

Terminology. There has been some ambiguity and confusion over terminology concerning plant - virus interactions. It is therefore necessary to explain such terminology like resistance as perceived in the context of the present investigation.

A plant in which a virus multiplies, and may cause visible disease symptoms, is regarded susceptible. The plant lacks resistant genes. Resistance is defined here very broadly as any inhibition of virus multiplication, or of its pathogenic effects on the host. Resistant individuals contain a gene or genes conferring resistance to TLCV, hence is heritable and is of interest in resistance breeding. Plants which support pathogen multiplication without pronounced disease symptom are referred to as tolerant or partially resistant. The usage of term immunity is not adopted here.

5.1 TLCV RESISTANCE SCREENING

5.1.1 Identification of TLCV resistant lines

The first step in an endeavour of development of resistant varieties is identification of stable resistant lines, and that is where the present investigation makes a begining. Tests employed for disease resistance screening necessarily have to be rigorous ones, lest a susceptible escapes creep in undermining subsequent resistance breeding programmes.

Bannerjee and Kalloo (1987a) reported that the sources of TLCV resistance recorded by Nariani and Vasudeva (1963), Varma *et al.* (1980) and Joshi and Chaudhury (1981) were no longer resistant. This may be due, among other factors, to faultless and thorough techniques not applied while screening. Such care as use of ELISA or back-inoculation to evaluate the symptomless carriers, graft-inoculation tests when *L. hir.* lines were involved, and progency tests to ensure the genetic nature of resistance were often lacking in the above referred studies.

Despite, Banerjee and Kalloo (1987a) observed that there was non-preference for whiteflies in L. hir. accessions studied by them. They employed vector inoculation for identification of TLCV resistant source.

Keeping the possible pitfalls in mind a comprehensive TLCV screening strategy was conceived with scrupulous approaches including assaying of test genotypes for virus to eliminate symptomless reactants and identify absolute genetic resistance.

5.1.2 Search material for TLCV resistance

Breeders maintain that in seeking specifically desired characters like disease resistance the easiest source of germplasm should be exploited first, the most distantly related forms as a last resort. Cultivated tomato species, L.esculentum, although represents several improved cultivars, F_1 hybrid varieties, obsolete cultivars and local types, but none is known to possess genes for TLCV resistance (Kalloo and Banerjee, 1990). Thus the option lies in searching for resistance in the related wild Lycopersicon species. Further, considering wild types in resistance scanning programmes is also justified as they rarely have epidemic diseases. This is because in nature both host and pathogen survive together in a complex equil ibrium and neither dominantes (Simmonds, 1979).

The genus Lycopersicon is relatively small with nine species (Warnock, 1988) having immense genetic reserve from which commercial tomatoes (L.esc.) can benefit. Resistance to many diseases and arthropods; tolerance to such environmental stresses as temperature extremes, deficit and excessive moisture and salinity; improved fruit quality in respect to pigment, sugar, acid, nutritional qualities; and an additional series of useful morphological and physilogical characters have been identified in these species (Rick, 1982). To exploit these potential resources comprehensively would be an enormous task. However, significant improvement has already been brought about by improving fruit quality and resistance to many diseases (Rick, 1987). To date resistance to 30 different diseases of cultivated tomatoes has been detected in wild species, and of these, 16 resistances have been bred into commercial cultivars (Rick and Yoder, 1988).

5.1.3 Screening techniques

Selected tomato cultivars and Lycopersicon species (Table 1) were subjected for a preliminary field screening during January-May (summer). High temperature, low humidity and low rainfall during summer coincides with the steep increase in the B.tabaci population and TLCV infection touching 100 per cent (Saikia and Muniyappa, 1989a). A strong positive correlation (r = +

0.970; P = 0.01) between the incidence of TLCV and the whitefly population density was recorded by these workers. Absence of any control measures like insecticidal spray, high incidence of the vector in the summer, several alternate crops and weeds hosting the vector and virus that surrounded the screening plot sown intermittently with spreaders (susceptible varieties) simulated an epiphytotic situation, and let no way - out for susceptible escapes. Diagnosis were made based on symptomatology and seralogical tests. In field, visual assessment for TLCV reaction was made with a premise that other viruses if any did not interfere in TLCV expression. Lines found promising for TLCV resistance based on preliminary field evaluation were re-grown in the next summer and ELISA carried out to locate symptomless carriers, and to discern TLCV from other possible viral infection that produce symptoms akin to or overlap TLCV symptoms. In order to ensure the validity of results obtained under field screening, whitefly - mediated inoculation of TLCV was resorted to under controlled conditions. This involved feeding of *B.tabaci* on TLCV infected *L.esc*. and later allowing viruliferous whiteflies to feed on test genotypes for inoculation. It was observed in *L.hir*. accessions, several whiteflies died due to entanglement in leaf trichomes or otherwise, resulting in dubious transmission of TLCV. This necessiated to seek further evidence for TLCV resistance of L.hir. by attempting to cause infection by graft inoculation.

Test genotypes were wedge grafted on the TLCV. infected L.esc. that displayed typical symptoms. Subsequently scions were examined by ELISA for virus antigen. Translocation of the virus from the stock was also verified by testing scion.

Simply observing the reaction of a given accession to imposition of TLCV stress is an inadequate basis upon which to conclude that useful heritable variation exists. Progency test, therefore, was conducted to make sure the true-to-type nature for reaction to TLCV.

5.1.4 Source of resistance

The present investigation for TLCV resistance was limited to L.esc. to which cultivated tomato belongs and three more exotic speices viz. L.pim., L.hir. and L.per.

The results obtained from TLCV screening indicated absence of viable resistance in cultivated species. L.pim. exhibited tolerance (moderate symptoms) in field, after graft and vector inoculation; L.hir. produced no symptoms both in field and even by vector inoculation. However, of the two accessions of L.hir., one accession ('58) showed mild signs of TLCV following grafting ; L.per. neither in field inspection nor upon grafting exhibited disease sensitivity.

When leaf samples from summer plantings were assayed by ELISA, very high virus titer for L.esc., weak reaction for *L.pim*, and negative reaction for *L.hir.* (both '58 and '59) and *L.per.* were revealed. Scions of *L.pim.* gave weak, *L.hir.* ('58) very weak and *L.hir.* ('59) negative ELISA absorbance values. Thus a good correspondence between disease symptoms and ELISA absorbance values was noticed. Further, based on the virus titer it appeared that genotypes *L.esc.* provided the best substrate for virus multiplication. In *L.pim.* although virus could spread systam .ically, but it showed relatively reduced virus replication and symptom severity. *L.hir.* ('58) also greatly supressed virus multiplication, but to levels which can still be detected. It appeared *L.hir.* ('59) and *L.per.* failed to support virus replication.

L.esc. cultivars were not grafted as they clearly confirmed their susceptibility to TLCV under field conditions and upon vector inoculation.

The above results provide evidence for the presence of resistant and tolerant genes for TLCV in *L.hir.* and *L.pim.* respectively. *L.pim.* unlike sensitive *L.esc.* could contain the virus. Affected *L.pim.* showed moderate symptoms, low virus titer, almost normal flowering, fruiting and growth. No notable difference was observed between plants infected by TLCV and those that had escaped infection i.e., healthy plants. But in TLCV affected *L.esc.*(A.Saurabh and A.Vikas), especially when infection occurred in early stage of plant growth, flower development and anthesis were seriously hampered, often producing no fruits worth harvesting (Fig. 1). Moreover, the mean number of days required for TLCV symptoms to appear was more by 2 weeks in L.pim. as was observed both in field and in greenhouse after vector inoculation. Thus it can be a good donor for TLCV tolerance. Kalloo and Banerjee (1990) also reported TLCV tolerance in L.pim, 'A 1921', and developed five breeding lines using this source. Same workers earlier (Banerjee and Kalloo, 1987a) reported resistance source for TLCV in two lines of L.hir. and one line of L.pim. and one line of L.per.

Scoring TLCV exclusively based on symptoms often is deceptive. At times plants observed to be free from TLCV, suddenly, at a later stage of plant growth may develop symptoms, and lines found to be free from symptoms may produce progenies developing TLCV symptoms. It is noteworthy that L. hir. ('58) remained free from infection both in summer planting and upon vector inoculation tests, but took mild infection during graft inoculation and even the ELISA of scion gave very weak reaction. This indicated grafting followed by ELISA is more trustworthy and helps identify true resistance. Possibly, because grafting transmits an intense and heavy dose of virus into the test genotype which unless has clear resistance, may lead to the development of symptoms and/or allow virus replication. Alternately, it cast suspicion on efficiency of TLCV transmission by *B.tabaci* to *L.hir* ('58).

5.2 INTERSPECIFIC HYBRIDIZATION

The second step in the endeavour of resistance breeding is making use of such exotic material in programmes for incorporating the resistance gene (s) into cultivated types. This can be achieved by the conventional backcross breeding technique involving sexual hybridization between the desired plant type and the source (donor) parent and successive backcrossing with desired type and selection in each generation for the desired plant type and disease resistance.

In this study, backcross method of interspecies gene transfer was attempted using TLCV tolerance of *L.pim.* and *L.hir.* ('58), and well-proven resistance of *L.hir.* ('59). However attempts to hybridize *L.per.* with *L.esc.* were unsuccessful. Cvs. A.Saurabh and A.Vikas were used as the reciepent parents. Both these cultivars (*L.esc.*) were hybridized with *L.pim.*, *L.hir.* (two accessions) and *L.per.* using the economic species as female parent. The reciprocal crosses (excepting *L.pim.* x *L.esc.*) were futile. Further, the F_1 hybrids were backcrossed to *L.esc*, by employing them as male, and as female with wild parent. The unilateral incompatibility of *L.hir.* is well attested (Rick, 1979b), so also the embryo abortion in *L.esc.* x *L.per.* crosses.

Pollen tube elongation of *L.esc.* is normally stopped before fertilization is effected, when hybridized with *L.per.* (Thomas and Pratt, 1981). In the reciprocal cross (i.e. when *L.per.* is male parent) the normal fruit development is observed but with no viable seeds possibly due to embryo abortion. Therefore, *in vitro* culture of embryo and immature seeds has been attempted by several workers with varying levels of success (Barbano and Topoleski, 1984). In the present study as it was difficult to recover culturable embryos when immature 35 days old seeds were dissected, attempts were made to induce callus using undeveloped seeds, with the hope of regenerating and salvaging some hybrids from such callus using standard cell culture techniques.

Although a few immature seeds produced some callus in both A.Saurabh x *L.per.* and A.Vikas x *L.per.*, it did not proliferate further but soon turned black and withered. According to Neal and Topoleski (1983) *in vitro* culture of particular stages of *L.esc.* x *L.per.* embryos has proved to be difficult since these stages have complex nutritional and hormonal requirements. Chances for successful *in vitro* culture of immature embryos can be improved by optimizing : the embryo stage, composition of the media, timing of media transfer and genotype of the *L.esc.* parent.

In backcross method of breeding usually 6-7 backcrosses are necessary to recover the recurrent parent in its essential features. But, the work here is confined to the first backcross to both parents and the study of F_2 generation in order to gain knowledge on the genetic bases of TLCV resistance and at the same time to intiate the TLCV resistance transfer.

5.2.1 Study of interspecific hybrids

The present investigation deals exclusively with the transfer of foreign genes for TLCV resistance across interspecific boundaries. In interspecific gene transfer, besides aiming at overcoming the hurdles in hybridization and securing viable F_1's, due consideration also needs to be given to *per se* behaviour of F_1 hybrids, although they are only a stepping stone in the ultimate development of a variety with specific attribute drawn from another spcies. Even when F_1's are produced after circumventing the obstacles encountered during interspecific hybridization, F_1 hybrid itself often may pose difficulties by way of high level of sterility, hybrid inviability, weakness of F_2 and later segregants (Stebbins, 1958).

All the interspecific hybrids viz. A.Saurabh x *L.pim*, A.Vikas x *L.pim*, A.Saurabh x *L.hir.* '58, A.Vikas x *L.hir.* '58, A.Saurabh x *L.hir.* '59 and A.Vikas x *L. hir.* '59 and their parents were studied. When fourteen attributes concerning vigour and reproductive aspects were evaluated (Table 5) in six interspecific F_1 hybrids, overwhelming evidence was available in favour of better crossability of L.esc. x L.pim. and poor crossability of A.Saurabh x L.hir. Rick and Butler (1956) also reported that L.pim. readily crosses with L.esc., the progeny in subsequent generations did not exhibit sterility, and this interspecitific cross was comparable to intervarietal cross. In the present study also low pollen sterility was recorded in L.esc. x L.pim. crosss.

The hybrid A.Saurabh x L.hir. '58 was most discouraging (Fig.5). This F_1 was not only weak, but suffered loss of reproduction capacity as revealed by poor pollen fertility, fruit set and seed germination. Thus apart from pre-mating barriers, (unilateral incompatability) observed in L.esc. x L.hir. cross, post-mating (post zygotic) barriers were also involved in free exchange of genes between these species. A. Vikas genome appeared to nick relatively well with both the accessions of L.hir. The F_1 hybrids between A.Vikas x L.hir. exhibited normal vegetative growth but high level of pollen sterility both in F_1 and F_2. But in general the sterility level was less than that observed in A.Saurabh x L.hir.

5.3 GENETIC BASES OF TLCV RESISTANCE

Some breeding for resistance can be carried out at a purely empirical level, without detailed undertanding of genetic basis. However, there can be little doubt that more efficient and systematic realization of available genetic resistance source is facilitated if the basic genetic mechanisms are known. Moreover, decisions about the strategies of resistance, gene deployment and predictions of the likely future patterns of interaction in the evolving genetic system of the virus can only be based on sound genetic knowledge. Likewise poor undertanding of how diverse types of resistance operate often at biochemical or physiological level, militates against complete and fast realization of the available potential. Knowledge of the genetic basis of resistance, and of the complementary genetic system controlling behaviour of the pathogen, and of their interactions, may provide clues to mechanisms involved.

The genetic basis of host resistance for TLCV studied by six generation mean method, is discussed hereunder.

5.3.1 **Field experiment**

An experiment (field study II) was laid out under heavy pressure of TLCV to study the reaction of *L.esc.*, *L.pim.*, *L.hir.*, F_1'S, F_2'S, BC'S in two independent CRBDs running adjacent ; one for *L.esc.* x *L.pim.* generations and other for *L.esc.* x *L.hir.* generations.

5.3.1.1 *L.esc.* x *L.pim.* crosses

Data from crosses involving A.Saurabh x *L.pim.* and A.Vikas x *L.pim.* were analysed for days to flower, days to TLCV symptom expression and disease severity. However, other attributes including plant height, average fruit weight were not considered for this analysis as their expression was largely influenced by intense TLCV incidence. Further, it was assumed that the TLCV had marginal influence on days to flower and such of the rare plants severely affected by TLCV prior to flowering were discounted while recording the observations.

High pollen fertility, good seed germination, normal plant growth and fruit set in different generations of A.Saurabh x *L.pim.* and A.Vikas x *L.pim.* indicated the possible absence of skewed segregation in these interspecific crosses. Therefore this interspecific cross might permit unbiased estimates of genetic parameters. According to Rick and Butler (1956) no barriers of any consequence exist to the hybridization of *L.esc.* and *L.pim.*, or in fact to the production of progency by the hybrid or to the gene recombination in the hybrid. Genes can be exchanged so freely between these species that most workers are inclined to view *L.pim.* as a variety of cultivated *L.esc.*

5.3.1.1a Performance of different generations

The total variance due to six generations of two crosses (11 entries) in a CRBD was partiond into that due to parents, F_1'S, F_2'S and BC'S and relevant interactions as shown in ANOVA (Table 8).

Generations of both the crosses were significantly different at 0.01 level for days to flower, days to express TLCV symptoms and disease score. Scope for studying crosses with more cultivars for securing better recombinants was evident from the differential response for all the three attributes by A.Saurabh and A.Vikas upon crossing with L.pim. Non significant variance obtained for parents vs F_1'S, F_2's vs. BC'S and segregating vs. non-segregating generations for days to TLCV symptom expression may be partly due to variation in days on which the natural TLCV inoculation occurred under field conditions.

For all the attributes significant differenence at lower level (defined as $P = 0.05$) was observed between A.Saurabh and A.Vikas, but the cultivars were different from L.pim. at 0.01 level. The time lag between the days to TLCV symptom appearence and the days to flower in respect of six generations is represented graphically in Fig.13. On an average about 7 days (A.Saurabh) and 4 days (A.Vikas) prior to flowering the TLCV symptom expression was noticed while in L.pim. and in the backcross B_2, symptom surfaced 25 days after flowering. In other words, infection was seen in L.pim. when the first fruits had attained 25 days. This was due to dual factors of earliness (54.2 days) and longer latent period (79.40 days). So, while making selection, due attention also needs to be given to early fruit maturity. Laboratory studies involving transfer of TLCV by B.tabaci also confirmed longer latent peirod of L.pim.

Transgressive segregants which took as low as 38 days to flower and certain segregants which expressed TLCV signs at an age of 98 days appeared in F_2 of A.Vikas x L.pim. and backcross of (A.Vikas x L.pim.) x L.pim. thereby providing opportunities for improving these attributes in directions favourable to effectively circumvent TLCV onslaught. Symptom manifestation when most of the fruits are on way to maturity does not cause serious crop losses. Saikia and Muniyappa (1989a) reported that age at which infection expresses had an important bearing on crop loss.

More importantly, selection of genotypes with no (score : 0) or very low (score : 1) TLCV incidence that were observed in F_2 and backcross generations should facilitate identifying lines with improved tolerance to TLCV. It was observed that several plants in segregating population went on producing flowers and fruits undeterred by TLCV infection (Fig.15). Therefore, by adopting comprehensive selection strategies it may be feasible to evolve genotypes that although may not intercept the pathogen, but can mature early, express symptoms late and react weakly to TLCV, and produce normal fruit yields. This approach appears to be better suited for determinate and semi-determinate types where cropping period is limited.

Kalloo and Banerjee (1990) developed five breeding lines by incorporating TLCV tolerance from *L.pim.* 'A 1921, which showed no disease signs till 90 days, but they observed certain degree of dilution of TLCV resistance during the process of transfer. Same workers (Banerjee and Kalloo, 1989) reported that TLCV tolerance was linked to late maturity and smaller fruit size. Therefore this necessiates raising of large population to increase the chance of getting desirable recombinants following unrestricted reshuffling of genes. Biparental mating is another way-out to overcome conservative influence of linkage.

5.3.1.1b Gene effects

In view of continuous variation observed in F_2 population of *L.esc.* x *L.pim.* for TLCV reaction and that reports of polygenic nature of rate-reducing resistance (Padgett *et al.*, 1990 and Nelson, 1984), of *L.pim.* it was decided to treat TLCV tolerance as a quantitative character controlled by a number of genes with individual small effects and also influenced largely by environment. This calls for a biometrical approach, rather than Mendelian analysis. Fraser (1990) opined that genetics of virus resistance in wild species may be complicated and not examined fully.

In this situation, assessment of contribution of individual gene is not possible and a geneticist/breeder has to be content with obtaining estimates of effects averaged over the whole genome, or sum total of genes involved, which is acheived byapartioning the genetic variablility into its broad components.

The six parameter model proposed by Hayman (1958) was used to estimate the additive ($\hat{d}$), dominance ($\hat{h}$) and digenic epistatic parameters [additive x additive ($\hat{i}$), additive x dominance ($\hat{j}$) and dominance x dominance ($\hat{l}$)] from the individual plant score of parents, F_1, 'S, F_2'S and backcross generation. The model is based on the assumption that trigenic and higher order epistatic effects are negligible. The scaling tests of Mather (1948) were done to detect the presence of non-allelic interaction.

This investigation characterized genetically days to flower in both the crosses as a trait governed by both additive, intra- and interallelic gene action (excepting additive x dominance) and duplicate epistasis. Therefore, the most appropriate and effective approach would be to mop up the additive genes simultaneously maintaining high degree of heterozygosity for exploiting non-additive component. Days to TLCV symptom expression was predominantly governed by additive genetic effects without epistasis. Hence, lines showing delayed expression of symptom may be isolated from the segregating population derived from the crosses in which TLCV tolerant L.pim. is involved. Further, it also indicates the possibility of early generation selection. But, a different picture of the gene action emerged when other genetic parameters were considered (see below, page 129).

TLCV disease score differed slightly in gene effects between the crosses studied. Analysis of genetic components of means evidenced that additive effect was mainly operative in both the crosses, while duplicate epistasis was found to influence in A.Vikas x L.pim. Other allelic interactions also had a role in the manifestation of TLCV resistance. Additive component of TLCV resistance can be salvaged from the segregating population by direct selection.

5.3.1.1c Genetic parameters

The data' on six generations was also further analysed for deriving heritability and genetic advance. In case of all the above discussed attributes, the cross A.Vikas x L.pim. registered more than 60 per cent heritability which according to Robinson et al. (1949) is very high while the other cross gave heritability between 30-60 per cent which is considered as moderate. According to Falconer (1981) a charecter that exhibits low heritability has either low genetic variability or is heavily influenced by environmental variables. But both the crosses recorded very low narrow sense heritability and negligible genetic advance indicating the major role of environment and insignificant contribution of additive gene effects. Even attributes like days to flowering in A.Vikas x L.pim. although showed very high (87.73%) broad sense heritability but the narrow sense estimate was negligible (0.1), suggesting the predominance of non-additive gene effects. Transgressive segregation as could be seen from the frequency polygons for days to flower (Fig. 9) and days to TLCV symptoms expression (Fig. 10), also reflect the significance of dominance and epistatic gene effects. While the major role of environment in the expression of these traits was evident from the larger variation noticed in F_1.

But these inferences are not in correspondence with the results of generation mean analysis presented in the preceding section, wherein it was found that the additive gene effects play an important role in the expression of most of the attributes. In generation mean analysis, inferences regarding magnitudes of additive and dominance effects are unreliable, due to the distribution of positive and negative gene effects in the parents, may result in different degrees of cancellation of effects in the expression of the means (Thompson et al., 1963). Therefore, in conclusion, it is felt that these three attributes in both the crosses were highly sensitive to environment, influenced by non additive effects and thus less amenable for improvement through simple selection based on phenotypic values.

The less divergent the wild or the uncultivated and adapted lines are, the more likely it will be that additive gene effects will play the primary role in the inheritance of quantitative traits (Isleib and Wynne 1983). As diversity between the alien and the adapted lines increases, dominance effects and epistatic variation may have significant roles in the inheritance of quantitative triats. This concept is well illustrated by the results of both the crosses in the present study. In contrast to uncultivated L. pim., the cvs A. Saurabh and A. Vikas (representing L. esc.) are highly evolved following intensive genetic upgradation for yield and several desirable attributes. However, according to Halward and Wynne (1991) even the expression of introgressed genes is also influenced by the genetic background of the cultivated parent.

Kalloo and Banerjee (1990) for TLCV and Pilowsky and Cohen (1974) for TYLCV reported that the tolerance of L.pim. was due to a single incompletely dominant gene, while Som and Choudhury (1976) adopting a biometrical method noted that polygenes and epistasis operate in determining incomplete dominance of TLCV tolerance. According to Yassin (1985) TLCV tolerance in L.pim. (LA1582) was dominant and governed by single major factor (or a group of factors tightly linked together) and behaving as one factor. These unparallel findings may be because of differences in a) L.pim. accessions tested b) TLCV isolate involved c) screening and disease scoring techniques d) methods of analysis.

5.3.1.1d Breeding methods

Cultivar improvement is a holistic activity in which disease and pest suppression is an integral component. It is not an adjunct to the mainstream breeding effort. In other words, resistance to diseases is but one character among the many of which the plant breeder must take into account. Thus resistance breeding should also involve the simultaneous realization of yield potential. There is no evidence to state that yield potential and biotic resistance are incompatible and even if so, it could be decoupled if the variability observed is sufficiently large to allow reshuffling of genes combining resistance, yield and other desirable attributes.

In tomato, as in any other crop, the effectiveness of selection for any quantitative trait is primarily determined by the nature of the genetic effects controlling its inheritance. Once the relative importance of the contributions from various genetic effects (additive, dominance, epistasis) are estimated for a particular trait in a given cross, the breeding objectives will dictate how the various effects will be exploited in the development of breeding lines or improved cultivars.

Thus, three aspects concerning TLCV resistance breeding [TLCV disease intensity, latent period, earliness] apart from yield and other attributes of intrest are to be dealt with concurrently by a breeder. Further as the three characters in question are quantitative in nature, it is desirable to advance the progenies to backcross F_2 and F_3 generations and backcrossing is alternated with progency testing. The number of backcross will depend on the extent of recovery of the desirable level of quantitative character under transfer. It is generally accepted three to four backcrosses are adequate and at this level transgressive segregants, combining the desirable genes from both the parents, would occur.

5.3.1.2 L.esc. x L.hir. crosses

Departure from normal segregation was suspected in the L.esc. x L.hir. interspecific crosses as high pollen sterility, poor fruit set, low seed germination, fast leaf senescence and premature mortality were often observed in the F_1 hybrids. The observation that deviations from expected segregations might have taken place in this interspecific cross is in keeping with several other reports in different crops (Stephens, 1949; Rick, 1963, 1971; Thomas and McGrath, 1988). Selective elimination of gametes or zygotes and/or reduced recombination, would disturb or upset the segregation of alleles according to expected Mendelian ratios and at times, specific alleles are nearly excluded. Any attempt to estimate the genetic parameters in generations developed from such interspecific crosses would be highly biased. Therefore only mean values along with the variance are presented for L.esc. x L.hir. crosses for three attributes in Table 11.

Cultivated L.esc. (A.Saurabh and A.Vikas) came to flowering about a fortnight before wild L.hir. accessions. Mean days for flowering in none of the other three generations (F_2, B_1 and B_2) for the four crosses was less than the early flowering parents, indicating making selections for earliness might be difficult.

Mean days to show TLCV symptoms under field conditions was not only lowest in the susceptible cultivars (60-62 days), but they also registered very high disease score (3.60-3.64). The corresponding variances were also very low. F_1's took 3-5 weeks more time to show up symptoms than the susceptible cultivars, while the mean disease score ranged from 1.51 to 1.72. Thus TLCV resistance in L.hir. appeared to be incompletely dominant. Cross A.Saurabh x L.hir. '59 clearly showed its superiority over other crosses by exhibiting highest mean and variance in F_2 for mean days to symptom expression. Likewise, for similar reasons this cross was found to be superior for mean disease score. It may be speculated that very high mean (1.88 to 2.43) and variance noticed in B_2 [(susceptible x resistant) x resistant] of all the four interspecific crosses might have resulted from strong, skewing for L.esc. genome.

5.3.2 Glasshouse studies

5.3.2.1 Minimum days required for TLCV symptom expression

Days required for symptom expression after inoculation with TLCV using B.tabaci was studied in parents, F_1's and F_2 S. The data thus gathered are more reliable than similar information obtained from field studies, as in this case there is no control over the amount of inoculum and the plant age at which the natural infection occurs.

Field studies also do not completely rule out susceptible escapes creeping in. However, it provides information on relative response of various genotypes for days to TLCV symptom expression under natural conditions.

Long latent period of L.pim. (23 days) and appearence of segregants in F_2 of A.Vikas x L.pim. (with 34 days latent period) showed that L.pim. has genes and could be a promising donor for postponing by a considerable period of time, symptom development. The F_1

vigour may be one factor that might have delayed symptom manifestation in L.esc. x L.hir. crosses. The information gathered from this study supports the results observed in field experiment II.

5.3.2.1 Inheritance of TLCV resistance

Symptom response to TLCV inoculation by B. tabaci of F_1, F_2 and backcross was considered for the study of resistance to TLCV in L. esc. x L. hir. crosses. Following inoculation the young seedlings posed ambiguity in scoring for disease. Therefore, to avoid possible misclassification, no attempt was made to grade for disease susceptibility. Instead, all such lines that showed any signs of TLCV were classified as susceptible and the remaining as resistant.

When observed under field conditions the F_1's of all the four L. esc. x L. hir. crosses displayed moderate TLCV symptoms suggesting lack of clear dominance either for resistance or susceptibility. When all the four crosses of L. esc. x L. hir. were considered together in F_2 (Table 13) TLCV reaction segregated in a ratio of 9 susceptible and 7 resistant indicating complementary modification of a dihybrid ratio (duplicate recessive genes). Thus it appeared that both the dominant alleles, when present together, complement each other resulting in a genotype susceptible to TLCV. The absence of either or both the dominant alleles provided protection from TLCV. This hypothesis was confirmed by segregation for almost all the progeny in favour of susceptible types in B_1 (F_1 x susceptible parent), but was not supported by distribution B_2 (F_1 x resistant parent). In B_2 the 59 suspectible : 40 resistant genotypes observed were by far different from the expected 1 susceptible : 3 resistant. Hence these results fail to favour a firm conclusion.

Failure to recover commonly recognised ratios in F_2 when individual crosses were considered, appearance of some resistant types in B_1 and the failure of B_2 to confirm the expected ratio may be because of conspicuous departure from the independent segregation observed in these interspecific crosses (page 132) and want of effective TLCV transmission by _B. tabaci_ in genotypes having trichomes of _L. hir._ (page 144). Further, drawback with scoring methods involving only visual symptom assessment is that they may be measuring effects some distance from the primary target of resistant gene action and at times symptoms may also be more likely to suffer from host's genetic background. Thus it appears that such influences might have obscured the simple inheritance ratios for TLCV resistance or it might be considerably more intricate.

The resistance to leaf curl was reported to be incompletely dominant over susceptibility and governed by polygenes (Som and Choudhury, 1976) and also as dominant and to follow the 13:3 digenic model (Banerjee and Kalloo, 1987b) in *L.esc.* x *L.hir.* crosses. Inheritance of tomato yellow leaf curl resistance derived from *L. hir.* was found to be dominant and controlled by more than one gene (Hassan *et al.*, 1984b). Thus, the genetics of resistance possibly may vary with lines and virus isolates involved.

5.3.3 Indexing, and useful material generated from the present work

Indexing of fourteen selected genotypes of F_2, B_1 and B_2 for TLCV by enzyme linked immunosorbant assay (ELISA) showed a good relationship between relative virus concentration as indicated by ELISA absorbance value and visible TLCV symptoms. Often, progenies with severe symptoms had higher ELISA values.

Certain progenies of *L.pim.* (R_1 24/1/3, R_1 34/1/1 and R_3 44/2/2) showed decline neither of fruit yield nor of plant growth. ELISA reaction for these genotypes was weak to very weak and the disease symptoms were moderate. Thus these infected genotypes appeared to support virus multiplication to a level that did not cause significant reduction in yield and vigour. Hence, breeders aiming at evolving cultivars that can help reduce or eliminate crop losses due to ravaging TLCV may select high yielding segregants possessing *L.pim.* tolerance (weak ELISA readings). Such resistance may go a long way, since durability of resistance does not necessarily mean that the cultivar is and remains free of a pathogen ; it may also mean that the cultivar puts a strong brake on the development of the pathogen (Zadoks and Schein, 1979).

The present investigation led to identification of three superior promising individual plants (Table 21) which deserved further attention in breeding for TLCV resistance. Selections were made based on general vigour of the plant, relatively higher yield, larger fruit size and low TLCV damage. It is to be stressed here that the yields in these selections were very impressive (Fig.15 and 16) when viewed in relation to the total failure of cultivated tomato and other segregating material under severe TLCV pressure. Selection R_3 35/2/4 yielded 119 per cent over a healthy A.Vikas plant yielding 1.5 kg with same cropping period. But smaller fruit size (about 10g) recorded in this selection called for further improvement of this trait among others. All selections had A.Vikas as one of the parents and were found to contain low virus levels without greatly hampering fruit yields. This material is being carried further by the Plant Breeding, and Virology sections of the University of Agricultural Sciences, for developing TLCV resistant/tolerant cultivars.

Table 21. Promising individual plant selections

Selection number #	TLCV symptom severity	ELISA reaction	Cropping period (d)	Fr. yield kg/plant	Av.fr.wt. (g)
R_1 24/1/3	mild	weak	135	1.20	8.2
R_1 34/1/1	mild	Very weak	140	1.38	28.0
R_1 35/2/4	moderate	weak	135	1.78	9.8
A.Vikas (healthy)	nil	negative	135	1.5	80.0
A.Vikas (infected)	severe	positive	135	*	*

* No yield or non-marketable fruits

\# R_1 24/1/3 A.Vikas x L.pim. - F_2
R_1 34/1/1 A.Vikas x (A.Vikas x L.pim.)-B_1
R_1 35/2/4 A.Vikas x (A.Vikas x L.hir.)-B_1

5.4 ROLE OF TRICHOMES IN VECTOR RESISTANCE

As TLCV is exclusively vector dependent for its dispersal, controlling B.tabaci would help in checking the disease. There are no effective chemical control for B. tabaci, therefore prevention is the best deterrent. The simple preventive measure is the use of tomato varieties not frequented by whiteflies, but as yet no variety is known to carry resistance to B.tabaci. Hence the need to identify source, factors associated with resistance and to develop varieties resistant to vector. B.tabaci fails to deliver the virus in such plants showing resistance to it. Thus indirect resistance to TLCV entails resistance to B.tabaci. L.hir. trichomes are implicated in resistance to several arthropods (Rick, 1982).

Trichomes or plant hairs are ubiquitous among terrestrial plants and often they provide defense against predators, and their role may present a physical barrier to predation or may be associated for certain modes of chemical defense. Among the seven types of trichomes recognised in Lycopersicon species (Luckwill, 1943), Type I, IV, VI and VII are glandular and rest all are non-glandular. The density, type and histochemistry especially of glands differ with the species. Among the seven types, Type IV, V and VI are often associated with arthropod resistance.

L. hirsutum is the most promising source of insect resistance with 14 out of 16 known resistances in wild species, and is also remarkable for it is the only known source of resistance to nine pests (Rick, 1987). Progress in exploiting these resistances lags far behind that of disease resistance because testing is far more complex and inheritance less well understood.

5.4.1 Trichome types, density and length

In the present study, the density (number/10 mm^2), type and length of trichomes was investigated on both adaxial and abaxial leaf surfaces of L. esc., L. hir. and the four F1 hybrids of L. esc. x L. hir. Total trichome density was found to be significantly different for adaxial surface, but not for abaxial surface.

Differences were not significant within L. hir. ('58 and '59), L. esc. (A. Saurabh and A. Vikas) entries, and the F1 hybrids between these two species were at par excepting A.Saurabh x L.hir. '58. But, mean trichome density was statistically not different for A. Vikas and L. hir. '59. Very dense trichomes for adaxial (432/10 mm^2) and abaxial (973/10 mm^2) surfaces were observed in L. hir. 58. The number of trichomes/unit area was denser on abaxial surface than on the adaxial in all the genotypes evaluated.

Among the five types (I, IV, V, VI and VII) measured, density of Type I on adaxial surface and Type VI and VII on abaxial surface differed significantly, while rest of the trichome types were at par. As was recorded by several workers (Luckwill, 1943; Snyder and Carter, 1985) Type IV trichomes in L. esc. and Type V in L. hir. were found to be absent. Morphologically, these two trichome types mainly differ (Fig. 25) in the presence (Type IV) or absence (Type V) of gland at the trichome tip. When the gland was damaged, it was difficult to distinguish these two types in F1 hybrids, hence, were counted together. On the leaves of all the species and hybrids tested, on the whole, Type VI (49 to 64%) on adaxial surface and Type IV/V (79 to 90%) on abaxial surface dominated (Table 16).

In L.hir. Type IV trichomes had single celled heads with viscous exudate. Type VI in L. esc. had four lobed tips, in L. hir. the tips were rounded, and in the Fl it was intermediate in shape (Fig.23 & 24). These observations correspond with those reported by Snyder and Carter (1985).

Although Type VI trichomes are present in both the species (L. esc. and L. hir.), but it has been recorded (Luckwill, 1943, Synder and Carter, 1985) to differ histochemically between the species. No significant difference was observed for Type VI trichome on adaxial leaf surface. Snyder and Carter (1985) also reported that type VI density did not differ between species. However, on the abaxial surface this type of trichome counted more in L.hir. ($127/10mm^2$) than in L.esc. ($63/10mm^2$) and showed significant difference among the entries. Type VII were relatively sparse (0-$9/10mm^2$) in the species and hybrids examined. Studies of Good and Snyder (1988) in $10F_2$ clones of L.esc. x L.hir. cross indicated wide range of 1.3 to 55.1 (Type IV), 1.5 to 69.6 (Type V) and 2.9 to 9.8 (Type VI) trichome number per mm^2.

Differences in trichome length were observed to be significant for Type I, IV and VI (adaxial surface) and Type I (abaxial surface). However, often one or the other cultivar of L. esc. failed to be different for trichome length from one or the other accession of L. hir. and the Fl hybrids. Thus, like trichome number, in case of trichome length also differences were significant at genotypic level, rather than at overall species or F1 hybrid level.

The greater resistance of *L. hir.* to several arthropods has been associated mainly with antibiotic properties of Type VI trichome tip (Aina *et al.*, 1972; Patterson *et al.*, 1975 ; Williams *et al.*, 1980; Kennedy *et al.*, 1981; Snyder and Carter, 1985). The type IV trichome is also a potential source of mite resistance in *L. hir.* (Snyder and Carter, 1985). The present study also revealed that excepting for the absence of Type IV trichomes in *L. esc.* and Type V in *L. hir.*, the other aspects concerning trichomes viz., density, type and length did not appear to have any decisive bearing in differentiating the species and F1 hybrids studied. Thus it appears that the most crucial factor determining the whitefly resistance in *L. hir.* might be the chemical composition of Type IV and VI glandular trichomes.

5.4.2 Whitefly preference

Studies on whitefly preference revealed that the whitefly activity was higher in fields with generations of *L. esc.* x *L. pim.* than in *L. esc.* x *L. hir.*, but no significant differences were observed within the six generations of *L. esc.* x *L. pim.* (Fig. 29), and within parents and F1's of *L. esc.* x *L. hir.* (Fig. 30). This indicated that *B. tabaci* preferred *L. esc.* and *L. pim.* to *L.hir.* These inferences, however, emerge from the data recorded on whiteflies resting during dawn when they are reported to be inactive. Hence, this conclusion necessarily may not reflect preference for feeding. Reports of evaluation of tomato plants which are resistant to whiteflies are scarce. Stoner *et al.* (1968) reported that Type VI glandular trichomes of *L. hir.* entrap the whiteflies.

Conspicuously higher whitefly counts observed in *L. esc.* x *L. hir.* Fl's (Fig. 31), when averaged over all the four hybrids, might suggest that whiteflies were attracted by the vegetatively vigorous Fl's, which possibly possessed lower levels of toxins than the *L. hir.* (Snyder and Carter, 1985).

5.4.3 Whitefly mortality in relation to trichome density

Whitefly mortality under controlled conditions was investigated by enclosing 35 whiteflies in a bottle having leaves of *L. esc.*, *L. hir.* and *L. esc.* x *L. hir.* With leaf expansion the trichome density reduces (Rodriguez *et al.*, 1972) hence young (3rd leaf) and old (10th leaf) were used for the study. Mortality was very high on third leaf both in *L. hir.* and *L. esc.* x *L. hir*, but in case of *L. esc.* mortality was more in case of tenth leaf than third leaf (Fig. 32). Dense trichomes with poisonous glands especially of Type IV and VI may explain to some extent such differential reaction in whitefly survival. Density of Type VI trichomes and mite resistance was demonstrated by Gentile et. al. (1969). Toxic contents of glandular trichomes may be mainly responsible for the higher casuality of flies in L. hir. and Fl hybrid than the total trichome density as lowest mortality was recorded in the 3rd leaf of L. esc. with equally dense trichomes. Rodriguez et. al. (1972) reported that substance toxic to spider mite would be more abundant on younger leaves than on older leaves of L.hir. Higher casuality observed in L. esc. older leaves than the younger ones, may be because such non-succulent leaves approaching senescence relatively faster (Table 5) may be unfit for whitefly feeding.

According to Heath (1991) detailed studies of basic resistance in truly wild plants are non-existant, comparisons of resistance responses of crop plants with their lesser cultivated relatives have shown that to be similar. Therefore, except, perhaps, for a greater prevelence of toxic secondary metabolites in non-cultivated plants, it seems unlikely that the mechanism of basic resistance in crop plants differ qualitatively from those in wild species. Presence of natural insecticidal substances like 2-tridecanone (Williams et al., 1980) and 2-undecanone (Kennedy et al., 1987) in Type VI trichomes of L. hir. has been reported. The wild L.hir. contains about 72 times as much 2 tridecanone as cultivars. Delicate cell walls of glands break open as the flies move around on leaves spilling the toxic contents. Such substance may paralyse the fly and thus 60 out of 81.4% (L. hir.) and 31.4 out of 51.5% (F1) flies were found entangled and dead (Fig. 3). Aphids on Solanum species with glandular trichomes, after coming in contact with the contents of the trichome glands became immobilized and quickly died (Gibson, 1971). Very few flies dead in case of older leaves may be because of several glands already damaged or the contents of the gland being diluted with age.

5.4.4 Whitefly oviposition

The influence of leaf age i.e. leaf expansion and the number of eggs per group is depicted through bar diagram (Fig. 33). On cotton, the most preferred host of the whitefly eggs were laid in larger groups (mean 4.15 eggs/group). Hair density in Varalaxmi variety of cotton is very low as compared to Lycopersicons. This may allow the female to oviposit freely. Stoner (1970) selected plants with higher concentration of trichomes, as he found that carmine spider mite oviposited fewer eggs on such tomato plants. Group size of the eggs, in the present study, was comparatively smaller in case of younger leaves than older, where the trichome density would be denser, thereby obstructing oviposition. Low level of toxic substances (Rodriguez et al., 1972) in older leaves may be another reason for such a difference. Non-survival of flies on the younger leaves of L. hir. did not allow to carry out eggs/group study in this case.

In the present study significantly lengthier eggs observed (Fig. 34) often on older leaves where the trichomes would be sparse lacked a convincing explanation. Variation in the structure of pupal cases of B. tabaci due to trichomes are reported (Mound, 1962 ; Estop, 1969), but it is not known how the fly pereives the hair density. Mound (1983) also showed that whiteflies often covered glandular hairs with wax prior to oviposition.

Under field conditions the very high pupal case count/100 cm^2 of leaf (1.95) observed in case of L. esc. x L. hir. (Fig. 35) may be possibly because of vigorous nature of plant and presence of reduced levels of toxins than L. hir. L. esc. x L. hir. hybrids were most preferred by B. tabaci was also evident from Fig. 31. The lowest number of pupal cases found on L. hir. leaves indicated that the B. tabaci did not prefer it for oviposition so much as L. esc. and L. esc. x L. hir..

To sum up, Type VI and IV trichomes on leaflets of L. hir. may account for observed differences in preference to whitefly, rather than the density (number) and length of trichomes. Mode of resistance was not non-preference, as (a) fly activity was recorded in L. hir. and populations derived from L. hir. germplasm (b) several flies were attracted and found on the foliage of these genotypes (c) flies were also found entrapped in the leaf trichomes (d) oviposition was found to occur on the leaves of L. hir. The toxic chemicals enclosed in the glands of type VI and IV trichomes, might be released only when they were damaged by the movement of the flies on the leaf surface. Subsequently the insect might be paralysed by the toxin (Gibson, 1971). This substance is in intense form on younger leaves than on the older, (Rodriguez et al., 1972) hence may reduce whitefly casuality on older leaves. Studies on oviposition showed that L. hir. was less favoured than L. esc. for egg laying. Thus these results together allow to define the nature of resistance for B. tabaci in L. hir. as L. hir. is not a preferred host for B. tabaci and it does not completely check feeding and oviposition.

But L. hir. may be a potentially valuable source in tomato breeding in developing cultivars not frequented by B. tabaci. As L. hir. is reported to have resistance to several arthropods, identification and introgression of a common factor like specific trichome types, should help tomato breeders to evolve cultivars with multiple pest resistance. The selection based on presence or absence of a specific plant attribute which is responsible for pest(s) resistance will greatly simplify introgression process.

5.5 ISOZYME STUDIES

Isozyme characterization lends itself to analyse the genetic structure of the individual genotypes as close as possible to the DNA level (Tanksley, 1983a). Isozymes are polypeptides whose amino acid sequences are transcribed directly from the nucleotide sequence of a gene. Through electrophoretic procedures isozymes (multiple forms of an enzyme) are separated according to size and charge. Isozymes coded by alleles of a single locus usually band within a specific region of the gel (Tanksley, 1983a).

As genetic markers, isozymes have several advantages over the other morphological traits such as, codominant gene expression, freedom from epistatic and environmental effects. More recently, however, according to Blake et al. (1991) quantitative trait loci (QTL) and according to Dixon and Harrison (1990) DNA hybridization (RFLPs) have greater promise in selecting and recovering genes from alien source in backcross progeny.

5.5.1 Importance of peroxidase in disease resistance

Plant resistance is usually of the passive type; most plants are non-hosts for most microbes. However, when plants are infected by parasites a variety of situations are encountered, ranging from very weak or no defence to very intense defence, ultimately leading to resistance. The hypersensitive response (TMV infecting tobacco cv. Sampson NN), one of the most natural mechanisms of defence, involves cell necrosis, thereby isolating the pathogen, and is often associated with accumulation of phenolic enzymes (Kosuge, 1969). Simons and Ross (1970) reported that peroxidase kill the infected cells, thus suppressing the multiplication of virus. Even induced resistance, according to Dixon and Harrison (1990), is related with rapid incorporation of phenolic material into plant cell walls by peroxidase to yield lignin. This may possibly change the membrane permeability and help localization of virus.

Although both quantitative and qualitative differences have been found in a number of isozymes (Retig, 1974), particular interest has centred on changes in the peroxidase system due to its possible involvement in biosynthesis of phytoalexin, ethylene, lignin and oxidation of phenolics (Simons and Ross, 1970; Vance et al., 1980 ; Burdon and Marshall, 1983 and Fernandez and Jouve, 1990).

5.5.2 Isoperoxidase in Lycopersicon

In the present study, an attempt was made to obtain a broad perspective into the differences in anodal isoperoxidases of resistant, late expressor and susceptible (healthy) parents, and their F_1 hybrids, in addition to TLCV infected susceptible parent.

The intense staining, thick bands in the TLCV infected L. esc. might indicate the increased activity of peroxidase (Fig. 37). Johnson and Lee (1978) attributed this to quantitative increase in the level of isoperoxidase. It has been argued that increase in the activity of the peroxidase is due to enhanced respiration and metabolic activity of diseased tissue (Sako and Stahmann, 1972) and it may be a consequence rather than determinant of resistance. Several workers reported synthesis of additional isoperoxidases (bands) apart from increased activity of the enzyme following infection (Andryushchenko and Dubinets, 1975; Yang et al., 1984; Park et al., 1985). However TLCV carrying samples, showed no additional bands. The banding position for Prx-3 isozyme differed among resistant (L. hir.), late expressor (L. pim.) and susceptible (L. esc.) parents. However, without the electrophoretical sampling of F_2 or backcross resistant and susceptible segregants, it would be questionable to conclude categorically the implication of Prx-3 in TLCV resistance.

Based on the regions of banding in the polyacrylamide gel stained for peroxidase, three putative alleles (Prx-1 to Prx-3) may be recognised for the anodal leaf peroxidase. Electrophoretic variants resolved for the isozyme Prx-3 showed 3 alleles for this locus in the Lycopersicon species examined. Rick et al.

(1974) identified two alleles each for four loci for anodal peroxidase in basal stem and root tissues of _L. esc._ and _L. pim_. Several workers (ex. Rick _et al_., 1974) have reported that each tissue possessed its characteristic set of isozymes.

The F_1 analysis for the slow migrating isozyme (Prx-3) indicated a single band in parents and two in hybrids corresponding to the parental types leading to inferences: (i) dimorphic nature of the locus (ii) the monomeric subunit structure of the peroxidase protein and (iii) codominant gene action. Such results (ii and iii) are in agreement with many reports in various crop plants including tomato (Crawford, 1983; Tanksley, 1983a). However, the interspecific polymorphism for Prx-3 loci was indicated.

Classically, recurrent backcrossing has been applied, inspite of the long time and effort it demands to transfer gene (s) from one background to another. Basing selection on isozymes in addition to morphological characters in backcross population will expedite the transfer process.

According to Tanksley (1983b), if enough isozymic markers could be found, it should be possible to mark the entire genome and select useful breeding lines or cultivars from interspecific crosses in one or two generations of backcross. Such material, subsequently, may be carried through one or two rounds of selfing and critical selections for plant type, date of flowering and maturity, fruit quality etc. may be made, to obtain releasable cultivar.

SUGGESTED APPROACHES FOR EFFECTIVE REALISATION OF TLCV RESISTANCE

The present study has revealed availability of most useful TLCV resistance in L.pim. and L.hir. Consideration is given herebelow to understand the nature of resistance and how such potential sources might be systamatically exploited.

In contrast to L.esc. low virus titer recorded in L.pim. both in field and graft infected plants may indicate the "restriction" or rate reducing resistance which means reduced replication, accumulation or spread of the virus in the plant. Delayed symptom expression observed in L.pim. is another characteristic nature of rate reducing resistance. Outwardly, moderate disease symptoms, less damage to the plant were the reaction of L.pim. to TLCV infection.

The TLCV resistance characterized in L.pim. herein may be likened to rate reducing or hypersensitive resistance reported in some hosts combating bacterial/ fungal invasion. Some forms of rate reducing resistance express their resistance in part in the form of lesions that are considerably larger than those associated with the hypersensitive resistance, but are smaller than those associated with a clearly susceptible reaction.

Slow leaf rusting of wheat and slow late blighting of wild Solanum species are some examples of diseases which are greatly checked by reduced size of infection sites. Similarly necrotic lesion response to TMV in Samsun NN occurs due to localization of virus around immediate site of infection.

In L.pim. complete localization of virus does not occur, it permits the spread of the TLCV within the plant systemically, but at the same time checks the inordinate virus multiplication. In some crops this type of systemically effective resistance mechanism has contributed significantly to virus restriction for example in control of BCMV in Phaseolus beans. The potential value of such resistance in managing plant disease at some economically acceptable threshold has been well established (Nelson, 1984) and it has also been shown to play an important role in integrated pest management (IPM) programmes (Padgett et al., 1990). Neverthless, the systemically effective types of resistance mechanism have been less studied than those clearly dependent on localization.

Epidemiologically, non-specific resistance is rate reducing in nature. Intensity is quantitative trait which is conditioned by several genes. According to Padgett et al. (1990) virus resistance in the host plant (rate reducing) is controlled polygenically, and has better stability than monogenically controlled resistance that is currently used in breeding programmes to control viruses (Ponz and Bruening, 1986).

L.hir. ('59), unlike L.pim. expressed neither symptoms (when attempted to infect by different means) nor allowed virus multiplication or accumulation. But L.hir. ('58) showed mild symptoms of TLCV upon graft inoculation. In practical crop protection, resistance mechanism which permits limited replications of the virus (L.pim.) is less attractive than that which effectively inhibit complete multiplication and, of course produces no disease symptoms (L.hir. '59). Firstly, systemic multiplication, although restricted, acts as a reservoir of infective virus which may affect susceptible cultivars grown closeby. Secondly, the ability of the virus to replicate, albeit to limited extents, increases the chance of production of resistance breaking strains. But, the realization of L.pim. resistance is by far easier than that from L.hir.

Two potential approaches applicable in the present context for exploiting the available opportunities for TLCV resistance breeding are : 1) use of rate reducing resistance of L.pim. or resistance located in L.hir. '59 2) stacking or pyramiding of genes. Although the second approach involves a greater amount of breeding effort and it also takes longer to breed such carriers of resistance, the longer lasting stability of this type of resistance neverthless would justify the expense.

The urgent need for bypassing the crop loss due to TLCV may be met by initiating short-term programmes for incorporating rate-reducing genes from L. pim. Approaches for incorporating components of TLCV resistance from L.pim. are discussed elsewhere (page 131). However, long term assignments involving testing of accessions of L. hir., and L. per. for different TLCV isolates may be taken up and subsequently several non-allelic genes conditioning such resistances mobilized and stacked in an acceptable tomato cultivar by attempting complex crosses involving 3-4 resistance donors. Such a strategy of diversity in resistance is expected to be more durable in providing better protection against the whole lot of virulent and avirulent TLCV forms. The importance of such an approach would be convincing when Flor's opinion (Pedersen and Leath, 1988) is considered "races are merely human's way of keeping track of pathogen and what really exists is a population of virulence/ avirulence genes and they are always in a state of flux".

Presently there is great interest in breeding for oligogenic resistance by introduction of individual resistance genes from wild sources (Fraser, 1986). Several researchers advocate combining of tolerance and other types of resistances. (Luthura and Rao, 1980; Pederson and Leath, 1988). Thus development of lines fortified with TLCV resistance genes from L.hir., L.per.

and rate reducing resistance genes from L.pim, is another possible potential approach for realization of available resistance. This calls for three concurrent backcross programmes involving the above three species, one in each programme and a common recurrent parent. The resulting three isogenic lines are then crossed together for construction of gene pyramids. In tomato, extensive RFLP, QTL and isozyme linkage maps have been developed (Chetelat, 1989) which should facilitate in consolidating quantitative resistance from various sources.

Lines bred for TLCV resistance may also serve as ready source for improvement of F_1 hybrid tomatoes which have become commercially most successful. The novel techniques being revealed by emerging biotechnological research are expected to benefit in asexual transfer of genes conferring TLCV resistance to the parents of F_1's.

SUMMARY

VI SUMMARY

For the tomato grower in India leaf curl virus (TLCV) has become a scourge. Sustained efforts on development of TLCV resistant cultivars is essential, as yet neither an effective control measure nor a resistant cultivar is available for this geminivirus vectored by *Bemisia tabaci* (Genn). The summary of this investigation that strove mainly to find a right donor for TLCV resistance/ tolerance, its usefulness in TLCV control, reveal genetics of resistance and to understand the role of trichomes in vector resistance, is presented hereunder.

Identification of TLCV resistance source

L. hirsutum (PI 390658 and PI 390659) and *L. peruvianum* (PI 127830) were found to possess gene(s) resistant to local strain of TLCV based on a critical evaluation involving approaches as exposing the test genotypes to intense inoculum pressure in summer, vector inoculation, graft transmission, ELISA and progeny tests.

By all means of screening, TLCV affected *L. pimpinellifolium* consistantly showed moderate symptoms, low ELISA readings, undeterred growth, flowering and fruiting. Additionally delayed appearance of symptom and also earliness in flowering made it a potential donor for TLCV tolerance.

ELISA provided evidence for the transmission of TLCV from stock to scion. It also demonstrated that the TLCV resistance of L. hirsutum (PI 390659) was an emphatic one as this genotype suppressed virus replication even when graft inoculation was attempted to cause infection.

Graft transmitted L. hirsutum (PI 390658) and L. pimpinellifolium recorded low ELISA absorbance values indicating that these genotypes restricted the multiplication of TLCV though allowing its systemic spread. Sensitive Arka Saurabh and Arka Vikas recorded very high virus levels.

Grafting followed by ELISA was observed to be the most sure way to test a genotype for TLCV resistance.

Progeny test revealed true-to-type breeding behaviour for TLCV reaction of tomato lines investigated.

Interspecific hybridization

Hybridization was successful when L.esculentum was pollinated with L.pimpinellifolium, L.hirsutum and L.peruvianum, but the reciprocal cross failed to set fruits excepting in case of L.pimpinellifolium x L.esculentum. Upon pollinating L.esculentum with L.peruvianum although fruit set was observed but the immature, small seeds failed to germinate. However, attempts to culture hybrid embryos on MS basal medium with some supplements resulted in callogenesis.

When aspects concerning vigour and fertility were measured on the interspecific hybrids, it was found that *L. esculentum* x *L. pimpinellifolium* F_1's were by far superior to *L. esculentum* x *L. hirsutum* hybrids.

Arka Saurabh genome failed to nick well with *L.hirsutum* especially *L. hirsutum* '58, these hybrids were not only weak but suffered loss of reproduction capacity as revealed by high pollen sterility (21.8%), poor fruit set (8.49%) and low seed germination (9.5%). Even in the F_2 generation maximum pollen sterility was recorded in Arka Saurabh x *L. hirsutum* (41.12%).

Genetics of TLCV resistance

Frequency polygons for six generations (P_1, P_2, F_1, F_2, B_1 and B_2) of *L.esculentum* x *L.pimpinellifolium* for days to flower and days to TLCV symptom expression indicated the importance of dominance and epistatic gene effects and the major role of environment in the expression of these attributes.

Highly significant differences (0.01 level) were observed for days to flower, days to TLCV symptom expression and TLCV disease score between the six generations of Arka Saurabh x *L. pimpinellifolium* and Arka Vikas x *L. pimpinellifolium*.

Under field conditions, *L. pimpinellifolium* exhibited low mean disease score (1.58), early flowering (54 days) and longer time to express TLCV symptoms (79 days). In the segregation population of L.esculentum x L. pimpinellifolium crosses transgressive recombinants which took as low as 38 days to flower and as high as 98 days to express TLCV symptoms were observed. It was also noticed that even segregants affected by TLCV were capable of producing normal flowers and fruits. By adopting appropriate selection strategies these attributes can suitably be manipulated as to develop lines that might circumvent the onslaught of TLCV and produce normal fruit yields.

Even in the laboratory studies when L. pimpinellifolium was subjected to screening for TLCV using B. tabaci, it displayed a longer latent period (minimum of 23 days) and ability to flower normally even after infection with TLCV at a very early stage (2-3 leaf stage) of plant growth while cvs Arka Saurabh and Arka Vikas showed TLCV symptoms eleven days after inoculation and the growth was adversly affected resulting in no flowering. Recombinants that had latent period upto 34 days were recorded in F2 generation of Arka Vikas x L. pimpinellifoliam.

In L.esculentum x L.hirsutum the F_1's were late in expressing TLCV symptoms than L.esculentum by 3-7 days. All the four F_1 hybrids between L. esculentum x L. hirsutum reacted moderately for TLCV. Segregation ratio in F_2 for TLCV resistance was in favour of a complementary modification of a dihybrid ratio. This was supported by B_1 (F_1 x susceptible parent), but not by distribution observed in B_2 (F_1 x resistant parent). This may be due to want of effective TLCV transmission by *B.tabaci* in genotypes with trichomes of *L.hirsutum* and departure from independent assortment in these interspecific hybrids or the inheritance of TLCV might itself be considerably more intricate in *L.esculentum* x *L.hirsutum* crosses.

The TLCV tolerance of *L. pimpinellifolium* appeared to be controlled quantitatively. Generation mean analysis revealed predominance of both additive and non-additive gene effects and presence of duplicate type of gene action for days to flower. But days to TLCV symptom expression and TLCV disease score showed predominance of additive effect in both Arka Saurabh x *L. pimpinellifolium* and Arka Vikas x *L. pimpinellifolium*.

However, it is inferred from genetic parameters and the frequency distribution pattern for six generations that the role of additive gene action was insignificant in determining the expression of days to flower, days to TLCV symptom expression and TLCV disease score in *L. esculentum* x *L. pimpinellifolium* crosses.

Trichomes and vector resistance

Significant differences were observed for the total trichome (number/10mm^2) for adaxial leaf surface of *L. hirsutum*, *L. esculentum* and *L. esculentum* x *L. hirsutum*.

However, trichome density for abaxial leaf surface was found to be at par in case of above two species and the F_1 hybrids.

Out of the five types of trichomes studied (I, IV, V, VI and VIII), Type I trichome number on adaxial and Type VI and VII on abaxial leaf surface showed statistical difference, while all other types were at par. Preponderance of Type VI (49 to 64%) on adaxial and IV/V (79 to 90%) on the abaxial surface was observed. Type IV trichomes in L.esculentum and Type V in L.hirsutum were absent.

Morphology of Type VI trichome gland showed that in L. esculentum it was four-lobed, in L. hirsutum it was rounded and the F_1 exhibited an intermediate shape.

Trichome length of Type I, IV and VI (adaxial surface) and Type I (abaxial surface) differed significantly in L.esculentum, L.hirsutum and the F_1 hybrids between these two species.

More critical analysis of the data collected on trichomes revealed that except for the absence of Type IV in L. esculentum and Type V in L. hirsutum, the other aspects such as density (number), type and length of trichomes had no decisive role to play in differentiating the two species and four F_1 hybrids (L. esculentum x L. hirsutum) studied.

Under field conditions it was observed that B. tabaci preferred L. esculentum and L. pimpinellifolium to L. hirsutum. No variation in the whitefly counts within the genetic populations of L. esculentum x L. pimpinellifolium and L. esculentum x L. hirsutum was noticed. However, whiteflies preferred L.esculentum x L.hirsutum F_1 hybrids to either of the parents, possibly because of reduced levels of toxins than L.hirsutum and improved vegetative vigour.

Whitefly mortality was high on leaves with dense trichomes as compared to leaves with sparse trichomes in L. hirsutum and F_1 (L. esculentum x L. hirsutum). Entrapment of whiteflies and death was mainly noticed on leaves of L. hirsutum, not on leaves of L. esculentum in which the trichomes were equally dense. Type IV and VI trichomes with poisonous glands were partly responsible for such differential response for whitefly mortality.

Under laboratory condition, the whitefly eggs/group were more on leaves with sparse trichomes than an leaves with dense trichomes suggesting that trichomes were hindrance for whitefly oviposition. Significantly lengthier eggs recorded on leaves with sparse trichomes lacked a convincing explanation. L. hirsutum leaves recorded least number of pupal cases under field conditions. Thus L. hirsutum was not a preferred host for B. tabaci.

Isozyme studies

Differences in the banding pattern for leaf isoperoxidase was observed in TLCV susceptible (L.

esculentum), resistant (L. hirsutum) and late expressor (L. pimpinellifolium) and the intermediate symptom producer (Fl) genotypes.

Thick intense staining bands in TLCV affected susceptible types suggested increased activity of peroxidase in diseased plants.

Additive banding pattern of F_1 zymograms showed monomeric form of peroxidase and dimorphic nature of isozyme Prx-3. But, the locus Prx-3 showed interspecific polymorphism.

The three factors of interest viz. late symptom expression, low disease score and early flowering of L. pimpinellifolium may be independently bred in three separate backcross schemes involving a single recurrent parent, and later merged into one line. As all the attributes are quantitative in nature, backcross may have to be altered with progeny testing.

The present investigation led to the identification of certain superior, promising individual plants. They produced impressive fruit yields (1.2 to 1.78 kg/plant) despite TLCV infection. In contrast, under such TLCV pressure in the field there was total failure of cultivated tomatoes.

The immediate need for bypassing the crop loss due to TLCV may be accomplished by initiating programmes for incorporating rate-reducing genes from L.

pimpinellifolium. Another possible approach is assembling of resistant genes from *L.pimpinellifolium*, *L.hirsutum* and *L.peruvianum* in a cultivar.

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* **Original not seen**

APPENDICES

Appendix I. Composition of the MS basal medium (Murashige and Skoog, 1962).

Ingredient	mg/lit.
KNO_3	1900.0
NH_4NO_3	1650.0
$CaCl_2$ $2H_2O$	440.0
KH_2PO_4	170.0
H_3BO_4	6.3
KI	0.8
$MgSO_4.7H_2O$	370.0
$MnSO_4.2H_2O$	22.3
$ZnSO_4.7H_2O$	8.6
Na_2MoO_4 $2H_2O$	0.25
$CuSO_4.5H_2O$	0.25
$CoCl_2.6H_2O$	0.025
$Na_2.EDTA.2H_2O$	37.3
$FeSO_4.7H_2O$	27.3
Agar	8,000.0

pH adjusted with 0.1 N KOH or 0.1 HCl to 5.8

MS basal salts were supplemented with BAP 8mg/l + NAA 1mg/l + PVP 600mg/l (Media No. 26) ; NAA 2ppm + BAP 2ppm (Media No. 32) ; NAA 6mg/l + Kinetin 6mg/ml (Media No. 47) ; 30g/l sucrose (Media No. 46) ; 5µm BAP + NAA 5um (Media No. 69). The media were autoclaved 120°C for 20min and when cooled sufficiently poured into tubes. 4-5 seeds were inoculated into each tube.

Appendix II. ANOVA table for six generations (schematic).

Source	d.f	MSS	F ratio
Replications	(r-1)	Mr	Mr/E
Entries or Treatment	(t-1)	Mt	Mt/E
a) Non-segregating	(ns-1)	MnS	MnS/E
1. Parents	(p-1)	Mp	Mp/E
2. F_1s	(F_1-1)	Mf	Mf/E
3. Parents V_s Hybrids	1	Mph	Mph/E
b) Segregating	(s-1)	Ms	Ms/E
1. Backcrosses	(b-1)	Mb	Mb/E
2. F_2s	(F_2-1)	Mf	Mf/E
3. Backcross V_s F_2s	1	Mbf	Mbf/E
c) Non-segregating V_s segregating	1	Mnss	Mnss/E
Error	(r-1) (t-1)	E	-

r = number of replications
t = treatments
p = parents

s = segregating generation
ns = non-segregating generation
b = backcross generation