

**MORPHOLOGICAL AND MOLECULAR VARIATION IN
MAIZE (*Zea mays* L.) WITH SPECIAL REFERENCE TO
BANDED LEAF AND SHEATH BLIGHT**

Thesis

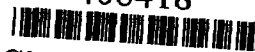
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By

BHAGWAN SINGH

***IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF***

Doctor of Philosophy

(PLANT PATHOLOGY)

August, 2004

Dedicated
to
my beloved
parents

Acknowledgement

Words, in my lexicon fail to elucidate my profound sense of veneration and indebtedness to Dr. S.C. Saxena, Professor & Sr. Maize Pathologist, Department of Plant Pathology and Chairman of my Advisory Committee for his meticulous guidance, out standing co-operation, encouragement and constructive criticism, amicable attitude throughout.

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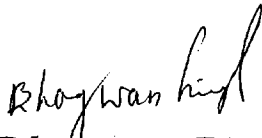
I am overwhelmed with love and care to evince my heartily appreciation to my amiable seniors, friends and juniors who helped me whole heartedly in every iota of thick and thin.

Finally, I express my heartiest scenes of reverence, respect and immense love to my most venerable parents for their support and love, I also wish my affectionate thanks to Harish bhaiya, Neetu bhabhi and Shikha.

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Pantnagar
August, 2004


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CERTIFICATE

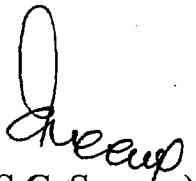
This is to certify that the thesis entitled "**MORPHOLOGICAL AND MOLECULAR VARIATION IN MAIZE (*Zea mays* L.) WITH SPECIAL REFERENCE TO BANDED LEAF AND SHEATH BLIGHT**" submitted in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** with major in **PLANT PATHOLOGY** and minor in **ENTOMOLOGY** of the College of Post-Graduate Studies, G.B. Pant University of Agriculture and Technology, Pantnagar, is a record of *bona fide* research carried out by **Mr. BHAGWAN SINGH, Id. No. 28825**, under my supervision, and no part of the thesis has been submitted for any other degree or diploma.

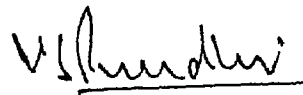
The assistance and help received during the course of this investigation have been acknowledged.

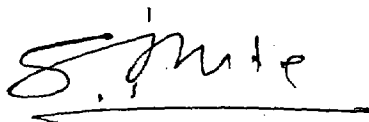

(S.C. Saxena)
Chairman
Advisory Committee

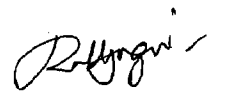
CERTIFICATE

We, the undersigned, members of the Advisory Committee of Mr. BHAGWAN SINGH, Id. No. 28825, a candidate for the degree of DOCTOR OF PHILOSOPHY with major in PLANT PATHOLOGY and minor in ENTOMOLOGY agree that the thesis entitled "MORPHOLOGICAL AND MOLECULAR VARIATION IN MAIZE (*Zea mays* L.) WITH SPECIAL REFERENCE TO BANDED LEAF AND SHEATH BLIGHT" may be submitted in partial fulfilment of the requirements for the degree.


(S.C. Saxena)
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Member


(S.J. Kolte)
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(Ram Ujagir)
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Ex-officio Member
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Introduction

Maize (*Zea mays* L.) is an important cereal crop in world agricultural economy. It belongs to the family Poaceae (Gramine) and is a cross pollinated crop. Maize is also called Drosophilla of genetics due to extensive use in basic studies in genetics.

It is grown throughout the temperature, tropical and subtropical zones and has a worldwide significance as human food, feed for livestock and for industrial and pharmaceutical sectors as every plant part of maize has one or the other economic values. The grains, leaves, stalk, tassel and even the cob are used to produce hundreds of food and non-food products (Watson, 1988). By virtue of its immense potentiality it is also called “Queen of cereals”.

In India, maize is grown throughout the country in varying environmental condition particularly in states like Madhya Pradesh, Punjab, Rajasthan, Haryana, Andhra Pradesh, Karnataka, Himachal Pradesh, Bihar and Uttar Pradesh. Maize is cultivated over an area of 139.02 million ha with production of 614.21 mt and average productivity of 4.45 tonnes per hectare (FAO, 2002). The total area under maize cultivation in India is 6.50 million hectare with total production of 13.30 metric tonnes with productivity 2018 kg/ha (Kallo *et al.*, 2003). During the year 2002-2003 the total area in the country under maize cultivation was 6.66 million hectare against 6.58 million hectare in 2001-2002 registering an increase of 0.08 million hectare over the previous year. The total production during the year was 11.16 million tonnes. The productivity during the year was 1676 kg. The increase in the area has mainly in the states of Gujarat, Bihar, A.P., M.P., Rajasthan, chhatishgarh, Karantaka, Maharastra, Tmil Nadu and Uttar Pradesh. While the increase in production was recorded in A.P., Chattisgarh, Rajasthan and Maharastra. The decrease in the overall production

has been mainly due to later arrival of monsoon, prolonged dry spell and drought in peninsular region during kharif and prolonged cold during winter season in northern states (Anonymous, 2004).

On global basis, about 112 diseases of maize have been reported so far from different parts of the world, of these over 70 are known to occur in India. Pantnagar is a hot spot for a number of diseases but particularly for three major diseases, viz., banded leaf and sheath blight (BLSB) caused by *Rhizoctonia solani* f.sp. *sasakii* (*Hypochonus sasakii*), brown stripe downy mildew (BSDM) caused by *Sclerophthora rayssiae* var. *zcae* and erwinia stalk rot (ESR) caused by *Erwinia chrysanthemi* pv. *zcae* (Anonymous, 2002). Banded leaf and sheath blight was first reported in India from Tarai region of Uttar Pradesh (Payak and Renfro, 1967). The importance of this disease was realized only 1972 when an epidemic occurred in warm and humid areas in foot hills, particularly the Mandi district of Himachal Pradesh (Thakur *et al.*, 1973). The disease is known under many name viz., sclerotial disease, banded sheath rot, banded sclerotial disease, sharp eye spot, oriental leaf and blight, sheath blight, sheath rot and corn sheath blight.

An important aspect is planning a programme of studying genetic variation through the knowledge of genetic behaviour of disease reactions by morphological and molecular markers, available in different genotypes.

Traditionally, the assessment of the genetic diversity of maize germplasm has been conducted on the basis of morphological phenotype marker characters. But major disadvantages associated with these are the limited number of morphological characters available for analysis further, these characters are also influenced by environmental factors. As a consequence different plant genotypes can not be distinguished (Staub and Meglie, 1993). In the last decade molecular markers, that are of heritable entities and are associated with economically important traits, have been used by plant breeders as selection tools (Backman and Soller, 1986). Molecular marker such as RFLP, RAPD, SCAR, AFLP etc.,

have been used to assess genetic diversity at the DNA level, allowing an estimation of degree of relatedness among individuals without the influence of environmental variation (Miller and Tanksley, 1990). Among the various technique available RAPD analysis is a potentially simple, rapid and reliable finger printing, which does not necessitate the use of radioactive labeling (Williams *et al.*, 1990). The protein markers also play a very important role in the identification of genotypes. Shah *et al.* (1989) evaluated genotypes and their mutant electrophoretically by using SDS-PAGE. Other most important protein marker is isozymes. The iso-electric focusing (IEF) is another protein electrophoretic technique with higher resolution (Radola, 1980).

In order to workout the genetic variation and disease reaction in respect of BLSB an experiment was conducted under epiphytic condition in field as well as glasshouse and laboratory. Relevant molecular and biochemical approaches were used in the present study. The specific objectives of the study are as follows.

1. To find out the various morphological characters and disease reaction of different resistant and susceptible genotype and which are associated with BLSB following field evaluation technique.
2. To find out the disease reaction of resistant/susceptible genotype through laboratory method.
3. To find out the resistant/susceptible genotype for BLSB through RAPD profile.
4. To find out resistant/susceptible genotype for BLSB through SDS-PAGE.

*Review
of
Literature*

Banded leaf and sheath blight (BLSB) of maize, caused by *Rhizoctonia solani* (Kuhn) is one of the most important disease in *tarai* region of Uttaranchal which reduces the yield coupled with its qualitative degradation. Literature pertaining to various aspects of the present study is reviewed under the following heads.

2.1 History, nomenclature and distribution

Banded leaf and sheath blight of maize, caused by *Rhizoctonia solani* f.sp. *sasakii* (Kuhn) Exner [*Thanatephorus cucumeris* (Frank) Donk] was reported for the first time from Sri Lanka (Bertus, 1927). The disease has been described under many names viz., banded leaf and blight, *Rhizoctonia* ear rot, leaf and sheath blight, sheath rot and corn sheath blight. Bertus (1927) as sclerotial disease of maize caused by *Rhizoctonia solani* Kuhn. Later on the disease was reported from many parts of the world; Siera Leone (Deighton, 1932), Philippines (Reyes, 1941), Ivory coast (Resplandy, 1954), Malaysia (Heath, 1956; Wiltshire, 1956), Nigeria (Van Eijanathen, 1961), England (Knight and Burrill, 1964), Japan (Kujware, 1968), China (Zhu, 1982), USA (Hirrel *et al.*, 1989) and Korea (Lee *et al.*, 1989). In India, the disease was first reported as banded leaf and sheath blight of maize caused by *Hypochochonus sasakii* by Ullstrup in 1960 from *Tarai* region of Uttar pradesh (Payak and Renfro, 1966). The disease was described as ear rot caused by *Corticium sasakii* on maize in 1977 from Meghalaya state (Maiti, 1978). Since then the disease has been reported from Himachal Pradesh, Haryana, Madhya Pradesh, Rajasthan and West Bengal (Anuja and Payak, 1988).

2.2 Symptomatology

Bertus (1927) reported the symptoms of the disease on all aerial parts of maize plant except the tassel, with its, frequent occurrence on lower leaf sheath and ears. Knight and Burrill (1964) reported sharp eye spots on the leaves both in lodged and unlodged plants. However, stalk lesions and rind spotting, stalk breakage, clumping and cracking of styles, horse shoe shaped lesion with banding on caryopses, and sclerotia formation on styles, glumes cupules and caryopses and sclerotia formation on styles, glumes cupules and caryopses are very rare (Ahuja and Payak, 1988; Sinha, 1992). Under natural conditions, disease appears at pre-flowering stage on 30 to 40 days-old plants but infection can also be seen on young plants (Baruah, 1979), which may subsequently result in severe blighting and death of apical region of growing plants.

2.2.1 On leaves

Lesions appears as irregular patches, more rapidly then on leaf sheath, covering greater portions with alternating dark bands. Many blotches interspersed with dark, 1-3 cm broad bands are produced perpendicularly to the long axis of leaves and sheaths become thin and papery, loose and can be torn easily. A slightly raised and spreading type of mycelial growth is apparent on both the surface of infected leaf (Plate 3B).

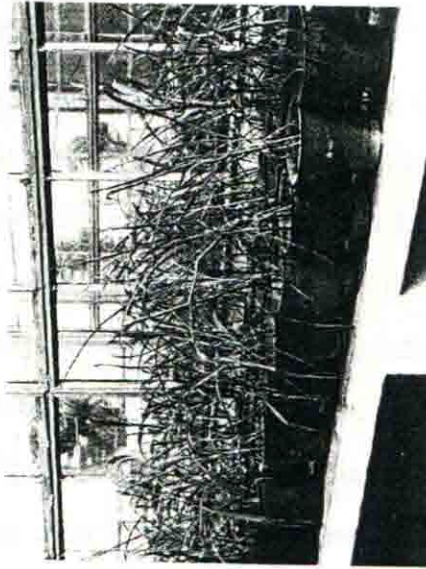
2.2.2 On leaf sheath

The symptoms are more common on sheath then on leaves. The disease appears on basal leaf sheaths as water soaked, straw coloured, irregular to round spots on both the surfaces. A sort of wave pattern of disease advancement can be seen not only on leaves but also on sheaths and husk leaves. In early stages marginal chlorosis and rotting of laminae can be observed which progresses inwards. As the disease advances numerous, sclerotial bodies are produced.

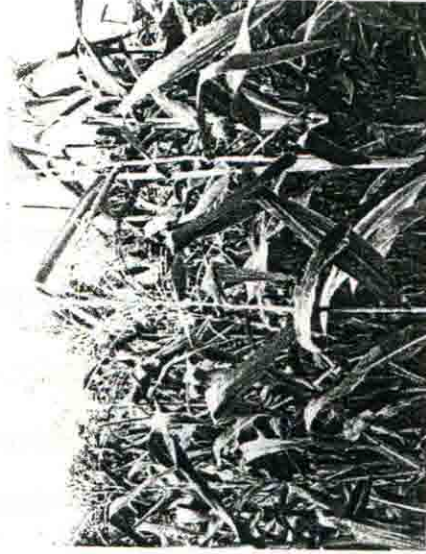
PLATE -3



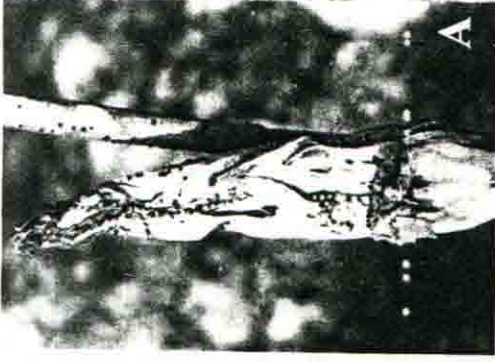
Morphological Character-CM Genotype



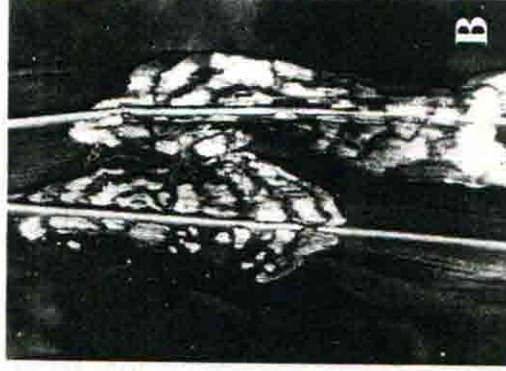
Glass House Experiment



Field Experiment



BLSB Symptom on cob



BLSB Symptom on Leaf

2.2.3 On stalk

The pathogen also causes elongated dark brown to black spots or lesions on the rind of the stalk under the affected sheaths. These spots coalesce to cover entire internode measuring from 2-10 × 3-15 mm (Plate 4 and 5). Sometimes these lesion are transformed into cankers. Generally, lesions develop lengthwise but a few girdle near the nodes under artificial inoculation conditions, sometimes the entire rind is affected making the stalk weak (Saxena, 1997).

2.2.4 On ear

The disease is observed on basal part of the outer most husk leaves expanding to sheath from where the ear emerges. The affected ears become brown and numerous sclerotia are observed on husk lightly attached to the cob. Whitish mycelium and sclerotia are seen frequently on silks between kernel rows and glumes (Plate 3A).

The affected grains show light grayish to dark brown discolouration drastically reduced in size, wrinkled and under severe conditions they become chaffy in appearance and light in weight (Saxena, 1997).

Three types of ear rot can be distinguished : (a) in case of early infection the cob formation is suppressed or remains rudimentary with bleaching of entire husk leaves, (b) when infection reaches ear shoots after ear emergence the silk fibres at the tip darken, become caked up and turn into hardened mass, leading to poor grain filling and (c) if ears infected after grain filling stage, the kernes become smaller in size, light in weight, chaffy, lustuerless and cupule infection is common. Kernels developing in such cupules show reddish brown, horse-shoe shaped lesion below the crown. Such disfiguration is useful in diagnosing the disease even at the harvest time. Infection after dough stage does not affect grains severely as they become hard due to maturity.

2.3 Causal organism

The causal organism of banded leaf and sheath blight disease has been reported more than 100 year ago. The German scientist Julius Kuhn observed a fungus on diseased potato tubers and named it as *Rhizoctonia solani* (Kuhn, 1958). Since then the fungus has gained the reputation of being a wide spread, destructive and versatile plant pathogen. It occurs in all parts of the world and is probably indigenous to uncultivated areas.

The genus *Rhizoctonia* was erected in the year 1915 (De Candolle, 1915) to accommodate the non-sporulating root pathogen *R. crocorum* D.C. ex. Fr. The fungus produces sclerotia of uniform texture with hyphal threads. As a result the genus contains a heterogeneous group of fungi of diverse relationships. The pathogen has a wide host range including maize causing seed decay, damping off, stem canker, root rots, aerial blight and seed/fruit decay. Due to its competitive saprophytic ability and high pathogenic potential, make *R. solani* a persistent and destructive plant pathogen. The perfect state, *Thanatephorus cucumeris* (Frank) Donk (Sakena, 1971) is the only sure means to recognize *R. solani* (Talbot, 1970). But the lack of standard fruiting method, makes the task difficult. *Corticium sasakii* (Shirai) Matsumoto was considered as a possible synonym of *T. cucumeris* (Tu and Kimbrough, 1973). Other possessions of a basidiomycotina perfect state referred to as *Corticium solani* (Prill and Deder), Bourd and Granz (Deighton, 1932; Heath, 1956; Wiltshire, 1956). *C. vagum* Berk and Curt (Van Eijanathen, 1961), *Hypochoyus sasakii* Shirai (Payak and Renfro) and *Pellicularia sasakii* Shirari (Kujware, 1968). This multiplicity of names has been caused due to occurrence of pathogen more frequently in an anamorphic stage (Gangopadhyay and Chakravorti, 1982) and availability of a few characters for differentiation of teleomorph stage (Exner, 1953; Tu and Kimbrough, 1973). On the basis of large size basidia and spore, frequent occurrence of adventitious septa in protosterigmata, more, intense KI reaction in

cytoplasm, presence of neutral lipids and primitive type of sclerotial development has been reported between the two fungi and proposed the sheath blight pathogen to be named as *T. sasakii*.

Sheath blight pathogen has been considered distinct from *R. solani* complex on the basis of cultural characters, symptomatology, pathogenicity (Exner, 1953) and compatibility (Ogoshi, 1975 a,b; Ogoshi, 1976; Parmeter *et al.*, 1969). Comparative studies on mycelial and sclerotial characters of basidia (teleomorph) and anamorph of *Corticium solani*, *C. microsclerotia* and *C. sasakii* led to the conclusion that these did not differ significantly as distinct species and considered as forma speciales of *Pellicularia filamentosa* (Pat.) Rogers, a synonym of *T. cucumeris* (Frank) Donk. An additional f.sp. *timissii* was created to have four strains f.sp. *sasakii*, *S. sp.*, *microsclerotia*, f.sp. *solani* and f.sp. *timssii*. From pathogenecity point of view three characters are significant (i) isolates may cause several types disease and symptoms, (ii) isolates may vary from avirulent to aggressively virulent, and (iii) host range among isolates may vary from few to extremely wide. Comparison of 200 isolates of *R. solani* from 59 crop plants on the basis of cultural, pathogenecity and saprophytic ability characters indicated that isolates belonged to specific groups.

Number of nuclei per cell provide valuable diagnostic criterion (Flenkje and Stretton, 1964; Flenkje *et al.*, 1963; Kim *et al.*, 1992). Actively growing hyphae are multinucleate and there is a great variation in the number of nuclei per cell.

2.3.1 Pathogenicity

From the taxonomic point of view three characteristics are significant :

- (i) Isolates may cause several types of disease including aerial blight, damping off, seed decay, fruit decay and root rot.
- (ii) Isolates may vary from a virulent to aggressively virulent.
- (iii) Host range among isolates may vary from limited to extremely wide.

Since *R. solani* has a very wide host range it is difficult to assume that a given isolate is a virulent if it fails to attack a number of hosts. Similarly, host specificity is also difficult to determine. Variation was shown by some isolates when tested repeatedly in wheat (Leclerg, 1939) and in different soils (Sanford, 1938). Prior nutrition may also influence virulence (Weinhodl and Bowman, 1967). Thus pathogenicity to a wide range of plants is characteristics of *R. solani* avirulence and restricted host range do not exclude isolates from *R. solani*.

2.3.2 Host range

The pathogen has a wide host range including over 32 families in 188 genera belonging to family Graminae, Papilionaceae and Solanaceae and genera *R. solani* f.sp. *sasakii* infects a number of crop plants, *Paspalum scrobiculatum*, *Panicum purpureum*, *Panicum miliaceum*, *Zea maxicana*, *Zea mays*, *Oryza sativa*, *Saceharum officinarum*, *Sorghum bicolar*, *Arachis hypogea*, *Glycine max*, *Pisum sativum*, *Vigna radiata* and *Lycopersium esculentum* (Baruah and Lal, 1981; Ahuja and Payak, 1988; Paul and Sharma, 1990; Singh *et al.*, 1994). Maize has also been found to be infected by isolates from rice, sugarcane arrow rot and some grasses (Haque, 1975; Ahuja and Payak, 1985). Cross inoculation tests indicated that rice and turmeric isolates did not differ in pathogenicity and morphological characters (Lal *et al.*, 1980). Cotton isolates showed a clean distinction from cereal isolates and within latter, wheat isolates were considered to be different from those of maize and rice isolates (Sohi *et al.*, 1963; Xia *et al.*, 1993). Rice and maize isolates are, however, indistinguishable on the basis of cross inoculation tests, host range, virulence, number of nuclei per hyphal cell, and other morphological characters including pathogenicity. Comparative studies of rice maize, sugarcane and sorghum isolates revealed that maize isolates were pathogenic on *Paspalum scrobiculatum*, whereas sugarcane and sorghum isolates failed to infect.

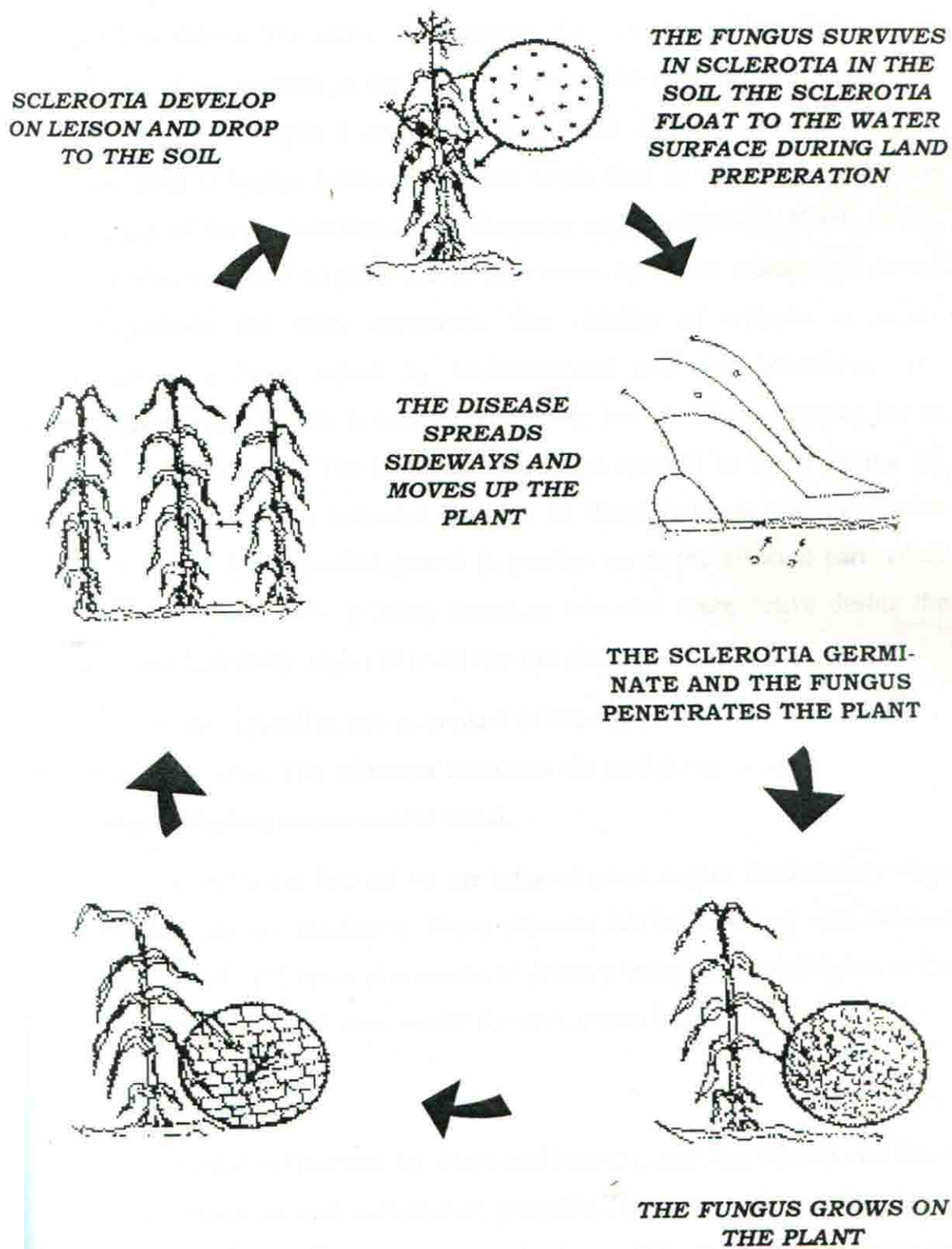


Fig. 5 : Disease cycle of banded leaf & sheath blight of maize

2.3.3 Source of primary and secondary inoculum

The primary source of inoculum are the sclerotia present in soil or in infected host debris. The active mycelium on the other grass hosts that grow in the vicinity of maize plant in the field may also serve as a source of inoculum. Sclerotia are present upto 2 cm depth (Roy, 1980). Population of sclerotia in ploughed field is higher between 6-12 cm depth than in upper layers and the sclerotia buried deep in soil have better bioyance and germinability (Dash, 1985). Sclerotia which survive on plant debris often come up on the soil surface during field preparation and other operations. The viability of sclerotia is greatly influenced to a large extent by environmental and soil conditions. The germinated sclerotia comes in contact with lower leaf sheath or dropping leaves touching to the ground. The infection progresses upward in bands killing the tissues in advance. The infection extends to distal half of the leaf lamina exhibiting buff while mycelial growth in patches on to the affected part within 48-72 h of infection. The primary inoculum becomes more active during the period of pre-flowering stage (30 to 40 day old plants).

Secondary spread is due to contact of healthy plants with infected leaf or sheath or vice-versa. The infection continues till mid-dough stage infecting the entire plant including the ear except tassel.

The sclerotia ear formed on the infected plant organs immediately when the conditions are not conducive. These sclerotai fall down in soil with infected host leaves/sheath and serve as a source of primary inoculum for infection on the other host crops in field or over winter for next season (Fig. 5).

2.3.4 Epidemiology

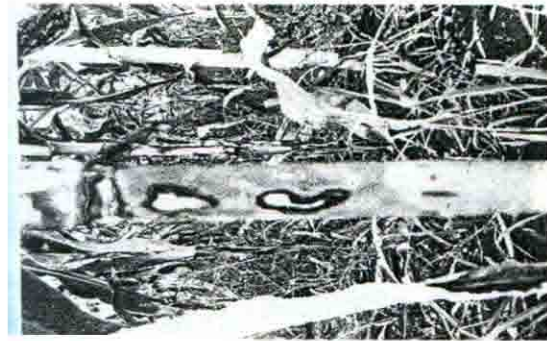
The disease is favoured by warm and humid conditions (Quazi and Iqbal, 1970). High moisture and temperature prevailing in north Indian plains during the month of mid July till August, a period when maize crop is in vulnerable for

attack. Studies on the meteorological factors in relation to disease progress revealed that relative humidity (88-90%) in first two weeks of inoculation favoured rapid disease development with an optimum temperature of 28°C and below 70 per cent RH, the disease development and spread is checked. Similarly rainfall over 100 mm in the first two weeks of inoculation favoured early infection and disease development (Ahuja and Payak, 1981). Positive correlation was noticed between rain fall and disease progress (Pandey, 1992) this led to partial regression coefficients to derive a similar action equation based on meteorological factors for the prediction of disease. However, it needs more data to standardize the model for practical utility.

2.3.5 Artificial inoculation technique

Various methods and techniques have been used for mass multiplication of inoculum (Ahuja and Payak, 1981; Amin, 1975; Lal *et al.*, 1980; Singh and Sharma, 1976). Fungal growth on agar medium has been used for field inoculation in paddy (Amin, 1975). Discs of actively growing mycelium sclerotia from fifteen day-old culture and tooth pick carrying mycelium for ear rot infection in maize have also been used. However, all these methods appear cumbersome and time consuming (Sinha, 1992). Of 18 substrate evaluated unhulled bajra, barely, oats and sorghum grains supported excellent growth of *R. solani* and be very well used as base. Several other grains including rice supported fairly good growth of the fungus, while the pathogen apparently did not grow on maize seed (Lal *et al.*, 1980). Rice was reported earlier as a good medium for mass multiplication of rice isolate of the pathogen (Anonymous, 1974). Another advantage in using bajra, barley, oats and sorghum grains was that the inoculum could be prepared easily and stored at room temperature for considerable period without any significant loss in viability of the pathogen. Most of these media tried by earlier workers were used mainly for soil inoculations rather than for direct use on growing plants (Papavizas and Ayers, 1961, 1965).

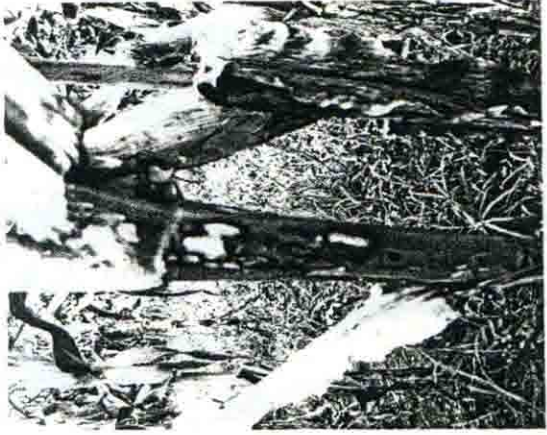
PLATE -4



CM104 (BLSB 1)



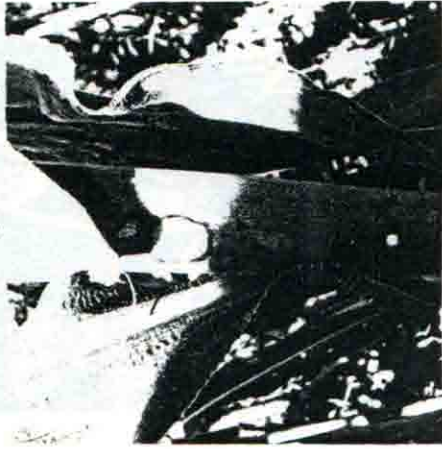
CM105 (BLSB 9)



CM500 (BLSB 19)



CMT1 (BLSB 21)



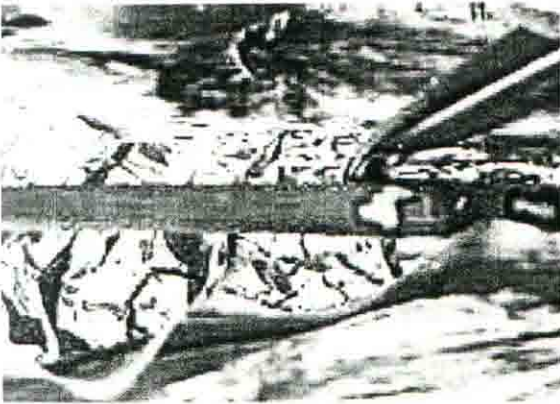
CM600 (BLSB 22)



CM104 (BLSB 23)

Disease Reaction of Resistant Maize Genotype

PLATE -S



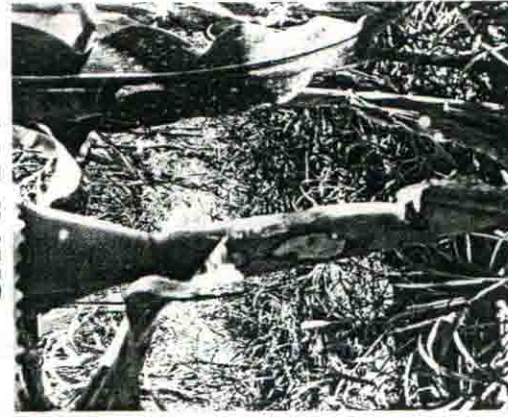
CA14509



CA00310



CA049Y04



(561)



CA00106



(556)

Disease Reaction of Resistant Maize Genotype

Most convenient method is the soaking of sorghum/barely/oat grains in water for 24-36 h after thorough washing in running tap water. The soaked grains (40 g) are dispersed in 250 ml Erlenmeyer flasks after removing excess water and plugged with cotton and aluminum foil tightly. Care should be taken to keep the ratio of grain while using the different capacity flasks, are subjected to autoclaving at 15 lb psi for 30 minutes. Before the second sterilization is attempted 24 h after the first one, each flask should be shaken to remove formation of grain clots. The grain medium is inoculated with actively growing homogenized culture suspension in sterile water. The flasks are incubated at 28°C for 7-10 days. During incubation the grains in flasks should also be shaken to provide uniform fungal growth on all grains. This grain culture can be used immediately for inoculations. Uniform mycelial growth is favoured by prolonged exposure of culture to visible light (Lokesh and Somashekar, 1988).

2.3.6 Screening of germplasm

Among the four inoculation levels, placing of 48 sorghum grains provide equally effective for causing maximum disease development in field. Inoculations made on all the leaf sheaths, upto ear placement produced heavy disease infection within five days. Inoculations made up to 4 to 6 lower leaves also resulted in good infection. Plants inoculated at 15 days old to 75 days old stage at 15 days intervals indicated that all the stages of plant growth were prone to disease development. The plant age from 30 to 60 days has shown significant relationship with disease development (Lal *et al.*, 1980). Plants infected before pre-flowering stage to silking resulted into complete infection upto ear causing few or no grain. The grains become hard as they approached maturity. Screening tests carried out on 6 inbred lines having different levels of resistance using 4 different methods of inoculation revealed that hill inoculation with soil cover resulted in high infected sheath ratio with small variance among inbred lines (Koinuma and Mockizuki, 1989). Therefore, in view of the above finding and the continuous work on germplasm evaluation at our place suggested that placing of

only 2 grains in between the two lower third and fourth leaf sheath and stem starting at pre-flowering stage resulted in fairly good disease outbreak in field. It is also suggested that the inoculations be repeated again within seven days to avoid escape from inoculations. Inbred lines, CM 104, CM 103, CM 300, CM 105, P 217 407, CM 600 and hybrid VL 43 have been found resistant under field and laboratory conditions (Ahuja and Payak, 1984). Of these, CM 104 and CM 105 have been reported earlier to be resistant source to banded leaf and sheath blight of maize. Out of 24 Expt. hybrid (St. Croix × BB × 4D) × (Cuadalupe 14 D × 16 D) was found to be resistant to ear rot (Singh and Sharma, 1975). In 15 crosses involving 6 inbred lines, CM 104 was the best general combiner and CM 104, Eto 182 and Aust 25 were recommended as source of resistance for incorporation into agronomically superior genotype (Vimla *et al.*, 1988). Of the 80 field sibs tested for multiple disease resistance, 37 were found resistance to leaf and sheath blight also (Kaiser and Chowdhuri, 1986) maize varieties, Junjuok Suwan 83, Suwan 87, Suwan 89, P-3055, P-3160, DK 689 and × CG 51 showed high tolerance levels to sheath blight disease in Korea (Lee *et al.*, 1989).

2.4 Laboratory evaluation

Excised leaves have been used to study various aspects of host pathogen interactions (Yarwood, 1946; Payak, 1955; Hooker and Yarwood, 1966). The use of excised leaves/plant parts has been extended to evaluate germination for disease reaction (Anonymous, 1965; Srivastava and Ahuja, 1973; Thind and Payak, 1976). On the basis, rating scale 1.0-5.0 which is used in laboratory method (Ahuja and Payak, 1981) categorize different inbred lines of maize in resistant (1.0-2.0) e.g. CM 104, CM 300, CM 105, CM 600 etc) and intermediate (2.1-3.0) (CM 201, CM 109, CM 112, etc.) (3.1-3.5) (Cuba-257, CM 500) (3.6-4.0) (VL 54) susceptible (Sweet corn, X-104, Hunius etc.).

Ahuja and Payak (1981) single grain culture is placed on adaxial surface of leaf pieces (8.0 × 5.0 cm), collected from 30 to 45 days old plants and floated

on kinetin solution (2 ppm) in petridishes. Inoculated leaf pieces are incubated at $27 \pm 1^{\circ}\text{C}$ for two day and intensity on the basis of extent of area affected is recorded.

During evaluation of over 200 maize germplasm including inbred lines specifically for banded leaf and sheath blight at Pantnagar. Only six inbred lines CM 105, CM 117, CM 600, CM 201, CM 205 and CML 267 showed moderate level of resistance (Anonymous, 1995). Total phenols and soluble proteins in CM 600 might be contributing to resistance than susceptible inbred line CM 202 (Gangopadhyaya Lal, 1986). Under screening tests in Orissa, India, inbred maize lines CM 122, CM 123, CM 500 and CM 600 were resistant to *Maydis* leaf blight and C 177 and CM 211 were resistant to banded leaf and sheath blight.

2.5 Type of markers

The markers have been categorized into two categories e.g. morphological markers and molecular markers.

2.5.1 Morphological markers

Morphological markers have been routinely used to analyse genetic diversity with the major disadvantages of the limited number of morphological characters available for analysis and also influenced by environmental factors. As a consequence different plant genotype can not be distinguished (Staub and Meglie, 1993). The genetic basis of most morphological variations are generally unknown and hence these markers could not provide desirable information for genetic differentiation.

2.5.2 Molecular markers

2.5.2.1 Protein markers

The most important protein markers in plant breeding are isozymes. Isozymes may have different charges and hence get separated during

electrophoresis (usually starch gels) (Mark and Meller, 1959). The isoelectric focusing (IEF) is another protein electrophoretic technique with higher resolution (Radela, 1980). Since enzymes catalyze specific biochemical reactions, the position of a particular enzyme on gel can be visualized by providing appropriate substrate and co-factors and the formation of a coloured enzymatic reaction product can be deposited on gel forming a visible band. The presence of specific enzymes visualized on gel have a genetic basis and can provide information as co-dominant markers. Application of isozymes in plant genetics have been reviewed by Tanksley and Orton (1983). Isozymes have been extensively used in germplasm characterization and tagging disease resistance. Polymorphic isozymes and storage proteins have been investigated for use in the classification of a wide range of crops (Cook, 1987) and maize (Cardy and Kanneberg, 1982).

Morphological description of plant cultivars often create problem in identification because the phenotypic difference within species are too minute with continuous addition of new varieties the phenotypic difference between them have become unclear.

Identification of crop cultivars through biochemical markers have been used to measure genetic diversity and genetic relationship among individual and populations. It has been widely used to establish phylogenetic relationship among taxa, genetic markers for hybrid confirmation and for convenient screening of seedling target character (Thorpe *et al.*, 1988).

Protein and allozyme markers provide an indirect assessment of nuclear DNA (nDNA) variability and are in routine ways and widely accepted as reliable method for determination of genetic identity of the crop varieties (Weir, 1990). The successful use of these markers however, depends on observed polymorphism in seed proteins.

According to Cook (1983) electrophoresis is the choicest method to solve protein polymorphism and polyacrylamide gel electrophoresis was introduced by

Raymond and Weintraub (1959). The rate of migration is affected by a large number of factors such as strength of field net charge, size and shape of molecule, ionic strength, viscosity, pH of the buffer system, type of buffer system and its concentration, concentration of acrylamide and bisacrylamide, running duration and temperature (Padloni and Vorier, 1993).

The majority of studies employing electrophoresis is polyacrylamide gel, use of buffer system designed to dissociate proteins to their individual subunits. SDS sodium dodecyl sulphate is one such dissociating agent. The protein mixture denatured by heating at 100°C in the presence of excess of SDS and ethanol reagent (to clear classified bands). Under these condition, most polypeptides bound SDS in constant weight ratio. The bound SDS-polypeptide complexes carries an identical charge density and migrates in polyacrylamide composition (Anisimora *et al.*, 1995).

Korangi (1989) have analysed inbred lines and hybrids by sodium dodecyl sulphate porosity gradient polyacrylamide gel electrophoresis (SDS-PAGE), a technique which dispenses with the need for germination and for parallel examination of many isoenzymes. Korangi (1989) SDS graded polyacrylamide gel electrophoresis allowed separation of upto 60 protein monomers of a homogeneous inbred maize sample. A heterogeneous inbred sample demonstrated alternatives of band intensities and positions, and biotypes could be differentiated, heterogenicity varied with sample but was 2 and 12:1. Shah *et al.* (1989) evaluated inbred lines and their mutant electrophoretically using grain protein, enzyme and glycoprotein patterns. *Helminthosporium turcicum* mutant (resistance to *Helminthosporium turcicum*) showed characteristics bands or spots, whereas a protein suitable as a marker gene was observed in the waxy mutant. The glycoprotein patterns can identify the lines but not mutants. Staining for peroxidases indicated heterogeneity in the lines. Frova *et al.* (1988) evaluated 35 inbred lines by the SDS-PAGE following *in vivo* labeling with ³⁵S. A standard HSP pattern of 9 bands present in all but one line,

was defined, through 6 of these bands were present and reduced level in a few lines. Various combinations of 10 additional band were found in 28 lines.

Qu *et al.* (1986) have isolated major proteins in lipid bodies from maize cv. MO 17, soybean cv. Coker 237, cotton, cucumber, rape, jojoba, mustard, sunflower cv. 894, safflower cv. 5541, oil seed flax cv. 10 and *Ricinus communis* cv. Hale Seeds were shown to be distinctly different by SDS-PAGE pattern. Bunce *et al.* (1985) estimates the variation of molecular weight of cereal prolamins by SDS-PAGE, the prolamins fractions from barley wheat, rye, maize, millet and sorghum grains were separated using 3 system of polyacrylamide gel electrophoresis in presence of SDS- and relative MW of the major components were determined by comparison with the mobilities of standard proteins.

Falk *et al.* (1983) uses various purified from greenhouse grown inoculated maize plants using a modified method of those used for sonchus yellow net virus and potato yellow dwarf virus. Electron microscopy of purified preparations showed virus 224 ± 21 × 6 ± 9 nm. Purified preparations injected into *peregrinus maidis*, the planthopper vector of MMV, were infectious to maize MMV had sedimentation coefficient of 774 as estimated by linear-log sucrose density gradient centrifugation.

Kolesnichenko *et al.* (2000) a search for proteins immunologically related to the 310 kDa cold shock protein from winter rye was undertaken in several grass species differing in tolerance low temperature, maize (*Zea mays* hybrid VIR 36) winter wheat (*Triticum aestivum* cv. Zalarinka) winter rye (*Secale cereale* cv. Dymka), and a highly resistant wild species, *Elymus sibiricus*.

Corcuera *et al.* (1999) have studied different inbreds endosperm texture through electrophoretic method. A modified SDS-PAGE technique was used to analyse the endosperm of 9 inbred maize lines classified as flint, opaque 2 or waxy types. Degree of affinity amongs the genotypes was calculated on the basis of the protein bands. Yu *et al.* (1998) have advance in identification technology

maize hybrids and inbreds. Subjects considered include morphological and chemical procedures electrophoretic techniques such as zein-IEF, iso enzyme, electrophoresis, SDS-PAGE and acidic-PAGE and molecular technique such as restriction fragment length polymorphism or random amplified polymorphic DNA analysis.

Highes *et al* (1981), three northern flints (RIW, WF, CM 48) and two corn belt inbreds (W 64A, Oh 43) differed by at least four polypeptides in the 80-90 kdalton range in pollen extract electrophoregrams. The variation in polypeptides, which different by only a few amino acids, is regarded as a reflection of genetic diversity in the gametophyte generation.

2.5.2.2 Molecular markers (DNA markers)

DNA markers are numerous in number and represent a milestone in genetics by providing a more or less complete converge of nuclear, mitochondrial and chloroplast genomes. These markers are stability inherited and are unaffected by environment and developmental stages (Brar, 1997).

A major achievement occurred in marker technology with the advent of marker based on DNA sequence (Botstein *et al.*, 1980). The genetic markers based on DNA provide a genetic diagnostic tool that permits direct identification of genotypes in environment in independent manner in any tissue and developmental stage. It has great benefits for plant breeders, when the DNA markers are closely linked to the traits of interest (Tanksley *et al.*, 1989).

Molecular markers are rapidly being adopted by crop improvement researchers globally as an effective and appropriate tool for basic and applied studies addressing biological components in agricultural production systems (Jones *et al.*, 1997; Mohan *et al.*, 1997; Prioul *et al.*, 1997). Molecular markers offer specific crop improvement. Use of markers in applied breeding programmes can range from facilitating appropriate choice of parents for crosses, to

mapping/tagging of gene blocks associated with economically important traits (often termed “quantitative trait loci” – QTLs). Gene tagging and QTL mapping in turn permits marker-assisted selection (MAS) in backcross, pedigree and population improvement programmes (Mohan *et al.*, 1997). This is especially useful for crop traits that are otherwise difficult or impossible to deal by conventional means. The hereditary products of marker assisted back crossing programs provide genetic tools for crop physiologists and crop protection scientists to use in improving our understanding of the mechanisms of various abiotic stress tolerance (Jones *et al.*, 1997; Prioul *et al.*, 1997) and resistances to biotic production constraints such as diseases, insect pests, nematodes and parasitic weeds like striga.

2.5.2.3 Characteristics of molecular markers

1. Molecular loci can be assayed at whole plant, tissue and cellular level.
2. Allele frequency tends to be much higher at molecular loci.
3. Molecular loci might exhibit a codominant mode of inheritance that allow the genotypic identification of individuals in segregating populations.

The basis of DNA sequence differences include single base pair changes, deletions, insertions and chromosomal inversions. A variety of PCR based marker systems are available including Random Amplified Polymorphic DNA (RAPD) (Williams, 1990), Amplified Fragment Length Polymorphism (AFLP) (Vos *et al.*, 1995), Simple Sequence Repeats (SSR) (Zietkiewicz *et al.*, 1994), Arbitrarily Primed PCR (APCR), Amplicon Length Polymorphism, Allele Specific PCR, DNA Amplification Finger Printing (DAF), Specific Amplicon Polymorphism (SAP), Sequence Characterized Amplified Region (SCAR), Single Strand Conformation Polymorphism, Simple Sequence Repeats (SSR), Sequence Tagged Sites (STS) and Single Nucleotide Polymorphism (SNP). A number of reviews have been published in different aspects of molecular markers

and their application in crop improvement. Recent reviews of molecular marker's usefulness in mapping plant genomes have been written by authors (Karp *et al.*, 1997; Malyshev and Kartel, 1997; Mohan *et al.*, 1997). Among various types of molecular (DNA) markers RFLP, RAPD, AFLP and SSR are the most important class of markers.

2.5.2.4 Random amplified polymorphic DNA (RAPD) markers

Williams *et al.* (1990) described a simple technique based on PCR amplification of genomic DNA with single primers of arbitrary nucleotide sequencing. The polymorphisms should be called Random Amplified Polymorphic DNA (RAPD). The initial experiments indicated that the percentage of G + C contents of primers should be greater than 50 per cent to generate detectable level of amplification products. The appropriate number of sequence of primer should be 10-12 nucleotides in length.

Reiter *et al.* (1992) stated that the primers for RAPD should be relatively short. Due to this reason, mismatch of even a single nucleotide can often prevent the primer from annealing giving rise to RAPD polymorphism. The RAPD polymorphisms result either from chromosomal changes like insertion, deletion or inversion or base changes at the primer binding sites i.e. point mutation, which alter the size or prevent the successful amplification of the target DNA.

The amplifiable distance exists within two primer binding sites, usually less than 3 kb pairs and the orientation of primer binding sites should be in reverse order (Whitkins *et al.*, 1994).

If the primer sites are much farther apart in the genome, the amplification will not occur. The choice of which single arbitrary primers to use is done operationally by testing in the plant species to be studied, and those which give simple and reproducible bands are selected (Kochert *et al.*, 1994).

RAPD markers are inherited as dominant/recessive characters and DNA fragment, amplified from homozygous and heterozygous loci can not be distinguished from each other. Therefore, in an F₂ generation only two classes of progeny can be distinguished.

Molecular markers are also used in breeding programmes which can range from facilitating appropriate choice of parents for crosses to mapping/tagging

2.5.2.5 Application of RAPD in maize

Meller *et al.* (1999) investigated genetic variation among 15 accessions of Native American maize from the great plains using random amplified polymorphic DNA (RAPD), revealed very high level of polymorphism among accessions. Banding pattern ranged in percentage polymorphism from 46.78 per cent to 86.2 per cent with an overall mean of 70.7 per cent for the primers analyzed. The construction of genetic relationship using cluster analysis and principle coordinates revealed that RAPDs are successful in confirming hypothesized relationship and in identifying miss classified specimen.

Sivolap *et al.* (2001) investigated maize genotypes by RAPD, SSR and ISSAR methods. Using DNA of 12 inbred lines and 6 respective hybrids.

Sieh *et al.* (2002) have used RAPD to evaluate the genetic diversity of 13 maize inbred lines to determine the correlation between genetic distance and single cross hybrid performance. Khan *et al.* (2002) used the feasibility of RAPD marker to carried out the study of salt tolerance maize cultivars. Polymorphism was sought in two salt tolerant accession and a small sample of their F₂ progeny using a limited number of random primers. Louie *et al.* (1991) have study the genetic basis of resistance in inbred Pu405 to strains A, B, D, E and F of maize dwarf mosaic poly virus. Investigation carried out by incorporating 26 translocation markers and morphological markers for white endosperm Y₁ on chromosome 6 into the inbred M 14. Linkage relationships with molecular markers were determined by the RAPD analysis.

Pe *et al.* (1993) have used RAPD in order to understand the genetic basis of resistance to *G. zea*, genetic linkage were estimated between available molecular markers and the character.

Chin *et al.* (1993) used 3 inbred lines of maize, the same DNA profile were directly amplified and generated from fresh leaf tissue as were obtained using extracts made from lyophilized leaf tissue from two week old seedlings. Amplifications obtained via classical polymerase chain reaction (PCR) using a pair of primer gave clear and repeatable banding profiles regardless of whether the DNA had been extracted from lyophilized flour or whether reaction occurred instead in the presence of an immersed fresh leaf disc as a source of DNA. RAPD banding profiles obtained from reactions that had DNA from lyophilized leaf flour also gave clear profiles. However, with fresh leaf disc as the source of DNA, the RAPD method did not result in any obvious bands following two round of amplification with RAPD. However, the second round resulted in clear and distinct banding profiles that were very similar to those obtained from a single round of amplification with RAPD.

Dinelli *et al.* (1993) have investigated genomic regions involved in resistance of maize to *G. zea* were located using regression analysis between each RFLP and RAPD locus and a character expressing the level of disease resistance (infected area/total area ratio in the inoculated internode CINF) or interval mapping (using map marker QTL).

Hunter *et al.* (1993) have defined that the RAPD and RFLP will helpful to identification of relatedness (difference) between the different maize lines.

McDonald *et al.* (1994) have describe a DNA extraction procedure from dry seeds of maize, cotton, soyabean, wheat and red clover that can be successfully employed in subsequent amplification of DNA fragments using the RAPD technique. This procedure proved to be inexpensive simple and fast, it avoids the use of growing plant, can be applicable to a number of crops.

Ting (1994) have uses the random amplified polymorphic DNA marker for analysis of the maize genotypes obtained through anther culture and also used this marker for investigating the map linkage.

Rafalski *et al.* (1997) have analyzed twelve flint and dent type maize inbreds for polymorphisms by using semi random primer against intron species junction sequence. Using semi random primers higher numbers of polymorphic bands and more complex polymorphic band patterns were revealed as compared to RAPD. Polymorphisms were located mainly in the transcribed region of the genome.

Abal Tawab *et al.* (1997), the molecular basis of tolerance of salinity was investigated in maize inbred line G 221-D, G 307-A, G-13, G-249, G-267 was chosen as the most salt tolerant line and G 278 as the most sensitive one. RAPD markers for salt tolerance was detected by bulked segregant analysis with 10-oligomer primer.

Zacchini *et al.* (1997) uses four callus lines from immature embryo of self crossed maize (*Zea mays*) hybrid cultivar were selected for RAPD analysis. Polymorphism was found, demonstrating a genetic difference between the lines.

Demeke *et al.* (1997) DNA based molecular marker have become important tools for developing improved cultivars and for studying phylogenetic relationships. The most commonly used molecular markers are restriction fragment length polymorphism and RAPD. Wang *et al.* (1997) have extracted DNA from 6 maize lines and was subjected to RAPD analysis using 5 primers. Zinzhi *et al.* (1997) utilize 15 maize inbreds which are widely used for hybrid production in China and subjected them for RAPD analysis. Sivolapyu *et al.* (1997) have uses 30 lines of maize with two type of molecular marker and the genetic distances between them were determined.

Lanza *et al.* (1997) have evaluated 18 maize inbred lines and to determine the correlation between genetic distance and single cross hybrids performance, by using random amplified polymorphic DNA (RAPD).

Matzir *et al.* (1996) have used RAPD analysis for identification of near-isogenic sweet corn lines and identified region of the maize genome associated with Sel (sugar enhancer) gene OPN 20-675 was on chromosome 3 near to UMC 50 and UBC 281-800 and UBC 425-700 were on chromosome 6 near to UMC 59. Zhang *et al.* (1996) have used RAPD markers for identification of soybean cultivars by using 30 primers. Agramma *et al.* (1996) have used random amplified DNA polymorphisms DNA marker to study the maize dwarf mosaic polyviruses (MDMV) resistance gene using segregation analysis.

Hetz *et al.* (1996) have used RAPD marker for identification of R + CS locus mapped to the short arm of chromosome in stem derived crown roots of the maize. Zhang *et al.* (1996) have described that RAPD markers are useful in identification of genotypes of various crops. In particular, this technology has important application in plant breeding and seed production, environment did not generally affect the stability of RAPD markers in maize seed. Gourmet *et al.* (1996) have used RAPD marker for differentiating the maize population which contains β chromosome or those devoid of β chromosome and he uses 320 RAPD primers. Beaumont *et al.* (1996) have used RAPD and RFLP marker to create a genetic linkage map. In both RAPD were shown to prove Mendelian type markers.

Kawata *et al.* (1995) have used 24 maize inbred lines and used 32 primers, some 128 fragments generated by these primers. Cluster analysis divided 24 inbred lines into 6 groups. Lebeel (1995). They surveyed and highlighted recent progress in application of biotechnology to resistance breeding in 3 main areas. Plant tissue and all culture genetically engineered resistance to viruses, bacteria and fungi, and use of molecular marker in plant breeding.

Montero *et al.* (1998) have studied the genetic diversity of populations via RAPD analysis have paid little attention to intra population genetic variability. Index of genetic variability is proposed based on the presence or absence of bands and the total number of bands per primer. This method was applied in 2 populations of maize.

Liu (1998) studied 15 major commercial inbred lines for heterosis breeding by using RAPD. RAPD clustering is seen as reliable approach to distinguishing heterotic groups of maize inbred lines. Pejic *et al.* (1998) made a comparison of DNA finger printing techniques by using a set of 33 maize inbred lines to evaluate this informativeness and applicability for the study of genetic diversity. The four assays (RFLP, RAPD, SSRs and AFLP) differed in the amount of polymorphism detected.

2.5.2.6 Advantage of RAPD markers

Whitkins *et al.* (1994) documented several advantages of RAPD based genetic analysis.

- (a) It is simple and fast.
- (b) It requires less labour and cost.
- (c) It is not necessary to construct or maintain a genomic library.
- (d) It requires small quantities of genomic DNA.
- (e) Same RAPD primers can be tested for different species.
- (f) It provides unlimited number of markers throughout the genome.
- (g) It reveals high level of polymorphism even within and among the species.

*Materials
and
Methods*

Field experiment were conducted at Crop Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar, where as glasshouse experiments were carried out in the Department of Plant Pathology, College of Agriculture at G.B.P.U.A.&T., Pantnagar (Udham Singh Nagar) Uttaranchal, India. Pantnagar falls in the humid subtropical climate zone known as Tarai and lies in foothills of Shivalik range of Himalayas. Geographically, it is situated at 29°N latitude and 79.30°E longitude at an altitude of 243.84 m above the mean sea level. The average relative humidity is highest (106.4-96.7) in August-September and December-January while lowest (60-33) May-June. Average rain fall in this area is about 1200 mm.

3.1 Field experiments

3.1.1 Preparation of field

Field preparations were begun in the second week of June crop season of 2003. The soil was well pulverized by one disc ploughing followed by three to four harrowings with a disc harrow. The field then leveled by tractor drawn leveler.

3.1.2 Fertilizer schedule

The fertilizers were applied at the rate of 100 kg N/ha, 60 kg P₂O₅/ha and 20 kg K₂O/ha, in the form of urea, single super phosphate, respectively. Nitrogen was splitted in three equal dosages. One third of N dose mixed thoroughly with all dose of phosphorus and potash applied as basal broad casting just before leveling of field. The second and third dosage of nitrogen were applied as top dressing along the row, one at the high stage and the other just before flowering.

3.1.3 Sowing

Seed of maize inbred lines given in Table 2 and 3 were used in field and glass house experiments. During 2003 sowing was done on 29th July, 2003. At optimum soil moisture level for germination, around 30 seeds were sown per 5 m row and 75 cm apart at 20 cm spacing and about 2.3 cm depth in shallow furrow. After planting, the seeds were covered with soil.

3.1.4 Interculture operation

After one week of germination, plants were thinned out keeping a plant to plant distance of 20 cm. Weeding and other operations were carried out as recommended for raising a good maize crop.

For disease assessment, epiphytotic conditions were created in field following artificial inoculations with causal agent *Rhizoctonia solani* at appropriate stage of the plant growth.

3.1.5 Preparation of inoculum

A pathogenic culture of *R. solani* isolated from fresh disc arid leaf sheaths of maize was used throughout the investigation. For preparation of inoculum, sorghum grains were soaked in water for 24 hours, after thorough washing in running tap water. The soaked 50 gm were filled in 250 ml Erlenmayer flask after removing excess water. These flasks were plugged with cotton and aluminium foil tightly. Grain filled flasks were then autoclaved twice at 15 lb pasi for 30 minutes. The second sterilization was repeated after 24 h of first one. Each flask was shaken to remove formation of grain clots. The grain medium was used for inoculation with actively growing culture of *R. solani* in PDA plates. One or two discs were seeded in each flask and then the flasks were incubated at $28 \pm 1^\circ\text{C}$ for 7-10 days. During incubation, the grains in flasks were also shaken to provide uniform fungal growth on all grains. These grains were then used for artificial inoculation in field.

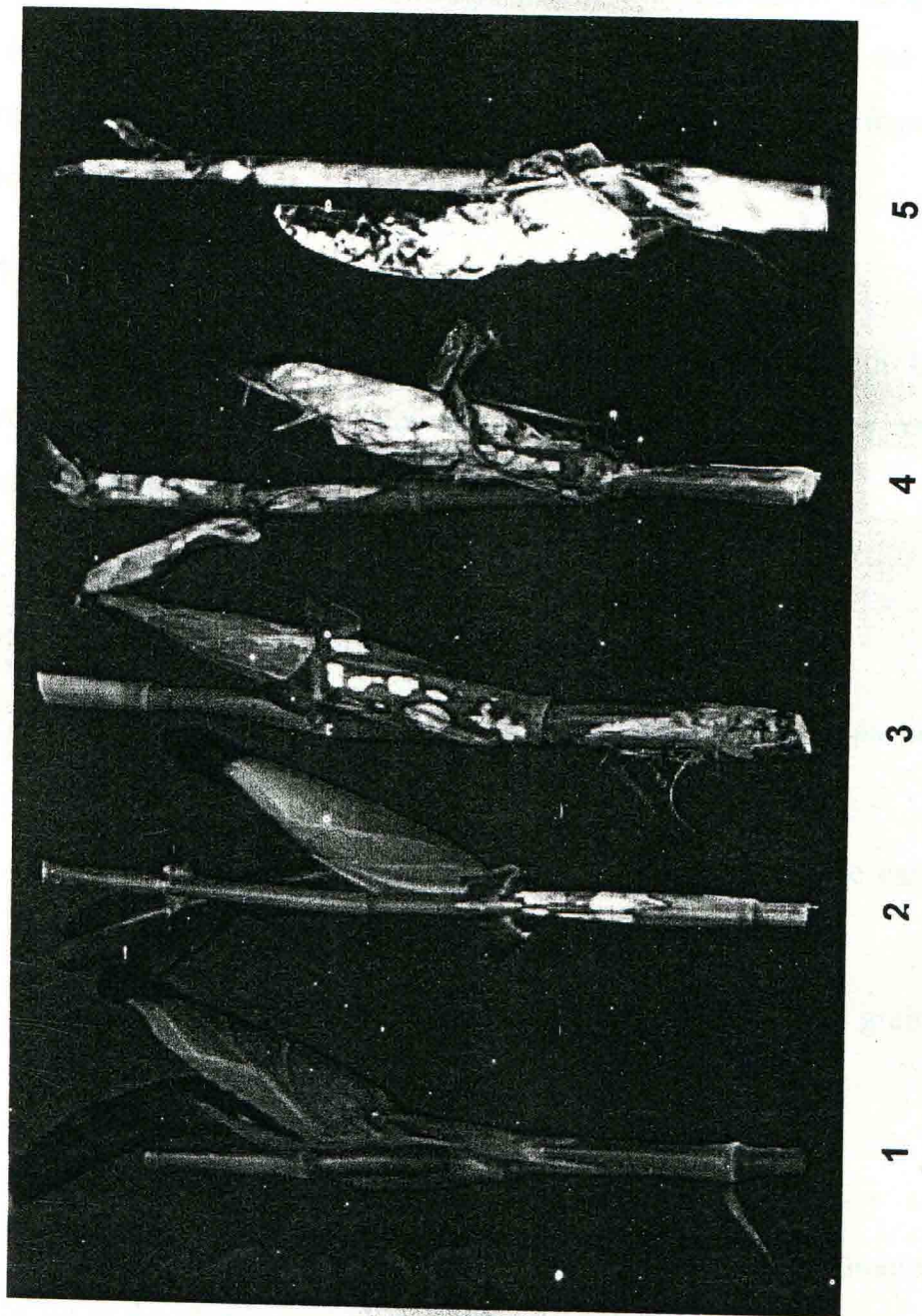


Fig. 1 : Field Disease Rating Scale 1-5

3.1.6 Field inoculation

Field inoculations were done by placing two sorghum grains between the leaf sheath and stem on lower third and fourth internode above the ground level just a week before the onset of tassel emergence stage of the crop. The inoculations were repeated after 3 days of first inoculation to safeguard against the escape.

3.1.7 Observations on disease severity

Observations on the severity of banded leaf and sheath blight were recorded two weeks after flowering following 1-5 disease rating scale (Butchaiah) as below :

- 1 = No infection
- 2 = Partial infection upto lower four leaf sheaths and leaves
- 3 = Heavy infection upto lower four leaf sheaths and leaves, partial on upper leaf sheaths below the our placement, no cob infection
- 4 = Heavy infection on all leaf sheaths and leaves below the ear placement partial infection on cobs
- 5 = Complete rating of cobs, very little or no grain formation, grains becomes chatty or may be rotten

3.2 Glasshouse experiments

In addition to genotype evaluation in field laboratory evaluation was also carried out in the glasshouse using sterilized sandy loam soil.

3.2.1 Pot filling

The pots were filled upto one fourth of height with well pulverized soil.

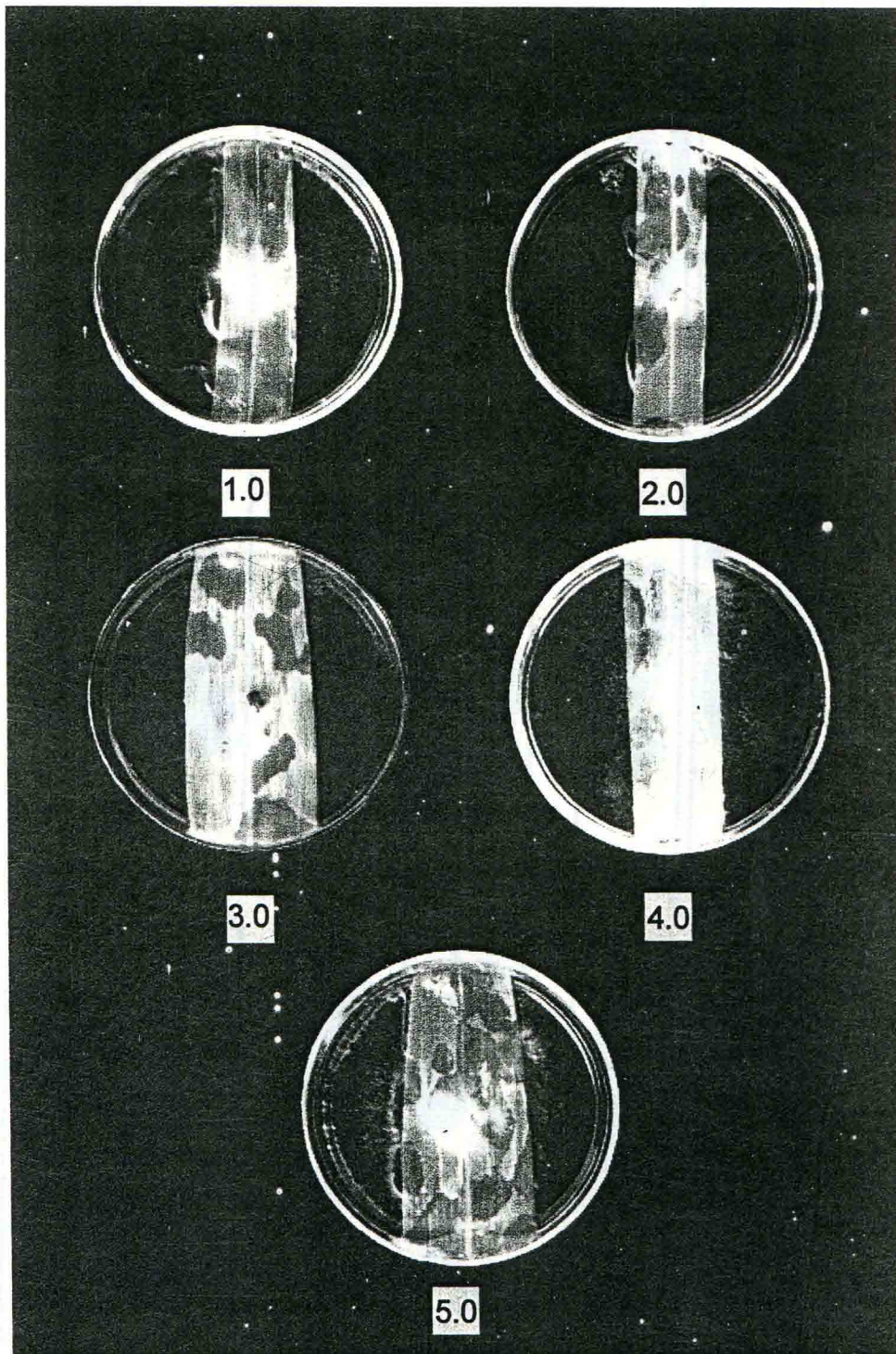


Fig. 3 : Laboratory rating scale for disease reaction to BLSB

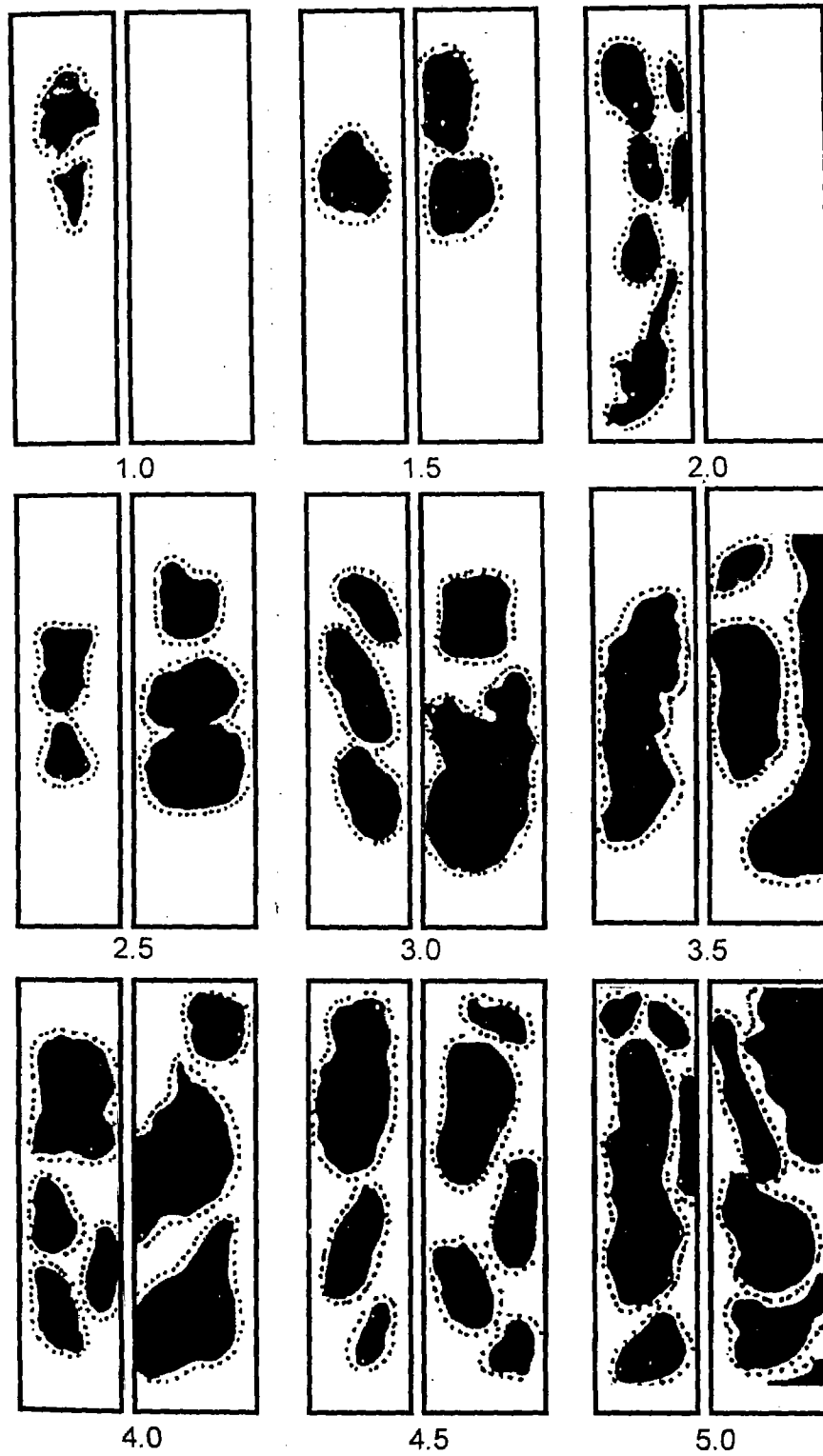


Fig. 4 : Disease rating Laboratory Method

3.2.2 Fertilizer application

The fertilizers were applied in the form of urea, single super phosphate and muriate of potash, respectively. Nitrogen was applied in three equal dosages.

3.2.3 Sowing

Seeds for glasshouse experiments are same which was used in field experiment. Of each genotypes 5-6 seeds were sown in one pots. After planting, the seeds were covered by the soil.

3.2.4 Interculture operation

After one week of germination, plants were thinned out for appropriate growth of plants. Weeding and other agronomic operation were also carried out.

3.2.5 Preparation of inoculum and inoculation

Preparation of inoculum and inoculation technique was same in glasshouse condition as used in field condition.

3.2.6 Laboratory method for evaluating maize germplasm

Rhizoctonia solani Kuhn. f. sp. *sasakii* was multiplied on autoclaved sorghum grains and leaves were collected from 30 day old plants in the afternoon for reason given by Payak (1955). Leaf pieces were cut into suitable size (8.0 × 5.5 cm), washed in tap water and floated on 8 ml of 2 ppm kinetin (6-furfuryl-amino purine) solution in petridishes. Five replicates having one leaf piece in each dish were maintained for all entries. These leaf pieces were inoculated by placing single grain culture on the midrib of adaxial surface and incubated at 27±1°C for two days.

Observations on disease development were recorded after 48 hr. Using Laboratory rating scale (1.0-5.0). Rating scale given in Fig. 4.

3.3 SDS-PAGE Analysis

The experiment for the study of polymorphism in seed proteins by SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) was carried out in the Department of Biochemistry, College of Basic Sciences and Humanities, G.B.P.U.A.&T., Pantnagar. The technique of SDS-PAGE as described by Laemmli (1970) and used with some modification as follows.

3.3.1 Experimental material

Experimental material for SDS-PAGE analysis is given in Table 2 and 3.

3.3.2 Equipments and accessories

- Vertical slab gel electrophoresis apparatus with all the accessories including glass plates, platinum electrodes, well forming combs, spacers and buffer tank.
- Power supply with adjustable constant current/voltage (200 mA, 500V) output capacity.
- Balance for weigh range of 1 mg to 250 mg.
- A bench top micro centrifuge.
- A platform rocker for staining and distaining.
- White light box for gel viewing.
- A scalpel for cutting the seeds, a hammer with smooth flat head and a smooth metal for crushing the seeds.
- A long blade for trimming the gel.
- Glassware for making stock and working solutions and eppendorf tubes for sample.
- Transparent plastic or glass trays preferably with lids for gel staining and distaining.

Table 2: Experimental Material

Pedigree of code lines used in BLSB 03 Trial

BLSB code	Code	Pedigree
BLSB-1	CA00106	EY-DMR-G-C5-S2-B-B-2-1-B-BBB-B
BLSB-2	CA049Y04	P49(Y)S5B-124-#-6-B-BBB-B
BLSB-3	CA03147	P31C4S5B-6-##-3-1-BBBB-B
BLSB-4	CA14509	SW92145-1P2S2-#-#-3-B-B-BB-B
BLSB-5	CA14518	SW92145-2EV-108-2-BBB-B
BLSB-6	CA03106	P31C4S5B-85-#-#-10-B-B-B-B-B
BLSB-7	CA003134	AMATLC0HS55-1-1-2E-2-2-1-B-B
BLSB-8	CA00370 (CML 469)	(AMATLC0HS133-1-F/R)-1-3-1-2-5-BBB-B
BLSB-9	CA34507	P345C5HS306-2-1-2-1-1-B-B-B
BLSB-10	CA00102 (CML 429)	EY-DMR-G-C5-S2-B-B-3-1-B-BBB-B
BLSB-11	CA00396	PIO3011F2-3-4-1-BBB
BLSB-12	CA00310 (CML 465)	AMATLC0HS71-1-1-2-1-1-1-B*6
BLSB-13	CA34506	P345C5S1B-15-4-2-1-2-1-2-B-BB-B
BLSB-14	CA03131	P31C4S5B-6-#-#-2-3-BB-B
BLSB-15	CA00334 (CML 433)	KTX3753F2-5-1-1-2-B-BBB-B
BLSB-16	CA34516	SW91345-1EV-100-3-BB-B
BLSB-17	CA03149	P31C4S5B-43-##-9-BBBB-B
BLSB-18	CA14510 (CML 428)	SW91145-2P3S2-#-#-3-B-B-BB-B
BLSB-19	CA00332 (CML 432)	KTX3752-7-1-1-1-B-BBB-B
BLSB-20	A00344	Pac777F2-6-1-1-BBBB
BLSB-21	CML 425	P31C4S5B-6-##-BBBB
BLSB-22	CML 430	AMATLC0HS169-1-1-1-1-2-2-1-B*3
BLSB-23	CA03124	P31C4S5B-99-#-#-B-B-B-B-B
BLSB-24	CA00384	(AMATLC0HS184-2-F/R)-2-3-1-2-4-BBB-B
BLSB-25	CA00378	AMATLC0HS115-1-2-3-3-1-2-BBBBB-B
BLSB-26	CML 421	P31DMR#1-55-2-3-2-1-BBBBBBB
BLSB-27	CA14711	Pop.1 47-F2#1 60-1-2-B-2-B-B
BLSB-28	Pop. 147-F2#161-3-2-B-1-B	Pop. 147-F2#161-3-2-B-1-B
BLSB-29	CA14519	SW92145-2 EV-55-1-B-B-B-B
BLSB-30	CA00104	EY-DMR-G-C5-S2-B-B-3-2-B-B-B-B
BLSB-31	CA03142	P31 C4S5B-23-#-#-4-B-B-B-B
BLSB-32	CA14527	SW89145-1 P7G-#-#-3-B-B-B-B
BLSB-33	CA03130 (CML 426)	P31C4S5B-38-#-#-2-B-B-B-B-B
BLSB-34	CA14502 (CML 427)	SW92145-2P9S2-#-#-4-B-B-B-B-B
BLSB-35	CA00304 (CML 431)	AMATLC0HS170-2-3-2-1-1-1-B-B-B-B-B
BLSB-36	CML 413	Sint Am. TSR-23-3-2-3-2-BB-f-##-BBBBB
BLSB-37	CA14501	SW92145-1P6S2-#-#-B-B-B-B
BLSB-38	CA14517	P145C4MH7-1-B-1-1-B-1-1-BBBB-B
BLSB-39	CA03116 (CML 470)	P31C4S5B-39-#-#-1-B-B-B-B-B
BLSB-40	CA14709 (CML 471)	P147-F2#110-1-2-B-2-BB
BLSB-41	CA14524 (CML 474)	SW92145-2EV-13-1-BBBB-B
BLSB-42	CA14520 (CML 475)	SW92145-2P4S2-#-#-11-BBBB-B
Check 1	CML 428	SW91145-2P3S2-#-#-3-BBBB
Check 2	CA00334 (CML 433)	KTX3753F2-5-1-1-2-B-BBB-B

Table 3 : Experimental Material

BLSB code	Code and Pedigree
CM 1	CM 104
CM 2	CM 105
CM 3	CM 111
CM 4	CM 115
CM 5	CM 119
CM 6	CM 120
CM 7	CM 121
CM 8	CM 123
CM 9	CM 125
CM 10	CM 500
CM 11	CM 400
CM 12	CM 600
CM 13	CM 209
CM 14	CM 210
S1	Surya
D1	D-995
D2	D-994
K1	Kanchan
A1	Amar
550	YHT Pant × 45-2-1-3-2-2-5-3
551	YHT Pant × 45-2-1-3-3-1-1-1
552	YHT Pant × 161-1-4-1-2-1-2-1
553	YHT Pant × 161-1-4-1-2-1-2-1
554	YHT Pant × 161-1-4-1-2-1-2-1
555	YHT Pant × 161-1-4-2-3-1
556	YHT Pant × 161-1-4-2-3-2
557	YHT Pant × 130-2-2-5-1-4-1-2
558	YHT Pant × 130-2-2-5-1-4-1-3
559	YHT Pant × 130-2-2-5-1-4-1-1
560	YHT Pant × 130-2-2-5-1-4-1-2
561	YHT Pant × 130-2-2-5-1-4-1-3
562	YHT Pant × 130-2-3-4-1-2-1-1
563	YHT Pant × 130-2-2-5-1-4-1-2
564	YHT Pant × 135-1-2-2-1-3-4-1
565	YHT Pant × 135-1-2-2-1-3-4-2
566	YHT Pant × 135-1-2-2-1-3-5-1
567	YHT Pant × 135-1-2-2-1-3-5-2
568	YHT Pant × 135-1-2-2-1-3-5-3
569	YHT Pant × 134-1-2-3-2-5-1-1
570	YHT Pant × 134-2-3-2-5-5-1-2

- Butter paper cut to 3" × 3" square pieces.
- Glass syringes for preparing and pouring solution.
- Latex gloves for handling toxic chemicals.
- Micropipettes with tips.
- pH meter.
- Gel documentation system to take photograph of gel.

3.3.3 Stock Solution

I. 2x sample buffer (pH 6.8)

Tris base (151 g) was dissolved with 8 g SDS, 30 ml glycerol, 2 mg bromophenol blue in 64 ml distilled water. The solution was heated to dissolve till it turned transparent. Concentrated HCl was added drop wise and pH was adjusted to 6.8. Total volume was adjusted to 100 ml. It was stored in an oven at 37°C.

II. 10% ammonium per sulphate

Dissolve 0.1 g ammonium per sulphate in 1 ml distilled water and was made fresh each time or kept for 5 day in a refrigerator.

III. 2x separating gel buffer (pH 8.9)

Tris base (45.5 g) was dissolved with 1 g SDS in 460 ml water. Concentrated HCl was added drop wise until pH fell to 8.88. The total volume was made to 500 ml and stored in a refrigerator.

IV. Stock acrylamide for separating gel (0.8% C)

Acrylamide (75 g) was dissolved with 0.6 g bis-acrylamide in 181 ml water. Total volume was made to 250 ml (30% acrylamide, 0.247 bis acrylamide and 0.8% C) and was stored in dark bottle at 4°C.

V. 2x stacking gel buffer (pH 6.8)

Tris base (6.06 g) was dissolved with 0.4 g SDS in 190 ml water. Concentrated HCl was added drop wise until pH fall to 6.8. Total volume was made to 200 ml. It was stored in a refrigerator.

VI. Stock acrylamide for stacking gel

In 36 ml water, 15 g acrylamide, 0.4 g bis-acrylamide were dissolved. Total volume was made to 50 ml (30% acrylamide, 0.8% bis acrylamide and 7% C). It was stored in dark bottle at 4°C.

VII. 10x electrode buffer (pH 8.3)

In 885 ml water, 30.3 g tris base, 144.2 g glycine, 10 g SDS were dissolved. The pH was approximately 8.6. The total volume was one litre and stored at room temperature. For use, one part of this solution was mixed with 9 parts of distilled water. The pH falls to approximately 8.4. A little more glycine is added to bring the pH to 8.3.

VIII. Staining Solution

Step-1 : In 25 ml water, 0.25 g of coomassie brilliant blue R-250 was dissolved.

Step-2 : In 720 ml water, 180 ml methanol and 60 ml glacial acetic acid, 60 g of trichloroacetic acid was dissolved in a big size beaker.

Step-3 : Step-1 solution was tipped into Step-2 solution and mixed by stirring total volume was made to one litre and stored in dark bottle at room temperature.

IX. Distaining solution

In 5 litre of water, 150 g NaCl was dissolves and stored at room temperature.

3.3.4 Total protein extraction

0.1 gm of leaf sample subjected into one eppendorf (1.5 ml) containing sample extraction buffer 1 ml.

SB : L : S : B + 10% B mercaptoethanol
(900 μ l + 100 μ l)

↓
Kept at 100°C/for 5 min. water bath

↓
Eppendorf tube were taken out and kept on ice

↓
Dilution of sample was done in eppendorf tube
(Sample 10 μ l + 19 μ l S : B)

↓
Spun briefly (5-10 sec.)
(10000 rpm)

↓
Supernatant collected

3.3.5 Gel Preparation

Before gel preparation, the glass pates were made to a leak proof gel cassette. All the glass plates were cleaned spotlessly and fresh grease was applied whenever necessary to prevent leakage. Slab gels, 1.5 mm thickness, were prepared from the stock solutions described earlier by mixing them in the following proportions.

The gels of various consistencies could be prepared as follows :

Solution	Gel consistency (%)				
	5	10	12.5	15	20
Acryl bis	1.67	3.33	4.20	5.00	6.67
Separating gel buffer	2.50	2.50	2.50	2.50	2.50
TEMED	0.005	0.005	0.005	0.005	0.005
APS	0.03	0.03	0.03	0.03	0.03
H ₂ O	5.80	4.14	3.30	2.47	0.8-1.0

All volumes in ml

12.5 per cent separating and 10 per cent stacking gels were used for this experiment.

Sl. No.	Solution	Quantity
1.	Acryl bis	16.80 ml
2.	Separating gel buffer	10.00 ml
3.	Distilled water	13.20 ml
4.	SDS (10%)	400 μ l
5.	TEMED	30 μ l
6.	APS* (10%)	120 μ l

*Prepared fresh and added just before pouring the gel.

These solutions were mixed and shaken well in a beaker before pouring into gel mold.

10 per cent stacking gel (10 ml for one gel)

Sl. No.	Solution	Quantity
1.	Acryl bis	3.30 ml
2.	Separating gel buffer	2.50 ml
3.	Distilled water	4.20 ml
4.	SDS (10%)	100 ml
5.	TEMED	10 μ l
6.	APS* (10%)	60 μ l

*Prepared fresh and added just before pouring the gel.

The glass plates were cleaned using detergent, water and finally wiped with ethanol. The notched glass plate was placed in the front support with side spacers facing upward. The gasket was placed on the glass plate, along the side spacers. It

was clamped with plain glass plate. The two corners of the glass plates should be inside the corner guides of the gasket. Finally, two plates were clamped with rear platen, engaging the guides of the platen and the guides of the front support. One ml of the distilled water was carefully over layered on the separating gel solution to real off he gel from air which inhibits gel polymerization. The gel could set in 30 minutes. It could be easily known by checking the gel solution left in the beaker. During this gel setting period electrode buffer (1x) was prepared. Once the gel was fully set, a clear interface would be visible between the top of the gel and the water over layered earlier on the gel. The excess water was removed from the top of the gel using one ml syringe.

The comb was inserted in the place between the glass plates leaving a 10 mm gap between the comb and the separating gel. Then, the stacking gel solution was poured between the glass plates using a 5 ml syringe following by immediate rinsing of the syringe. Care was taken that there should not be any air bubble in the gel. The stalking gel could set in about 15 minutes. The gel left for one hour for complete polymerization. The comb was removed once the gel was set. The walls were cleaned/rinsed with a syringe using electrode buffer. The gel sandwiched between the two plates was taken out from the casting system. The side gaskets were removed.

The platen was placed between the gel sandwich and the front panel of the lower buffer tank, until it stopped. About 480 ml of electrode buffer was poured gently in the upper buffer tank. Now the electrophoresis apparatus was connected with power supply and voltage was adjusted at 100 V. Now it was switched off and 5 μ l (microlitre) of prepared protein samples were loaded in the individual wells. The power supply was switched on and the run was performed until the bromophenol blue dye migrated to about 0.5 cm from the bottom of the separating gel. It took about four hours after completion of the run, the power supply was switched off.

The upper tank buffer was discarded. The lower tank buffer was used for two/three runs. The gel along with supporting plates, taken out from the chamber. The upper plates was removed by separating the two glass plates with the help of spatula. The position of first sample of gel was marked by cutting the left corner of the gel at the bottom side of the first sample below the tracking dye. The gel was transferred to staining solution immediately. Staining continued overnight. Thereafter, the gel was placed into distaining solution, containing 500 ml of 3 per cent HaCl. It took three to twelve hours to distain the gel a clear background. The electrophoregrams of the seed protein profiles were prepared and the gels were photographed and stored in sealed plastic bags in fridge after a brief rinse in distilled water to remove excess slat solution from the gel. The gel documentation system (syngene) was also used for gel documentation and photography.

3.3.6 Gel analysis

The distance between origin and tracking dye of gel was divided into three equal zones. In each of the zone, the total number of bands were counted and further classified into following group according to their thickness and intensity.

1. Thick : That is relatively thick as compared to other protein bands.
2. Medium : That is, in between the thick and thin protein bands.
3. Thin : That is relatively thin as compared to other protein bands.
4. Faint : That is slight appearance of the protein bands.

3.3.7 Relative mobility (Rf)

Total length of lane was measured in CM on the gel and relative mobility Rf of each bands was calculated according to formula given below :

$$\text{Relative mobility (Rf)} = \frac{\text{Distance between origin and migrating band}}{\text{Distance between origin and tracking dye}}$$

For this investigation each lane of electrophoregram was supposed to be 150 mm in length and 0.7 mm thick. Each relative mobility (Rf) was multiplied by 150. It gave the value in mm i.e. the distance of band from origin. Accordingly, each band on the electrophoregram strip was marked by using scale for the preparation of the electrophoregram.

Data analysis of electrophoretic gel patterns.

The gel patterns were extrapolated on the basis of relative mobility of each protein band.

3.3.8 Jaccard's coefficient (JC)

Jaccard's coefficient (similarity coefficient) was used for calculating the genetic similarity between different maize genotypes

$$J.C. = \frac{\sum \text{of common bands}}{\sum \text{of common bands} + \sum \text{of different bands}}$$

3.4 RAPD profile

The present study on RAPD based molecular characterization of various *Zea mays* L. was conducted in Department of Biochemistry, College of Basic Sciences and Humanity, G.B.P.U.A.&T., Pantnagar.

3.4.1 Experimental materials

Different inbreed line of *Zea mays* L. were used for the present study. The accession number assigned to these cultivars are given in Table 2 and 3. The list and source of chemicals, glasswares and instruments used for study are given in Appendix 1.

3.4.2 DNA extraction

I. Germination of seeds

- Seeds of *Zea mays* L. were obtained from the Department of Genetics and Plant Breeding, G.B. Pant University of Agriculture and Technology, Pantnagar and from CIMMYT.
- The seeds were soaked in water for 5 hrs and then subsequently washed with 70 per cent alcohol and sterilized water for three times in the laminar flow chamber. Then they were soaked in 0.1 per cent HgCl₂ for 10 minute followed by washing three times with sterilized water.
- After washing seeds were kept for germination on 2 per cent agar media in jam bottles.
- Jam bottles were kept in dark for on day in tissue culture lab and later were transferred in light for healthy leaves development.

3.4.3 Genomic DNA isolation

The method described by Dellaparta (1983) was followed for extraction of genomic DNA. The list of buffers and stock solution is given in Appendix II.

The isolated DNA was quantified by measuring its absorbance at A_{260}/A_{280} and was used for RAPD analysis.

I. Dellaparta SDS method for DNA isolation

Steps involved were as follows :

1. Weighed 1 gm of green leaves. Transferred the tissue rapidly to liquid nitrogen. Ground to powder with pastel mortar.
2. The frozen powder was transferred into a 50 ml centrifuge tube containing 15 ml of DNA extraction buffer (100 mM Tris HCl pH 8.0; 50 mM EDTA pH 8.0; 500 mM NaCl).

3. One ml of 20 per cent SDS was added, mixed thoroughly by vigorously shaking each tubes. The tubes were then incubated in a water bath at 65°C for 10 min.
4. 5 ml of (5M) potassium acetate was added to the mixture and incubated at 0°C for 20 min.
5. Centrifuged at 9000 rpm for 15 min. The supernatant was poured into clear 50 ml tube containing 10 ml of chilled isopropanol mixed gently.
6. The tubes were then incubated at -20°C for 30 min or overnight. The tubes were centrifuged at 9000 rpm for 15 min at 4°C to pellet the DNA. Gently, poured off the supernatant and dried the pellet with 70 per cent alcohol.
7. Redissolved the DNA pellet in 1.4 ml Tris-EDTA (TE, pH 8) buffer and transferred the solution of eppendorf tubes.
8. centrifuged the tubes at 8000 rpm at 4°C for 10 min and transferred the supernatant to another eppendorf tube and precipitate with 0.6 volume isopropanol and 0.1 volume 3 m sodium acetate (pH 5.2).
9. The tubes were centrifuged again and pellet obtained was washed with 70% C₂H₅OH and dried for 10 minutes.
10. Finally redissolved the DNA in 100 µl TE buffer and kept at 20°C.

3.4.5 DNA quantification

DNA quantification was done by using spectrophotometer (Beckman Du 6804). Quantification measurements were done by adding 2 µl of DNA to 98 µl TE. The concentration (mg/ml) was calculated by using following formula.

$$\text{DNA concentration (mg/ml)} = A_{260} \times \text{Dilution factor} \times 60$$

3.4.6 PCR amplification of genomic DNA

PCR conditions were standardized into two steps.

- (1) By taking primer, DNA concentration ratio 1 : 1 (constant) and applying the temperature gradient from 30.0°C to 49.0°C.
- (2) Applying the constant annealing temperature of 35.2°C and primer, DNA concentration ratio was taken as 1 : 1 (i.e. 20 ng primer, 20 ng, DNA).

PCR amplifications were performed in a volume of 25 μ l reaction mixture that was set up as detailed below.

Reaction mixture for PCR amplification

Component (stock)	Final concentration	Single tube (μ l)
DNA template (20 ng/ μ l)	20 ng/ μ l	1 μ l
DNTPs (10 mM each)	10 μ M each	0.5 μ l
Taq polymerase (3 U/ μ l)	1 U	1/3 μ l
Assay buffer (10x)	1x	2.5 μ l
Primer (10 ng/ μ l)	10 ng/ μ l	2 μ l
Triple distilled sterile water	17.67	17.67
	Total	25.0

A set of ten decanucleotide random primers were employed for PCR amplification. The specific primer selected were got custom synthesized from Bangalore Geni Pvt. Ltd. The details of lot number, sequence of primers and GC content are given in Table 1.

A master mixture containing no template DNA was prepared for specific number of tubes to reduce repeating error. The master mixture was then distributed in different tubes (24 μ l). Finally 1.0 μ l of different template DNA

Table 1 : Random 10-mer nucleotide primers

Sl. No.	Genic Lot No.	Operon code	Sequence 5'-3'	GC contents (%)
1.	BGC 02	7SS10A1	CGGAGAGCGA	70
2.	BGC 02	8SS10C2	GAGAGGCACC	70
3.	BGC 02	9SS10C3	CGGAGAGCCC	80
4.	BGC 02	10SS10T4	CGGTCACTGT	60
5.	BGC 02	11SS10T5	CGGCCACTGT	70
6.	BGC 02	12SS10T6	CGGCCCTGT	80
7.	BGC 02	13SS10T7	AGGCCCTGT	90
8.	BGC 02	14SS10C8	AGGCGGGTAC	70
9.	BGC 02	15SS10C9	CGGCCCCGGC	100
10.	BGC 02	16SS10C10	CGTCGTTTAACC	60
11.	BGC 02	17SS10C11	GTCCTCAGTG	60
12.	BGC 02	18SS10G12	GCAGACTGAG	60
13.	BGC 02	19SS10C13	TCTTAGTGCC	50
14.	BGC 02	20SS10A14	GACATGAGCA	50
15.	BGC 02	21SS10A15	CTTGGATGGA	60
16.	BG 80 GB 10 AA/201	UBC 18	GGGCCGTTTA	60
17.	BG 86 GB 10 G7/201	UBC 535	CCACCAACG	60
18.	BG 81 GB 10 G2/201	UBC 337	TCCCGAACCG	70
19.	BG 87 GB 10 G8/201	UBC 552	CTAAATGGCG	50
20.	BG 90 GB 10 G10/201	UBC 600	GAAGAACCGC	60
21.	BG 84 GB 10 G5/201	UBC 532	TTGAGACAGG	50
22.	BG 88 GB 10 C9/201	UBC 572	TTCGACCATC	50
23.	BG 85 GB 10 C6/201	UBC 534	CACCCCTGC	80
24.	BG 82 GB 10 C3/201	UBC 350	TGACGCGCTC	70
25.	BG 83 GB 10 G4/201	UBC 386	TGTAACGTCG	50

PCR protocol

Cycle	Denaturation	Annealing	Extension
First cycle	94°C 3 min		
44 cycle	94°C 1 min	35.2°C 2 min	72°C 2 min
Last cycle			72°C 10 min

The amplified products were stored at -20°C till further use.

was added to each tube. The mixture was gently mixed by centrifuging for 10 seconds.

The PCR amplification was done in Biomutra DNA thermocycler as per the following protocol.

3.4.7 Agarose gel electrophoresis

Submerged gel electrophoresis unit was used for fractionating RAPD markers on agarose gel. Agarose gel (1.2%) was prepared by dissolving appropriate amount of agarose in $0.5 \times$ TBE buffer. DNA sample and DNA loading dye were mixed 5 : 1 ratio for each and loaded with the help of a micropipette. Electrophoresis was conducted at 50 V for 5 hrs in $0.5 \times$ TBE electrophoresis buffer. The gel was then stained in ethidium bromide solution ($0.5 \mu\text{g ml}^{-1}$). After destaining in distilled water, the gel image was viewed and stored in gel documentation system (Alpha Imager).

3.4.8 Data analysis

All gels were viewed twice manually and independently. Each RAPD band was treated as independent character of locus and assigned numbers on the basis of decreasing order of their molecular weight. The size of each band was estimated using DNA molecular weight marker (100 bp ladder and λ DNA ECOR I/Hind III double digest). A band was scored 1, and when present zero when absent.

Pair genetic similarity matrix was generated among 10 accessions using Jaccard's similarity coefficient (Jaccard, 1908; cited in Mumm and Dudley, 1995). The Jaccard's similarity coefficient is given as :

$$S_{ij} = \frac{a}{a + b + c}$$

Where 'a' is the total number of bands shared between accessions I and J; 'b' is the total number of bands present in accession I but not in accession J; and 'c' is total number of bands present in accession J but not in accession I. The similarity coefficient of Jaccard's is equivalent to Gower's (1971). Similarly coefficient for binary characters i.e. characters that are either present or absent. The main feature of this similarity coefficient is that it does not consider negative matches (Gower, 1971). Since the absence of particular band may have several causes, therefore, it is reasonable not to consider similarity arising from mutual absence of bands (Viriling *et al.*, 1994; Vicrling and Nguyen, 1992).

Using the appropriate similarity matrix, cluster analysis was performed using the unweighted pair-group method with arithmetic averages (UPGMA) (Sheath and Sokal, 1973) and dendogram was constructed for the 8 accessions. The analysis was done using NTSYS-PC (Rholff, 1973).

A. Solutions for DNA extraction

1. 1M Tris buffer pH 8.0

Dissolve 12.11 g Tris base in 80 ml autoclaved distilled water. Adjust the pH to 8.0 with 1N HCl. Adjust to 100 ml with distilled water. Autoclave the buffer, then store at room temperature.

2. 0.5 M EDTA

Dissolve 18.612 g Na₂ EDTA (by adding 2 g of NaOH pellets). Stir vigorously on a magnetic stirrer for several hrs to ensure that all the solutes have dissolved. Adjust the volume upto 100 ml.

3. DNA extraction buffer	500 ml
1 M Tris buffer	50 ml
0.5 M EDTA	50 ml
NaCl	14.61 g

Adjust the pH to 8.0 with HCl. Make up volume upto 500 ml. Autoclave at 151 b (Psi) for 20 min and store at 4°C.

4.	70% ethanol	100 ml
	Absolute ethyl alcohol	70 ml
	H ₂ O	30 ml

5.	5M potassium acetate	100 ml
	Potassium acetate	49.07 g
	Glacial acetic acid	11.5 ml

Make upto 100 ml. Autoclave and then store at room temperature.

6.	20% (w/v) SDS	100 ml
	SDS	20 g
	H ₂ O	80 ml

Heat to 60°C to dissolve properly. Adjust pH to 7.2 by adding HCl. Make upto 100 ml. Autoclave and store at room temperature.

7.	3M sodium acetate	100 ml
	Sodium acetate	24.6 g
	H ₂ O	40 ml

Adjust pH to 5.2 with glacial acetic acid. Make upto 100 ml. Autoclave and store at room temperature.

8.	TE buffer	100 ml
	1M Tris buffer	1.0 ml
	0.5 M EDTA	0.2 ml

Make upto 100 ml. Autoclave and store at room temperature.

9.	T₅₀E₁₀ buffer	100 ml
	1M Tris buffer	0.5 ml
	0.5 M EDTA	2 ml

10. **Isopropanol** 100 ml

Store at -20°C in ml dark coloured bottle.

B. Solution for DNA Purification

1. **Phenol : chloroform : isoamyl alcohol** 500 ml

Chloroform 240 ml

Isoamyl alcohol 10 ml

Phenol 250 ml

Store in brown bottle or ordinary bottle covered with two folds of silver foils. Store at -20°C .

Note : Mixing chloroform with other solvents may cause serious hazards. Don't mix chloroform with acetone and strong base. Isoamyl alcohol should be handled carefully. Its vapours are poisonous.

2. **RNase** 1 ml

RNase A 10 mg

5M sodium acetate (pH 7.5) 3 μl

1M Tris Cl (pH 7.5) 10 μl

Heat to 100°C for 15 min. Allow it cool. Study to room temperature. Adjust pH 7.5 with 1M Tris (pH 7.5). Adjust into 1 ml eppendorf tubes. Store at -20°C .

C. Solutions for Gel Electrophoresis

1. **DNA loading dye (single dye)** 10 ml

Bromophenol blue (0.25% w/v) 0.025 g

Sucrose (40% w/v) 4.0 g

Dissolve properly, make up volume to 10 ml. Store at -20°C . Use sterilized distilled water.

2. Electrophoresis buffer (50x TAE)	100 ml
Tris base	24.2 g
Glacial acetic acid	5.7 ml
EDTA (0.5 M)	10 ml

Dissolve in min volume of H₂O. Adjust pH to 8.0. Make upto 100 ml.
Autoclave and store at room temperature.

3. Ethidium bromide (10,000x)	10 ml
Ethidium bromide	100 mg
H ₂ O	10 ml

Store at 4°C. Ethidium bromide is highly carcinogenic. Use gloves while handling. Working solution for staining gel is made by dissolving 60 µl ethidium bromide stock (10 mg/ml) in 300 ml water.

With the view of workout the relationship of the morphological and molecular markers and banded leaf and sheath blight (BLSB) caused by *Rhizoctonia solani* F. sp. *sasakii*. eighty six maize genotypes were screened against *R. solani* in field, glasshouse as well as in laboratory at Pantnagar, under artificial epiphytotic conditions. The result obtained under different field/laboratory condition including the use of molecular markers in relationship of banded leaf & sheath blight are presented in following paragraphs.

A. Field experiment

4.1 Morphological character and disease reaction

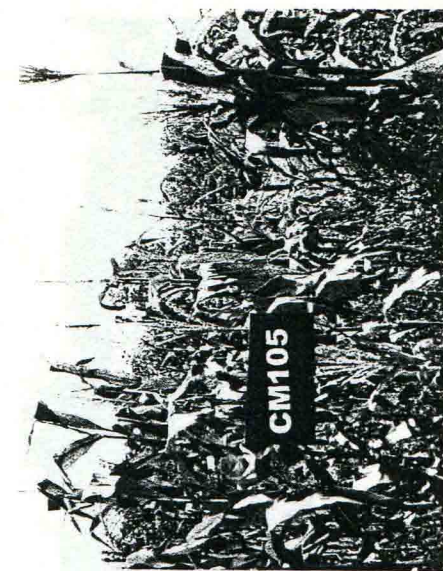
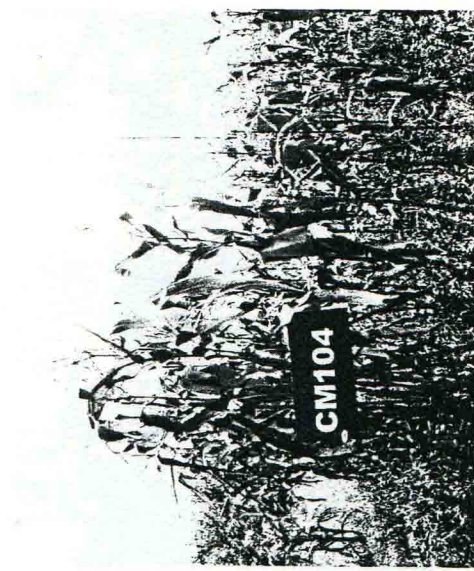
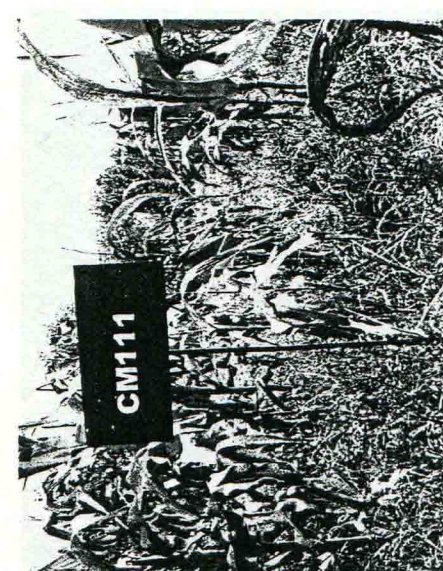
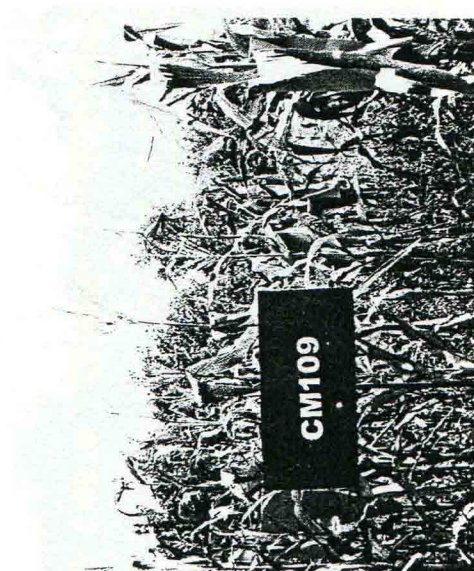
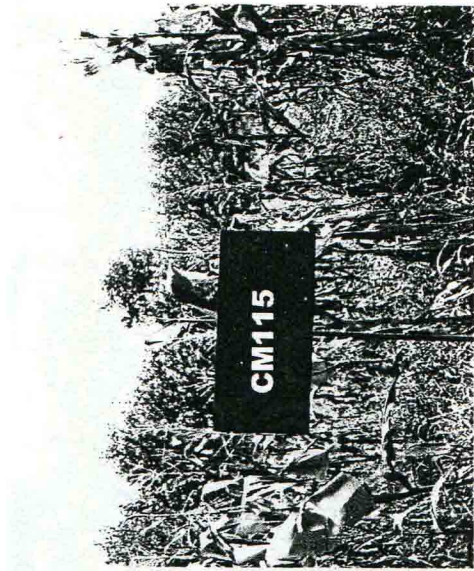
In the present investigation, different morphological characters, like plant height cob placement incubation period and blighting size, blighting sphere and blighting colour and tassel colour, tassel texture, silk colour and tassiling day were observed and the result obtained on their morphological character associated with the banded leaf and sheath blight and are given below character wise.

4.1.1 Plant height and disease reaction

Plant height was measured in all the replication of experimental material of the three group, 44 CIMMYT lines, 14 CM lines and 26 Pantnagar lines. Mean height (cm) was analysed for co-relation with disease reaction. The data are presented in Table 4 and Fig. 8.

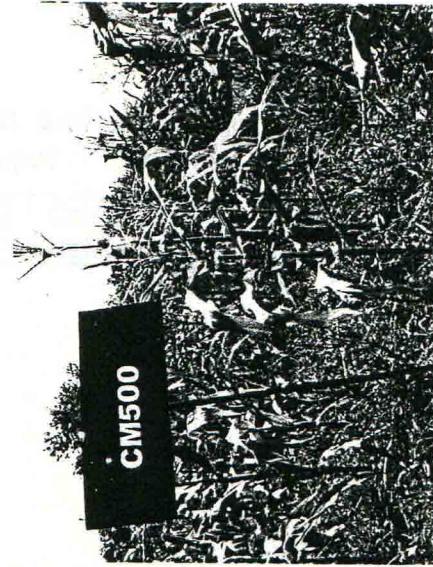
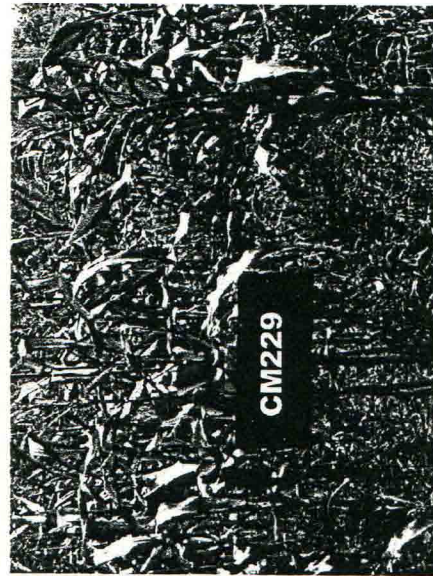
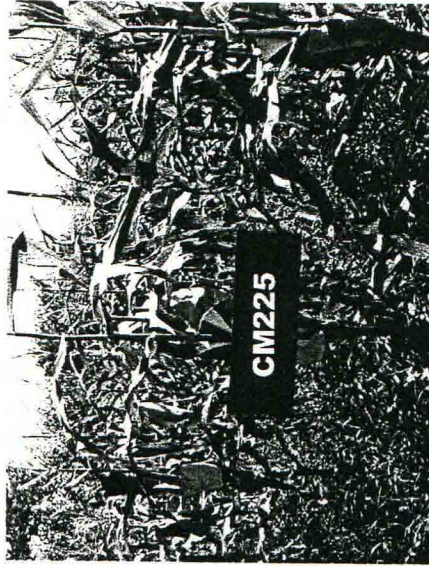
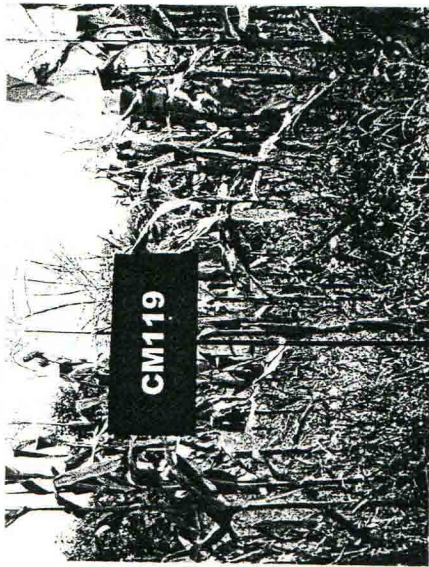
In forty four genotypes which were obtained from CIMMYT, categorized in to three group on the basis of the disease reaction, resistant susceptible and intermediate. The data obtained from the field experiment, represent that all the

PLATE -1



Morphological Character-CM Genotype

PLATE -2



Morphological Character-CM Genotype

Table 4 : Field experiment plant height and cob placement and disease reaction of 44 CIMMYT genotypes

Inbred Lines (Code)	Plant height (cm) mean	Cob placement height (cm) mean	Disease reaction (mean)
CA00106	162.6	69.6	1.3
CA049Y04	177.6	78.3	1.5
CA03147	167.6	61.6	1.1
CA14509	164.6	62.0	1.4
CA14518	175.6	66.6	1.2
CA03106	161.0	60.0	3.6
CA003134	149.6	57.6	2.4
CA00370 (CML 469)	142.0	59.0	3.2
CA34507	174.3	77.0	3.1
CA00102 (CML 429)	165.6	69.6	4.8
CA00396	133.6	52.3	2.3
CA00310 (CML 465)	175.0	60.3	1.1
CA34506	190.0	81.0	4.1
CA03131	137.3	50.6	3.9
CA00334 (CML 433)	151.6	58.0	2.7
CA34516	167.3	60.0	4.1
CA03149	180.3	75.6	3.6
CA14510 (CML 428)	169.3	64.6	3.7
CA00332 (CML 432)	180.3	62.6	3.6
A00344	140.6	46.6	4.2
CML 425	161.0	70.0	3.5
CML 430	162.3	60.3	3.7
CA03124	176.6	74.3	1.6
CA00384	150.6	52.0	4.6
CA00378	191.6	88.0	2.1
CML 421	140.0	65.0	3.8
CA14711	148.0	46.0	4.1
Pop. 147-F2#161-3-2-B-1-B	180.3	75.6	4.1
CA14519	160.6	62.6	4.2
CA00104	152.0	45.0	3.6
CA03142	165.6	79.0	3.7
CA14527	180.3	74.3	4.2
CA03130 (CML 426)	175.0	81.3	4.4
CA14502 (CML 427)	169.3	69.3	4.1
CA00304 (CML 431)	176.0	57.6	3.8
CML 413	159.3	77.0	3.1
CA14501	176.0	72.3	4.6
CA14517	156.0	52.0	2.9
CA03116 (CML 470)	149.6	70.6	3.3
CA14709 (CML 471)	167.0	60.6	3.0
CA14524 (CML 474)	161.0	66.3	3.5
CA14520 (CML 475)	171.0	59.3	2.9
CML 428	138.0	57.6	4.5
CA00334 (CML 433)	161.0	59.0	4.7

maize genotypes , CA 00106, CAO49YO4, CA03147, CA14509, CA14518 and CA00310 (CML 465), CA03124 showed mean disease score between (1.0 to 2.0) in resistant, category when the mean plant height of this group of maize genotypes , comprises between 160-180 Cm and which is greater in comparison to the maize inbred lines which are categorized in intermediate and susceptible group. The maize genotypes which comprised in intermediate group on the basis of disease reaction between (2.0-4.0), CA03106, CA0031, CA00370 (CML 469), CA34507, CA00396, CA03131, CML 433, CAO3149, CML 428, CML 432, CML 425, CML 430, CA00378, CML 421, CA00104, CA03149, CML 431, CML 413, CA14517, CML 470, CM471, CML 474, CML 475 and maize genotypes with mean plant height between 160-170 are eight and between 130 to 160 are ten. The maize genotypes which showed the mean disease score (4.0-5.0) represent highly susceptible group were CA00102 (CML 429), CA34506, CA34516, A00344, CA00384, CA14711, PoP147-F2#161-3-2-B-1-B, CA14516, CA14527, CA03130 (CML 426), CML 427, CA14501, CML 428 and CML 433. From this susceptible group, mean plant height ranged between 160-170 cm included only four maize genotypes .

In field experiment fourteen CM lines were categorized into three groups resistant susceptible and intermediate on the basis of the mean disease score. CM 104, CM 105 and CM 500, CM 600 comprises into resistant group (1.0-2.0), genotypes CM 120, CM 121, CM 125, CM 400, CM 209 in the intermediate group (2.0-4.0) and CM 111, CM 115, CM 123, CM 210 in susceptible group (4.0-5.0).

The plant height of all the inbred lines were categorized in three groups on the basis of their mean plant height (cm). The maize genotypes these which represented plant height 160 to 170 cm. were CM 105, CM 119, CM 121m, CM 400, CM 209, between 170-180 included CM 115, CM 123, CM 179 and CM 600 and between 180-190 cm were CM 104, CM 111, CM 120, CM 125, CM 210. The data on the above parameters are presented in Table 5 and Fig. 9.

Table 5 : Field experiment plant height and cob placement and disease reaction of 14 CM genotypes

Inbred Lines (Code)	Plant height (cm) mean	Cob placement height (cm) mean	Disease reaction (mean)
CM 104	187.0	76.6	1.9
CM 105	164.0	68.0	1.7
CM 111	188.6	70.3	4.6
CM 115	172.3	74.0	4.4
CM 119	168.3	65.0	4.6
CM 120	186.6	76.6	3.0
CM 121	166.0	70.3	3.7
CM 123	171.6	68.6	4.8
CM 125	181.0	74.6	2.6
CM 500	159.3	66.0	3.0
CM 400	179.3	70.6	2.0
CM 600	176.6	72.3	1.9
CM 209	160.0	63.6	3.9
CM 210	186.3	66.3	4.4

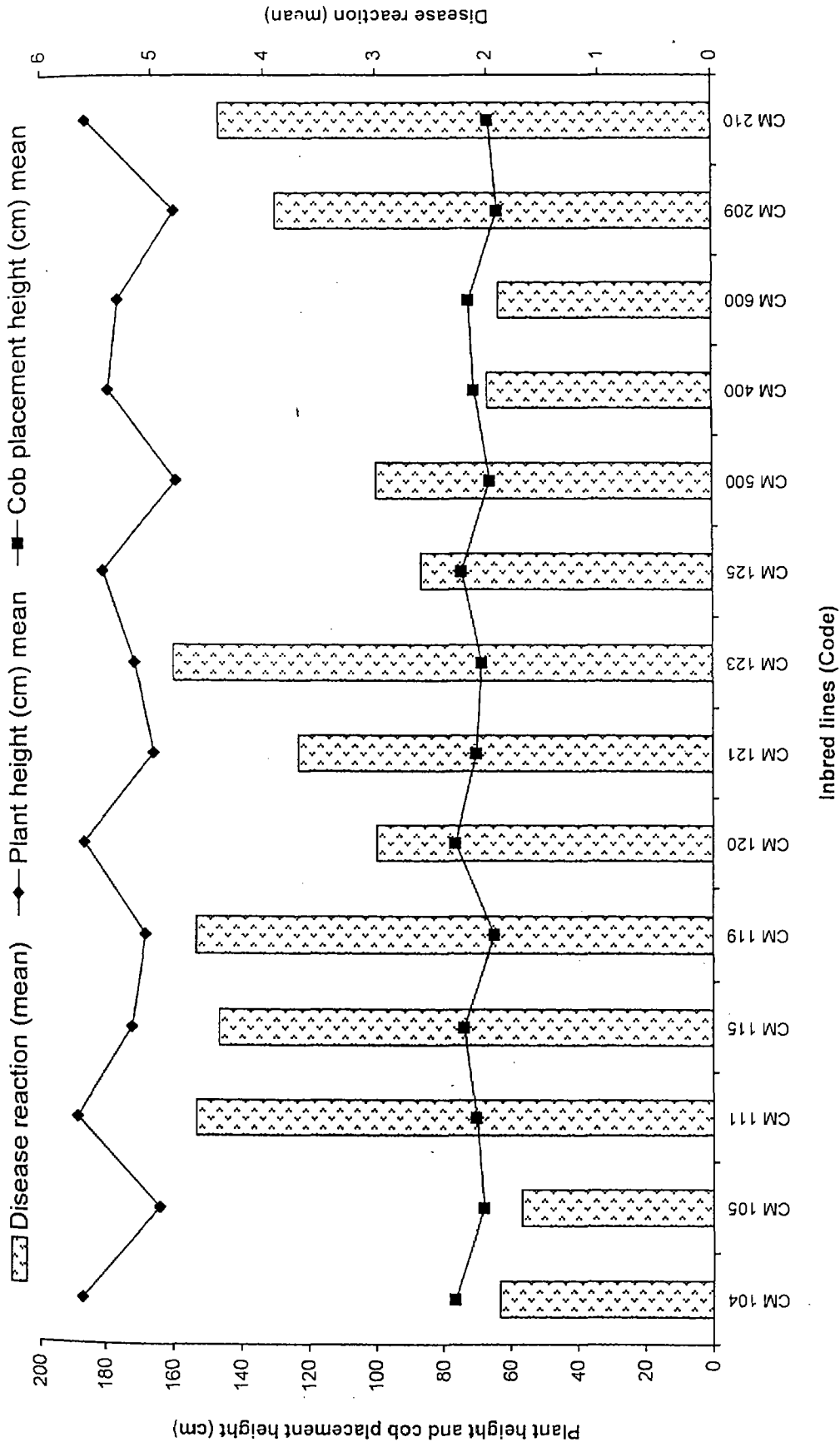


Fig. 9 : Field experiment plant height and cob placement and disease reaction of 14 CM genotypes

The twenty Pantnagar genotypes along with some released maize varieties were categorized on the basis of mean plant height and disease reaction. Maize genotypes which showed plant height between (130 to 160 cm) were 553, 560, 566 and 569 between (160 to 180 cm) were D 995, Amar, 550, 553, 555, 556, 557, 558, 559, 561, 563, 562, 564 and between plant height (180-190) were Surya, D 994, Kanchan, 552, 554, 565, 567, 568 and 570. Similarly, on the basis of field disease rating mean score the maize genotypes were categorized into three groups, resistant 561 and 556 with mean disease score (1.0-2.0) and susceptible with mean disease score (4.0 to 5.0). Resistant group included, moderately resistant group (2.0 to 4.0) included, Surya, D 995, Kanchan, 550, 553, 554, 555, 556, 559, 562, 563, 564, 567. The data on disease reaction Pantnagar lines experiment given in Table 5. The plant height and all three groups and their disease reaction also graphically represented in Fig 10.

4.1.2 Cob placement and disease reaction

Cob placement was taken as a morphological character with assumption that it might be associated with the disease severity. Cob placement of all the three groups CIMMYT, CM and Pantnagar genotypes were measured. On the basis of the mean cob placement the maize genotypes (Table 4, 5 and 6) which showed the cob placement (40 to 60 cm) were CA003134, CML 469, CA00396, CA03131, CM 433, A00344, CA00384, CA14711, CA00104, CA00304 (CML 431), CA 14517, CML 475, CML 428 and CML 433. Cob placement between (60 to 80 cm). Included CA00106, CA049Y04, CA03147, CA14509, CA1458, CA03106, CA34507, CML 465, CA34516, CA03149, CML 428, CML 432, CML 425, CM 430, CA03124, CML 421, PoP147-F2#161-3-2-B-1-B, CA14519, CA03142, CA14527, CML 427, CML 413, CA 14501, CML 470, CML 474 and cob placement between (80 to 90 cm) were CA34506, CA00378, CML 426.

Cob placement of 14 CM lines measured under field condition were also categorized in which cob placement between (60 to 80 cm) include, CM 104,

Table 6 : Field experiment plant height and cob placement and disease reaction of 26 genotypes

Inbred Lines (Code)	Plant height (cm) mean	Cob placement height (cm) mean	Disease reaction (mean)
Surya	190.3	92.3	2.6
D-995	169.0	54.3	2.3
D-994	186.0	81.0	4.1
Kanchan	189.3	78.0	2.8
Amar	170.6	77.3	4.6
550	161.0	58.6	3.4
551	165.3	67.6	4.6
552	189.3	85.0	2.8
553	159.6	79.0	3.1
554	182.3	79.3	2.9
555	174.0	69.6	3.7
556	169.6	70.6	2.0
557	161.0	65.3	4.1
558	165.3	67.3	4.2
559	162.0	75.9	3.7
560	141.0	63.3	4.6
561	173.3	77.0	1.5
562	178.3	67.6	3.8
563	170.3	65.6	3.5
564	169.0	79.3	3.6
565	188.0	69.6	4.4
566	138.6	73.0	4.3
567	181.6	78.3	2.8
568	181.3	78.0	4.6
569	143.6	67.3	4.5
570	181.6	82.6	4.8

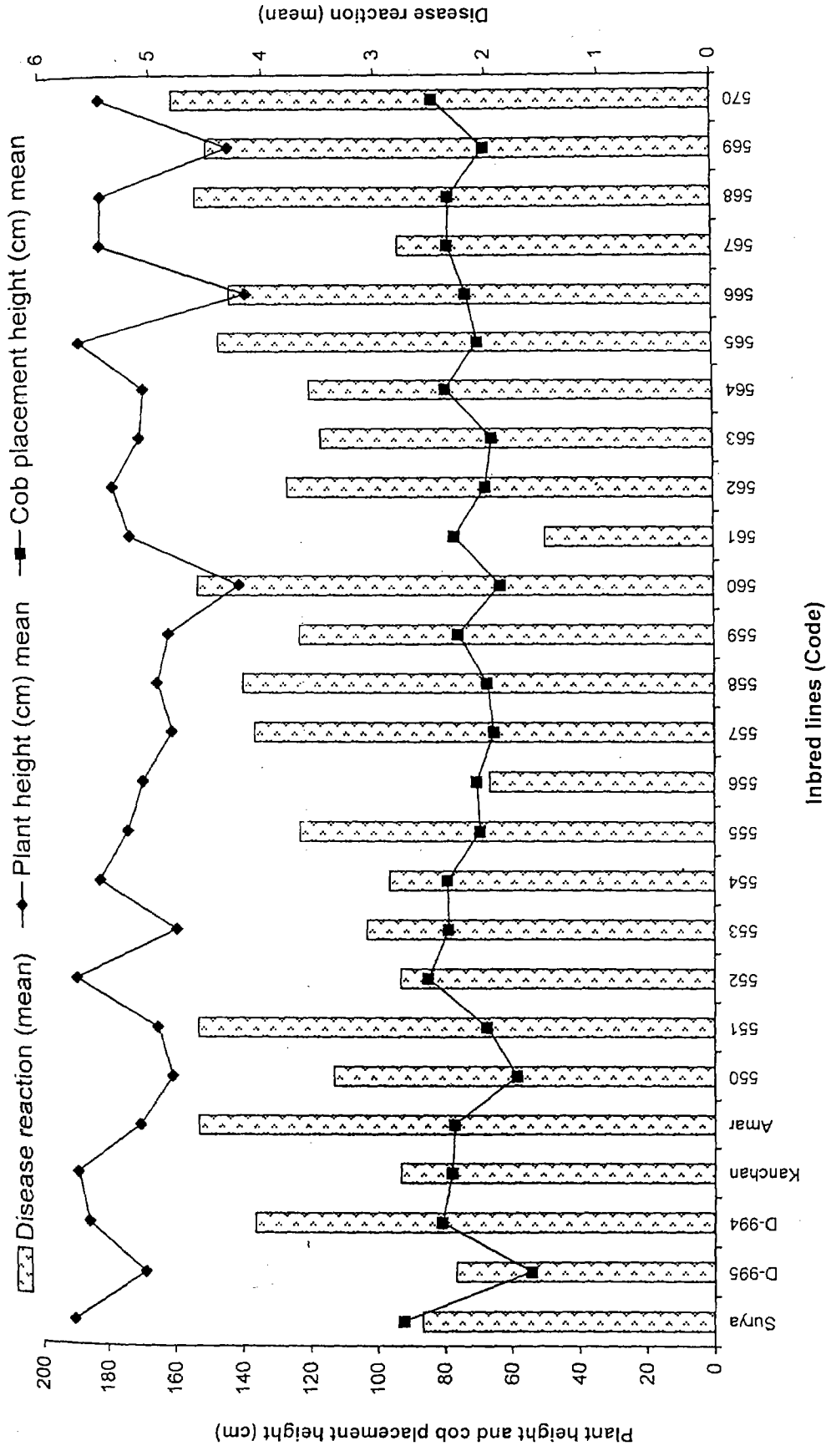


Fig. 10 : Glasshouse experiment plant height and cob placement and disease reaction of 26 genotypes

CM 105, CM 111, CM 115, CM 119, CM 120, CM 121, CM 123, CM 125, CM 400, CM 500, CM 600, CM 209, CM 210. The data on cob placement along with disease reaction of these CM lines are presented in Table 4.

The cob placement of Pantnagar genotypes along with their disease reaction are given in Table 5. On the basis of field experiment, the Cob placement of all the genotypes were categorized into 50 to 70 cm (D 995, D 994, 551, 555, 557, 560, 562, 560, 563, 565, 569) and 80 to 90 cm (Surya, 552 and 570) (Fig. 8, 9 and 10).

4.1.3 Incubation period

Incubation period was taken as a morphological character might be associated with the disease severity and disease reaction of different maize genotypes. Incubation period of all the maize inbred line, was recorded at different interval. On the basis of mean incubation period all the 44 CIMMYT lines were categorized in following three group (Table 7). The genotypes which showed incubation period of 24 to 48 hrs were CA03106, CA003134, CML 469, Ca345078, CML 429, CA00396, CML 465, CA34506, CA03131, CML 433, CA34516, CA03149, CML 428, CA00332 (CML 432), A00344, CM 425, CML 430, CA03124, CA00384, CA00378, CML 421, CA14711, CA14519, CA00104, CA03142, CA14527, CML 426, CML 427, CML 431, CML 413, CA14501, CA14517, CML470, CML 471, CML 474, CML 475, CML 428 and CML 433 of 48 to 72 hr were CA00106, CA049Y04, CA03147, CA14509, CA14518, CML 469, PoP147-F2#161-3-2-B-1-B (Fig. 11).

Incubation period of 14 CM lines was categorized into two group (Table 8 and Fig. 12), genotypes which showed incubation period between (24 to 48 hr) were CM 111, CM 115, CM 119, CM 120, CM 121, CM 123, CM 125, CM 400, CM 600, CM 209, CM 210 between 48 to 72 hrs included, CM 104, CM 105 and CM 500.

Table 7 : Incubation period and spot size and disease reaction of 44 CIMMYT genotypes (Field experiment)

Inbred Lines (Code)	Mean incubation period	Mean spot size (cm)	Disease reaction (mean)
CA00106	47.6	54.3	1.3
CA049Y04	46.6	52.0	1.5
CA03147	46.0	54.3	1.1
CA14509	46.0	51.0	1.4
CA14518	45.3	48.6	1.2
CA03106	46.3	49.3	3.6
CA003134	40.6	51.0	2.4
CA00370 (CML 469)	45.0	50.6	3.2
CA34507	44.0	51.6	3.1
CA00102 (CML 429)	42.2	51.3	4.8
CA00396	44.6	52.0	2.3
CA00310 (CML 465)	45.6	51.3	1.1
CA34506	45.6	54.0	4.1
CA03131	44.6	54.3	3.9
CA00334 (CML 433)	46.0	51.0	2.7
CA34516	44.6	51.0	4.1
CA03149	45.6	51.0	3.6
CA14510 (CML 428)	43.6	51.3	3.7
CA00332 (CML 432)	43.6	49.0	3.6
A00344	44.0	49.6	4.2
CML 425	44.6	50.0	3.5
CML 430	43.3	52.0	3.7
CA03124	44.0	50.3	1.6
CA00384	45.3	49.6	4.6
CA00378	42.0	50.0	2.1
CML 421	45.6	52.0	3.8
CA14711	42.6	52.0	4.1
Pop. 147-F2#161-3-2-B-1-B	45.3	49.0	4.1
CA14519	43.0	50.0	4.2
CA00104	46.0	49.3	3.6
CA03142	44.6	50.6	3.7
CA14527	44.0	48.3	4.2
CA03130 (CML 426)	44.3	53.0	4.4
CA14502 (CML 427)	46.0	51.0	4.1
CA00304 (CML 431)	44.0	51.3	3.8
CML 413	45.0	51.0	3.1
CA14501	45.3	51.3	4.6
CA14517	42.6	49.0	2.9
CA03116 (CML 470)	46.3	52.3	3.3
CA14709 (CML 471)	43.3	51.0	3.0
CA14524 (CML 474)	45.0	51.6	3.5
CA14520 (CML 475)	45.6	48.6	2.9
CML 428	41.0	47.0	4.5
CA00334 (CML 433)	42.0	51.6	4.7

Table 8 : Incubation period and spot size and disease reaction of 14 CM genotypes (Field experiment)

Inbred Lines (Code)	Incubation period (hrs)	Spot size (cm)	Disease reaction
CM 104	72.0	1.2	1.9
CM 105	72.0	1.6	1.7
CM 111	48.0	1.3	4.6
CM 115	48.0	1.6	4.4
CM 119	48.0	1.1	4.6
CM 120	32.0	1.7	3.0
CM 121	40.0	1.2	3.7
CM 123	48.0	1.3	4.8
CM 125	48.0	1.4	2.6
CM 500	32.0	1.7	3.0
CM 400	72.0	1.7	2.0
CM 600	48.0	1.5	1.9
CM 209	48.0	2.5	3.9
CM 210	48.0	1.2	4.4

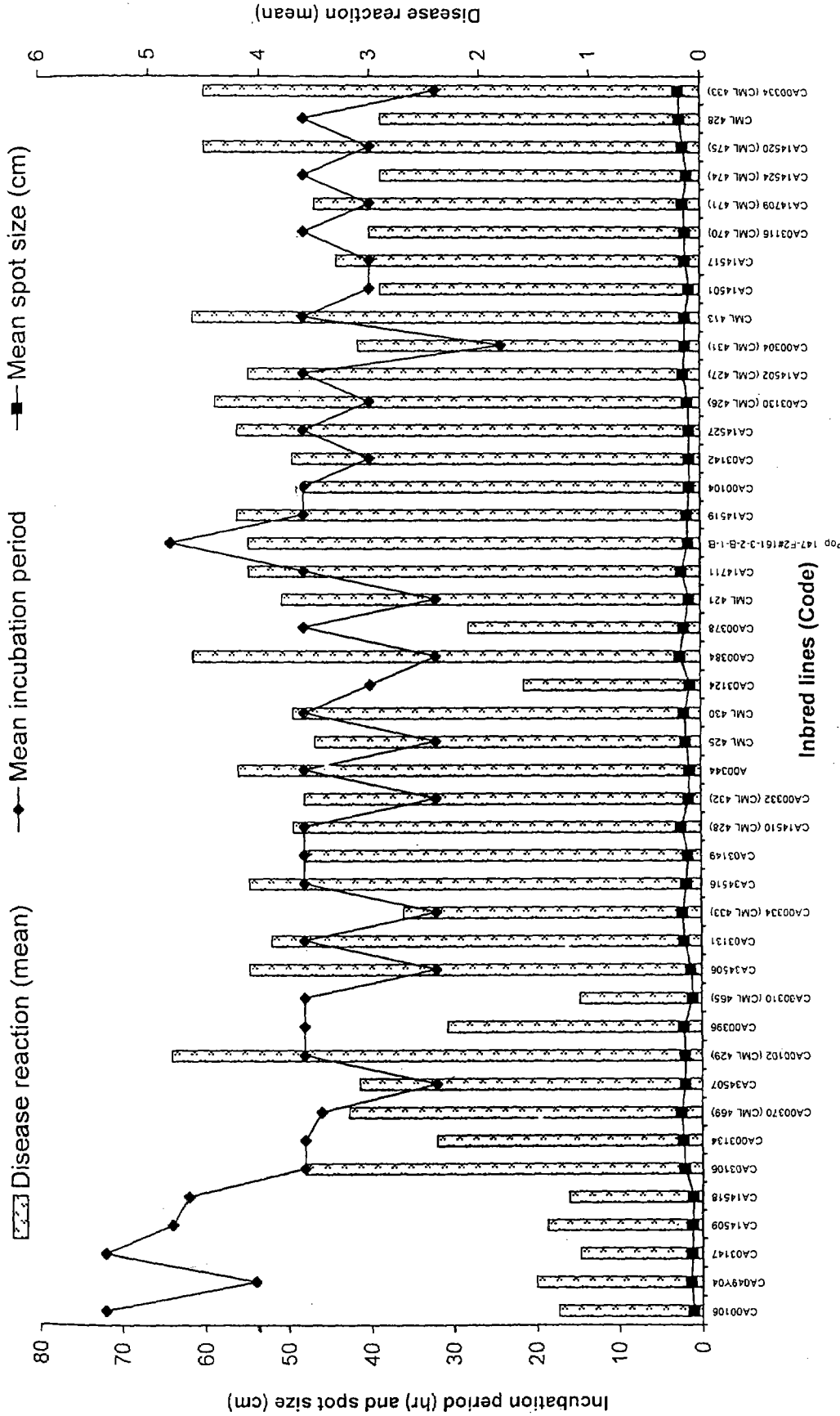


Fig. 11 : Incubation period and spot size and disease reaction of 44 CIMMYT genotypes (Field experiment)

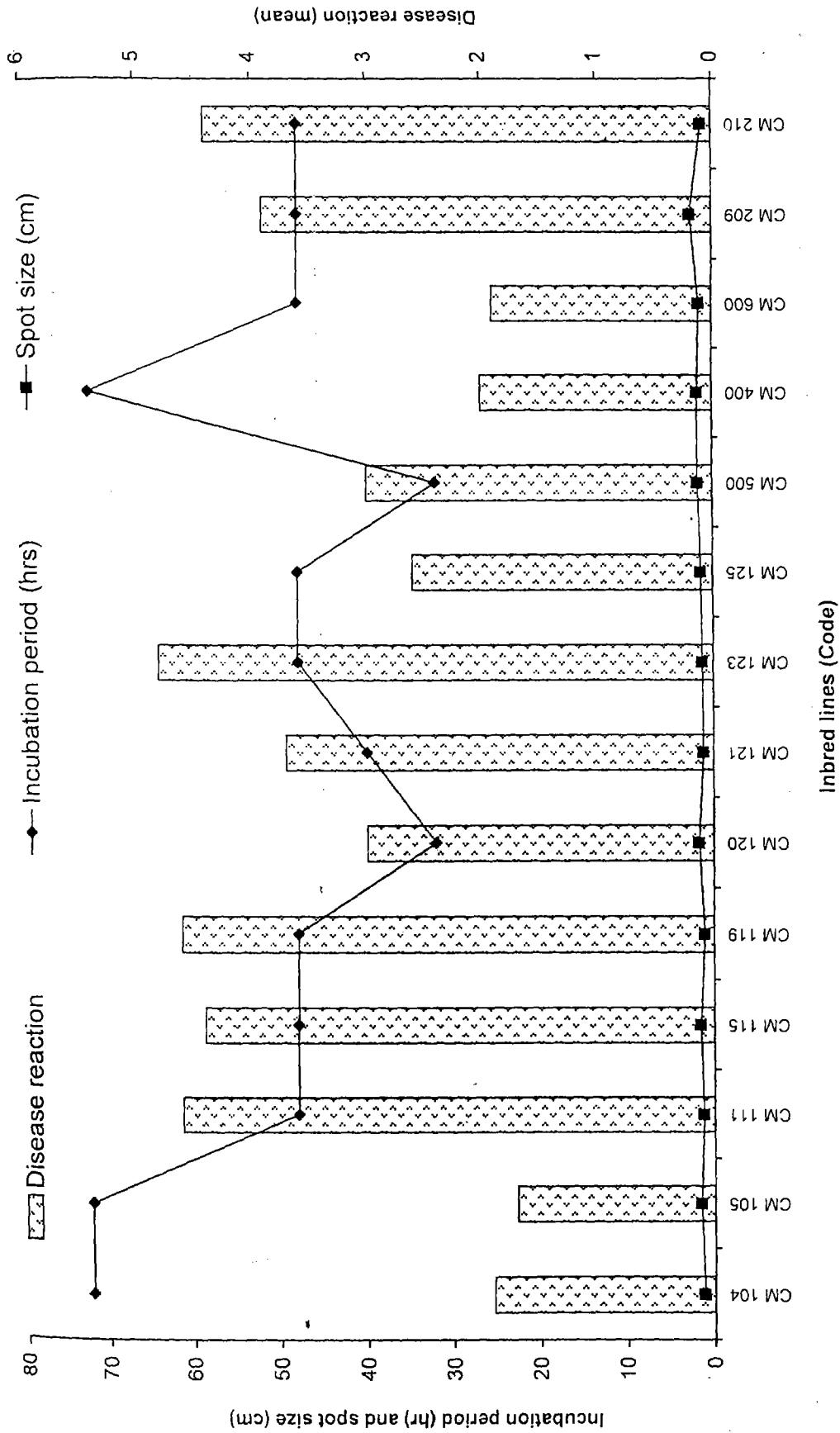


Fig. 12 : Incubation period and spot size and disease reaction of 14 CM line (Field experiment)

Observations on the incubation period of Pantnagar genotypes is presented in Table 9 and Fig. 13. On the basis of incubation period all the maize genotypes were categorized in one group between (24 to 48 hr).

In the resistant maize inbred line, CA00106, CA03147, CM 104, CM 105 and CM 600, the mean incubation period was 70 to 80 hrs and moderately resistant maize inbred line, CA049Y04, CA14509, CA14518, the mean incubation period was in between 50 to 70 hrs and only CML 465, CA03124, 561, 556, CM 600 showed incubation period in between 40 to 50 hrs in susceptible category.

4.1.4 Spot size (cm)

Spot size of all the maize genotypes were recorded after 48 hrs and the data are presented in Table 7. On the basis of mean spot size the forty-four lines was grouped into spot mean size (1.0 to 1.5 cm) which included CA00106, CA049Y04, CA03147, CA14509, CA14518, CML 465, CA34506, CA34516, CA03149, A00344, CML 425, CA03124, CML 421, PoP147-F2#161-3-2-B-1-B, CA14519, CA00104, CA03142, CA14527, CML 426, spot-size between (1.5 to 2.51 cm) included CA 03106, CA003134, CML 469, CA34507, CML 429, CA00396, CA03131, CML 433, CML 428, CML 430, CA00384, CA00378, CA14711, CML 427, CML 431, CML 413, CA14517, CML 470, CML 471, CML 474, CML 475, CML 428, CML 433.

Spot size of all the maize genotypes of CM group were and the data are presented in Table 8. On the basis of mean spot size, these lines were categorized into two groups. First group, comprises spot size of (1.0 to 1.5 cm) included CM 104, CM 111, CM 119, CM 121, CM 123, CM 125, CM 600 and CM 210 and second group of maize inbred line which showed spot size of (1.5 to 2.0), include CM 106, CM 115, CM 120, CM 400, CM 500 and CM 209.

Table 9 : Incubation period and spot size and disease reaction of 26 genotypes (Field experiment)

Inbred Lines (Code)	Incubation period (hrs)	Spot size (cm)	Disease reaction
Surya	48.0	1.4	2.6
D-995	48.0	1.3	2.3
D-994	48.0	2.0	4.1
Kanchan	48.0	1.6	2.8
Amar	48.0	1.3	4.6
550	48.0	1.4	3.4
551	48.0	1.5	4.6
552	48.0	1.9	2.8
553	48.0	1.7	3.1
554	48.0	1.3	2.9
555	48.0	1.3	3.7
556	48.0	1.6	2.0
557	48.0	1.7	4.1
558	48.0	1.3	4.2
559	48.0	1.3	3.7
560	48.0	1.9	4.6
561	48.0	1.5	1.5
562	48.0	1.7	3.8
563	48.0	1.9	3.5
564	48.0	2.0	3.6
565	48.0	1.5	4.4
566	48.0	1.4	4.3
567	48.0	1.1	2.8
568	48.0	2.0	4.6
569	48.0	1.2	4.5
570	40.0	1.3	4.8

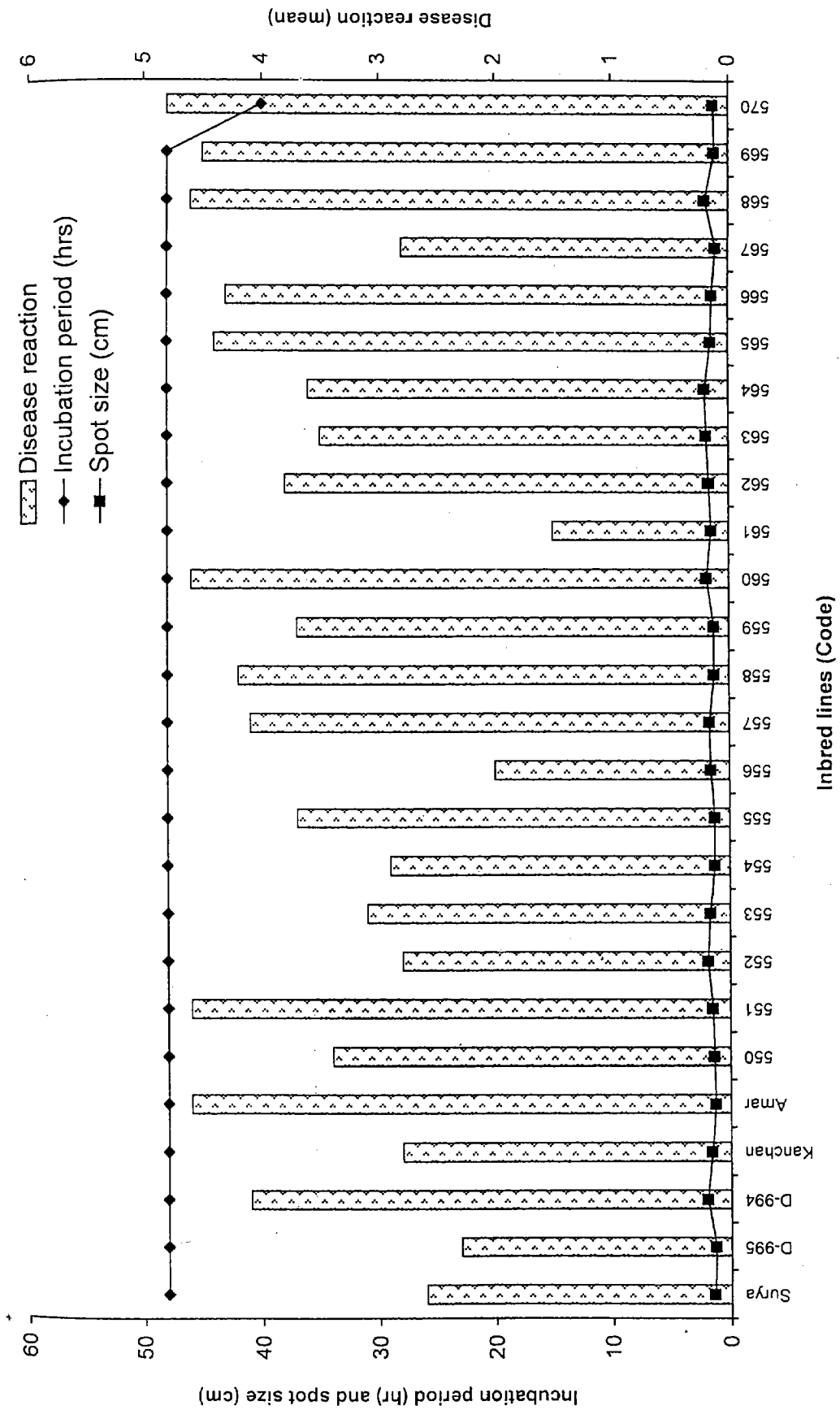


Fig. 13 : Incubation period and spot size and disease reaction of 26 inbred lines (Field experiment)

Spot size of Pantnagar genotypes are given in Table 9, Suray, D 995, D 994, Kanchan, Amar, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 567, 568, 569, 570 showed spot size in between 1.0 to 1.5 cm and genotypes 566 showed mean spot size of 1.6 cm.

Spot size of maize genotypes of CIMMYT group which showed resistant reaction in field condition was compare with the susceptible inbred lines. Inbred line, CA00106, CA049Y04, CA03147, CA14518 and CA00310 (CML 465), CA03124, 561, and CM 104, CM 600 showed mean spot size between (1.0 to 1.5 cm). Whereas CM 105, CM 500, 556 showed mean spot size between (1.5 to 2.0 cm) indicated that the average mean spot size of resistant maize genotypes was less than susceptible ones (Fig. 7, 12 and 13).

4.1.5 Tassel texture

Tassel texture of all maize genotypes was observed in field condition. Tassel texture is another morphological character which might be associated with the disease reaction. Tassel texture of all the lines were observed in field condition. The tassel texture of all CIMMYT genotypes are presented in Table 11. Inbred lines CA00106, CA049Y04, CA031147, CA14509, CA03106, CA003134, CA34507, CML 429, CA00396, CML 433, CML428, CML 430, CA03124, CML 421, CA03142, CA14527, CML 426, CML 431, CML 413, CML 428, CML 433 showed textured tassel, CA14518, CA34506, CA03131, CA34516, CML432, CML 425, CA00378, CA14711, CML 427, CML 470 showed medium textured tassel and CML 465, CA03149, A00344, CA00384, PoP147-F2#161-3-2-B-1-B, CA14519, CA00104, CA145001, CA14517, CML 471 showed light textured tassel.

The data on tassel texture of all fourteen CM inbred liens are presented in Table 12. Maize genotypes , CM 104, CM 105, CM 111, CM 119, CM 120, CM 121, CM 123, CM 125, CM 500, CM 600, CM 210 showed dense texture tassel. Whereas CM 115, CM 400 and CM 209 showed medium texture tassel.

The data on tassel texture of Pantnagar genotypes, given in Table 13. Genotypes 556, 561, 568 and varieties D 995, Kanchan, Amar showed medium texture tassel 550, 551, 552, 553, 554, 555, 557, 558, 559, 560, 562, 564, 565, 566, 567, 569 and 570 and composite D 994 and Surya showed dense texture tassel.

The resistant genotypes of all the three groups, which showed mean disease reaction (1.0 to 2.0) of which, CA00106, CA049Y04, CA03147, CA14509, CA03124, CM 104, CM 105, CM 500, CM 600 represented dense tassel texture, only CA00310 (CML 429) showed lax tassel texture, and two resistant lines, 556, 561 showed medium texture tassel.

4.1.6 Tassel colour

4.1.6.1 Tassel anther glum colour

Tassel A-G colour of all forty four CIMMYT genotypes are given in Table 11. CA00106, CA049Y04, CA049Y04, CA14518, CA03106, CML 469, CML 429, CML 465, CM 432, CM 425, CML 430, CA144711, CA14519, CML 470, CML 475, CML 427, CA00396, CA34507 showed light purple tassel colour, CA03147, CA03106, CA003134, CA03149, CM428, A00344, CA00384, CML 421, PoP147-F2#161-3-2-B-1-B, CA00104, CML 426, CML 413, CA14501, CA14517, CML 474 showed light green tassel colour and CA34506, CA03131, CML 433, CA034516, CA03124, CA00378, CA03142, CA14527, CML 431 and CML 471, showed purple tassel colour.

Tassel colour of CM lines are given in Table 12 . CM 104, CM 105, CM 500, CM 600, showed light purple tassel colour, CM 111, CM 115, CM 119, CM 120, CM 121, CM 123, CM 125, CM 400, CM 209, CM 210 showed purple tassel colour. The tassel colour of Pantnagar genotypes are given in Table D 994, Kanchan, Amar, 551, 552, 553, 555, 556, 557, 559, 561, 567, 568, showed the light purple tassel colour, Surya, D 995, 550, 558, 565, 566, 570

Table 10 : Morphological character of 44 maize genotypes (CIMMYT)

Inbred line	Tassel texture	Tassel A-G colour	Tassel G-B colour	Silk colour at emergence	Grain shape	Grain size	Blighting shape	Blighting colour
1.	Dense	Light purple	Purple	Green	1	7	Round	Yellow/dark brown
2.	Dense	Light purple	Purple	Green	1	7	Round	Yellow/straw
3.	Dense	Green	Absent	Purple	1	7	Round	Yellow/dark brown
4.	Dense	Light purple	Purple	Purple	1	5	Round	Yellow/dark brown
5.	Medium	Light purple	Purple	Green	3	5	Banded	Yellow/straw
6.	Dense	Green	Absent	Purple	1	5	Round	Yellow/brown
7.	Dense	Green	Absent	Purple	1	7	Banded	Yellow/straw
8.	Light	Light purple	Purple	Purple/green	1	7	Banded	Yellow/water soaked
9.	Dense	Purple	Purple	Purple/green	1	5	Round	Yellow/dark brown
10.	Dense	Light purple	Absent	Green	1	5	Round	Yellow/water soaked
11.	Dense	Purple	Purple	Purple	1	5	Round	Yellow/dark brown
12.	Lax	Light purple	Purple	Purple	3	5	Round	Yellow/straw colour
13.	Medium	Purple	Absent	Purple	3	5	Banded	Yellow/water soaked
14.	Medium	Purple	Absent	Green	1	5	Banded	Yellow/water soaked
15.	Dense	Purple	Purple	Green	3	7	Banded	Yellow/water soaked
16.	Medium	Purple	Absent	Purple	3	7	Banded	Yellow/straw colour
17.	Lax	Purple/green	Purple	Purple	1	5	Banded	Yellow/water soaked
18.	Dense	Green	Purple	Purple	3	5	Banded	Yellow/water soaked
19.	Medium	Light purple	Absent	Purple	2	3	Banded	Yellow/straw
20.	Lax	Green	Absent	Green	1	5	Round	Yellow/dark brown
21.	Medium	Light purple	Purple	Purple	1	7	Banded	Yellow/straw colour
22.	Dense	Light purple	Absent	Green	3	7	Banded	Yellow/water soaked
23.	Dense	Purple	Purple/green	Purple	3	5	Round	Yellow/straw
24.	Light	Green	Purple	Green	2	3	Banded	Yellow/water soaked
25.	Medium	Purple	Absent	Green	1	5	Round	Yellow/straw colour
26.	Dense	Green	Absent	Purple	3	5	Banded	Yellow/straw colour
27.	Medium	Light purple	Purple	Purple	2	3	Banded	Yellow/water soaked
28.	Lax	Green	Absent	Purple	1	7	Banded	Yellow/water soaked
29.	Lax	Light purple	Absent	Green	3	7	Banded	Yellow/straw colour
30.	Lax	Green	Absent	Purple	3	5	Banded	Yellow/water soaked
31.	Dense	Purple	Purple	Green	3	7	Round	Yellow/straw colour
32.	Dense	Purple	Purple	Purple	2	7	Banded	Yellow/dark brown
33.	Dense	Green	Absent	Green	1	7	Banded	Yellow/straw
34.	Medium	Purple	Purple	Green	1	7	Banded	Yellow/straw
35.	Dense	Purple	Purple	Purple	1	5	Banded	Yellow/straw colour
36.	Dense	Green	Purple	Purple	1	5	Banded	Yellow/straw colour
37.	Lax	Green	Purple	Purple green	1	5	Banded	Yellow/water soaked
38.	Lax	Green	Purple	Green	3	5	Round	Yellow/water soaked
39.	Medium	Light purple	Absent	Green	1	7	Banded	Yellow/dark brown
40.	Lax	Purple	Purple	Purple	3	7	Banded	Yellow/straw
41.	Dense	Green	Purple	Purple	2	3	Banded	Yellow/water soaked
42.	Dense	Light purple	Absent	Green	1	7	Banded	Yellow/water soaked
43.	Medium	Green	Absent	Green	3	5	Banded	Yellow/straw
44.	Medium	Green	Absent	Green	3	5	Banded	Yellow/straw

Table 11 : Morphological character of 14 genotypes (CM lines)

Inbred line	Tassel texture	Tassel A-G colour	Tassel G-B colour	Silk colour at emergence	Grain shape	Grain size	Blighting shape	Blighting colour
1.	Dense	Light purple	Purple	Green	1	7	Round	Yellow/covered by brown ring
2.	Dense	Light purple	Purple	Green	1	7	Round	Yellow/covered by brown ring
3.	Dense	Purple	Purple	Green	3	5	Banded	Yellow/covered dark brown
4.	Medium	Purple	Purple	Green	1	7	Banded	Yellow/straw
5.	Dense	Purple	Absent	Purple	3	5	Banded	Yellow/dark brown
6.	Dense	Purple	Purple	Purple	3	5	Banded	Yellow/straw
7.	Dense	Purple	Absent	Green	1	5	Round	Yellow/straw soaked
8.	Dense	Purple	Purple	Green	3	5	Banded	Yellow/dark brown
9.	Dense	Purple	Absent	Purple	1	5	Banded	Yellow/dark brown
10.	Medium	Purple	Purple	Purple	3	7	Round	Yellow/dark brown
11.	Dense	Light purple	Absent	Green	1	7	Round	Yellow/brown
12.	Dense	Light purple	Purple	Green	1	7	Round	Yellow/brown
13.	Medium	Purple	Absent	Green	1	5	Banded	Yellow/straw
14.	Dense	Purple	Purple	Green	1	7	Banded	Yellow/water soaked

The data on tassel texture of Pantnagar genotypes, given in Table 13. Genotypes 556, 561, 568 and varieties D 995, Kanchan, Amar showed medium texture tassel 550, 551, 552, 553, 554, 555, 557, 558, 559, 560, 562, 564, 565, 566, 567, 569 and 570 and composite D 994 and Surya showed dense texture tassel.

The resistant genotypes of all the three groups, which showed mean disease reaction (1.0 to 2.0) of which, CA00106, CA049Y04, CA03147, CA14509, CA03124, CM 104, CM 105, CM 500, CM 600 represented dense tassel texture, only CA00310 (CML 429) showed lax tassel texture, and two resistant lines, 556, 561 showed medium texture tassel.

4.1.6 Tassel colour

4.1.6.1 Tassel anther glum colour

Tassel A-G colour of all forty four CIMMYT genotypes are given in Table 11. CA00106, CA049Y04, CA049Y04, CA14518, CA03106, CML 469, CML 429, CML 465, CM 432, CM 425, CML 430, CA144711, CA14519, CML 470, CML 475, CML 427, CA00396, CA34507 showed light purple tassel colour, CA03147, CA03106, CA003134, CA03149, CM428, A00344, CA00384, CML 421, PoP147-F2#161-3-2-B-1-B, CA00104, CML 426, CML 413, CA14501, CA14517, CML 474 showed light green tassel colour and CA34506, CA03131, CML 433, CA034516, CA03124, CA00378, CA03142, CA14527, CML 431 and CML 471, showed purple tassel colour.

Tassel colour of CM lines are given in Table 12 . CM 104, CM 105, CM 500, CM 600, showed light purple tassel colour, CM 111, CM 115, CM 119, CM 120, CM 121, CM 123, CM 125, CM 400, CM 209, CM 210 showed purple tassel colour. The tassel colour of Pantnagar genotypes are given in Table D 994, Kanchan, Amar, 551, 552, 553, 555, 556, 557, 559, 561, 567, 568, showed the light purple tassel colour, Surya, D 995, 550, 558, 565, 566, 570

Table 12 : Morphological character of Pantnagar lines

Inbred line	Tassel texture	Tassel A-G colour	Tassel G-B colour	Silk colour at emergence	Grain shape	Grain size	Blighting shape	Blighting colour
1.	Dense	Purple	Purple	Green	3	5	Banded	Yellow/dark brown
2.	Medium	Purple	Purple	Purple	1	7	Banded	Yellow/straw colour
3.	Dense	Light purple	Absent	Purple	1	5	Banded	Yellow/dark brown
4.	Medium	Light purple	Purple	Green	2	5	Banded	Yellow/dark brown
5.	Medium	Light purple	Purple	Green	2	5	Round	Yellow/water soaked
6.	Dense	Purple	Absent	Green	1	7	Banded	Yellow/straw colour
7.	Dense	Light purple	Purple	Green	1	5	Banded	Yellow/straw
8.	Dense	Light purple	Purple	Purple	1	7	Banded	Yellow/straw colour
9.	Dense	Light purple	Absent	Green	1	5	Banded	Yellow/water soaked
10.	Dense	Green	Absent	Purple	3	5	Banded	Yellow/water soaked
11.	Dense	Light purple	Purple	Green	1	5	Banded	Yellow/straw colour
12.	Medium	Light purple	Purple	Purple	2	5	Banded	Yellow/water soaked
13.	Dense	Light purple	Purple	Purple	1	5	Banded	Yellow/straw colour
14.	Dense	Purple	Absent	Purple	1	5	Banded	Yellow/water soaked
15.	Dense	Light purple	Purple	Purple	1	5	Banded	Yellow/water soaked
16.	Dense	Green	Absent	Purple	1	7	Banded	Yellow/straw colour
17.	Medium	Light purple	Purple	Purple	1	5	Banded	Yellow/water soaked
18.	Dense	Green	Purple	Green	1	5	Banded	Yellow/water soaked
19.	Dense	Green	Absent	Green	3	5	Banded	Yellow/dark brown
20.	Dense	Green	Absent	Purple	1	5	Banded	Yellow/water soaked
21.	Dense	Purple	Purple	Purple	3	5	Banded	Yellow/water soaked
22.	Dense	Purple	Absent	Purple	1	5	Banded	Yellow/straw
23.	Dense	Light purple	Purple	Green	3	5	Round	Yellow/dark brown
24.	Medium	Light purple	Purple	Green	3	5	Banded	Yellow/straw colour
25.	Dense	Green	Absent	Purple	1	5	Banded	Yellow/dark brown
26.	Dense	Purple	Absent	Purple	2	5	Banded	Yellow/water soaked

Table 13 : Days to tasseling and silking and disease reaction (Field experiment)

Inbred Lines (Code)	Day to tasseling (mean)	Day of silking (mean)	Disease reaction (mean)
CA00106	52.3	55.6	1.2
CA049Y04	49.6	54.6	1.1
CA03147	50.3	54.0	1.8
CA14509	50.0	53.3	1.4
CA14518	51.3	54.1	1.6
CA03106	46.0	52.6	3.3
CA003134	47.6	52.6	3.5
CA00370 (CML 469)	46.0	50.0	3.8
CA34507	45.3	51.3	3.5
CA00102 (CML 429)	47.0	52.6	3.7
CA00396	48.6	52.6	4.1
CA00310 (CML 465)	49.0	51.0	1.6
CA34506	43.6	53.6	3.0
CA03131	48.0	52.6	3.8
CA00334 (CML 433)	48.3	53.0	2.7
CA34516	49.3	58.0	2.8
CA03149	49.6	51.6	3.5
CA14510 (CML 428)	45.3	53.6	3.7
CA00332 (CML 432)	46.6	50.6	3.2
A00344	48.6	52.3	3.9
CML 425	49.6	52.0	4.0
CML 430	49.0	53.3	4.5
CA03124	48.0	53.0	1.5
CA00384	44.6	51.6	3.6
CA00378	44.3	52.3	3.5
CML 421	50.3	55.0	3.7
CA14711	48.6	53.3	3.1
Pop. 147-F2#161-3-2-B-1-B	46.3	52.6	2.8
CA14519	46.8	50.6	2.2
CA00104	48.3	51.6	2.0
CA03142	50.3	54.3	4.1
CA14527	48.6	52.3	4.1
CA03130 (CML 426)	49.0	53.6	2.7
CA14502 (CML 427)	48.3	53.2	4.1
CA00304 (CML 431)	48.6	54.2	4.1
CML 413	49.6	54.3	3.9
CA14501	49.6	52.0	4.5
CA14517	48.3	51.8	3.9
CA03116 (CML 470)	46.6	50.3	3.6
CA14709 (CML 471)	49.6	53.8	3.2
CA14524 (CML 474)	49.6	54.6	3.1
CA14520 (CML 475)	49.6	52.8	2.0
CML 428	48.3	51.9	4.5
CA00334 (CML 433)	47.0	53.3	4.5

showed purple tassel colour and 554, 560, 562, 563, 564 and 569, showed green tassel colour.

4.1.6.2 Tassel glum base colour

The observations on tassel glume base colour are presented in Table 11. CA00106, CA049Y04, CA14509, CA141518, CML 469, CA34507, CML 429, CA00396, CML 465, CML 433, CA03149, CML 428, CML 425, CA00384, CA14711, CA03142, CA14527, CML 427, CML 431, CML 413, CA14501, CA14517, CML 471, CM 474, all these genotypes showed purple glume base colour, whereas in CA03147, CA03106, CA003134, CML 429, CML 429, CA34506, CA03131, CA34516, CML 432, A00344, CML 430, CA00378, CML 421, PoP147-F2#161-3-2-B-1-B, CA14519, CA00104, CML 426, CML 470, CML 433, glume base colour was absent. Tassel glum-base colour of all the CM is given in Table 12. CM 104, CM 105, CM 111, CM 115, CM 120, CM 123, CM 400, CM 600, CM 210 showed purple glum base colour, whereas in CM 119, CM 121, CM 125, CM 500, CM 209 glum-base colour was absent.

The observation on the tassel glum-base of Pantnagar genotypes are given in Table 13. Surya, D 995, Kanchan, Amar, 550, 551, 555, 556, 557, 559, 560, 564, 565, 567, 568, showed purple glum-base colour whereas glume base colour was absent in D 994, 550, 553, 558, 560, 563, 566, 569, 570.

Tassel colour of resistant lines, which showed mean disease reaction in between (1.0 to 2.0) was CA00106, CA049Y04, CA14509, CA14518, CA00310 (CML 465), CA03124, CM 104, CM 105, CM 500, CM 600, 556, 561, which showed purple colour and only CA03147, showed the green tassel colour.

4.1.6 Silk colour

Silk colour is also a morphological character and the data on different CIMMYT genotypes are given in Table 11. Genotypes, CA00106, CA049Y04, CA14518, CML 429, CA03131, CML 433, A00344, CML 430, CA00384,

CA00378, CA14519, CA03142, CML 426, CML 427, CA14517, CA03116, (CML 470), CML 475, CML 428 and CML 433 showed green silk colour at emergence. Whereas CA03147, CA14509, CA14518, CA03106, CML 469, CA34507, CA00396, CA00310, CML 465, CML 433, CA34316, CA03149, CML 428, CML 432, CML 425, CA00384, CA14711, CA03142, CA14527, CML 427, CML 431, CML 413, CA14501, CA14517, CML 471, CML 474 showed purple silk colour.

The observations on the silk colour at emergence of different CM lines are given in Table 12. Genotypes CM 104, CM 105, CM 111, CM 115, CM 121, CM 123, CM 500, CM 600, CM 209, CM 210 showed green colour whereas. CM 119, cm 120, cm 125, cm 400 showed purple colour.

The observations on the silk colour at emergence of different, maize genotypes of Pantnagar genotypes are given in Table 13. Maize inbred line, Surya, Kanchan, Amar, 550, 551, 553, 555, 562, 563, 567, 568 showed green colour, D 995, D 994, 552, 556, 557, 558, 559, 560, 561, 564, 565, 566, 569, 570 showed purple colour at emergence.

4.1.7 Blighting shape

Blighting shape might be a good morphological marker for differentiating the resistant and susceptible maize inbred line. The observation recorded on the blighting shape of different CIMMYT maize genotypes are given in Table 11. Genotypes, CA00106, CA049Y04, CA03147, CA14509, CA03106, CA34507, CML 429, CML 429, CA00396, CA00310 (CML 465), CA03124, CA03142, CA1417 showed round blighting, while, CA14518, CA003134, CA003134, CA344506, CML 433, CA34516, CA03149, CML 428, CML 432, 100344, CML 425, CM 430, CA00384, CA00378, CML 421, CA14711, PoP147-F2#161-3-2-B-1-B, CA14519, CA00104, CA14527, CML 426, CML 427, CML 431, CML 413, CA14501, CML 470, CML 471, CML 474, CML 475, CML 428, CML 433, showed banded blighting shape.

Blighting shape of all CM lines also observed in field condition and are presented in Table 12, CM 104, CM 105, CM 121, CM 400, CM 500, CM 600 showed rounded pattern of blighting shape. Whereas, CM 111, CM 115, CM 119, CM 123, CM 125, CM 209, CM 210 showed banded pattern of blighting pattern.

Blighting shape of all Pantnagar genotypes were observed and the data on presented in Table 13. only Amar, 567 exhibited blighting shape in rounded pattern, whereas Surya, D 995, D 994, Kanchan, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 568, 569, 570 how rounded pattern.

Resistant maize inbred line, CA00106, CA049Y04, CA03147, CA14509, CA00310 (CML 465), CA03124, CM 104, CM 105, CM 500, CM 600 showed the rounded blighting shape in comparison to the susceptible genotypes which showed banded blighting shape predominantly.

4.1.8 Blighting colour

Blighting colour of all maize genotypes of CIMMYT are given in Table 11. CA00106, CA14509, CA14518, CA34507, CA00396, A00344, CA14527, CA14517, CA03106, showed yellow spot surrounded by dark brown ring, CA14518, CA049Y04, CA03106, CA003134, CML 432, CA03124, CML 430, CA00384, CA00378, CA03142, CA14527, CML 426, CML 427, CML 431, CML 471, CML 428, CML 433, showed yellow (straw colour) spot and CML 469, CML 429, CML 465, CA 34506, CA03131, CA34516, CA03149, CML 428, CML 425, CA003124, CA14711, Pop147-F2#161-3-2-B-1-B, CA00104, CA14501, CA14517, CML 474, CML 475, showed yellow water soaked spot.

Blighting colour of genotypes of CM group is given in Table 12. Inbred lines, CM 104, CM 105, CM 111, CM 119, CM 123, CM 125, CM 400, CM 500, CM 600 gave yellow spot surrounded by brown ring, CM 115, CM 120, CM 209,

showed yellow (straw colour spot), while only CM 210 showed yellow/water soaked spot.

Blighting colour of Pantnagar genotypes are given in Table 13. Genotypes which showed yellow spot surrounded by dark brown included Surya, D 994, Kanchan, 562, 567, 569 and which showed yellow straw colour symptom were D 995, 550, 551, 555, 557, 560, 566, 568. Water soaked symptom were, present in 553, 554, 558, 559, 561, 562, 564, 566, 570.

Resistant inbreds, CA00106, CA03147, CA14509, CM 104, CM 105, CM 500, CM 600 showed yellow blighting colour which is surrounded by browning in later stage, while CA049Y04, CA00310 (CML 465), CA03124, 556, showed yellow straw coloured blighting and, only one maize genotypes 561 showed yellow water soaked blighting colour.

4.1.10 Days to tassel

Days to tassel is another morphological character, which is taken as a morphological character during the present investigation (Table 14 and Fig. 14). The resistant maize inbred line, which showed the diseases reaction in between (1.0 to 2.0), were CA00106, CA049Y04, CA03147, CA14509, CA14518, CA00310, CML 465, CA03124 and CM 104, CM 105 showed average days to tassel (50 to 55) and genotypes CA03124, CM 500, CM 600, 561 and 556 showed average days to tassel (44 to 50) which were moderately susceptible, with field disease rating (2.0 to 4.0). The genotypes CA03106, CA003134, CA00370 (CML 469), CA34507, CA00102 (CML 429), CA00396, CA34506, CA03131, CA00334 (CML 433), CA34516, CA03149, CA14510 (CML 428), CA00332 (CML 432), A00344, CML 425, CA00384, CA00378, CML 421, CAI1711, PoP147-F2#161-3-2-B-1-B, CA03130 (CML 426), CML 413, CA14517, CA03116 (CML 470), CA14709 (CML 471), CA14524 (CML 474), CA14520 (CML 475), CM 120, CM 121, CM 125, CM 400, CM 209, Surya, D 995, D 994, Kanchan, 550, 552, 553, 555, 559, 562, 564, 567, showed days to

**Table 14 : Day of tasseling, silking and disease reaction of 14 CM genotypes
(Field experiment)**

Inbred Lines (Code)	Day of tasseling	Day of silking	Disease reaction (mean)
CM 104	50.0	54.6	1.9
CM 105	48.0	53.6	1.7
CM 111	46.0	53.3	4.6
CM 115	40.0	50.3	4.4
CM 119	45.3	52.3	4.6
CM 120	46.6	53.0	3.0
CM 121	44.3	52.6	3.7
CM 123	44.6	52.0	4.8
CM 125	45.3	51.6	2.6
CM 500	46.6	51.6	3.0
CM 400	46.0	52.3	2.0
CM 600	44.3	52.6	1.9
CM 209	45.3	52.0	3.9
CM 210	40.3	52.0	4.4

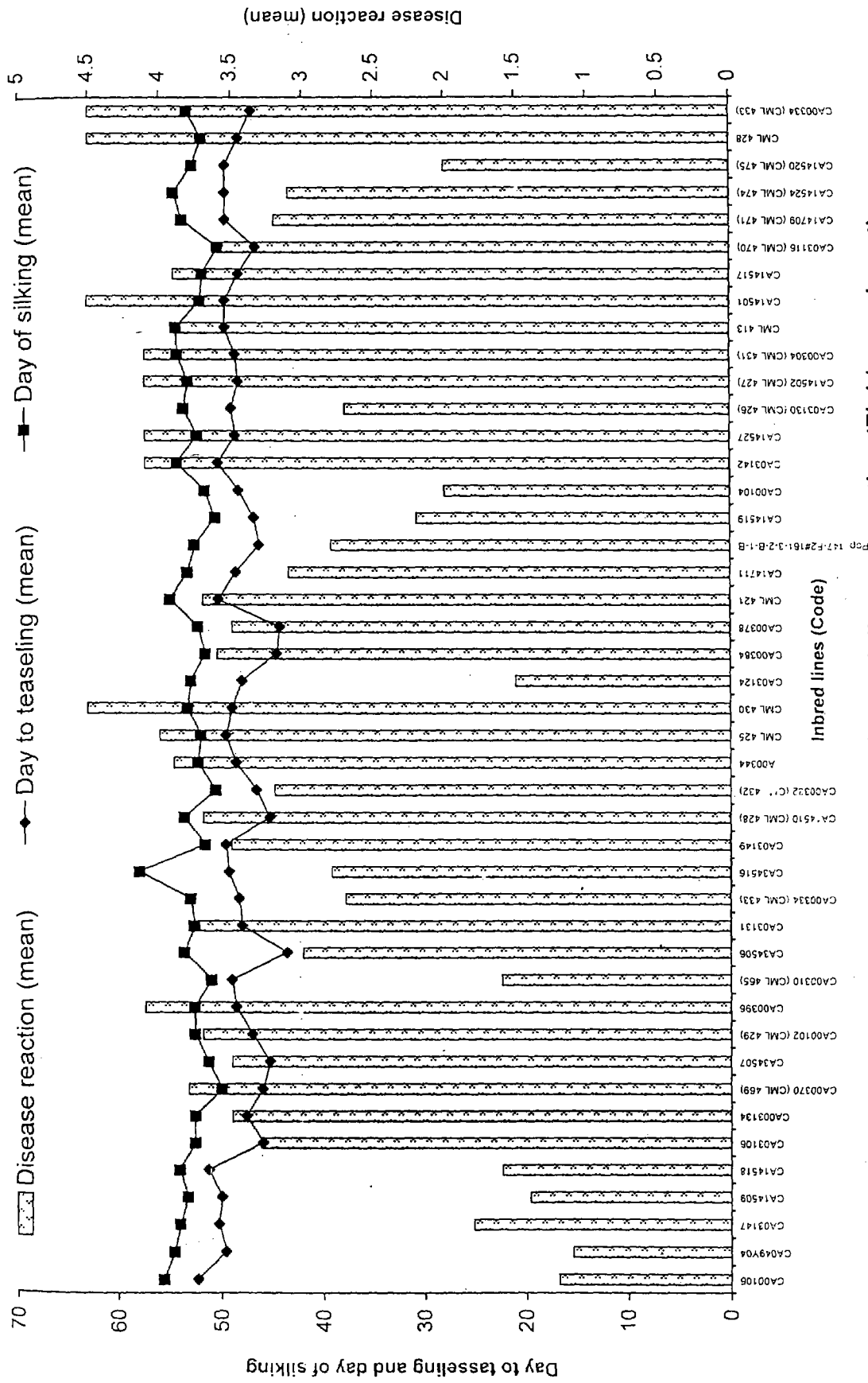


Fig. 14 : Days to tasseling and silking and disease reaction (Field experiment)

tassel between (40 to 50 days). The genotypes which showed high susceptibility (4.5 to 5.0), CA00396, CML 430, CA03142, CA14527, CA14502 (CML 427), CA00304 (CML 431), CA14501, CML 428, CA00334, (CML 433), CM 11, CM 115, CM 119, CM 123, CM 210, D 994, Amar, 551, 557, 558, 560, 465, 465. 468, 469, 570 showed average days to tassel (40 to 50) days.

4.1.11 Days to silk

Maize genotypes which showed disease reaction mean in between (1.0 to 2.0), CA00106, CA049Y04, CA03147, CA14509, CA14518, CA00310 (CML 465), CA03124, CM 104, CM 105, CM 500, CM 600, 56, 561, all showed the days to silk in between (50 to 55) (Table 13, 14, 15 and Fig. 14, 15, 16). Moderately susceptible group showed disease reaction in between (2.0 to 4.0) were CA03106, CA003134, CA00370 (CML 469), CA 344507, CA00102. (CML 429), CA00396, CA34506, CA03131, CA00334 (CML 433), CA34516. CA03149, CA14510, (CML 428), CA00332 (CML 432), A00344, CML 425, CA00384, CA00378, CML 421, CA14711, PoP147-F2#161-3-2-B-1-B, CA03130 (CML 4261), CML 413, CA14517, CA03116 (CML 470), CA14709 (CML 471), CA14524 (CML 474), CA14520 (CML 475), CM 120, CM 121. CM 400, CM 209, Surya, D 995, Kanchan, 550, 552, 553, 555, 559, 562, 564, 567, all these genotypes showed silking average day also in between (50 to 55).

4.2 Glasshouse experiment

4.2.1 Plant height and disease reaction

Maize inbred lines, CA00106, CA049Y04, CA03147, CA14518, CA00310 (CML 465), CA00104 and CM 104, CM 105, CM 500 CM 600, 561, which showed the average mean disease reaction in glasshouse condition in between (1.0 to 2.0), the average plant height varied between (140 to 165 cm). The moderately susceptible genotypes which showed mean disease reaction (2.0 to 4.0), CA03106, CA003134, CA00370 (CML 469), CA34507, CA00102

Table 15 : Field experiment day of tasseling, day of selking and diseases rating

Inbred Lines (Code)	Day tasseling mean	Day of selking mean	Disease reaction mean
Surya	46.0	50.6	2.6
D-995	45.0	52.6	2.3
D-994	47.0	52.6	4.1
Kanchan	46.3	51.0	2.8
Amar	46.3	54.3	4.6
550	47.0	51.3	3.4
551	46.0	53.3	4.6
552	46.0	51.0	2.8
553	46.0	52.6	3.1
554	47.6	52.0	2.9
555	46.6	54.6	3.7
556	48.0	53.0	2.0
557	46.6	54.6	4.1
558	45.0	53.3	4.2
559	47.3	53.0	3.7
560	47.0	52.0	4.6
561	44.6	54.0	1.5
562	45.0	55.0	3.8
563	44.0	53.3	3.5
564	46.6	52.3	3.6
565	47.6	52.3	4.4
566	49.3	53.0	4.3
567	44.6	52.6	2.8
568	46.3	54.0	4.6
569	47.3	53.3	4.6
570	44.3	52.6	4.8

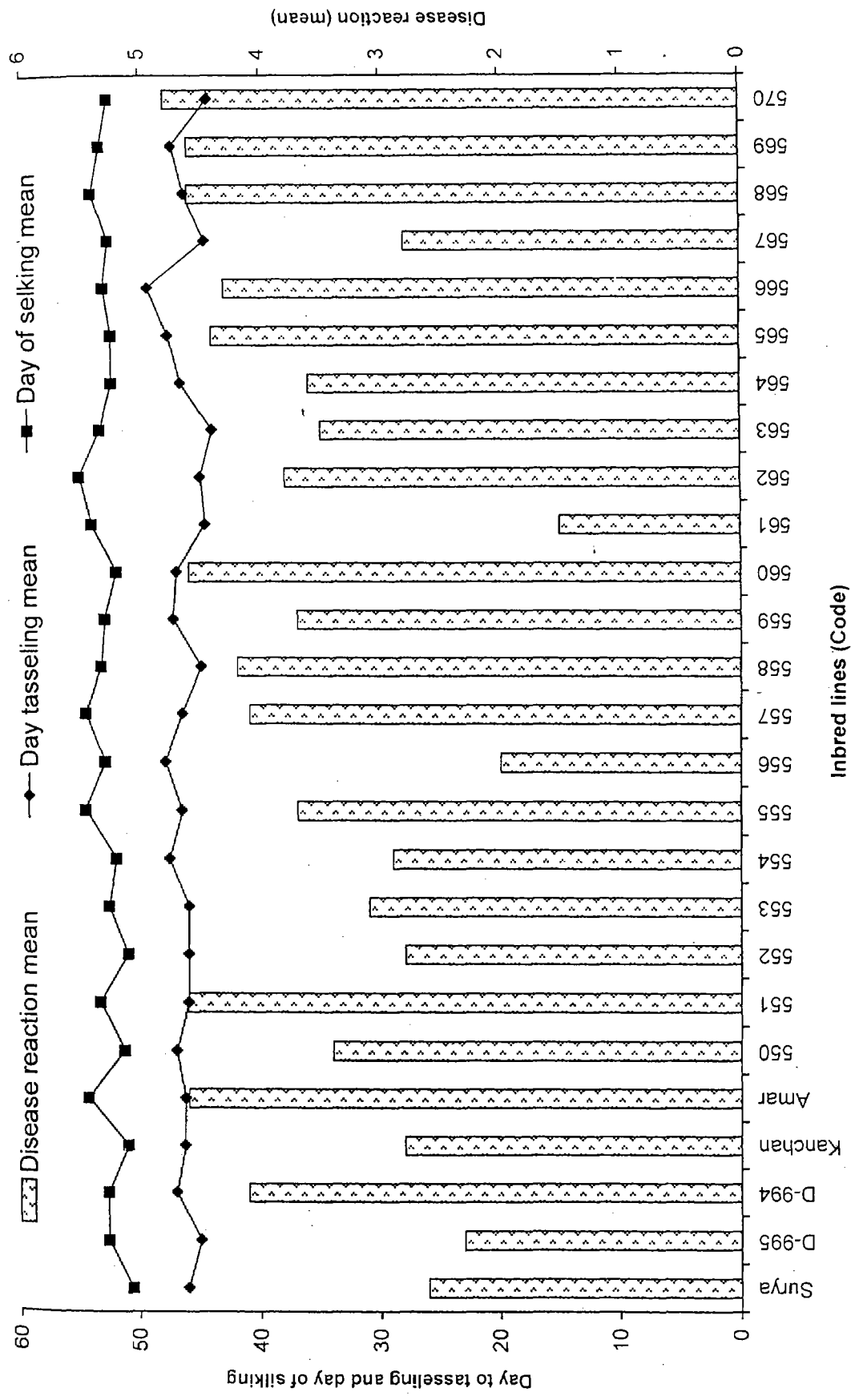


Fig. 15 : Field experiment day of tasseling, day of silking and diseases rating

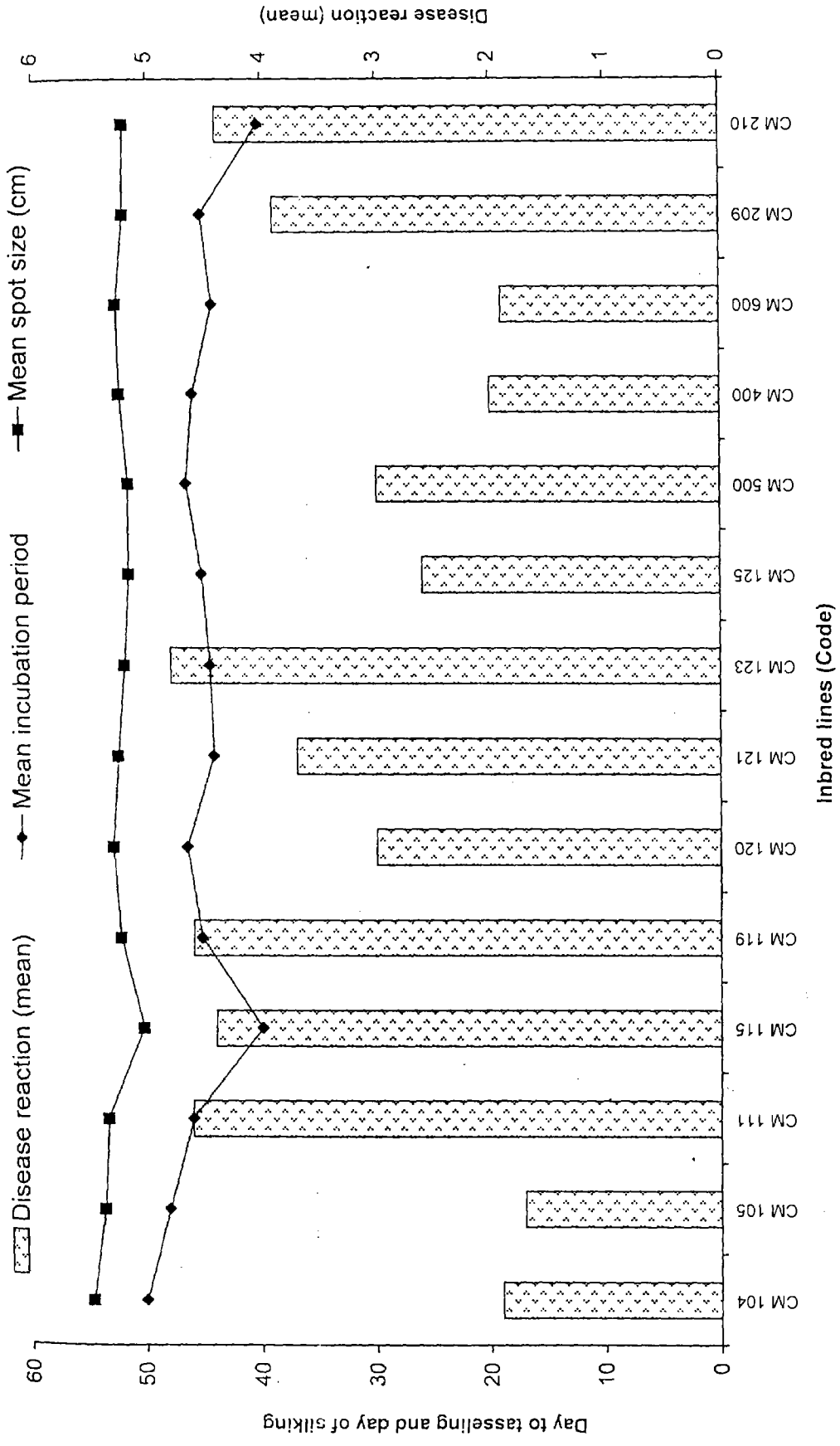


Fig. 16 : Day of tasseling, silking and disease reaction of 14 CM lines (Field experiment)

(CML 429), CA34506, CA03131, CA00334 (CML 433), CA34516, CA03149, CA14510 (CML 428), CA00332 (CML 432), A00344, CML 425, CA00384, CA00378, CML 421, PoP 147-F₂# 161-3-2-B-1-B, CA14519, CML 426, CML 413, CA14517, CML 470, CML 471, CML 474, CML 475, CM 111, CM 115, CM 119, CM 120, CM 121, CM 123, CM 125, CM 400, CM 209, Surya, D 995, Kanchan, 552, 553, 554, 556, 560, 562, 563, 564, 567, 570 showed the mean average plant height in between (100 to 150 cm). The material which were highly susceptible, with disease reaction (4.0 to 5.0), CA00396, CML 430, CA03142, CA14527, CA03142, CA14527, CML 427, CML 431, CA 14501, CML 428, CML 433, CM 210, D 994, Amar, 500, 551, 555, 557, 558, 559, 566. of the where the average plant height varied in between (75 to 150 cm) (Table 16, 17, 18 and Fig. 17, 18, 19).

4.2.2 Cob placement and disease reaction

Resistant maize material which showed average mean disease reaction in between (1.0 to 2.0) and cob placement at CA00106, CA049Y04, CA03147, CA14509, CA14518, CA00310 (CML 465), CA03124, CM 104, CM 105, CM 500, CM 600, 561, genotypes CA03106, CA003134, CA00370 (CML 469), CA34507, CA00102 (CML 429), CA34506, CA03131, A00344, CM 425, CA00384, CA00378, CML 421, PoP 147-F₂# 161-3-2-B-1-B, CA14519, CML 426, CML 413, CA14517, CML 470, CML 471, CML 474, CM 111, CM 115, CM 119, CM 120, CM 121, CM 123, CM 125, CM 400, CM 209, Surya, D 995, Kanchan, 552, 553, 554, 556, 560, 512, 563, 564, 564, 567, 570, showed disease reaction in between (2.0 to 4.0) and the cob placement average mean height varied in between (30 to 50 cm). The genotypes which was highly susceptible (4.0 to 5.0) were CA00396, CML 430, CA03142, CA14527, CA03142, CA14527, CML 427, CML 431, CA14501, CML 428, CML 433, CM 210, D-994, Amar, 550, 551, 555, 557, 5578, 559, 566, exhibited the average cob placement (30 to 45 cm) (Table 16, 17, 18 and Fig. 17, 18, 19).

Table 16 : Glasshouse experiment plant height and cob placement and disease reaction of 44 CIMMYT genotypes

Inbred Lines (Code)	Plant height (cm) mean	Cob placement height (cm) mean	Disease reaction (mean)
CA00106	144.3	40.3	1.2
CA049Y04	154.3	51.3	1.1
CA03147	144.6	53.6	1.8
CA14509	137.6	40.0	1.4
CA14518	139.0	37.3	1.6
CA03106	134.3	35.6	3.3
CA003134	131.0	33.3	3.5
CA00370 (CML 469)	122.6	46.6	3.8
CA34507	133.0	47.6	3.5
CA00102 (CML 429)	125.0	46.6	3.7
CA00396	111.6	33.3	4.1
CA00310 (CML 465)	145.0	53.6	1.6
CA34506	133.6	35.0	3.0
CA03131	129.6	39.0	3.8
CA00334 (CML 433)	125.0	35.0	2.7
CA34516	125.0	34.6	2.8
CA03149	125.0	40.0	3.5
CA14510 (CML 428)	120.0	35.0	3.7
CA00332 (CML 432)	118.0	39.0	3.2
A00344	132.6	48.0	3.9
CML 425	127.3	35.0	4.0
CML 430	108.3	32.5	4.5
CA03124	142.3	30.0	1.5
CA00384	126.0	31.6	3.6
CA00378	138.3	37.0	3.5
CML 421	115.0	35.0	3.7
CA14711	109.0	34.3	3.1
Pop. 147-F2#161-3-2-B-1-B	115.0	41.0	2.8
CA14519	121.3	30.0	2.2
CA00104	115.0	38.0	2.0
CA03142	114.0	32.3	4.1
CA14527	110.0	35.0	4.1
CA03130 (CML 426)	116.0	32.0	2.7
CA14502 (CML 427)	128.3	35.0	4.1
CA00304 (CML 431)	100.0	31.6	4.1
CML 413	100.0	39.3	3.9
CA14501	110.0	38.3	4.5
CA14517	108.0	37.6	3.9
CA03116 (CML 470)	105.0	32.3	3.6
CA14709 (CML 471)	76.0	35.0	3.2
CA14524 (CML 474)	115.0	33.6	3.1
CA14520 (CML 475)	98.6	37.6	2.0
CML 428	95.6	34.0	4.6
CA00334 (CML 433)	75.0	42.3	4.9

Table 17 : Glasshouse experiment plant height and cob placement and disease reaction of 14 CM genotypes

Inbred Lines (Code)	Plant height (cm) mean	Cob placement height (cm) mean	Disease reaction (mean)
CM 104	147.6	57.3	1.6
CM 105	145.0	58.3	1.2
CM 111	129.0	55.6	3.8
CM 115	115.0	41.6	3.5
CM 119	114.6	38.3	3.8
CM 120	113.3	41.3	3.1
CM 121	127.3	34.0	3.3
CM 123	137.3	42.3	3.6
CM 125	125.0	42.3	2.7
CM 500	152.0	43.3	2.6
CM 400	156.3	57.0	1.6
CM 600	165.0	61.6	1.8
CM 209	125.0	43.6	3.3
CM 210	108.3	38.3	4.3

Table 18 : Glasshouse experiment plant height and cob placement and disease reaction of 26 genotypes

Inbred Lines (Code)	Plant height (cm) mean	Cob placement height (cm) mean	Disease reaction (mean)
Surya	125.0	40.0	3.2
D-995	142.3	43.3	3.4
D-994	131.6	45.3	4.1
Kanchan	125.0	43.3	2.8
Amar	145.0	52.6	4.6
550	148.3	52.0	4.5
551	147.0	46.0	4.3
552	125.0	40.0	3.5
553	133.0	40.6	3.3
554	145.0	45.0	3.5
555	136.0	47.0	4.5
556	138.3	45.3	2.5
557	116.6	44.0	4.3
558	128.3	51.3	4.5
559	128.3	44.6	4.5
560	125.0	53.6	3.4
561	147.0	42.3	1.9
562	113.3	41.0	3.7
563	131.6	39.6	3.7
564	137.0	47.6	3.3
565	130.0	39.6	2.9
566	135.0	42.6	4.5
567	129.0	50.3	3.0
568	137.6	55.3	4.1
569	112.0	44.6	4.2
570	126.6	51.6	3.8

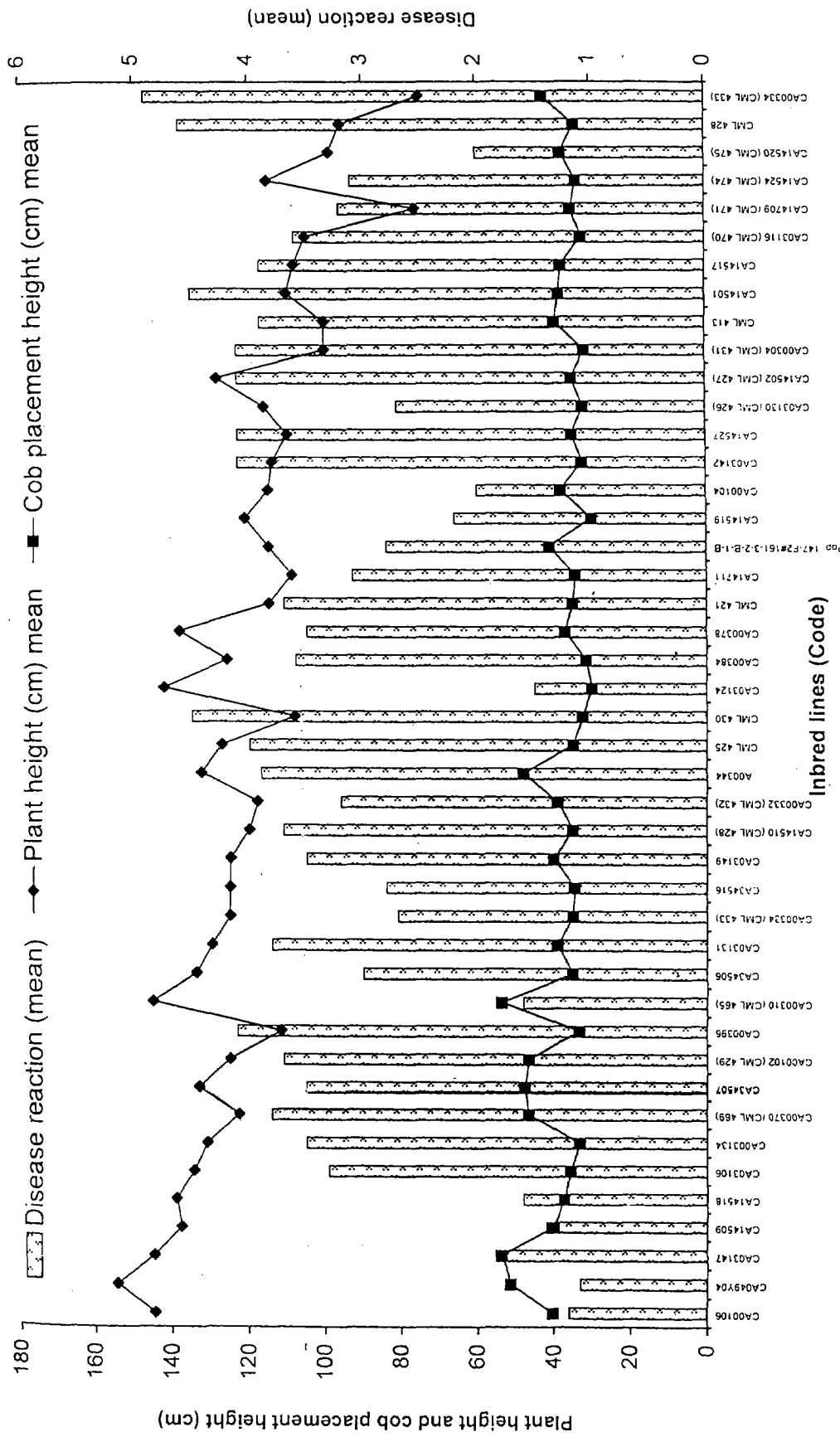


Fig. 17 : Glasshouse experiment plant height and cob placement and disease reaction of 44 CIMMYT genotypes

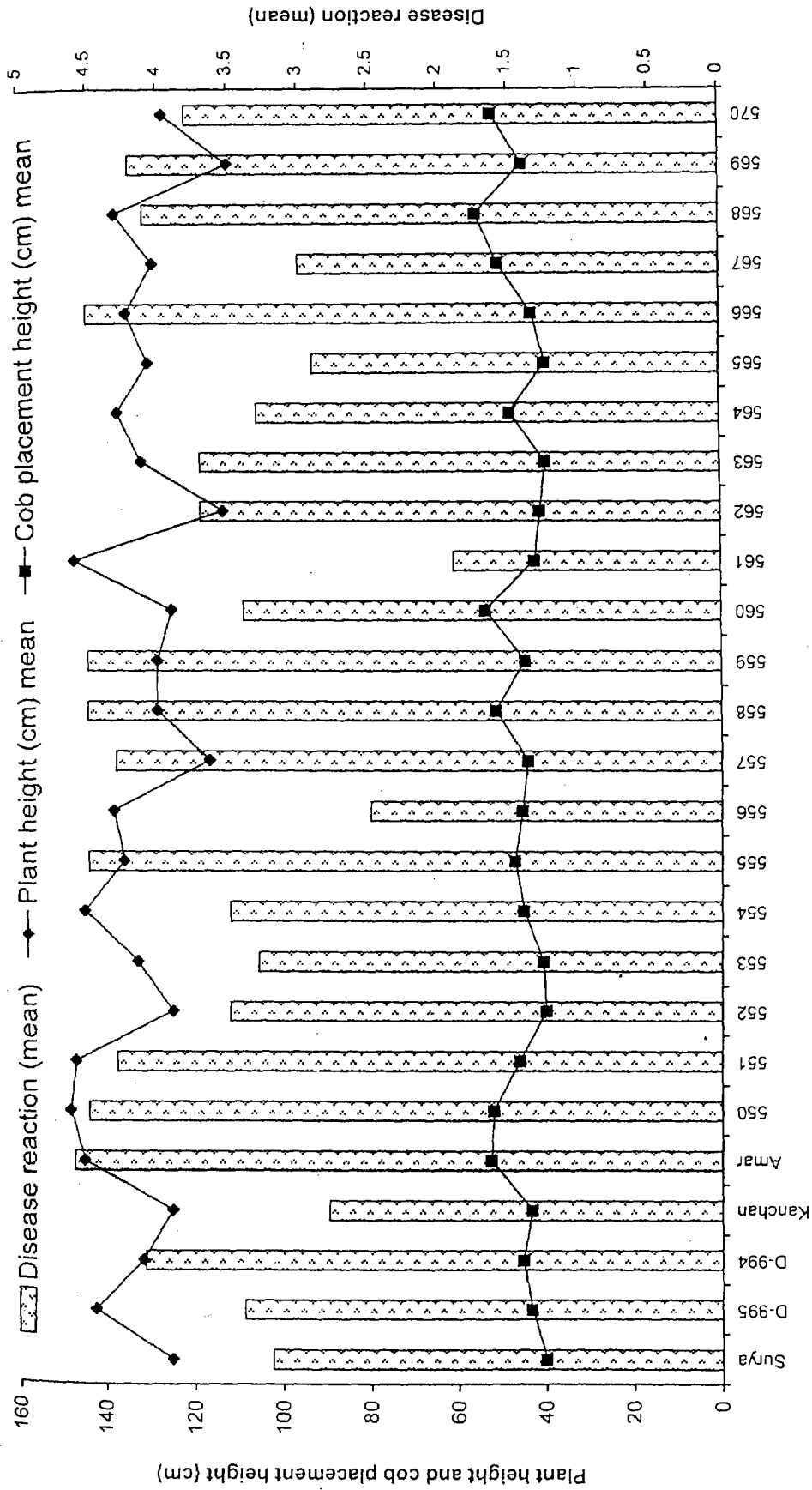


Fig. 18 : Glasshouse experiment plant height and cob placement and disease reaction of 26 genotypes

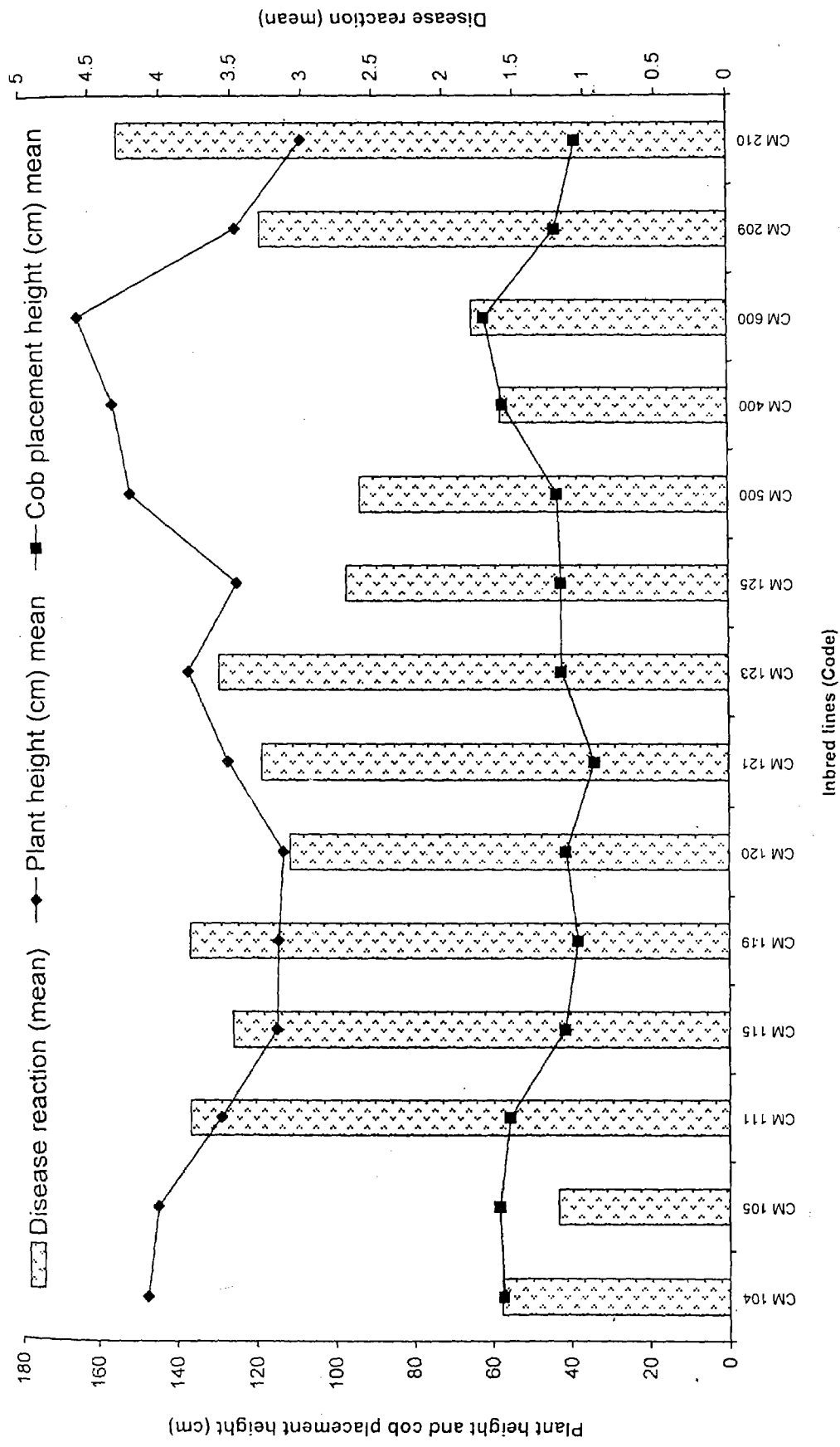


Fig. 19 : Glasshouse experiment plant height and cob placement and disease reaction of 14 CM genotypes

4.2.3 Incubation period

Resistant maize genotypes which depicted the mean disease reaction in between (1.0 to 2.0), CA00106, CA049Y04, CA03147, CA14509, CA14518, CA00310 (CML 465), CA00104, CM 104, CM 105, CM 500, CM 600, 561, showed average incubation period, (40 to 72 hrs). Maize genotypes which were moderately susceptible i.e. the average reaction mean (2.0 to 4.0), CA03106, CA003134, CA00370, (CML 469), CA34507, CA00102, (CML 429), CA34506, CA03131, CA00334 (CML 433), CA 34516, CA03149, CA14510 (CML 428), CA00332 (CML 432), A00344, CML 425, CA00384, CA00378, CML 421, PoP 147-F₂# 161-3-2-B-1-B, CA14519, CML 426, CML 413, CA14517, CML 470, CML 471, CML 474, CML 475, CML 111, CML 115, CM 119, CM 120, CM 121, CM 123, CM 125, CM 400, CM 209, Surya, D 995, Kanchan, 552, 553, 554, 556, 560, 562, 563, 564, 567, 570 showed average incubation period in between (24 to 48 hrs). Highly susceptible material showed mean disease reaction (4.0 to 5.0) were CA00396, CML 430, CA03142, CA14527, CA03142, CA14527M, CML 427, CML 431, CA14501, CML 428, CML 433, CML 210, D 994, Amar, 550, 555, 557, 558, 559, 566, and exhibited average incubation period in between 24 to 48 hrs (Table 19, 20, 21 and Fig. 20, 21, 22).

4.2.4 Spot size

Inbred lines A00106, CA049Y04, CA03147, CA14509, CA14518, CA00310 (CML 465), CA00104, C, 104, CM 105, CM 500, CM 600, 561, were resistant under glasshouse condition and showed mean disease reaction (1.0 to 2.0) and the spot size of these genotypes varied in between (0.6 to 1.6 cm). Whereas, genotypes CA03106, CA003134, CA00370 (CML 469), CA34507, CA00102 (CML 429), CA3450-6, CA03131, CA00334 (CML 433), CA34516, CA03149, CA14510, CML 428, CA00332 (CML 432), A00344, CML 425, CA00384, CA00384, CA00378, CML 421, PoP 147-F₂# 161-3-2-B-1-B,

Table 19 : Incubation period and spot size and disease reaction of 44 CIMMYT genotypes (Glasshouse experiment)

Inbred Lines (Code)	Mean incubation period	Mean spot size (cm)	Disease reaction (mean)
CA00106	64.0	0.9	1.2
CA049Y04	56.0	1.1	1.1
CA03147	72.0	0.8	1.8
CA14509	48.0	0.9	1.4
CA14518	62.0	0.8	1.6
CA03106	48.0	1.0	3.3
CA003134	48.0	0.7	3.5
CA00370 (CML 469)	40.0	1.1	3.8
CA34507	48.0	1.4	4.5
CA00102 (CML 429)	32.0	1.0	3.7
CA00396	24.0	1.8	4.1
CA00310 (CML 465)	56.0	0.7	1.6
CA34506	32.0	0.9	3.0
CA03131	32.0	1.8	3.8
CA00334 (CML 433)	48.0	1.2	2.7
CA34516	48.0	1.1	2.8
CA03149	48.0	1.1	3.5
CA14510 (CML 428)	24.0	1.0	3.7
CA00332 (CML 432)	48.0	0.8	3.2
A00344	48.0	0.9	3.9
CML 425	24.0	1.5	4.0
CML 430	40.0	1.5	4.5
CA03124	32.0	1.4	1.5
CA00384	24.0	1.3	3.6
CA00378	48.0	1.1	3.5
CML 421	24.0	1.6	3.7
CA14711	40.0	1.1	3.1
Pop. 147-F2#161-3-2-B-1-B	32.0	1.3	2.8
CA14519	24.0	2.0	2.2
CA00104	48.0	1.6	2.0
CA03142	24.0	1.3	4.1
CA14527	40.0	1.1	4.1
CA03130 (CML 426)	32.0	1.1	2.7
CA14502 (CML 427)	24.0	1.2	4.1
CA00304 (CML 431)	40.0	0.8	4.1
CML 413	40.0	1.6	3.9
CA14501	40.0	1.1	4.5
CA14517	48.0	1.3	3.9
CA03116 (CML 470)	48.0	1.6	3.6
CA14709 (CML 471)	48.0	1.5	3.2
CA14524 (CML 474)	24.0	1.2	3.1
CA14520 (CML 475)	48.0	1.3	2.0
CML 428	40.0	1.1	4.6
CA00334 (CML 433)	48.0	1.5	4.9

Table 20 : Incubation period and spot size and disease reaction of 14 CM genotypes (Glasshouse experiment)

Inbred Lines (Code)	Incubation period (hrs)	Spot size (cm)	Disease reaction
CM 104	64.0	0.8	1.6
CM 105	64.0	0.6	1.2
CM 111	32.0	1.5	3.8
CM 115	32.0	1.5	3.5
CM 119	40.0	0.9	3.8
CM 120	24.0	1.6	3.1
CM 121	48.0	1.5	3.3
CM 123	40.0	1.5	3.6
CM 125	48.0	2.0	2.7
CM 500	24.0	2.1	2.6
CM 400	40.0	1.1	1.6
CM 600	48.0	0.9	1.8
CM 209	32.0	1.4	3.3
CM 210	24.0	1.4	4.3

Table 21 : Incubation period and spot size and disease reaction of 26 genotypes (Glasshouse experiment)

Inbred Lines (Code)	Incubation period (hrs)	Spot size (cm)	Disease reaction
Surya	56.0	0.9	3.2
D-995	32.0	1.1	3.4
D-994	32.0	1.5	4.1
Kanchan	48.0	1.1	2.8
Amar	24.0	0.9	4.6
550	48.0	1.0	4.5
551	48.0	1.5	4.3
552	32.0	1.1	3.5
553	40.0	1.1	3.5
554	40.0	1.3	3.5
555	32.0	1.1	4.5
556	48.0	1.1	2.5
557	48.0	1.1	4.3
558	48.0	1.3	4.5
559	40.0	1.3	4.5
560	32.0	1.0	3.4
561	40.0	1.5	1.4
562	40.0	1.4	3.7
563	32.0	1.1	3.7
564	40.0	1.3	3.3
565	40.0	1.3	3.9
566	48.0	1.6	4.5
567	40.0	1.1	3.0
568	48.0	1.1	4.1
569	32.0	1.5	4.2
570	40.0	1.3	3.8

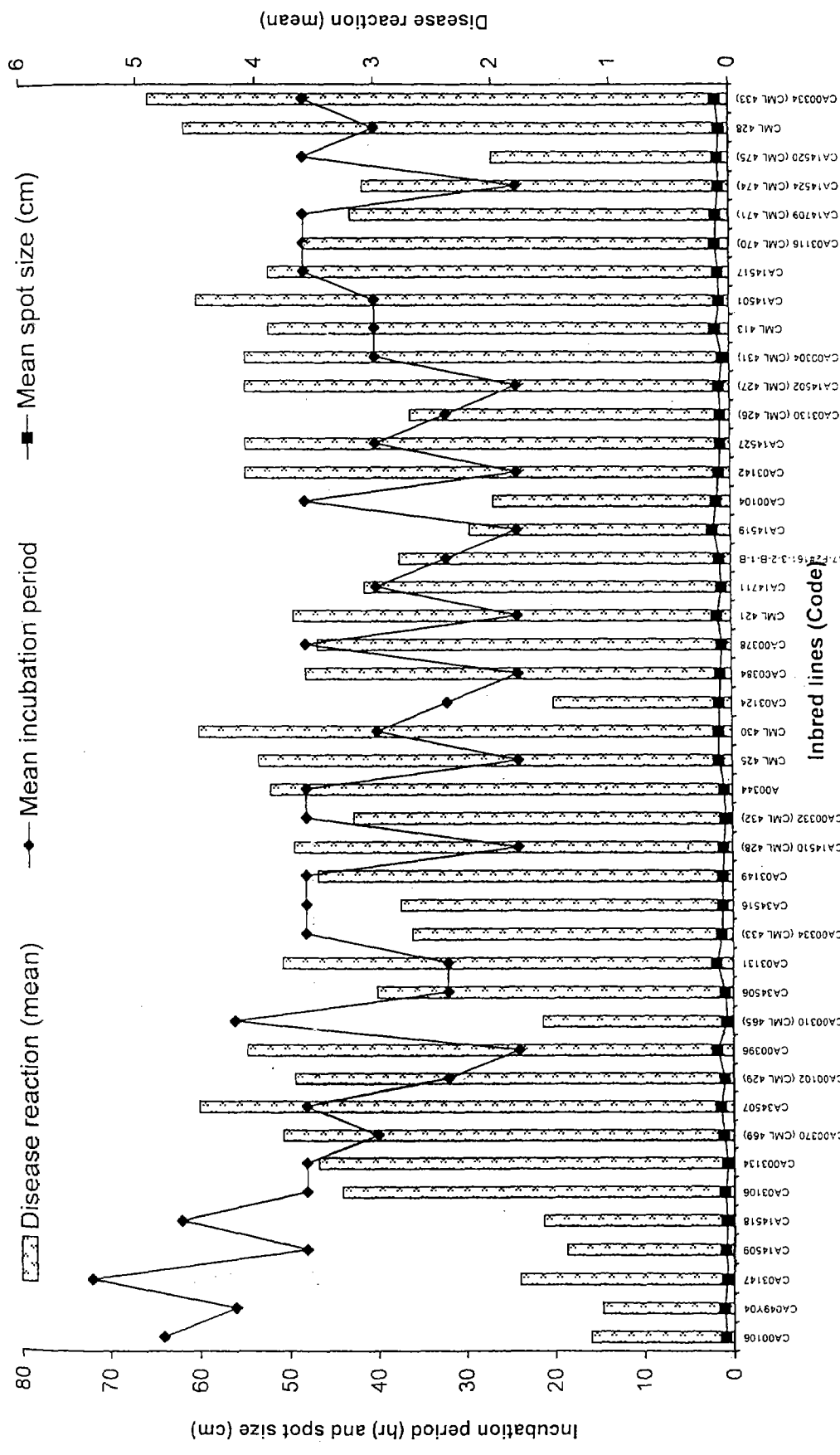


Fig. 20 : Incubation period and spot size and disease reaction of 44 CIMM lines (Glasshouse experiment)

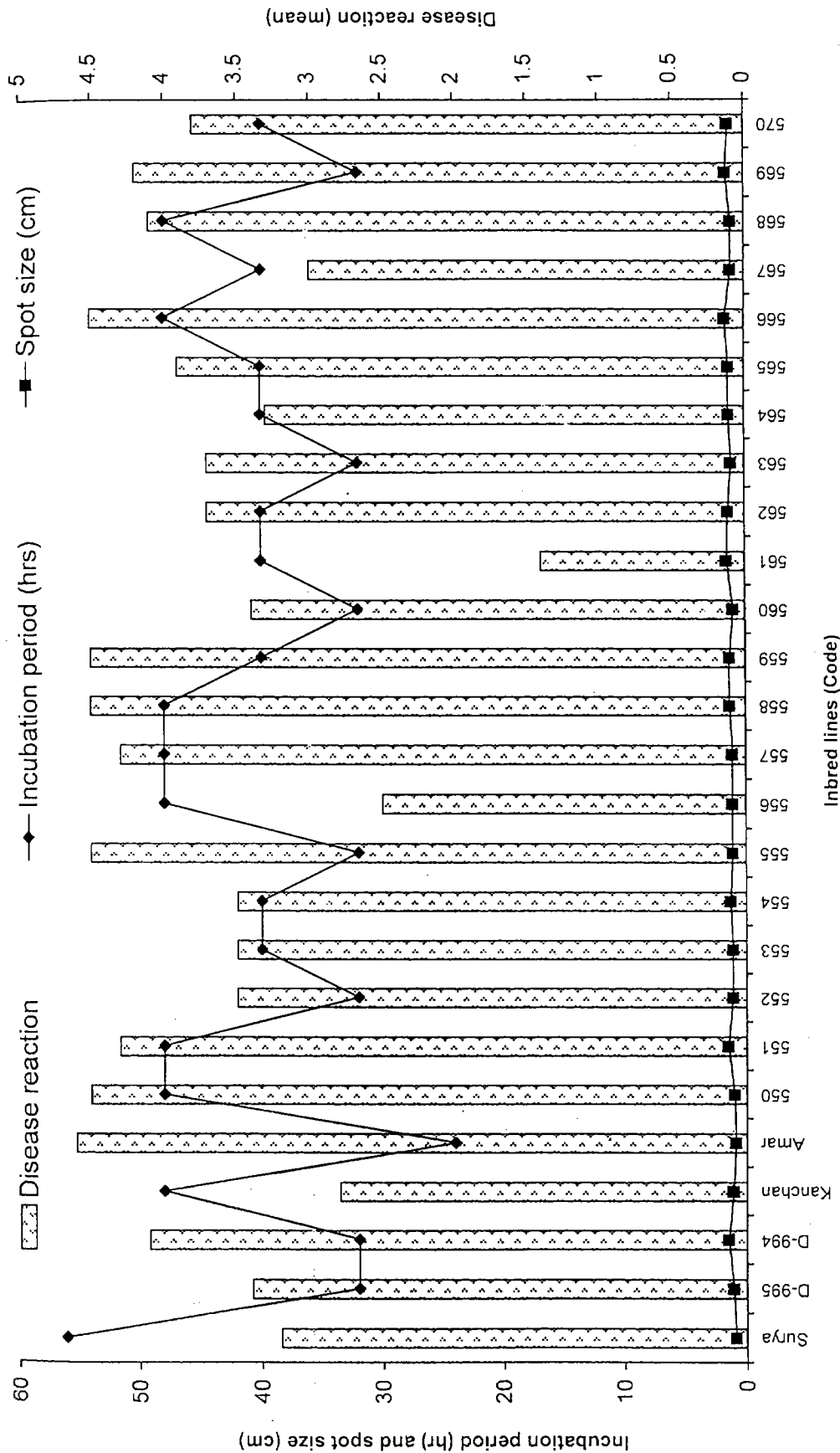


Fig. 21 : Incubation period and spot size and disease reaction of 26 inbred lines (Glasshouse experiment)

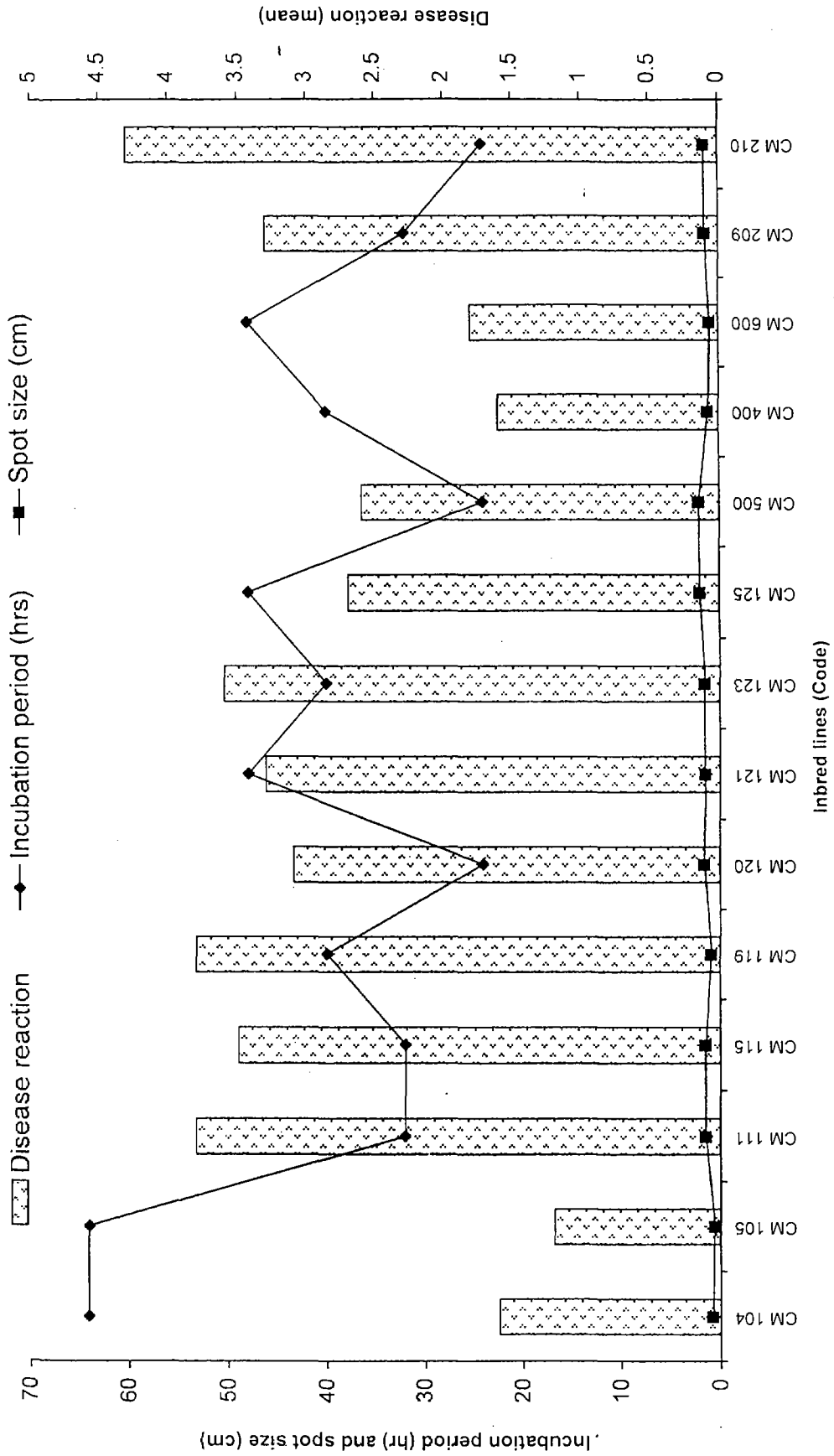


Fig. 22 : Incubation period and spot size and disease reaction of 14 CM line (Glasshouse experiment)

CA14519, CML 426, CML 413, CA14157, CML 470, CML 471, CML 474, CML 475, CM 111, CM 115, CML 119, CM 120, CM 121, CM 123, CM 125, CM 400, CM 209, Surya, D 995, Kanchan, 552, 553, 554, 556, 560, 562, 563, 564, 567, 570, exhibited showed mean disease reaction in between (2.0 to 4.0) and the spot size vary (1.0 to 2.0 cm) and materials which was highly susceptible and showed mean disease reaction (4.0 to 5.0) were CA00396, CML 430, CA03142, CA14527, CA03142, CA14527, CML 427, CML 431, CA14501, CML 428, CML 433, CML 210, D 994, Amar, 550, 551, 555, 557, 558, 559, 566 with a average mean spot size (1.0 to 1.5 cm) (Table 19, 20, 21 and Fig. 20, 21, 22).

4.2.5 Day of tasseling

Genotypes, CA00106, CA049Y04, CA03147, CA14509, CA14518, A00310 (CML 465), CA00104, CM 104, CM 105, CM 500, CM 600, 561, represented resistant reaction in glasshouse condition and the mean disease reaction was between (1.0 to 2.0) and days to tassel in these line were varied in between (45 to 50 days). Genotypes which were moderately susceptible (2.0 to 4.0), CA03106, CA03134, CA00370 (CML 469), CA34507, CA00102 (CML 429), CA 34506, CA03131, CA00334, (CML 433), CA34516, CA03149, CA14510, CML 428, CA00332 (CML 432), A00344, CML 425, CA00384, CA00378, CML 421, PoP 147-F₂# 161-3-2-B-1-B, CA14519, CML 426, CML 413, CA14517, CML 471, CML 474, CML 475, CM 111, CM 115, CM 119, CM 120, CM 121, CM 123, CM 125, CM 400, CM 209, Suray, D 995, Kanchan, 552, 553, 554, 556, 560, 562, 563, 564, 567, 570, showed days to tassel in between 40 to 45 days. Whereas, the susceptible group CA00396, CML 430, CA03142, CA14527, CA03142, CA14527, CML 427, CML 431, CA14501, CML 428, CML 433, CML 210, D 995, Amar, 550, 551, 555, 557, 558, 559, 566, the average days to tassel varied in between 40 to 45 days (Table 22, 23, 24 and Fig. 23, 24, 25).

Table 22 : Incubation period and spot size and disease reaction of 44 CIMMYT genotypes (Field experiment)

Inbred Lines (Code)	Mean incubation period	Mean spot size (cm)	Disease reaction (mean)
CA00106	72.0	1.1	1.3
CA049Y04	54.0	1.4	1.5
CA03147	72.0	1.3	1.1
CA14509	64.0	1.2	1.4
CA14518	62.0	1.1	1.2
CA03106	48.0	2.1	3.6
CA003134	48.0	2.3	2.4
CA00370 (CML 469)	46.0	2.5	3.2
CA34507	32.0	2.0	3.1
CA00102 (CML 429)	48.0	2.0	4.8
CA00396	48.0	2.2	2.3
CA00310 (CML 465)	48.0	1.1	1.1
CA34506	32.0	1.3	4.1
CA03131	48.0	2.1	3.9
CA00334 (CML 433)	32.0	2.3	2.7
CA34516	48.0	1.8	4.1
CA03149	48.0	1.6	3.6
CA14510 (CML 428)	48.0	2.4	3.7
CA00332 (CML 432)	32.0	1.5	3.6
A00344	48.0	1.4	4.2
CML 425	32.0	1.8	3.5
CML 430	48.0	2.0	3.7
CA03124	40.0	1.3	1.6
CA00384	32.0	2.5	4.6
CA00378	48.0	2.0	2.1
CML 421	32.0	1.4	3.8
CA14711	48.0	2.3	4.1
Pop. 147-F2#161-3-2-B-1-B	64.0	1.5	4.1
CA14519	48.0	1.6	4.2
CA00104	48.0	1.3	3.6
CA03142	40.0	1.3	3.7
CA14527	48.0	1.3	4.2
CA03130 (CML 426)	40.0	1.5	4.4
CA14502 (CML 427)	48.0	2.0	4.1
CA00304 (CML 431)	24.0	1.8	3.1
CML 413	48.0	1.8	4.6
CA14501	40.0	1.3	2.9
CA14517	40.0	1.8	3.3
CA03116 (CML 470)	48.0	1.8	3.0
CA14709 (CML 471)	40.0	2.1	3.5
CA14524 (CML 474)	48.0	1.6	2.9
CA14520 (CML 475)	40.0	2.1	4.5
CML 428	48.0	2.5	2.9
CA00334 (CML 433)	32.0	2.6	4.5

**Table 23 : Day of tasseling, silking and disease reaction of 14 CM genotypes
(Glasshouse experiment)**

Inbred Lines (Code)	Day of tasseling	Day of silking	Disease reaction (mean)
CM 104	46.0	53.6	1.6
CM 105	44.0	53.0	1.2
CM 111	43.3	47.6	3.8
CM 115	41.0	50.3	3.5
CM 119	42.6	51.3	3.8
CM 120	42.0	47.6	3.1
CM 121	43.0	52.6	3.3
CM 123	42.3	53.0	3.6
CM 125	40.6	48.3	2.7
CM 400	43.3	51.0	2.6
CM 500	45.3	50.6	1.6
CM 600	43.3	52.3	1.8
CM 209	41.6	51.8	3.3
CM 210	40.0	50.6	4.3

Table 24 : Glasshouse experiment day of tasseling, day of selking and diseases rating

Inbred Lines (Code)	Day tasseling mean	Day of selking mean	Disease reaction mean
Surya	39.6	44.6	3.2
D-995	40.3	45.0	3.4
D-994	42.6	49.0	4.1
Kanchan	44.3	50.3	2.8
Amar	41.6	52.0	4.6
550	37.0	48.3	4.5
551	35.3	50.3	4.3
552	44.0	49.6	3.5
553	44.3	48.0	3.3
554	41.6	50.3	3.5
555	42.6	49.0	4.5
556	42.6	49.6	2.5
557	41.0	54.3	4.3
558	43.6	49.0	4.5
559	43.6	49.0	4.5
560	43.6	49.3	3.4
561	40.6	54.6	1.9
562	40.6	54.3	3.7
563	43.3	49.0	3.7
564	44.0	51.6	3.3
565	46.0	47.0	2.9
566	41.6	50.3	4.5
567	41.3	48.0	3.0
568	44.0	50.1	4.1
569	40.0	48.3	4.2
570	42.6	50.3	3.8

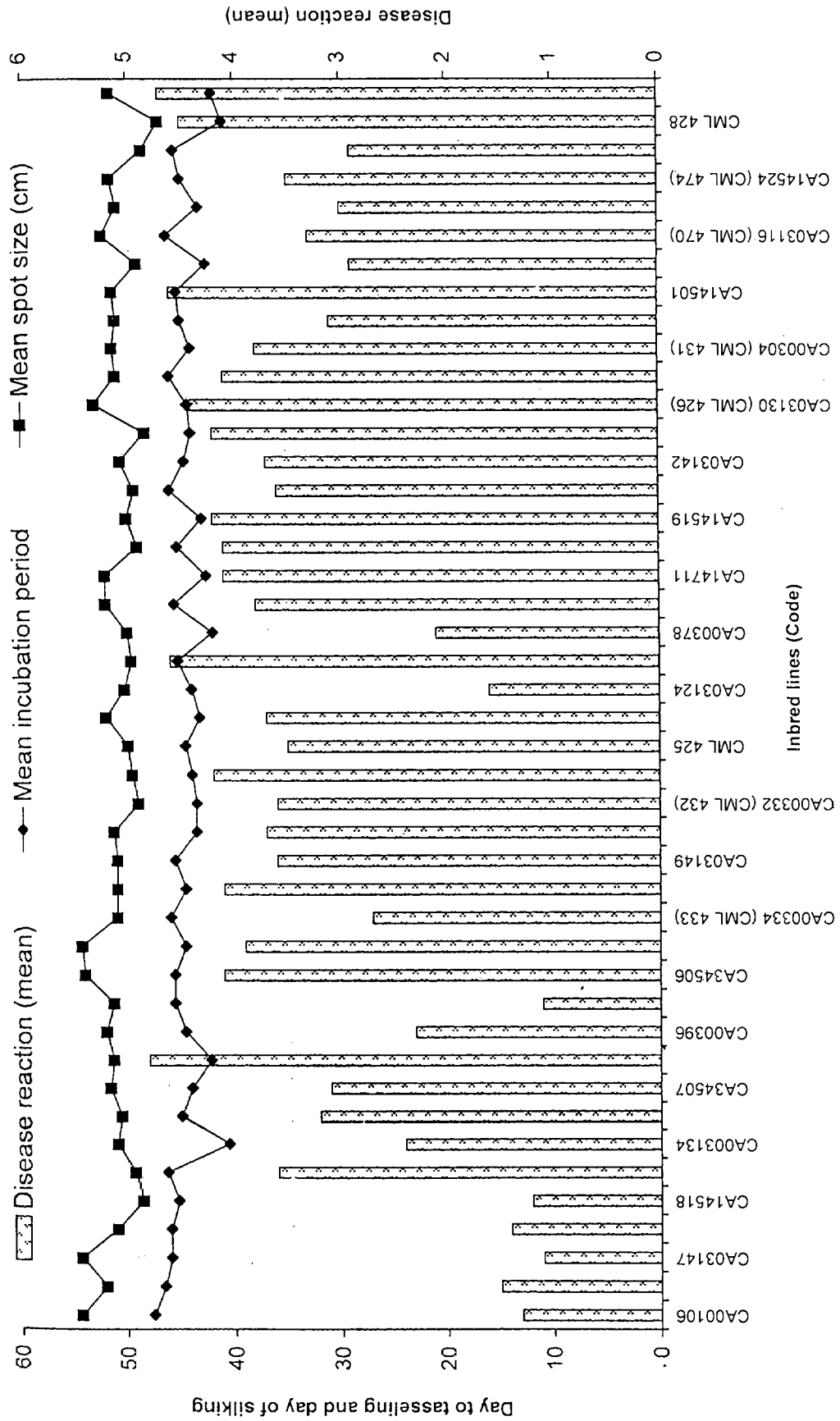


Fig. 23 : Incubation period and spot size and disease reaction of 44 CIMMYT lines (Field experiment)

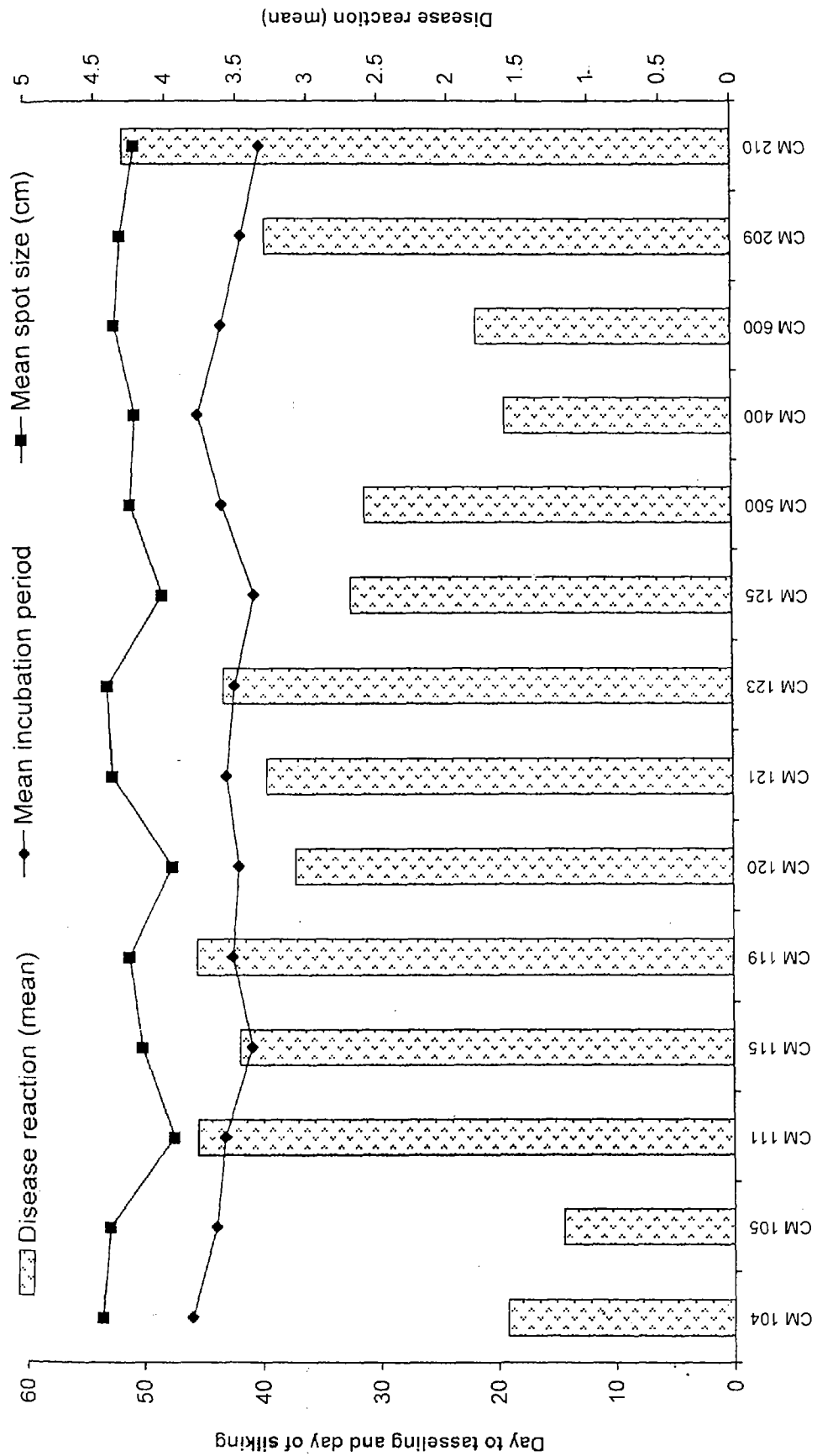


Fig. 24 : Day of tasseling, silking and disease reaction of 14 CM lines (Glasshouse experiment)

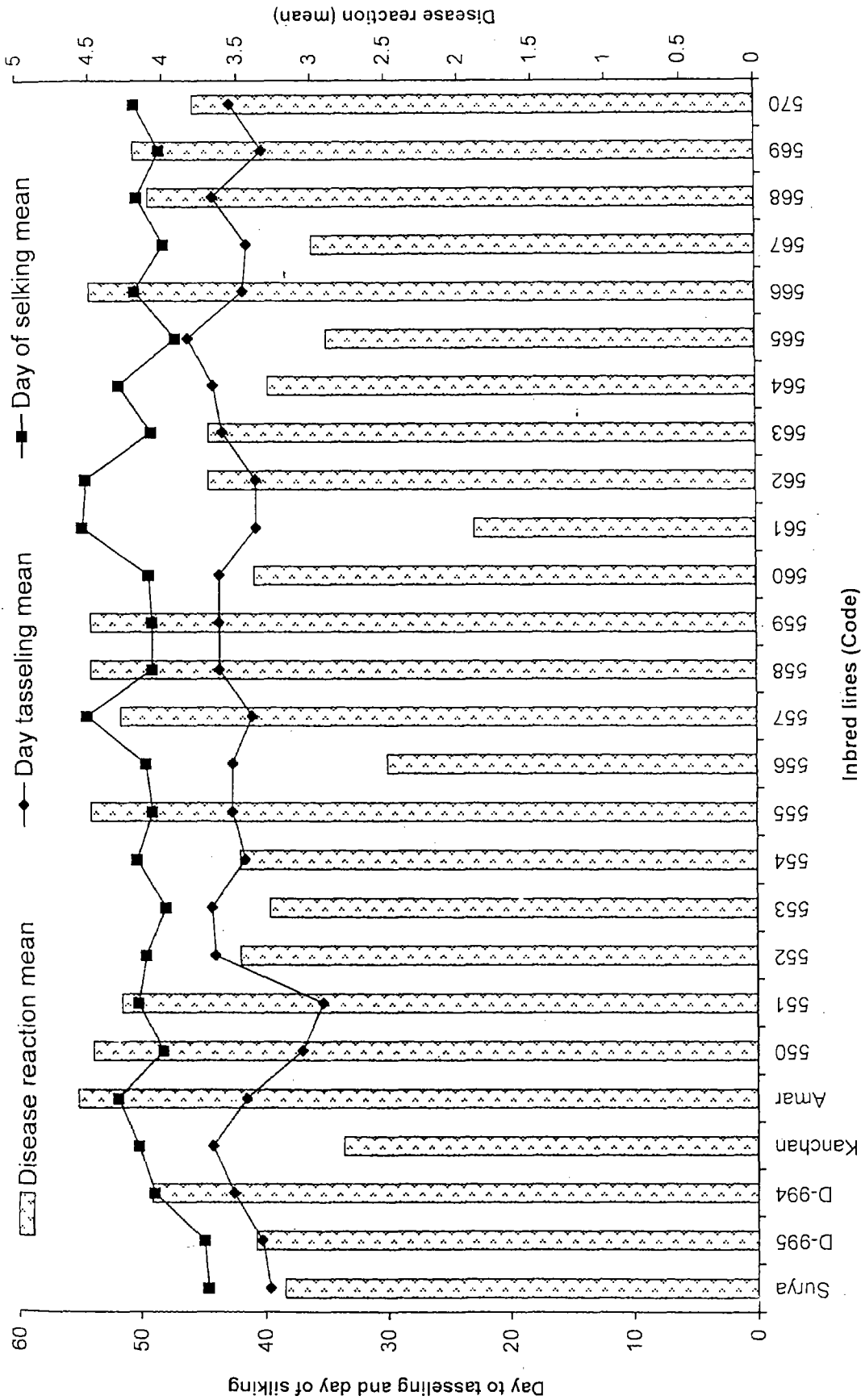


Fig. 25 : Glasshouse experiment day of tasseling, day of silking and diseases rating

4.2.6 Days to silk

Genotypes, these categorized into the resistant group on the basis of mean disease reaction (1.0 to 2.0) were CA00106, CA049Y04, CA03147, CA14509, CA14518, CA00310 (CML 465), CA00104, CM 104, CM 105, CM 500M, CM 600, 561, all these genotypes showed the average days to silk in between (50 to 55), whereas genotypes, CA03106, CA03134, CA00370 (CML 469), CA34507, CA00102 (CML 429), CA34506, CA03131, CA00334 (CML 433), CA34516, CA03149, CA14510, CML 428, CA00332 (CML 432), A00344, CML 413, CA14517, CML 470, CML 471, CML 474, CML 475, CM 111, CM 115, CM 119, CM 120, CM 121, CM 123, CM 125, CM 400, CM 209, Surya, D 995, Kanchan, 552, 553, 554, 556, 562, 563, 564, 567, 570, categorized into moderately susceptible group (2.0 to 4.0), the average days to varied between (50 to 55). Highly susceptible maize genotypes which showed mean disease reaction (4.0 to 5.0), CA0039, CML 430, CA03142, CA14527, CA03142, CA14527, CML 427, CML 431, CA14501, CML 428, CML 433, CML 210, D 994, Amar, 550, 551, 557, 558, 559, 566, the average days to silk varied between 45 to 55 days (Table 22, 23, 24 and Fig. 23, 24, 25).

4.2.7 Tassel texture

Tassel texture of resistant maize inbred line, which showed the average mean disease reaction (1.0 to 2.0), in all three group, CIMMYT, CM and Pantnagar genotypes, showed similar tassel texture, as in field condition, and same result obtained in case of moderately susceptible (2.0 to 4.0) and highly susceptible (4.0 to 5.0) maize inbred line. The tassel texture of all three group is given in Table 11, 12 and 13)

The tassel A-G and tassel G-B colour of all the three groups, CIMMYT, CM, Pantnagar genotypes are given in Table 11 and 12, and the result obtained from glasshouse experiment was similar to those obtained in for all field experiment, resistant (1.0 to 2.0) and moderately susceptible (2.0 to 4.0) and highly susceptible (4.0 to 5.0).

4.2.8 Silk colour

The silk colour of maize genotypes of all three groups, CIMMYT, CM, Pantnagar genotypes are given in Table 11, 12 and 13. The silk colour of all the maize genotypes resistant (1.0 to 2.0) and moderately resistant (2.0 to 4.0) and highly susceptible (4.0 to 5.0) similar to those as recorded under field conditions.

4.2.9 Blight shape and colour

Blighting shape in all these group, CIMMYT, CM, Pantnagar genotypes are given in Table 11, 12, 13 and all three type of maize genotypes resistant (1.0 to 2.0) and moderately susceptible (2.0 to 4.0) and highly susceptible (4.0 to 5.0) showed similar blighting shape in glasshouse condition as in field condition.

Blighting colour of all three type of maize genotypes resistant (1.0 to 2.0) and moderately susceptible (2.0 to 4.0) and highly susceptible (4.0 to 5.0) showed similar pattern of blighting colour as given in field condition. The blighting colour of CIMMYT, CM and Pantnagar genotypes are given in Table 11, 12 and 13).

4.3 Disease severity

The disease severity of all the eighty four maize genotypes recorded on three day interval and disease progressive curve was prepared. The disease severity of resistant maize genotypes in CA00106, CA049Y04 and CA14518. showed disease severity in between 20-30 per cent and it remain constant till the end of 36 days. But in case of susceptible genotype CA14501, CM 428 and CA00334 the disease severity starting from 20 per cent it reaches 90 to 100 per cent till the end of 36 days. Same result obtained in case of CM genotypes and Pantnagar genotypes and the disease progressive curve given in Fig. 26.

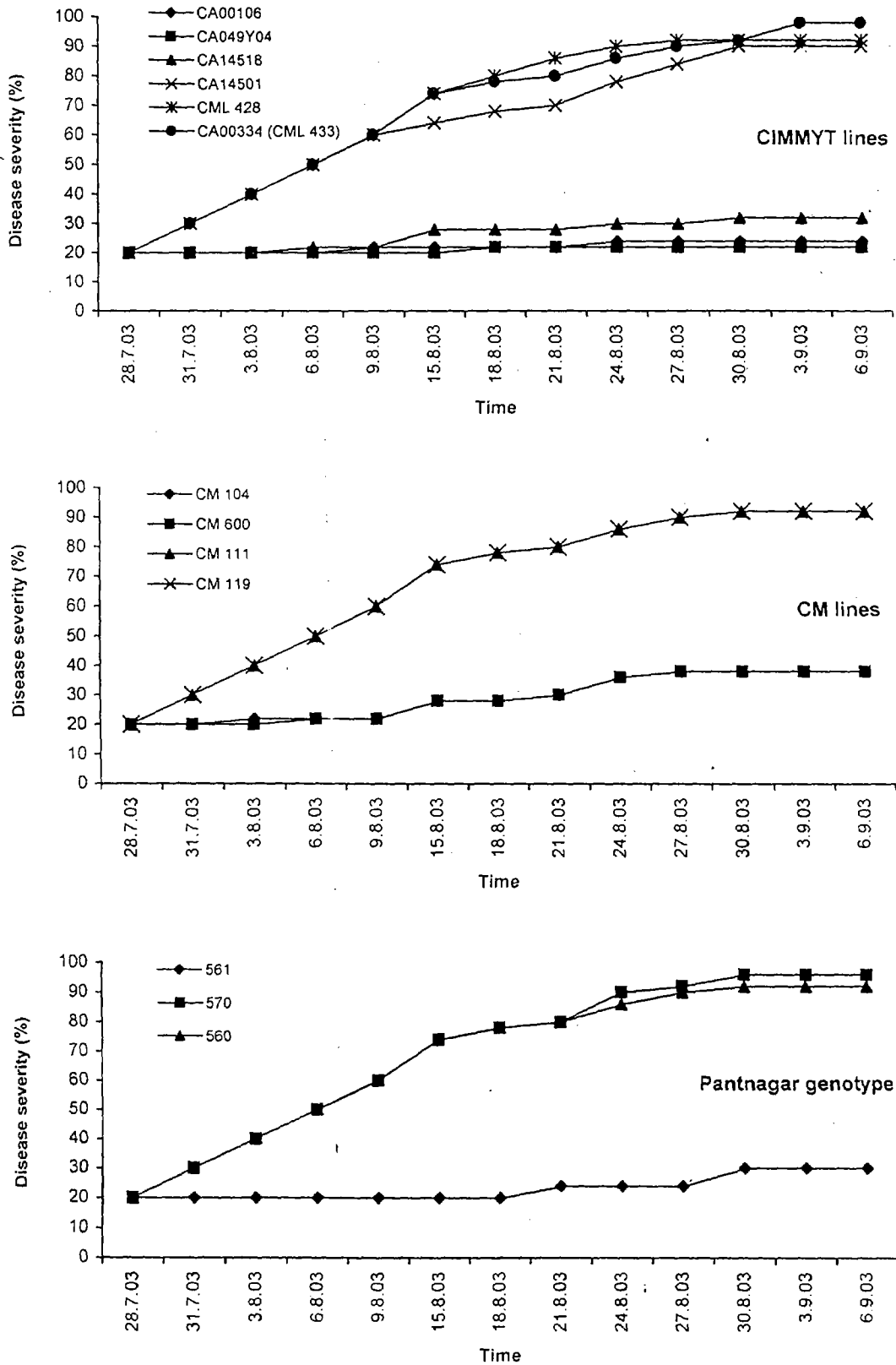


Fig. 26 : Disease progressive curve

4.4 Disease frequency

On the basis of field, glasshouse and laboratory experiment, the maize genotypes of all the three group viz. Pantnagar, CIMMYT and CM were categorized into four groups. One genotype which showed the frequency in between 1.0-2.0 and second 2.1 to 3.0 and third which showed the frequency in between 3.1-3.5 and fourth and fifth group which showed disease frequency in between 3.6 to 4.0 and 4.1 to 5.0. The frequency and number of maize genotypes given in Table 25, 26, 27 and Fig. 27, 28,29), on the basis of field, glasshouse and laboratory experiment.

4.5 RAPD marker analysis for disease reaction

4.5.1 Isolation and quantification of DNA

Genomic DNA was isolated from seeds of genotypes used in the present study for disease reaction to BLSB. An SDS method was followed with some modification. The genomic DNA isolated was compared for quality and quantity of 0.8 per cent agarose gel. The quantity of genomic DNA was good and intact without any smear.

The quantification of DNA was done indirectly by intensity of bands as well as by direct method by taking absorbance at 260 nm (OD_{260}). The isolated DNA was of high molecular weight. These DNA samples were placed for RNAase treatment. After RNAase treatment, there was no RNA left the genomic DNA. The bands of all genotypes were intact and did not showed any smearing. The purity of DNA samples was subsequently enhanced by RNAase treatment. RNA are subsequently enhanced the purity of DNA sample.

4.5.2 PCR amplification of DNA

Amplification reaction contained DNA template for single tube μ l, dNTPS (0.5 μ l) and Taq polymerase (3U/ μ l) 1/3 μ l, assay buffer (1X) 2 μ l and primer 2 μ l, and triple distilled sterile water 17.67. Final concentration make 25 μ l/

Table 25 : Frequency of glasshouse disease rating scale in different maize genotypes

Disease reaction (rating scale 1-5)	CIMMYT lines	CM lines	Pantnagar genotypes
1.0-2.0	9	4	1
2.1-3.0	6	2	4
3.1-3.5	9	4	7
3.6-4.0	11	3	3
4.1-5.0	9	1	10

Table 26 : Frequency of field disease rating scale in different maize genotypes

Disease reaction (rating scale 1-5)	CIMMYT lines	CM lines	Pantnagar genotypes
1.0-2.0	7	4	2
2.1-3.0	8	3	6
3.1-3.5	6	—	3
3.6-4.0	9	2	4
4.1-5.0	14	5	11

Table 27 : Frequency of laboratory disease rating scale in different maize genotypes

Disease reaction (rating scale 1-5)	CIMMYT lines	CM lines	Pantnagar genotypes
1.0-2.0	10	4	2
2.1-3.0	6	1	8
3.1-3.5	10	2	2
3.6-4.0	9	1	3
4.1-5.0	9	6	11

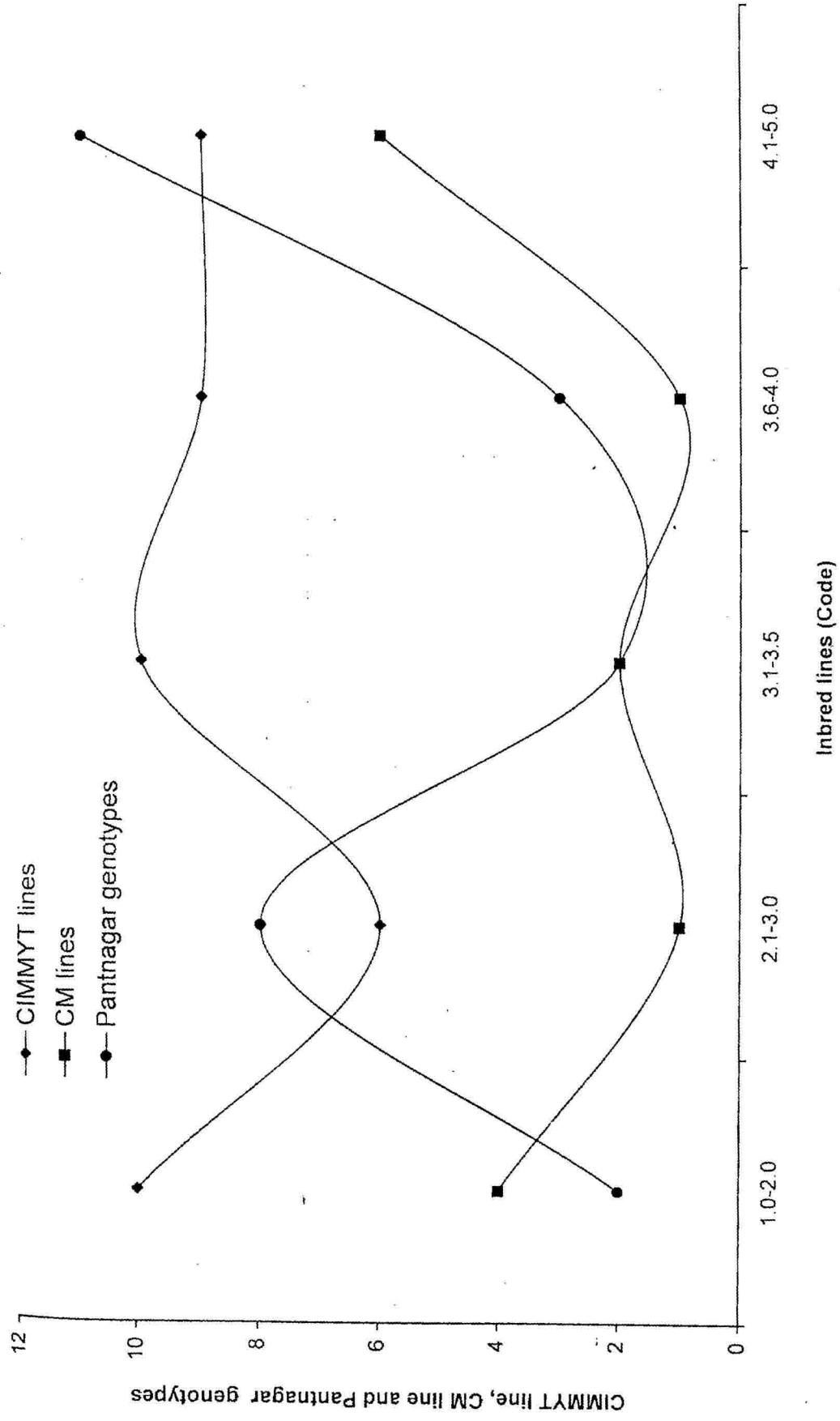


Fig. 27 : Frequency of laboratory disease rating scale in different maize genotypes

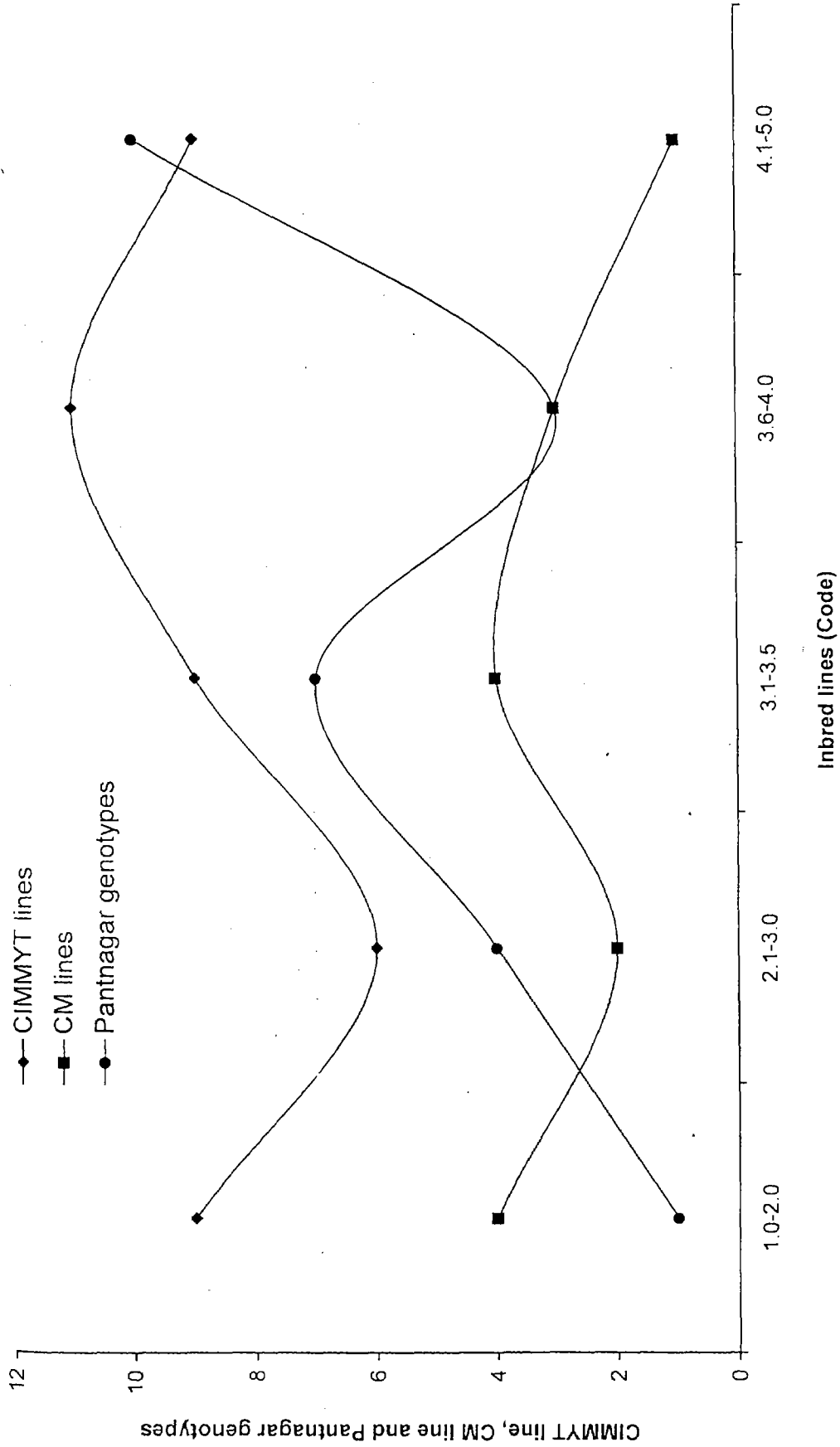


Fig. 28 : Frequency of glasshouse disease rating scale in different maize genotypes

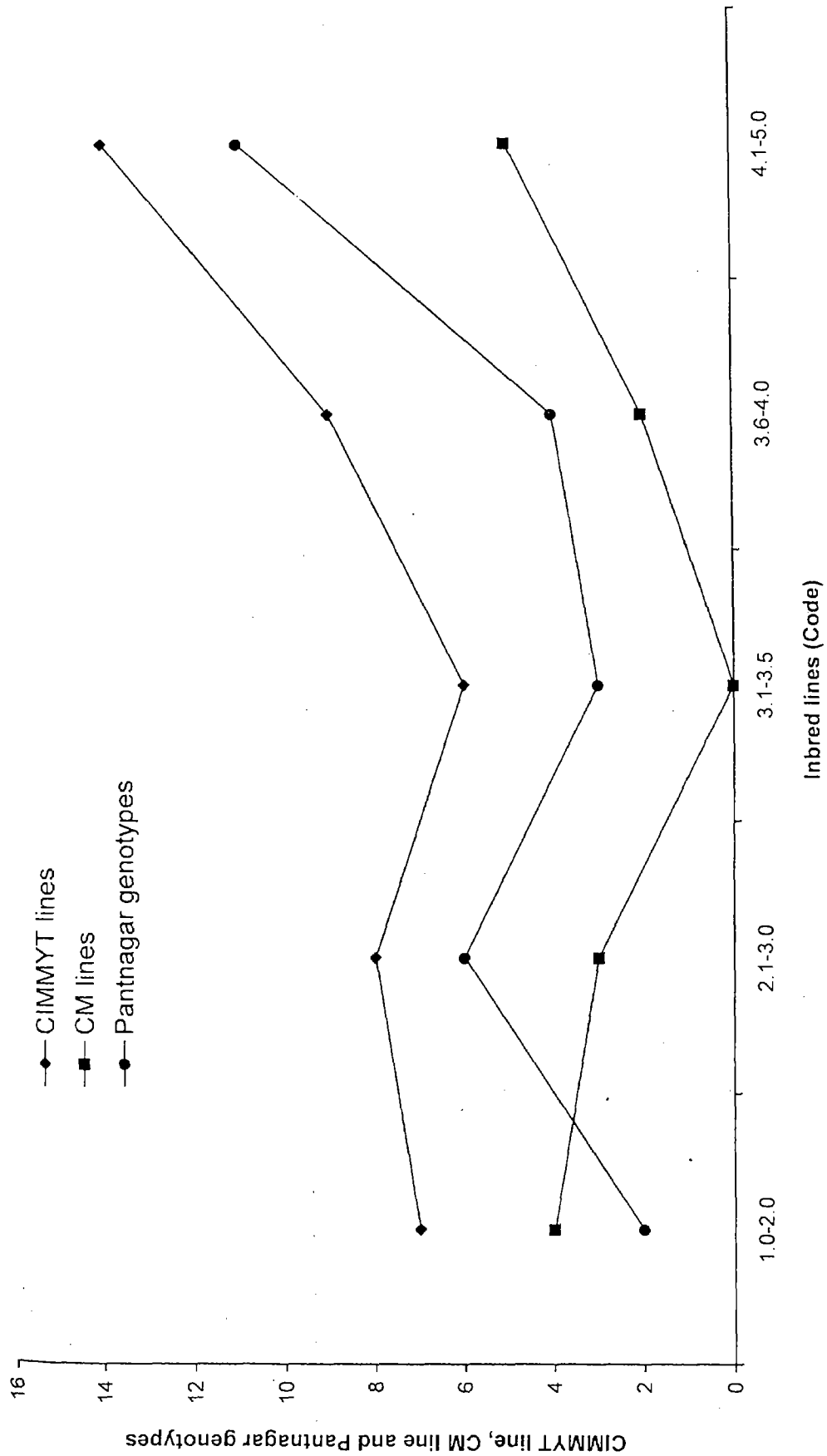


Fig. 29 : Frequency of field disease rating scale in different maize genotypes

The template DNA concentration for the optimum amplification was found to be 1 μ l per reaction for nearly all primers. The PCR amplification was carried out for DNA extracted from sixty nine genotypes using twenty five primer. The bands after the PCR amplification were manually scored on agarose gel. The amplification profile generated by each primer was compared with bands of DNA size marker (Lambda DNA/EcoRit Hind III 1000 bp) and 100 bp DNA ladder.

4.5.3 RAPD amplification

RAPD analysis was done to identify the markers associated with resistance to BLSB and RAPD profile for categorizing resistant and susceptible inbreds based on amplicons obtained on agarose gel. The number of RAPD markers generated by primer represented in Table 28 to 35 with remaining 14 primers no amplicons were observed. The total number of RAPD loci amplified by 11 primers with group I genotypes was 89 with average of 8.09 per primer and 72, with group II genotype was 98 with 8.9 per primer, out of which 65 were polymorphic with group III and IV and total RAPD loci was 92 and 90 out of which 61 with group III and 66 with group IV were polymorphic. All the 11 primers used were informative and gave polymorphic bands for one or more genotypes.

4.5.3.1 Primer 12SS10T4

On agarose gel, this primer, with group 1 (CM lines 15) amplified total 8 RAPD loci, out of which 8 found polymorphic (100%). Among these locus, four no locus unique. If all genotypes was consider. But it gave four unique locus with (0.5 kb) for CM 115, and (1.5 kb) for CM 500, (>1.5 kb) for CM 105, (>0.5 kb) for CM 104, This primer with 18 genotypes of group II, amplified total 12 RAPD loci, of which 9 found polymorphic (75%). Among these three locus was unique (1.3 kb) for CML 471 (>0.9 kb) for CML 433 (0.5) for CM 428. Among resistant

Table 28 : RAPD pattern on agarose among genotypes used in disease reaction to BLSB

Primer code	Total no. of RAPD loci	Polymorphic loci		Unique loci among all inbred studies	Unique loci between susceptible and resistance group
		Number	Percentage		
12SS10T4	8	8	100	—	4
UBC 18	6	5	83	—	1
UBC 535	5	3	75	3	5
UBC 337	11	6	54	3	4
UBC 552	7	7	100	—	3
UBC 600	5	4	80	—	2
UBC 532	8	8	100	—	—
UBC 572	12	7	58	2	6
UBC 534	11	9	81	1	3
UBC 350	9	9	100	1	4
UBC 386	7	6	85	1	3
Total	89	72			
Average	8.09	6.5			

Table 29 : RAPD analysis of unique loci amplified by primers on agrose gel among all genotypes and between susceptible and resistant groups

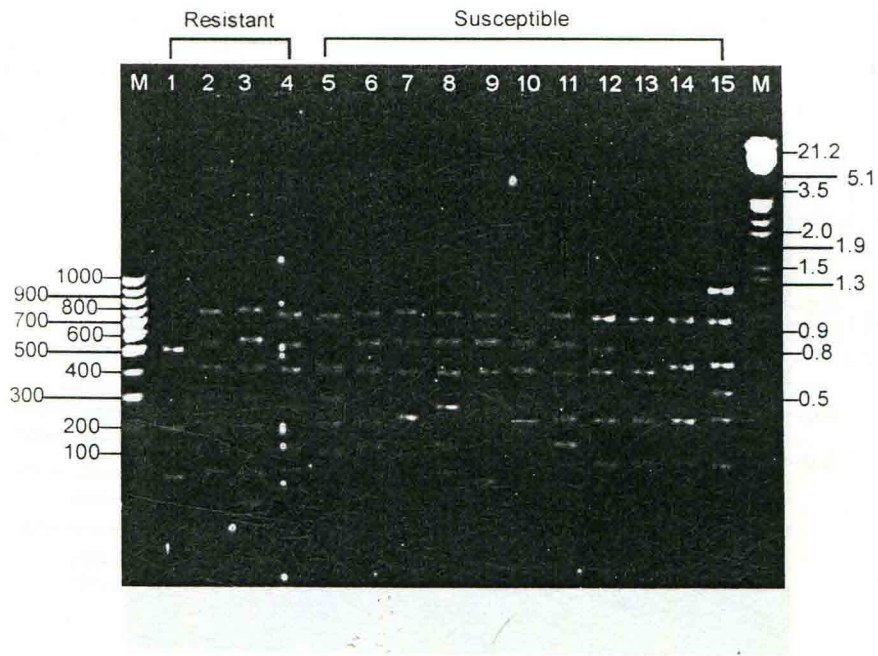
Primer code	Unique loci among all inbred s			Unique loci between susceptible and resistant groups		
	Unique loci	Size (kb)	Inbred having these loci	Unique loci	Size (kb)	Group having these loci
12SS10T4	—	—	—	4	1.5, <1.5, >0.5 >0.5	Resistant Susceptible
UBC 18	—	—	—	1	0.5	Susceptible
UBC 535	3	1.9, 0.5, <0.5	CM 210, CM 121, CM 104	5	<0.8, >0.5, <0.5	Resistant
UBC 337	4	>2.0, <2.0, <0.5, <0.5	Amar, CM 115, CM 111, CM 209	4	1.9, 1.3 2.0, 1.9; >1.5, <0.5	Susceptible Susceptible
UBC 552	—	—	—	3	1.3, <1.3, >0.9	Resistant
UBC 600	—	—	—	2	<0.5 <0.5	Susceptible Resistant
UBC 532	—	—	—	—	—	—
UBC 572	2	1.9, 1.5	CM 120	6	<1.5, <1.5, 1.3 1.9, <1.9, 1.5	Resistant Susceptible
UBC 534	1	1.9	CM 209	3	1.3, <0.5 1.9	Resistant Susceptible
UBC 350	1	<0.5	CM 500	4	>0.5, <0.5, <0.5 <1.3	Resistant Susceptible
UBC 386	1	1.5	CM 121	3	0.5 1.5, <0.5	Resistant Susceptible

Group-I

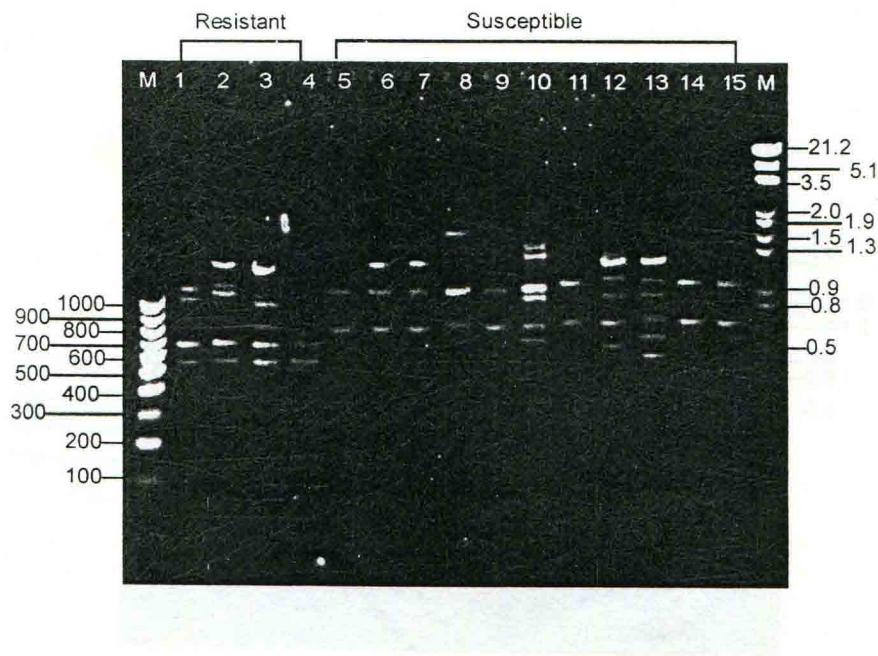
Marker-Lamda DNA/ Ecor1+Hind 111

1. CM104
2. CM105
3. CM500
4. CM600
5. CM111
6. CM119
7. CM120
8. CM121
9. CM123
10. CM125
11. CM400
12. CM209
13. CM210
14. CM115
15. Amar

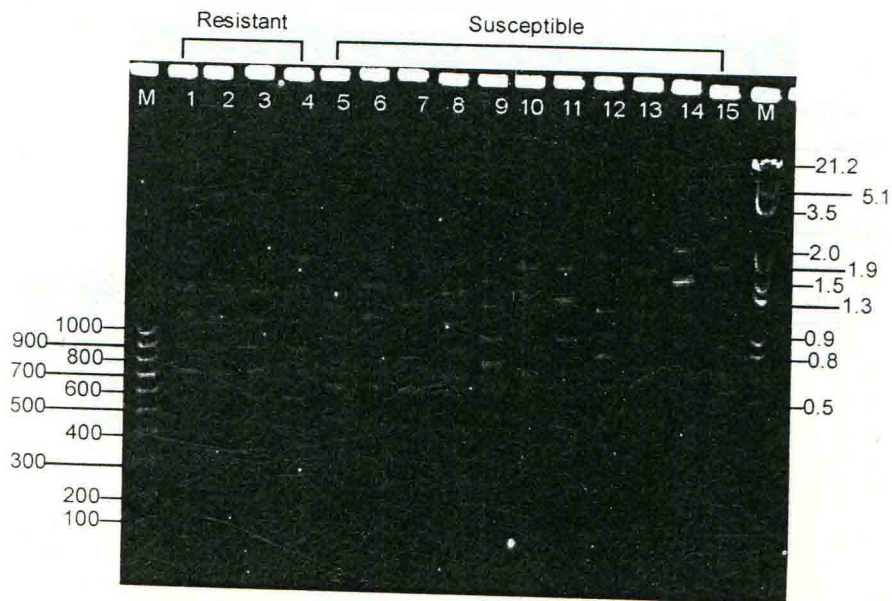
Marker-100bp



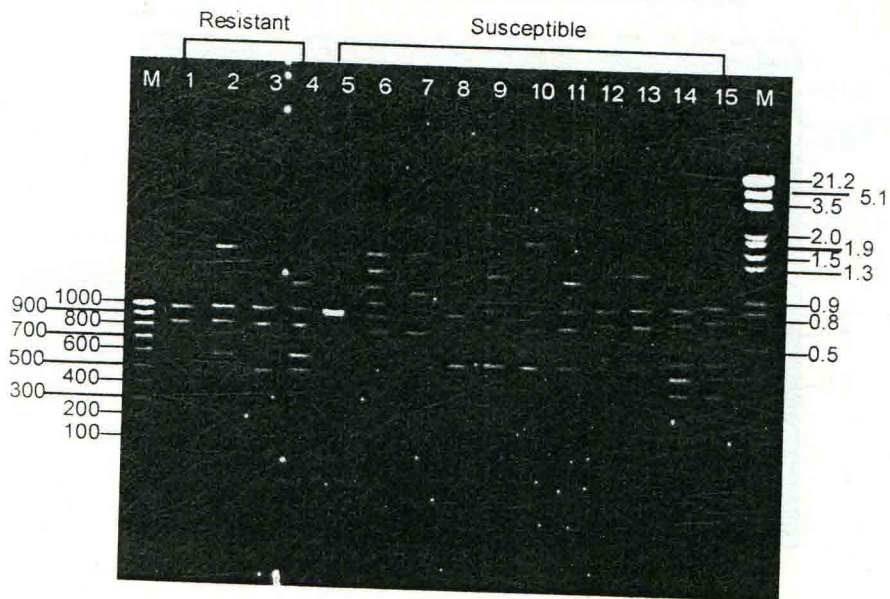
Primer -UBC 350



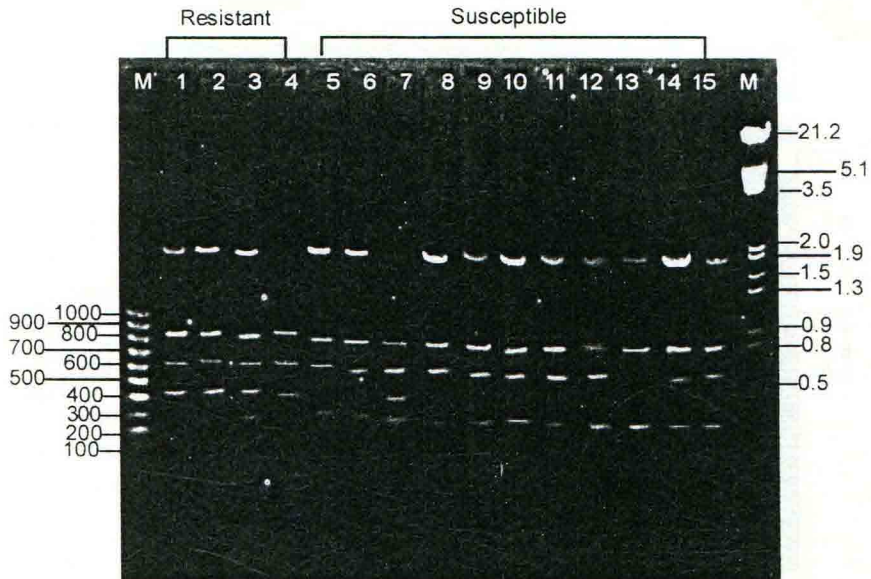
Primer -UBC 386



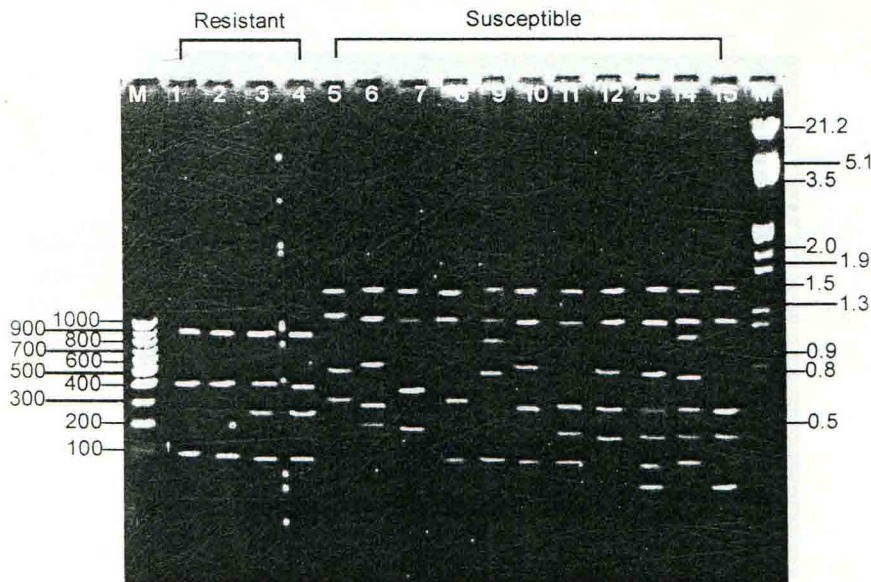
Primer -UBC 337



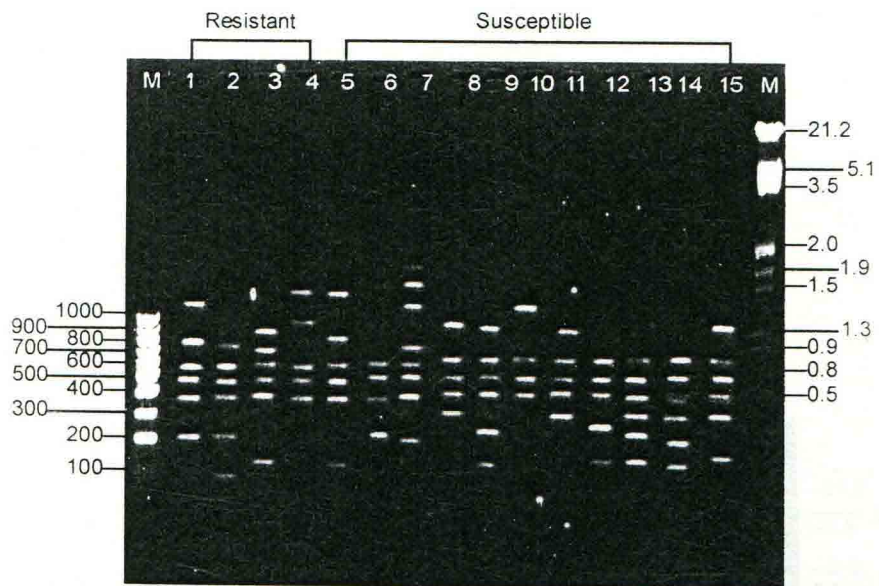
Primer -UBC 552



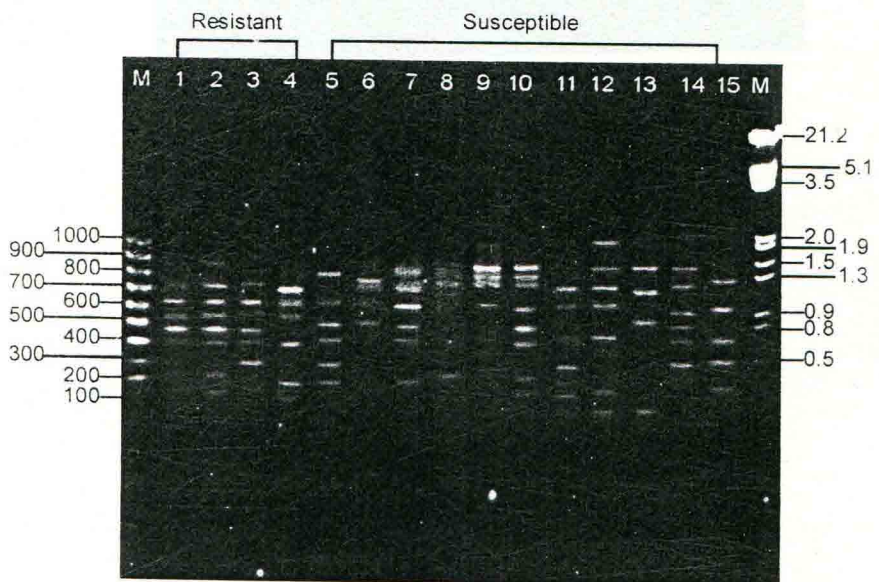
Primer -UBC 600



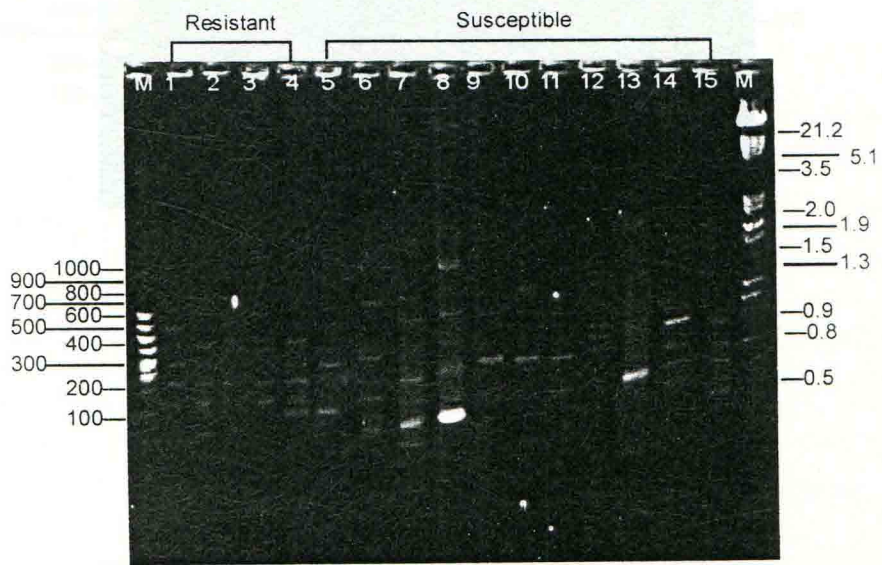
Primer -UBC 532



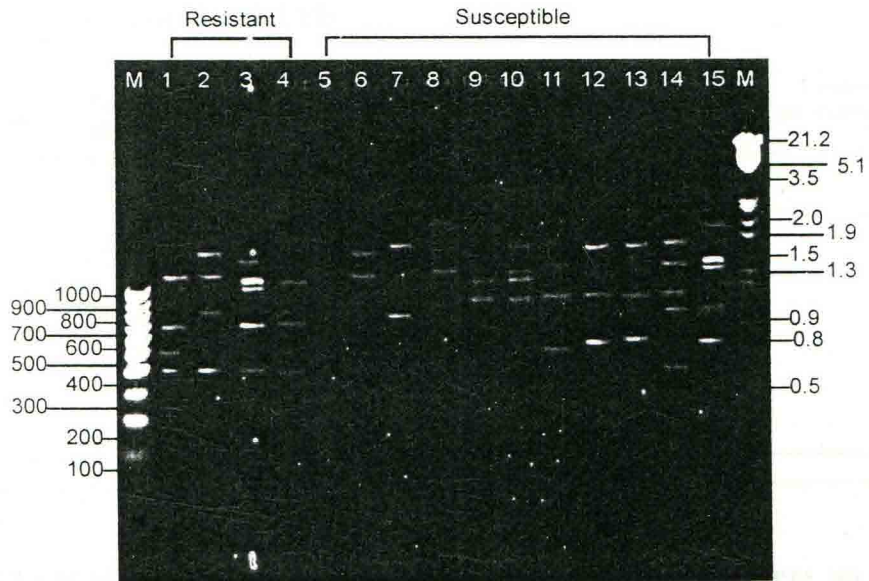
Primer -UBC 572



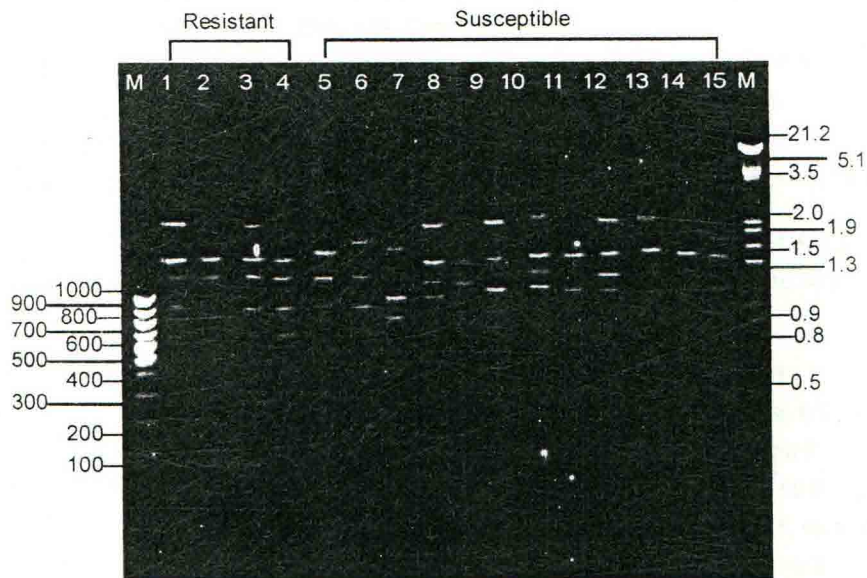
Primer -UBC 534



Primer -UBC 335



Primer -12SS10T6



Primer -UBC18

Table 32 : RAPD patten on agrose gel among genotypes used in disease reaction to BLSB

Primer code	Total no. of RAPD loci	Polymorphic loci		Unique loci among all inbred studies	Unique loci between susceptible and resistance group
		Number	Percentage		
12SS10T4	10	6	60.0	4	6
UBC 18	7	4	57.1	1	3
UBC 535	10	9	90.1	1	1
UBC 337	9	4	44.4	5	8
UBC 552	8	5	62.5	3	4
UBC 600	6	4	66.6	2	3
UBC 532	8	6	75.0	2	4
UBC 572	9	7	77.7	2	7
UBC 534	8	6	75.0	2	2
UBC 350	8	6	75.0	2	3
UBC 386	9	8	88.8	1	3
Total	92	61			
Average	8.36				

Table 33 : RAPD analysis of unique loci amplified by primers on agarose gel among all genotypes and between susceptible and resistant groups

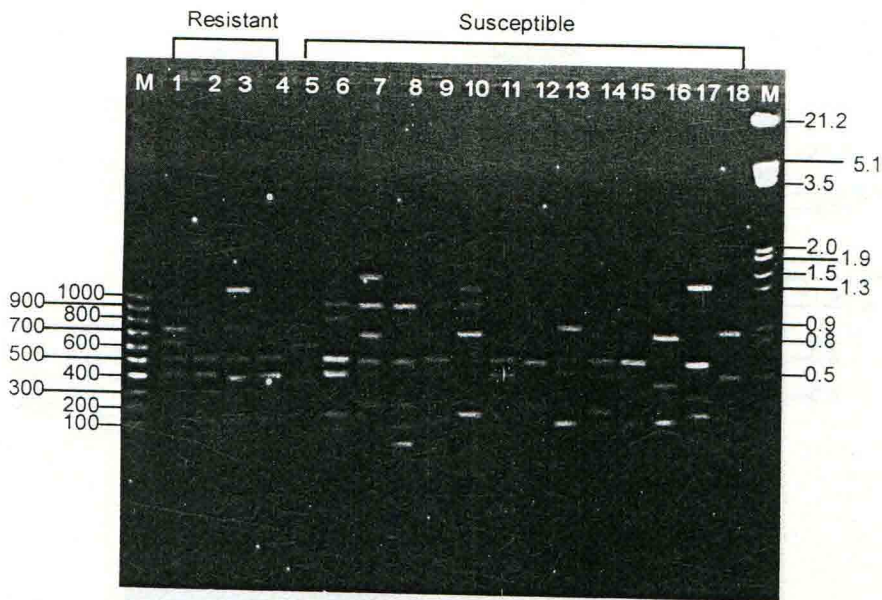
Primer code	Unique loci among all inbred s			Unique loci between susceptible and resistant groups		
	Unique loci	Size (kb)	Inbred having these loci	Unique loci	Size (kb)	Group having these loci
12SS10T4	4	1.5, <0.5	CML 428	6	1.5	Susceptible
		<1.3	CA14518		<1.3, 1.3, 0.8, >0.5, <0.5	Resistant
		0.8	CML 465, CML 465			
UBC 18	1	0.8	Ca03124	3	1.3, >0.9 0.8	Resistant Susceptible
UBC 535	1	<0.5	Ca00310	1	<0.5	Resistant
UBC 337	4	2.0, 1.9, >1.3	CA003134, CA003134, CML 428	8	<0.5, <0.5, <0.5, <0.5	Resistant
		<0.5	CML 475		2.0, 1.9, 1.5, 1.3	Susceptible
UBC 552	3	1.0, <1.9, 1.5	CA003134 (CML 465), CA00310 (CML 465), CA00396	4	1.9, <1.9, <0.5	Resistant
					1.5	Susceptible
UBC 600	2	1.5, <0.5	CA03124, CA00310	3	1.5, 0.8, 0.9	Resistant
UBC 532	2	>0.9, >0.5	CA03449, CA03124	4	0.9, >0.9	Resistant
					>0.9, <0.5	Susceptible
UBC 572	2	>0.9, <0.5	CA34516, CA14520	7	0.8, >0.5, <0.5, <0.5, <0.5	Resistant
					>0.5, >0.9	Susceptible
UBC 534	2	>0.9, >0.9	CA03106, CML 433	2	>0.9, >0.9	Susceptible
UBC 350	7	2.0, >0.8	CA00310, CA03124	3	2.0, 0.8, 0.5	Resistant
UBC 386	1	<0.5	CA03106	3	0.5,	Resistant
					<0.5, <0.5	Susceptible

Group-II

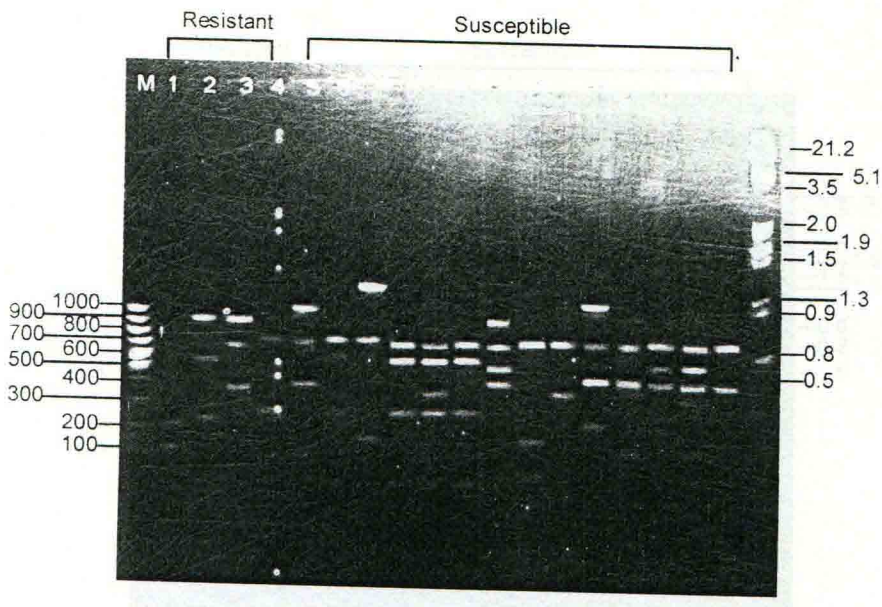
Marker-Lamda DNA/ Ecor1+Hind 111

1. CA00106
2. CAO49Y04
3. CA03147
4. CA14509
5. CML469
6. CML429
7. CML433
8. CML428
9. CML438
10. CML425
11. CML421
12. CML426
13. CML427
14. CML431
15. CML413
16. CML470
17. CML471
18. CML474

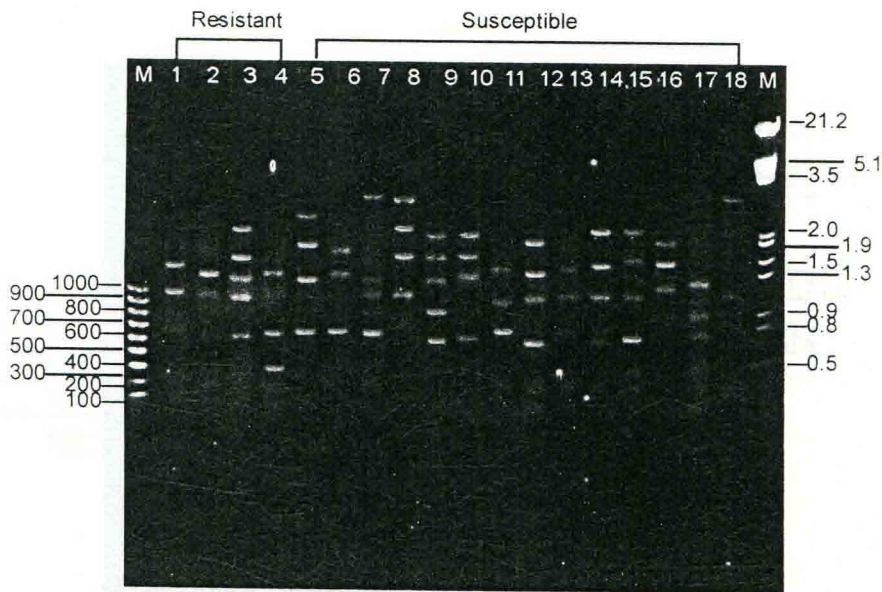
Marker-100bp



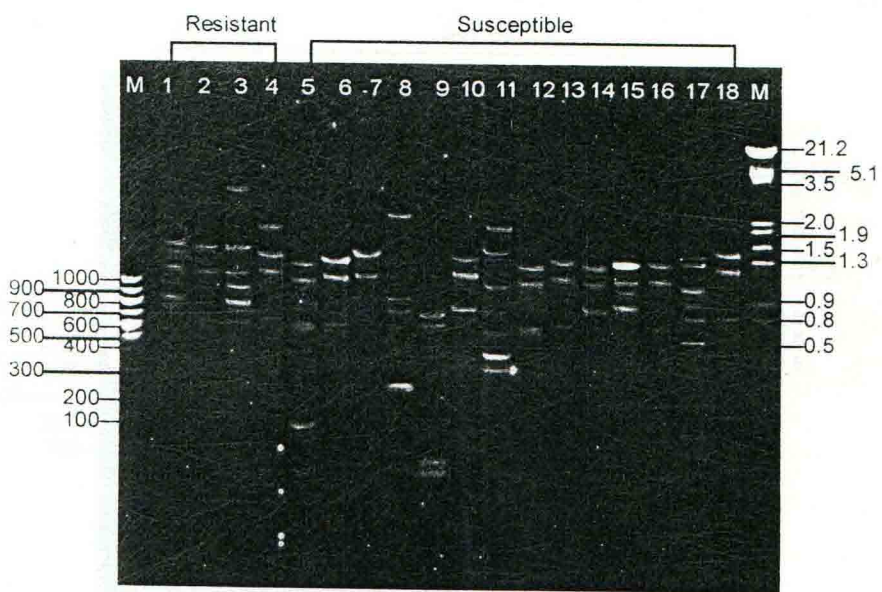
Primer -12SS10T6



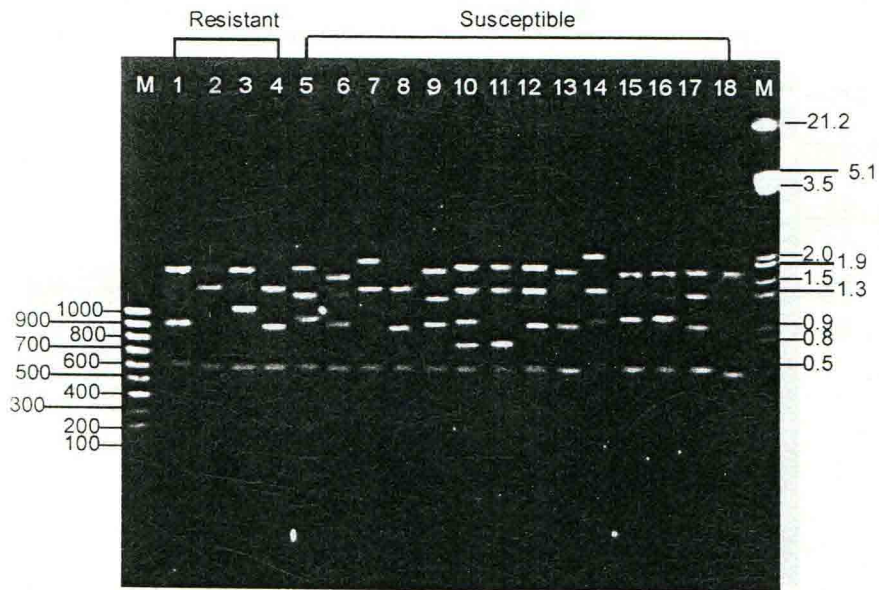
Primer -UBC18



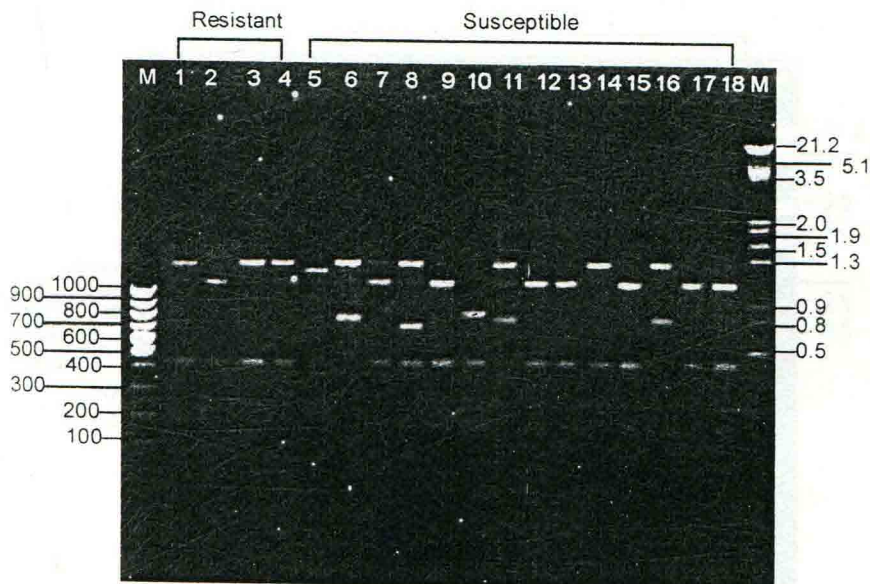
Primer - UBC 535



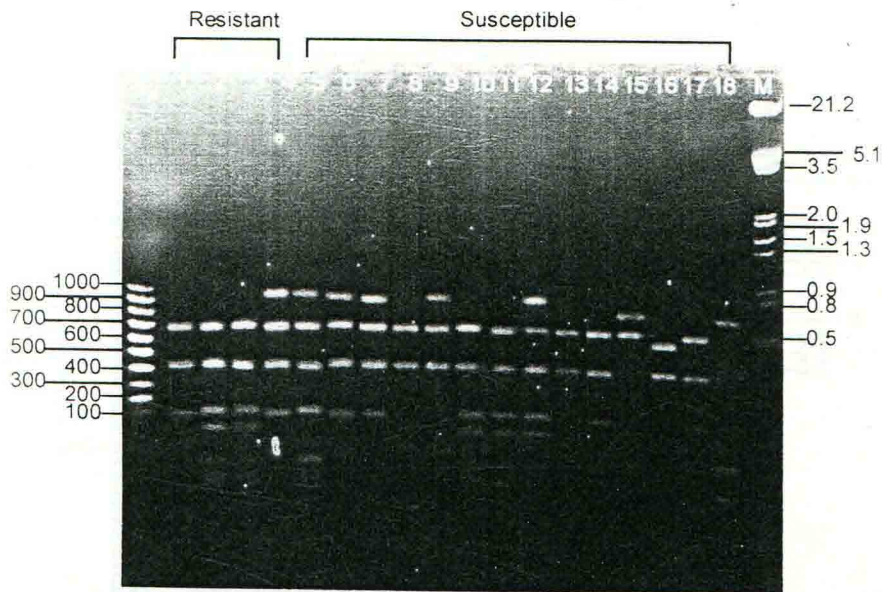
Primer-UBC 337



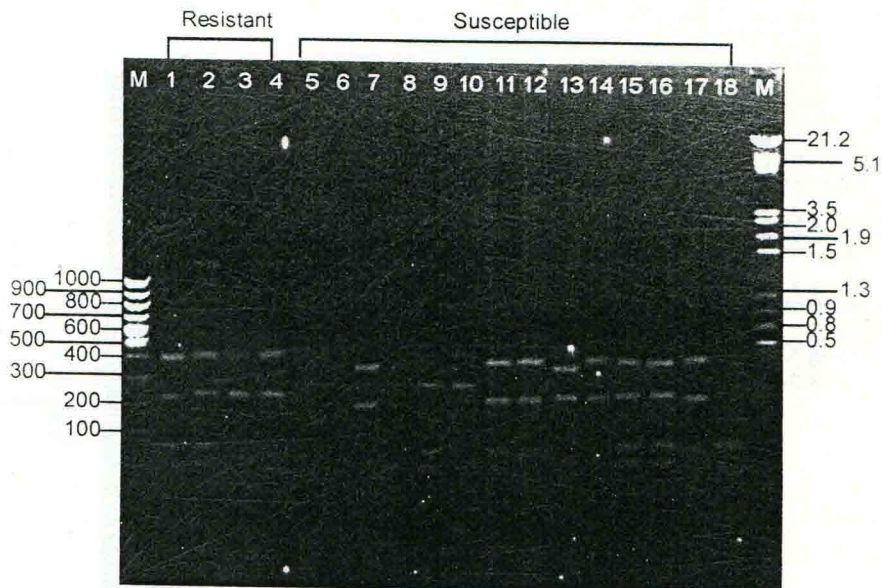
Primer -UBC 552



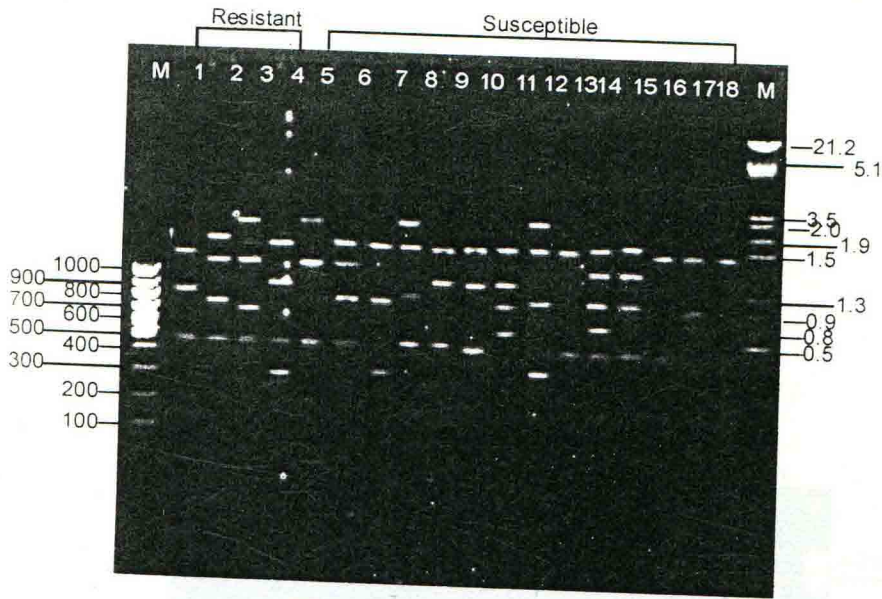
Primer-UBC 600



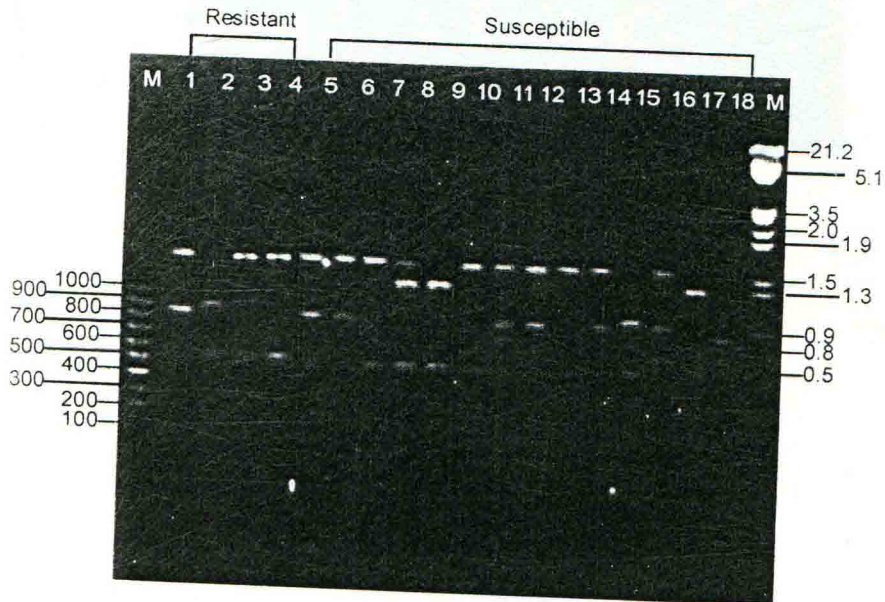
Primer -UBC 532



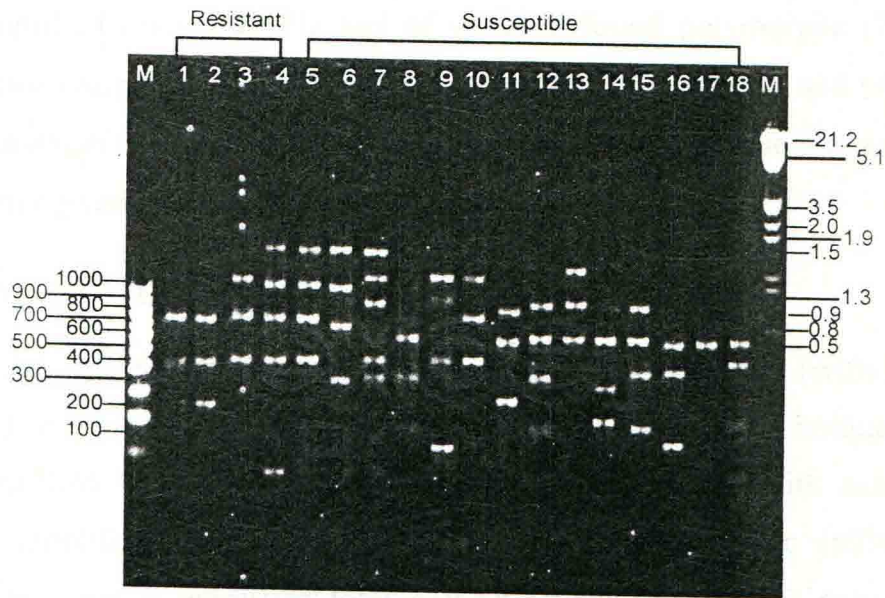
Primer-UBC 572



Primer -UBC 534



Primer- UBC 350



Primer -UBC 386

and susceptible genotype, it amplified 7 unique loci, (1.3 kb) for CML 471 (<1.3 kb) for CML 433 (>0.9 kb) for CML 425 (0.97) for CML 429 (>0.5 kb) for CML 469 and (<0.05 kb) for CML 428 and (>0.9 kb) for resistant CA03147 (>0.5) for CA00106. With group III (18 line). It amplified total 10 RAPD loci, of which 6 were polymorphic (60%). It amplified 4 unique loci among all genotypes, (1.50 kb) for CML 428 (1.3 kb) for CA 14518 (0.8 kb) for CML 465 (<0.5 kb) for CML 465. It amplified 6 unique loci, in resistant and susceptible genotypes (1.5 kb) for susceptible genotypes CML 428 and (<1.3 kb) for CML 475 (1.3 kb) for CA14518 (0.8 kb) for CML 465 (<0.5) for CML 475. Whit group IV (18 inbred line). It amplified total 8 RAPD loci of which 6 found polymorphic (75%). It amplified two unique loci in resistant genotypes (1.5 kb) for 556 and (<1.3 kb) for 561. Total number of RAPD loci, polymorphic loci and unique loci amplified by this primer given group wise in Table 36 to 39).

4.5.3.2 Primer 2 (UBC 18)

On agarose gel, this primer amplified total 6 RAPD loci (with group I inbred line) of which 5 found polymorphic (83%). It amplified a unique loci of (0.5 kb) for CM 121, a susceptible genotypes. This primer with susceptible genotypes, amplified total 5 RAPD loci, 3 found polymorphic (60%), with resistant genotypes it amplified total 6 RAPD loci, of which 2 polymorphic (33%). This primer amplified total 10 RAPD loci of which 8 found polymorphic (80%) (with group II inbred line). It amplified 2 unique loci in all inbred line. and 7 unique loci. If resistant and susceptible genotypes considered separately. It amplified unique loci of 1.3 kb with CML 433, and <0.5 kb for CA00106. It amplified unique loci of 1.3 kb with CML 433 and >0.8 kb with CML 421, susceptible genotypes and it amplified 4 unique loci with resistant genotypes >0.5 kb for CA049Y04 0.5 kb for CA03147 and <0.5 kb for CA14509 <0.5 kb for CA00106. It amplified total 7 RAPD loci with (group III inbred line) of which 4 where found polymorphic (57%). It amplified 1 unique loci among all genotypes and 3 unique loci between resistant and susceptible inbred line. One

Table 36 : RAPD pattern on agarose gel among 11 susceptible and 4 resistant inbred used in disease reaction to BLSB

Primer code	Total no. of RAPD loci	Polymorphic loci		No. of unique loci among susceptible genotypes	Total no. of RAPD loci	Polymorphic loci		No. of unique loci between resistant genotypes
		Number	Percentage			Number	Percentage	
12SS10T4	8	7	87.5	1	7	2	28.5	3
UBC 18	5	3	60.0	1	6	2	33.0	-
UBC 535	4	2	50.0	2	3	-	0.0	3
UBC 337	10	6	60.0	4	5	1	20.0	-
UBC 552	7	7	100.0	-	7	2	28.0	3
UBC 600	5	2	40.0	1	5	1	20.0	1
UBC 532	8	6	75.0	-	7	2	28.5	-
UBC 572	12	6	50.0	3	9	3	33.1	3
UBC 534	11	10	90.0	1	7	1	14.2	2
UBC 350	7	7	100.0	1	8	3	37.5	3
UBC 386	7	3	42.80	2	6	3	50.0	1

Table 37 : RAPD pattern on agarose gel among susceptible genotypes and between resistant genotypes used in disease reaction to BLSB

Primer code	Total no. of RAPD loci	Polymorphic loci		No. of unique loci among susceptible genotypes	Total no. of RAPD loci	Polymorphic loci		No. of unique loci between resistant genotypes
		Number	Percentage			Number	Percentage	
12SS10T4	11	5	45.4	6	5	1	20.0	2
UBC 18	9	5	55.5	2	6	2	33.3	4
UBC 535	8	7	87.5	1	6	2	33.3	3
UBC 337	8	5	62.5	3	5	1	20.0	3
UBC 552	5	2	40.0	1	4	1	25.0	1
UBC 600	5	2	40.0	1	3	1	33.0	1
UBC 532	5	4	80.0	—	4	1	25.0	1
UBC 572	4	3	75.0	—	6	2	33.0	2
UBC 534	5	5	100.0	—	4	1	25.0	—
UBC 350	4	4	100.0	—	5	2	40.0	1
UBC 386	7	7	100.0	—	7	1	14.2	3

Table 38 : RAPD pattern on agarose gel among susceptible genotypes and between resistant genotypes used in disease reaction to BLSB

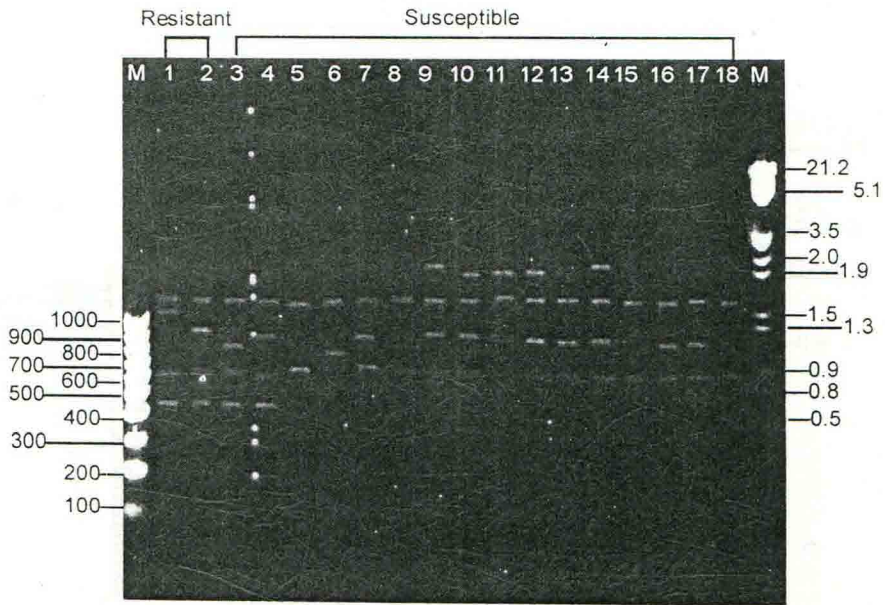
Primer code	Total no. of RAPD loci	Polymorphic loci		No. of unique loci among susceptible genotypes	Total no. of RAPD loci	Polymorphic loci		No. of unique loci between resistant genotypes
		Number	Percentage			Number	Percentage	
12SS10T4	6	5	83.0	1	9	1	11.1	6
UBC 18	6	4	66.6	1	4	–	–	2
UBC 535	7	7	100.0	–	6	2	33.3	1
UBC 337	6	2	33.3	4	6	4	66.6	4
UBC 552	6	4	66.8	1	5	2	40.0	3
UBC 600	4	4	100.0	–	5	3	60.0	3
UBC 532	7	5	71.4	3	5	–	–	3
UBC 572	7	2	28.5	2	7	5	71.4	5
UBC 534	7	2	28.5	2	3	1	33.3	–
UBC 350	8	7	87.5	–	6	4	66.6	3
UBC 386	9	7	77.7	2	4	–	–	1

Group-IV

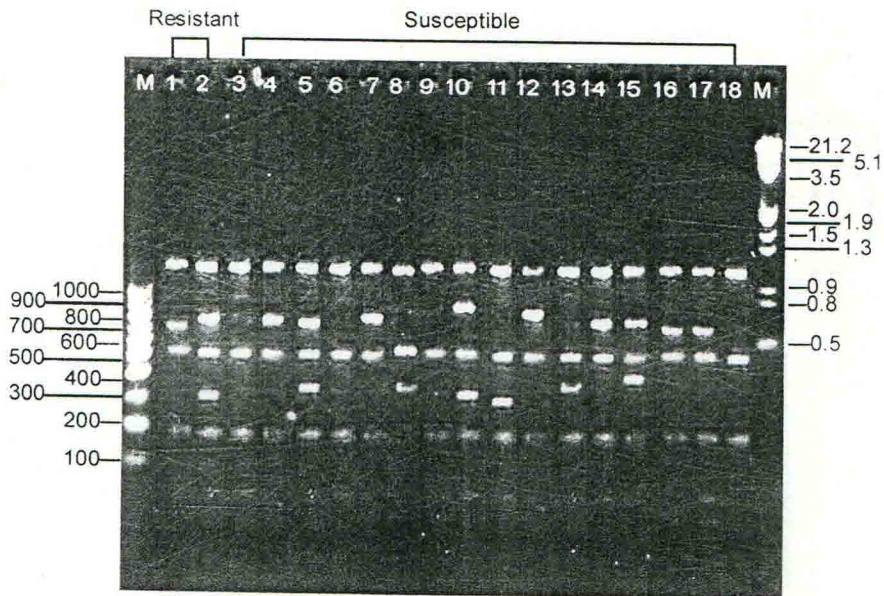
Marker-Lamda DNA/ Ecor1+Hind 111

1. 556
2. 561
3. CA14519
4. CA00104
5. CA03142
6. CA14527
7. CA14517
8. CA14501
9. D-995
10. D-994
11. 550
12. 551
13. 552
14. 558
15. 560
16. 565
17. 569
18. 570

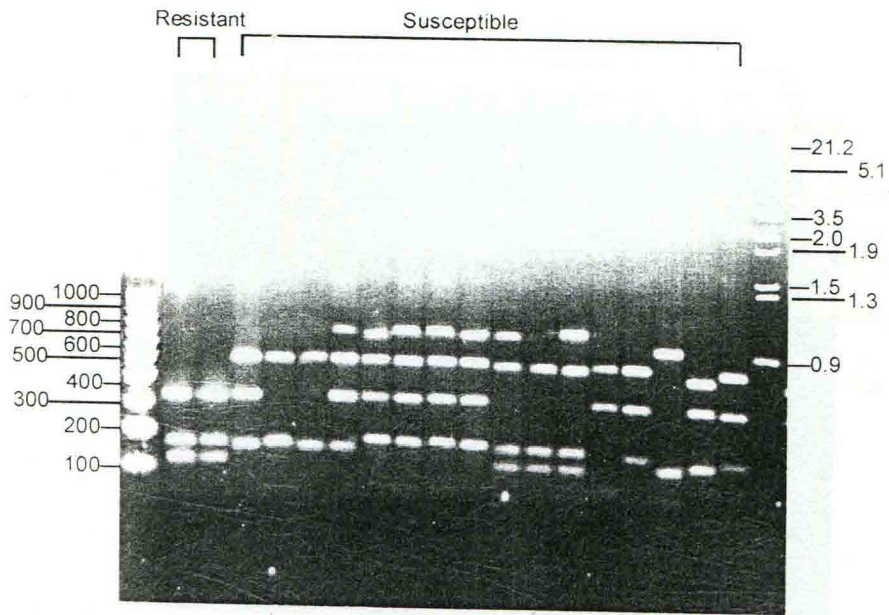
Marker-100bp



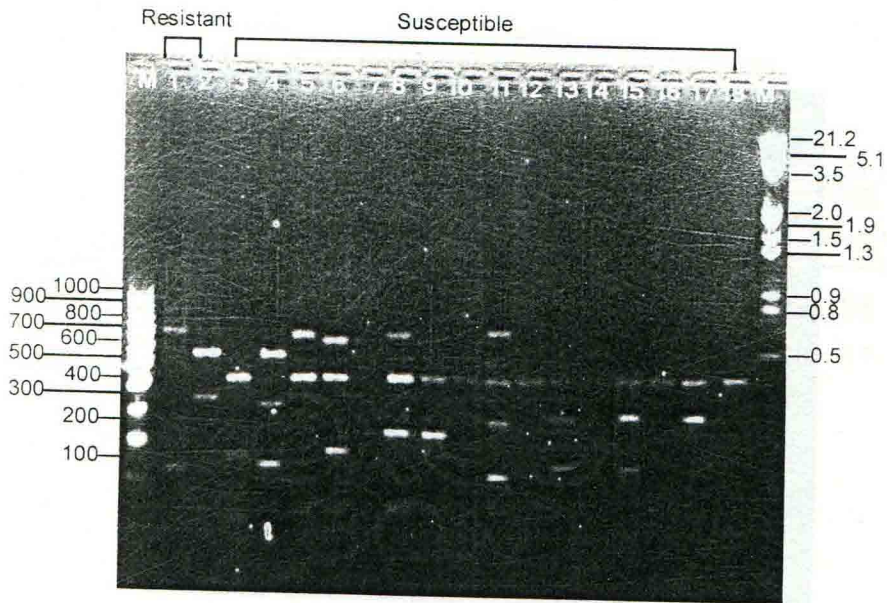
Primer-12SS10T6



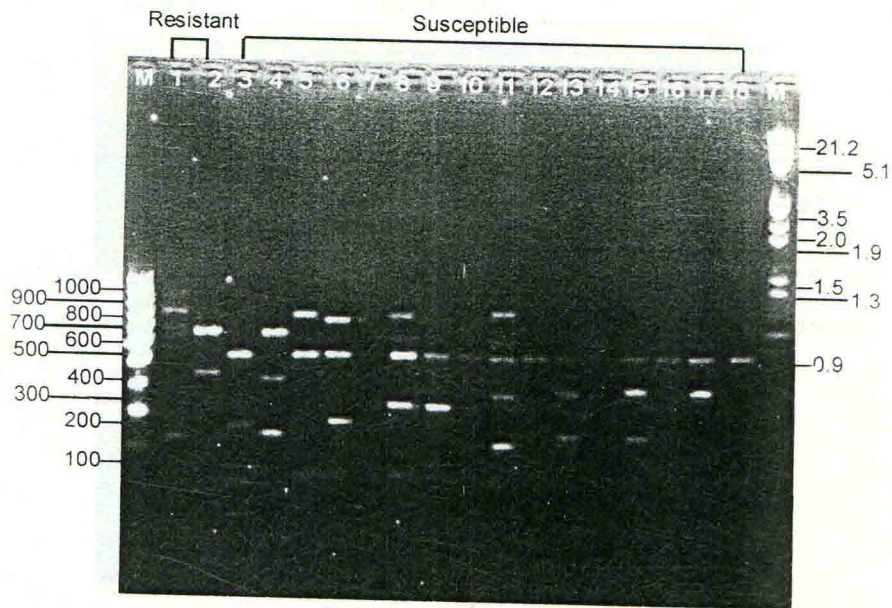
Primer-UBC 18



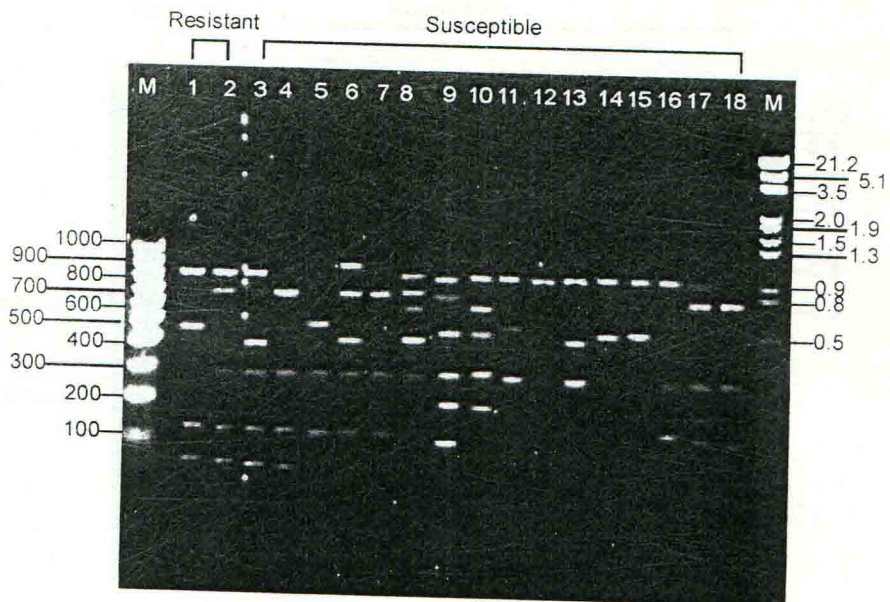
Primer-UBC 535



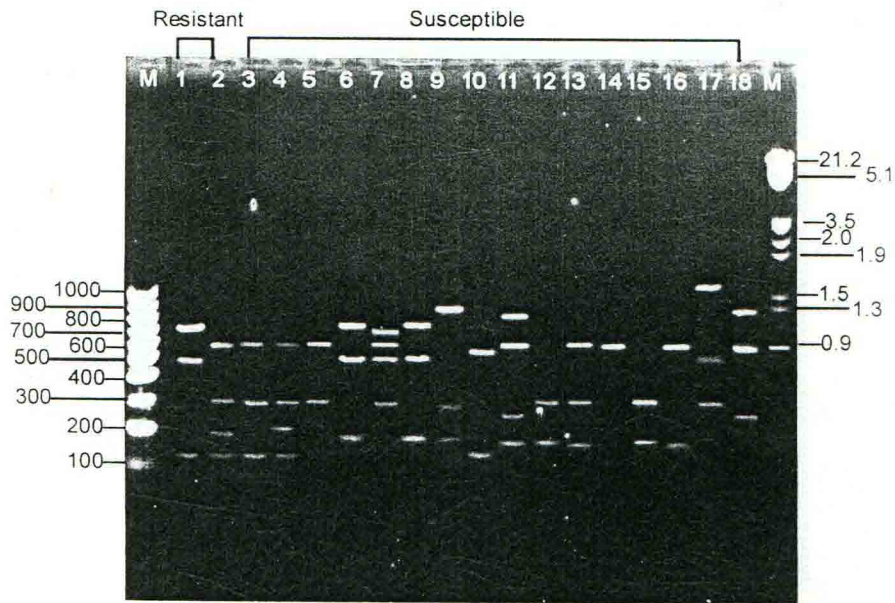
Primer-UBC 337



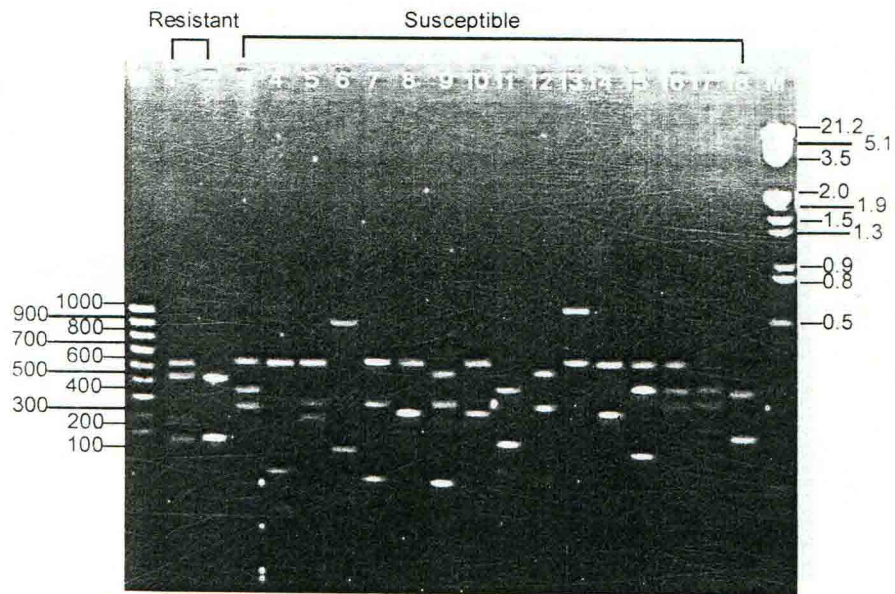
Primer-UBC 552



Primer-UBC 600



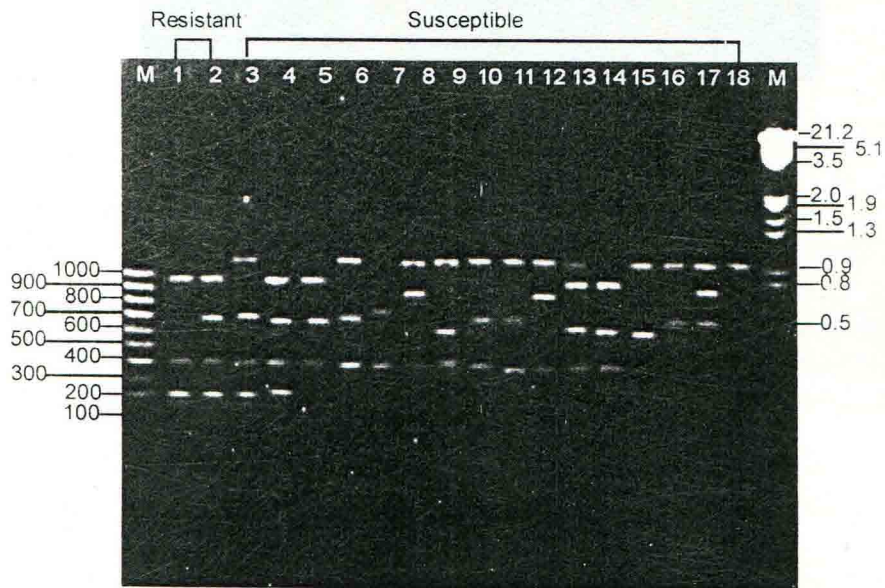
Primer-UBC 532



Primer-UBC 572



Primer-UBC 534



Primer-UBC 350



Primer-UBC 386

Table 34 : RAPD patten on agrose gel among genotypes used in disease reaction to BLSB

Primer code	Total no. of RAPD loci	Polymorphic loci		Unique loci among all inbred studies	Unique loci between susceptible and resistance group
		Number	Percentage		
I2SS10T4	8	6	75.0	—	2
UBC 18	5	2	40.0	—	1
UBC 535	7	5	71.42	1	1
UBC 337	9	6	66.6	3	5
UBC 552	6	4	66.6	2	3
UBC 600	10	9	90.0	2	6
UBC 532	8	7	87.5	1	6
UBC 572	9	8	88.8	2	3
UBC 534	11	7	63.6	5	5
UBC 350	7	4	57.1	3	4
UBC 386	10	6	60.0	4	7
Total	90	66			
Average	8.18				

Table 35 : RAPD analysis of unique loci amplified by primers on agrose gel among all genotypes and between susceptible and resistant groups

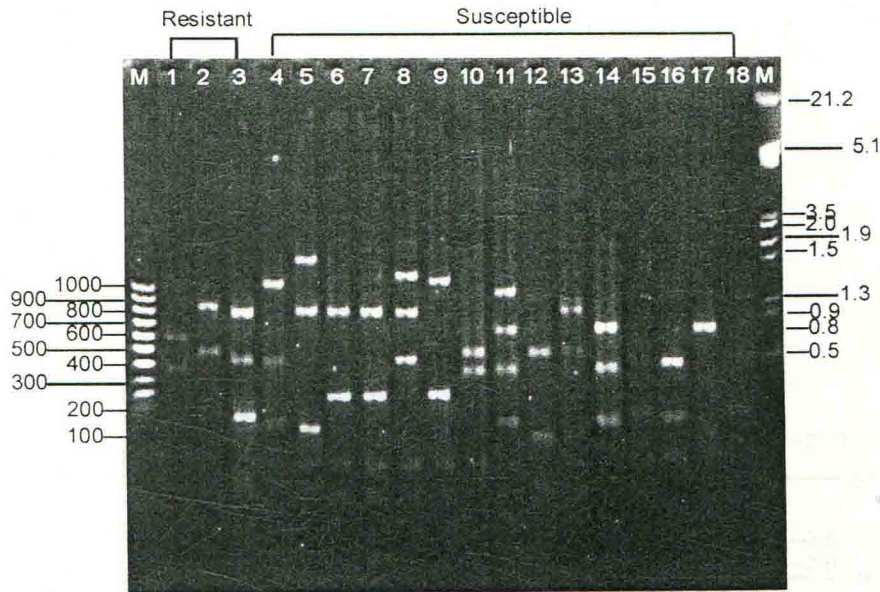
Primer code	Unique loci among all inbred s			Unique loci between susceptible and resistant groups		
	Unique loci	Size (kb)	Inbred having these loci	Unique loci	Size (kb)	Group having these loci
I2SS10T4	—	—	—	2	1.5, <1.3	Resistant
UBC 18	—	—	—	1	<0.5	Resistant
UBC 535	1	>0.9	565	1	>0.9	Susceptible
UBC 337	3	<0.8, <0.5	556, CA14517	5	0.8, >0.5, <0.5	Resistant
		<0.5	556		<0.5, <0.5	Susceptible
UBC 552	2	<0.5, <0.5	556, 556	3	>0.9, <0.5, <0.5	Resistant
UBC 600	2	<0.5, >0.5	558, 556	6	0.9, >0.5, <0.5	Resistant
					>0.9, <0.5, <0.5	Susceptible
UBC 532	1	1.5	569	6	0.3, <0.9, <0.9, 0.5	Resistant
					1.5	Susceptible
UBC 572	2	>0.5, <0.5	CA14527, CA00104	3	<0.5, <0.5	Resistant
					>0.5, <0.5	Susceptible
UBC 534	5	>1.3, <1.3, <1.3	550, 570, CA00104	6	0.5, 0.5, <0.5	Resistant
		0.8, <0.5	552, 556		1.3, <1.3, <0.9	Susceptible
UBC 350	3	>0.5, >0.5, <0.5	CA03142, CA03142, 551	4	<0.5	Resistant
					0.9, <0.9, <0.5	Susceptible
UBC 386	4	<0.5, <0.5	561, CA00104	6	<0.8, >0.5, <0.5, <0.5	Resistant
		<0.5, <0.5	CA00104, CA14519		0.5, <0.5	Susceptible

Group-III

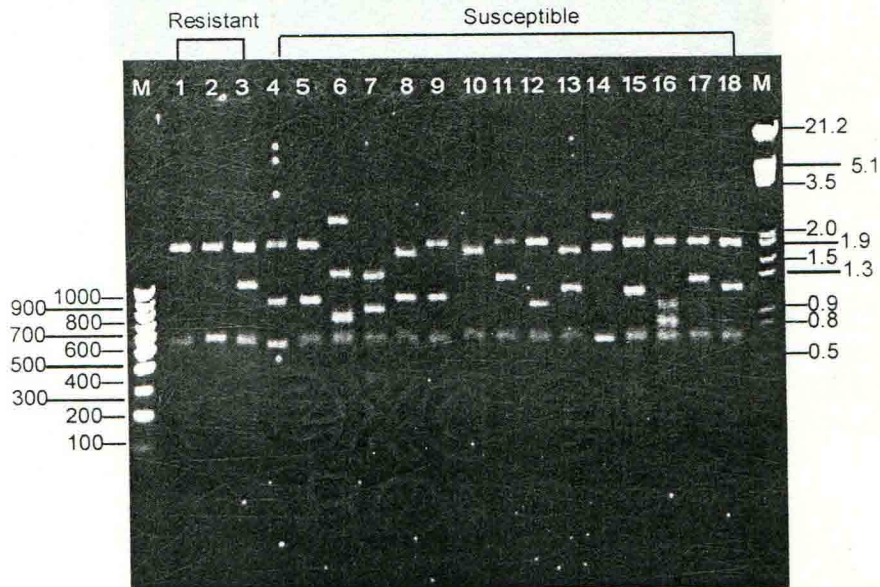
Marker-Lambda DNA/ Ecor1+Hind 111

1. CA00310
2. CA03124
3. CA14518
4. CA14520
5. CML428
6. CA00334
7. CA03106
8. CA003134
9. CA34507
10. CA00396
11. CA34516
12. CA03131
13. CA34516
14. CA3149
15. A00344
16. CA03124
17. CA00384
18. POP. 147-F2#161-3-2-B-1-B

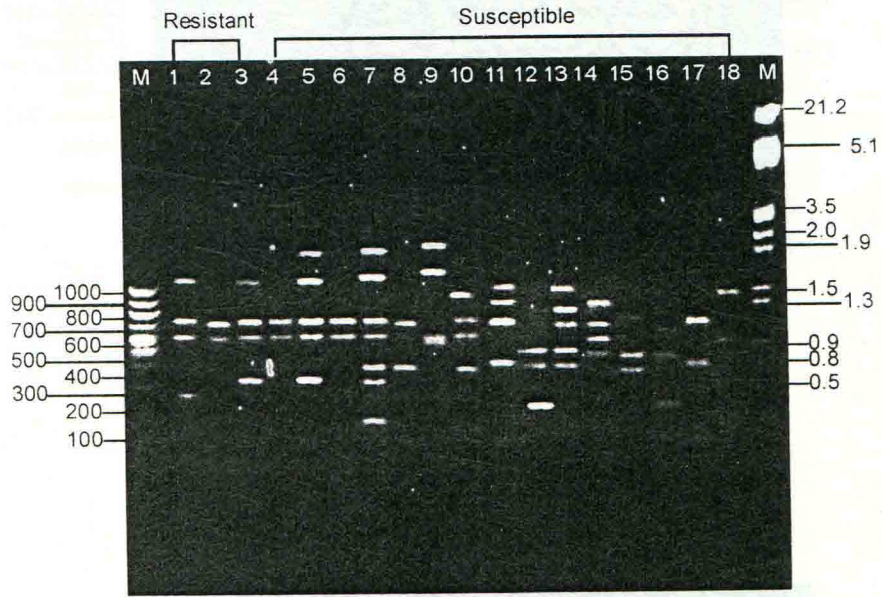
Marker-100bp



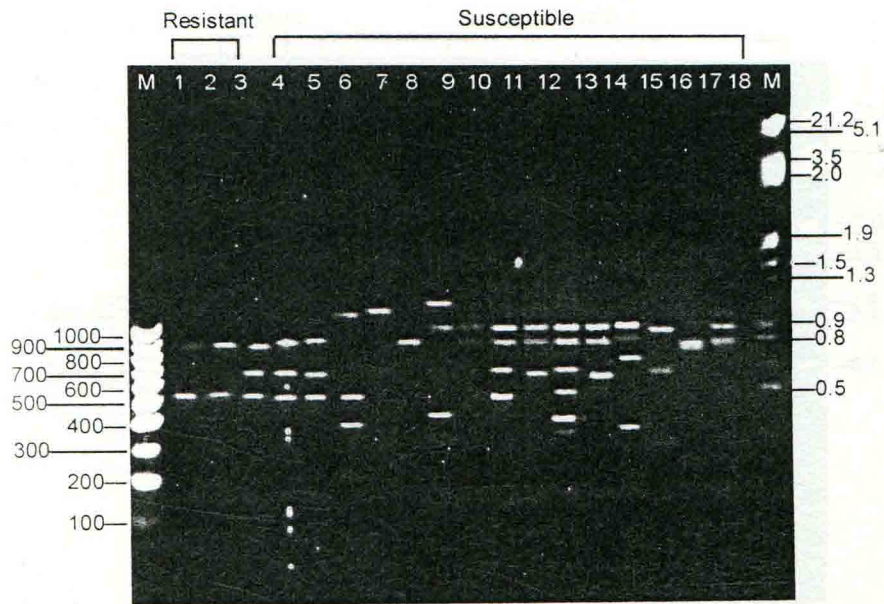
Primer - 12SS10T6



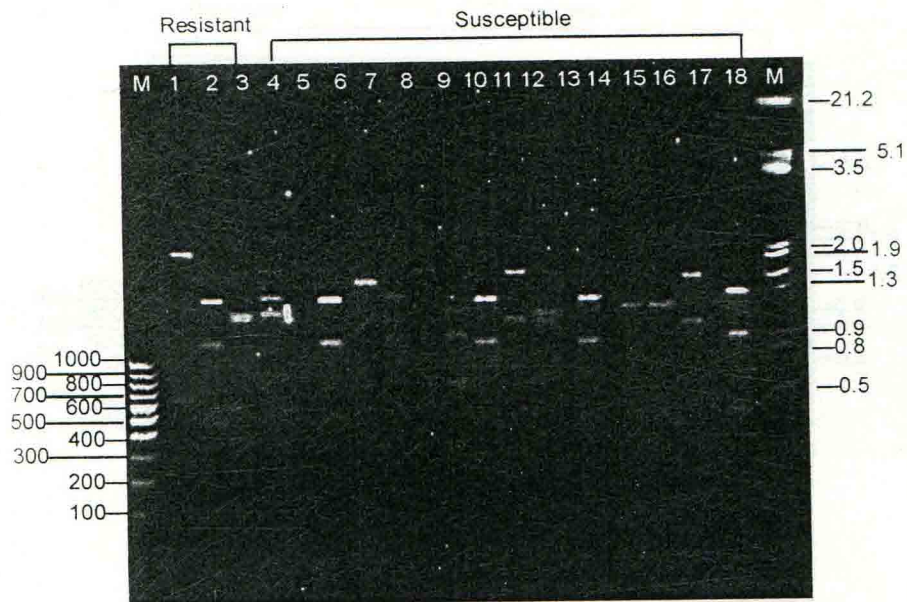
Primer-UBC 18



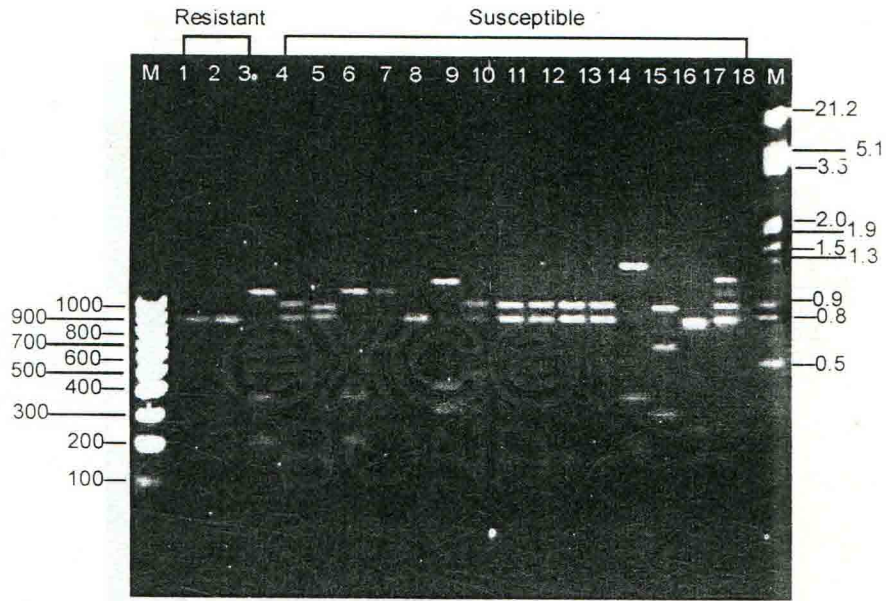
Primer-UBC 386



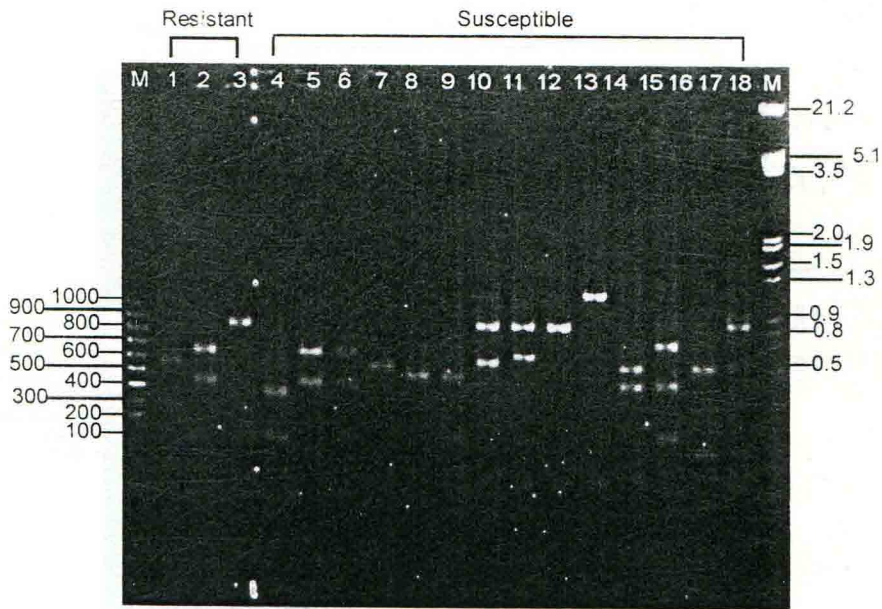
Primer-UBC 534



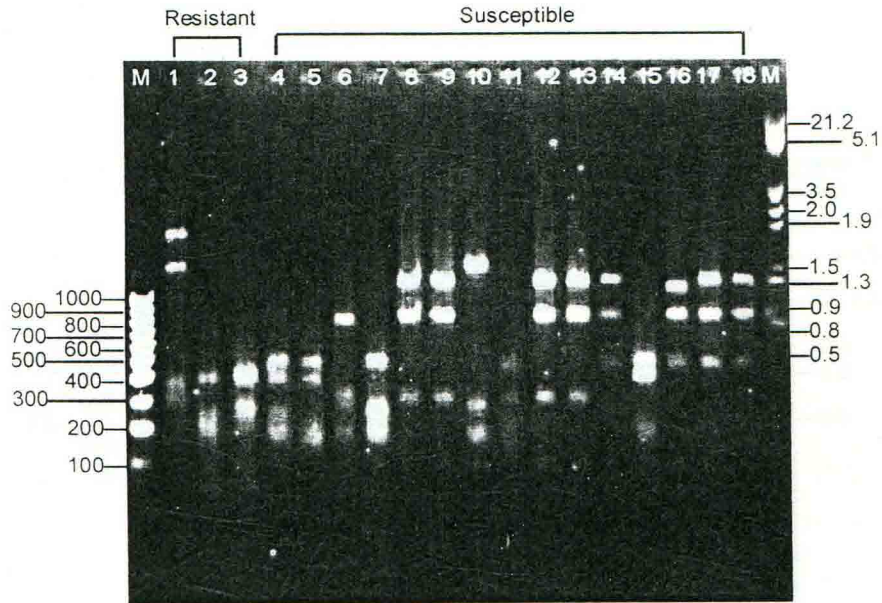
Primer-UBC 350



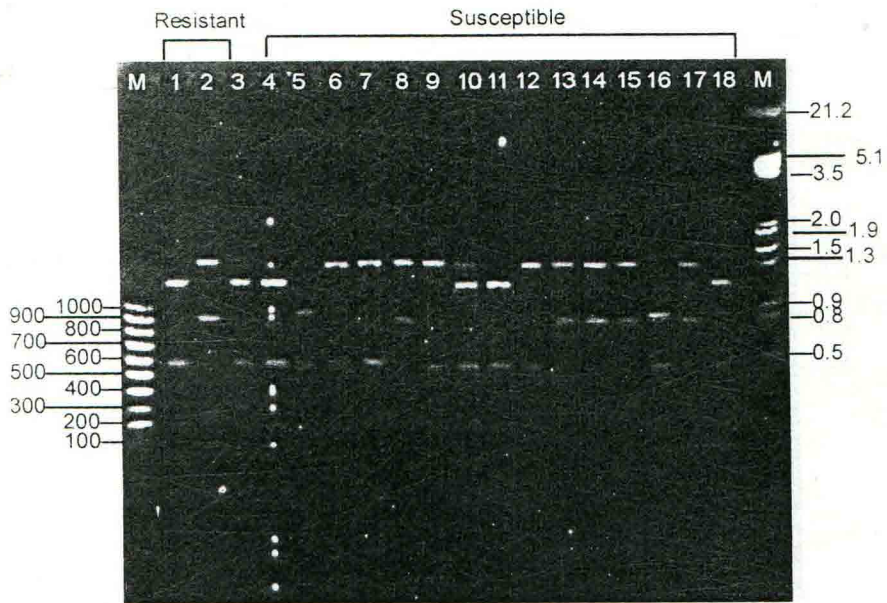
Primer -UBC 532



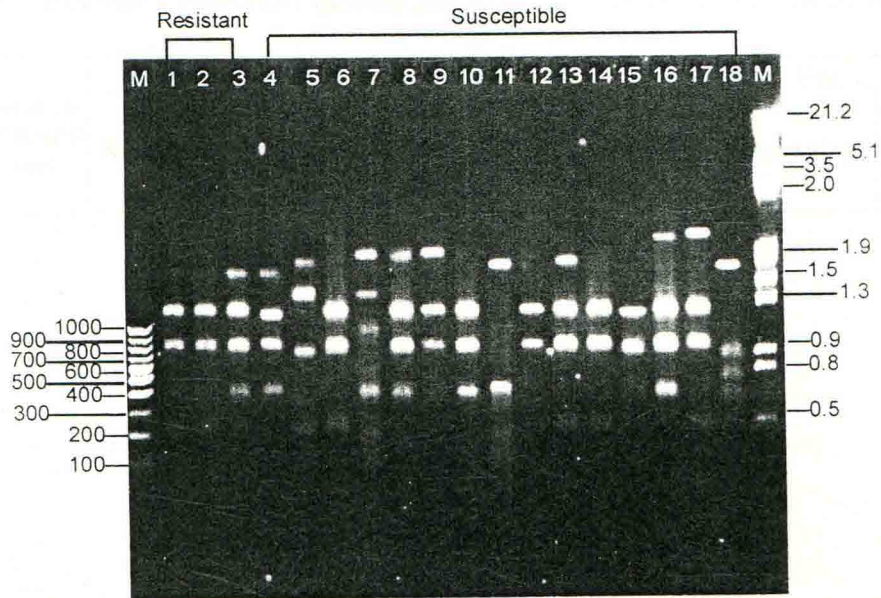
Primer-UBC 572



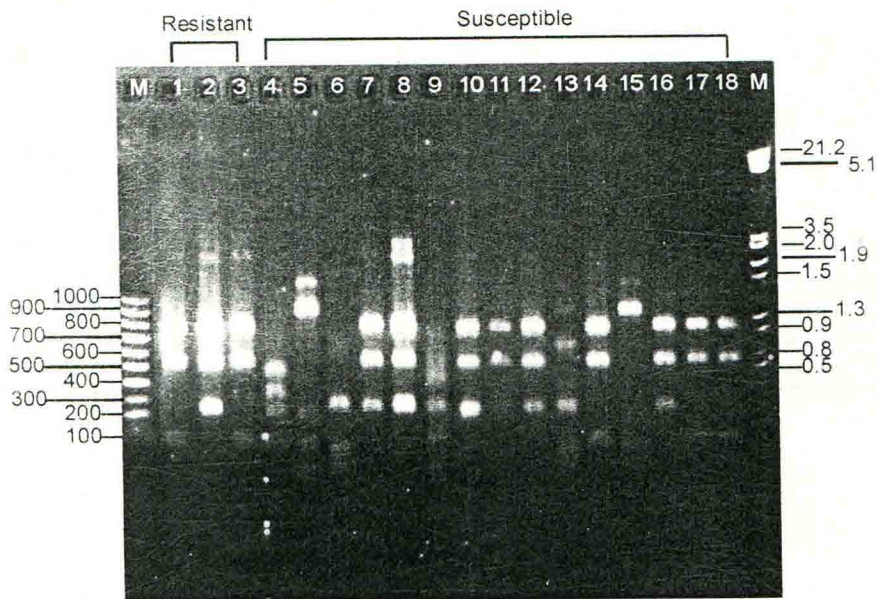
Primer -UBC 552



Primer-UBC 600



Primer -UBC 535



Primer-UBC 337

Table 39 : RAPD pattern on agarose gel among susceptible genotypes and between resistant genotypes used in disease reaction to BLSB

Primer code	Total no. of RAPD loci	Polymorphic loci		No. of unique loci among susceptible genotypes	Total no. of RAPD loci	Polymorphic loci		No. of unique loci between resistant genotypes
		Number	Percentage			Number	Percentage	
12SS10T4	6	5	83.3	-	5	-	-	2
UBC 18	5	2	40.0	-	5	-	-	1
UBC 535	6	4	66.6	1	3	-	-	2
UBC 337	5	4	80.0	2	4	-	-	3
UBC 552	4	3	75.0	-	4	-	-	3
UBC 600	10	7	70.0	3	6	-	-	3
UBC 532	-	7	87.5	1	6	-	-	5
UBC 572	8	6	75.0	2	4	-	-	2
UBC 534	9	5	55.5	3	5	-	-	3
UBC 350	6	2	33.3	3	4	-	-	1
UBC 386	8	5	62.5	2	5	-	-	5

unique loci of 0.8 kb for CA03124 and 0.3 kb for CA14518 and <0.9 kb for CML 413. It amplified total 5 RAPD loci (with group IV genotype) of which 2 were found polymorphic (40%) and one unique loci amplified of (<0.5 kb) for 561 resistant genotypes. Total number of RAPD loci, polymorphic loci and unique loci in all genotypes and unique loci in resistant and susceptible genotypes amplified by primer 2 (UBC 18) given in Table 28-35 and 36 to 39.

4.5.3.3 Primer 3 (UBC 535)

On agrose gel this primer amplified total 5 RAPD loci with (group I inbred line) of which 3 where found polymorphic (75%). It amplified 3 unique loci among all genotypes and 5 unique loci in resistant and susceptible genotypes if they consider separately. It amplified 3 unique loci of (1.9 kb) for CM 210, and 1.5 kb for CM 121, and 05 kb for CM 104, >2.0 kb for CM 500 and it amplified unique loci of 1.9 kb for CM 209 and 1.3 kb for CM 121 of susceptible inbred line. It amplified 3 loci of >0.3 kb of total 10 RAPD loci with (group III inbred line) of which 7 polymorphic (70%) and it amplified 3 unique loci in all inbred line, 2.0 kb for CML 469 and 1.9 kb for CA031747 <0.5 kb for CA14509 and it amplified 4 unique loci among resistant and susceptible inbred line, 2.0 kb for CM 469 and 1.9 kb for CA 1047, 1.3 kb for CA 03147 and <0.5 kb for CA14509. It amplified total 10 RAPD loci with (group III genotypes of which 9 where found polymorphic (90%). It amplified one unique loci among all genotypes and one unique between resistant and susceptible inbred line. It amplified <0.5 kb unique and for CA 00310 and one unique loci of 0.5 kb for CML 465. It amplified total 7 RAPD loci (with group IV inbred line) of which 5 were found polymorphic. It amplified one unique loci with all genotypes and one unique loci with resistant and susceptible inbred line. It amplified one unique loci of >0.9 kb for 565 and one unique loci of 0.9 kb for 565 resistant genotypes if compared separately. Total number of RAPD loci, in resistant and susceptible genotypes amplified by primer 3 (UBC 535) are given in Table 28 to 35 and 41 to 44).

Table 40 : RAPD analysis on agrose gel among susceptible genotypes and resistant genotypes

Primer code	Unique loci among susceptible genotypes			Unique loci between resistant genotypes		
	No. of unique loci	Size (kb)	Genotypes having these loci	No. of unique loci	Size (kb)	Genotypes those having
12SS10T4	1	<0.5	CM 115	3	>1.5, >0.5,>0.5	CM 500, CM105, CM 104
UBC 18	1	>0.5	CM 121	–	–	–
UBC 535	2	1.9	CM 209	3	<0.5	CM 500
		1.3	CM 121		<0.5	CM 104
UBC 337	4	>2.0	Amar	–	–	–
		2.0	CM 115	–	–	–
		<2.0	CM 111	–	–	–
		<2.0	CM 209	–	–	–
UBC 552	–	–	–	3	1.5	CM 105
					<1.3	CM 105
					>0.9	CM 600
UBC 600	–	–	–	–	–	–
UBC 532	–	–	–	–	–	–
UBC 572	3	1.9	CM 120	3	<1.5	CM 600
		1.9	–		<1.5	CM 104
		>1.5	CM 111		1.3	CM 105
UBC 534	1	1.9	CM 209	2	1.3	CM 105
					<0.5	CM 500
UBC 350	1	1.9	Amar	3	>0.5	CM 600
					<0.5	CM 600
					<0.5	CM 500
UBC 386	2	1.5	CM 121	–	–	–
		<0.5	CM 210	–	–	–

Table 41 : RAPD analysis on agrose gel among susceptible genotypes and resistant genotypes

Primer code	Unique loci among susceptible genotypes			Unique loci between resistant genotypes		
	No. of unique loci	Size (kb)	Genotypes having these loci	No. of unique loci	Size (kb)	Genotypes those having
12SS10T4	6	1.3	CML 471	2	>0.9	CA03147
		<1.3	CML 433		>0.5	CA00106
		>0.9	CML 425			
		0.9	CML 429			
		>0.5	CML 469			
		<0.5	CML 428			
UBC 18	2	1.3	CML 433	4	>0.5	CA049Y04
		>0.8	CML 421		<0.5	CA03147
					<0.5	CA14509
					<0.5	CA00106
UBC 535	1	2.0	CML 469	3	1.9	CA03147
					1.3	CA03147
					<0.5	CA14509
UBC 337	3	2.0	CML 428	3	>2.0	CA00106
		1.9	CML 421		1.9	CA14509
		>0.9	CML 413		<0.5	CA03147
UBC 552	1	2.0	CML 431	1	>0.9	CA03147
UBC 600	1	>0.5	CM 428	1	>0.9	CA049Y04
UBC 532	-	-	-	1	0.8	CML 433
UBC 572	-	-	-	2	1.3	CA049Y04
					>1.3	-
UBC 534	-	-	-	-	-	-
UBC 350	-	-	-	1	<0.5	CA00106
UBC 386	-	-	-	3	1.9	CA03147
					<0.5	CA00106
					<0.5	CA14509

Table 42 : RAPD analysis on agrose gel among susceptible genotypes and resistant genotypes

Primer code	Unique loci among susceptible genotypes			Unique loci between resistant genotypes		
	No. of unique loci	Size (kb)	Genotypes having these loci	No. of unique loci	Size (kb)	Genotypes those having
I2SS10T4	1	0.5	CML 428	4	<1.3	CML 475
					1.3	CA14518
					0.8	CML 465
					>0.5	CA03124
UBC 18	1	0.8	CA03124	2	<0.5	CML 475
					1.3	CA14518
					>0.9	CML 475
UBC 535	-	-	-	1	<0.5	CML 465
UBC 337	4	2.0	CA003134	4	<0.5	CML 475
		1.9	CA003134		<0.5	CML 475
		1.5	CML 428		<0.5	CA03124
		1.3	A00344		<0.5	CML 475
UBC 552	3	1.5	CA00396	3	1.9	CA00.310 (CML 465)
					<1.9	CA00310 (CML 465)
UBC 600	-	-	-	3	<0.5	CA14520 (CML 475)
					1.5	CA00334 (CML 433)
					0.8	CA00334 (CML 433)
					0.9	CA00310 (CML 465)
UBC 532	2	>0.9	CA03149	2	>0.9	CA14518
		<0.5	CA03124		<0.5	CA14520 (CML 475)
UBC 572	2	>0.5	CA03149	5	0.8	CA14518
		>0.9	CA03124		>0.5	CA03124
					<0.5	CA03124
					<0.5	CML 475
UBC 534	2	>0.9	CA00316		<0.5	CML 475
		>0.9	CA00334		-	-
UBC 350	-	-	-	3	2.0	CML 465
					0.8	CA03124
					0.5	CA03124
UBC 386	2	<0.5	CA03131	1	0.5	CA03124
		<0.5	CA03106		0.5	CML 465

Table 43 : RAPD analysis on agrose gel among susceptible genotypes and resistant genotypes

Primer code	Unique loci among susceptible genotypes			Unique loci between resistant genotypes		
	No. of unique loci	Size (kb)	Genotypes having these loci	No. of unique loci	Size (kb)	Genotypes those having
12SS10T4	-	-	-	2	1.5 <1.3	
UBC 18	-	-	-	1	<0.5	561
UBC 535	1	>0.9	565	-	-	-
UBC 337	2	<0.5	CA00104	3	0.8	556
		<0.5			>0.5	561
					<0.5	556
UBC 552	-	-	-	3	>0.9	561
					<0.5	556
					<0.5	556
UBC 600	3	>0.9	CA14519	3	>0.9	561
		<0.5	CA14519		<0.5	556
		<0.5	558		<0.5	556
UBC 532	1	>1.5	569	5	>0.9	556
					<0.9	561
					<0.9	556
					<0.5	561
					<0.5	561
UBC 572	2	>0.5	CA14527	2	>0.5	556
		<0.5	CA00104		<0.5	556
UBC 534	3	1.3	550	3	0.5	556
		<1.3	CA00104		0.5	556
		<0.9	552		<0.5	556
UBC 350	3	0.9	CA14501	1	<0.5	561
		<0.9	CA14501			
		<0.5	CA14519			
UBC 386	2	0.5	CA00104	4	0.8	556
		<0.5	CA14519		>0.5	561
					<0.5	561
					<0.5	556

4.5.3.4 Primer 4 (UBC 337)

On agrose gel, this primer detected total 11 RAPD loci (with group one inbred line) of which 6 found polymorphic (51%). It also amplified 3 unique loci among all inbred line, like (>2.0 kb) for Amar and <2.0 kb for CM 111, <0.5 kb for CM 209. It also amplified 4 unique loci of all inbred line compared between resistant and susceptible group. it amplified, unique loci of >2.0 kb for Amar and 2.0 kb for CM 115 and <2.0 kb for CM 111 and <2.5 kb for CM 209. It amplified total of 10 RAPD loci (which group II, inbred line) with 4 of which found polymorphic (40%). It also amplified 2 unique RAPD loci if all genotypes were considered and 6 unique loci for resistant and susceptible inbred line. It amplified two unique loci of >2.0 kb for CA03147 and 2.0 kb for CML 428. It amplified 6 unique loci, of which, 2.0 kb for CM 428 and 1.9 kb for CML 421 and >0.9 kb for CMI 413 all three were susceptible and 1.0 kb for CA 03147, 1.3 kb for CA03147 and <0.5 kb for CA14509 all three genotypes were resistant. it amplified total 9 RAPD loci (with group III inbred line) of which 4 found polymorphic (44.41). It also amplified 5 unique loci in all inbred line, 2.0 kb for CA003134 and 1.9 kb for CA003134 and >1.3 kb for CML 428 and <0.5 kb for CML 475. It amplified 8 unique loci in resistant and susceptible inbred line. 2.0 kb for CA003134, 1.9 kb for CA003134, and 1.5 kb CML 428, and 1.3 kb for A00344, all four were susceptible and 0.5 kb for CML 475, <0.5 CML 475, <0.5 CA03124, <0.5 CML 475 all four were resistant genotypes. It amplified total 9 RAPD loci with (group IV) of which 6 where found polymorphic (66.6%). It amplified 3 unique loci in all genotypes, <0.8 kb for 556 and <0.5 kb for CA14517. It also amplified 5 unique loci two in susceptible inbred and three in resistant genotypes, <0.5 kb for CA00104 and <0.5 kb for CA14517 and 0.8 for 556 and >0.5 kb for 561 and <0.5 kb for 556. Total number of RAPD loci, in resistant and susceptible genotypes amplified by primer 4 (UBC 537) given in Table 28 to 35 and 41 to 44).

4.3.3.5 Primer UBC 552

On agarose gel total number of amplified loci given by this primer were 7 (with group I, inbred line) of which 7 found polymorphic (100%). This primer also amplified the unique loci in three resistant inbred line, 1.3 kb for CM 105, <1.3 kb for CM 105 and >0.9 kb for CM 600. This primer amplified total number of 6 RAPD loci with (group II, genotypes) of which 4 found polymorphic. This primer also amplified 1 unique loci among all genotypes and two unique loci, one in resistant and one in susceptible genotypes. One unique loci amplified by this primer of 2.0 kb for CML 431. One unique loci in susceptible genotypes CML 431 of 2.0 kb and one loci of >0.9 kb of CA03147 resistant genotypes. It amplified total 8 RAPD loci with (group III genotypes) of which 5 found polymorphic (62.5%). It also amplified 3 loci among all inbred line, and found unique loci between resistant and susceptible inbred line. Three unique loci amplified by this primer where 1.0 kb for CA00310 (CML 465) and <1.9 kb for CML 465 and 1.5 kb for C00310. One unique loci of 1.5 kb for CA00396 and three loci, of 1.9 kb of CA00310 (CML 465), <1.9 kb of CA00210, <0.5 kb CA 14526 were amplified by this primer. It amplified total 6 RAPD loci with (group IV genotypes) 1 of which 4 found polymorphic (66.6%). It also amplified two unique loci, of <0.5 kb for 556 and <0.5 kb for 556 inbred line. It also amplified 3 unique loci, >0.9 kb for 561 and <0.5 kb for 556 and <0.5 kb for 556. The total number of RAPD loci in resistant and susceptible genotypes amplified by primer UBC 552 are given in Table (28 to 35 and 41 to 44).

4.3.3.6 Primer UBC 600

On agarose gel, this primer amplified a total of 5 RAPD loci with (group-I genotypes) of which 4 were found polymorphic (80%). It also amplified two unique loci of <0.5 kb for CM 120 and one unique of <0.5 kb for CM 500. This primer amplified total 6 RAPD loci with group-II of which 5 found to be polymorphic (83.3%). It also amplified two unique loci of <0.5 kb for CM 120

and one unique loci of <0.5 kb for CM 500. This primer amplified total 6 RAPD loci with group II of which 5 found to be polymorphic (83.3%). It also amplified one unique loci among all genotypes of <0.5 kb for CM 428 and two unique loci, for one susceptible genotypes CM 428 to >0.5 kb and it amplified one unique loci of 1.9 kb for CA 14509. This primer amplified at total of 6 RAPD loci of which 4 found polymorphic (66.6%) with group III. It also amplified two unique loci of 1.5 and <0.5 kb for CA03124 and CA00310 and it also amplified three unique loci of 1.5 kb, <0.8 kb, <0.9 kb for CA00334, CML 433, CA00334, and CML 465. It also amplified total of 10 RAPD loci with group IV genotypes of which 9 found polymorphic (90%). It also amplified two unique loci among all genotypes of <0.5 kb for 558 and >0.5 kb for 556. It also amplified 6 unique loci among resistant and susceptible genotypes when they were compared separately. Three unique loci of >0.9 kb for CA14519 and <0.5 kb for CA14519 and <0.5 kb for 558 susceptible genotypes. It amplified tree unique loci for resistant genotypes of >0.9 kb for 564 and <0.5 kb for 556 and <0.5 kb for 556. The total number of RAPD loci and polymorphic loci among all genotypes and unique loci among all inbred and resistant and susceptible genotypes, amplified by this primer UBC 600 given in Table 28 to 35 and 41 to 44.

4.3.37 Primer UBC 532

On agarose gel, this primer amplified total 8 RAPD loci with group I genotypes, out of these 8 found polymorphic (100%). This primer would not amplified any unique loci among all genotypes as well as between resistant and susceptible genotypes separately. This primer amplified total of 5 RAPD loci of which 5 were polymorphic 100% with group II genotypes. It also amplified a unique loci in resistant line, CML 433 of 0.8 kb. This primer amplified total of 8 RAPD loci with group III inbred line out of which 6 were found polymorphic (75%). It also amplified two unique loci among all genotypes and 4 unique loci among susceptible and resistant inbred line. The two amplified unique band of

>0.9 kb of CA0349, and >0.5 kb of CA03124 maize genotypes and it also amplified two unique loci in susceptible inbred line, CA03149 with unique loci of size >0.9 kb and CA00334 with size of >0.9 kb and two unique loci with resistant genotype with size of >0.9 kb for CA14518, and >0.5 kb after CA14520 (CML 475). This primer amplified total of 8 RAPD loci with group IV genotypes and out of which 7 were polymorphic (87.5%). It also amplified a unique loci among all inbred studies. It also amplified total of 6 unique RAPD loci between resistant and susceptible group. Unique loci in all genotypes of size 1.5 kb for 56.9 and unique loci for susceptible genotypes of >1.5 kb for 569 and unique loci for resistant genotypes of >0.9 kb for 556 and <0.98 kb for 561 and <0.9 kb for 556 and <0.5 for 561 and <0.5 kb for 561 (Table 28 to 35 and 41 to 44).

4.3.3.8 Primer UBC 572

On agarose gel, this primer amplified total 12 RAPD loci group I genotypes of which 7 were found polymorphic (581%). It also amplified two unique loci among all genotypes and six unique loci among resistant and susceptible genotypes. It amplified two unique loci of size 1.9 kb for CM 120 and 1.5 kb for CM 120. This primer also amplified 3 unique loci of size 1.9 kb for CM 120 and 1.9 kb for CM 120 and >1.5 kb for CM 111. This primer also amplified three unique loci in resistant genotypes <1.5 CM 600, <1.5 kb for CM 105. This primer also amplified total of 7 RAPD loci with group II genotypes of which 5 were found to be polymorphic (71.4%). It also amplified two unique loci of size 1.5 kb for CA049Y04 and >1.3 kb for CA049Y04. It also amplified 2 unique loci among resistant genotypes of 1.3kb for CA049Y04 and >1.3 kb for CA049Y04. This primer also amplified total of 9 RAPD loci of which seven were polymorphic (77.7%). It also amplified two unique loci among all genotypes and 7 unique loci among resistant and susceptible genotypes. Two unique loci of size >0.5 kb for CA 34516 and CA14520. Among susceptible genotypes the unique loci of size >0.5 kb for CA03149 and >0.9 kb for

CA003124, among resistant inbred line, unique loci of size 0.8 kb for CA14518, and >0.5 for CA03124 and <0.5 for CA03124 and <0.5 kb for CML 475. This primer also amplified total of 9 RAPD loci of which 8 polymorphic (88.8%), with group IV genotypes. It also amplified two unique loci among all genotypes of >0.3 kb for CA14527 and <0.5 kb for CA00104. It also amplified 4 unique loci between resistant and susceptible inbred line. Unique loci, among susceptible genotypes of >0.5 kb for CA14527 and <0.5 kb for CA00104 and two unique loci among resistant genotypes of size >0.5 kb for 556 and <0.5 kb for 556. Total number of RAPD loci, polymorphic loci and unique loci in all inbred line, and unique loci in resistant and susceptible genotypes amplified by primer UBC 572 given in Table 28 to 35 and 41 to 44.

4.3.3.9 Primer UBC 534

On agarose gel, total 11 RAPD loci amplified by this primer with Group I genotypes out of which 9 were polymorphic (81%). It also amplified one unique loci among all genotypes, and unique loci also amplified in between resistant and susceptible inbred line. The unique loci of 1.9 kb amplified for CM 209. It amplified one unique loci of 1.9 kb for CM 209 and two unique loci of 1.3 kb and <0.5 kb for CM 105 and CM 500 respectively. This primer also amplified total of 6 RAPD loci, with (group II) out of which 6 found polymorphic (100%). This primer did not give any unique loci in among all inbred or between resistant and susceptible inbred line. This primer amplified total of 8 RAPD loci with group III genotype, out of which 6 found polymorphic (75%). It also amplify two unique loci among all and 2 unique loci in between resistant and susceptible inbred line. Two unique loci of >0.9 kb and >0.9 kb for CA03106, CML 433 and it also amplified two unique loci among susceptible genotype of <0.9 CA00316 and >0.9 of CA00334. this primer amplified total of 11 RAPD loci with group IV genotypes, out of which 7 found polymorphic (63%). It amplified five unique loci among all genotypes, of >1.3 kb size for 550, and <1.3 kb for 570, <1.3 for

CA00104 and 0.8 kb size for 552, and <0.5 for 556. It also amplified 6 unique loci among resistant and susceptible genotypes of 1.3 kb size of 550 and <1.3 kb CA00104 and <0.9 kb for 552 and in three resistant genotypes of size 0.05 kb for 556 and 0.5 kb for 566 and <0.5 for 556. The total number of RAPD loci, polymorphic loci and unique loci in all genotypes and unique loci in resistant and susceptible genotypes amplified by primer UBC 534 given in Table 28 to 35 and 41 to 44.

4.3.3.10 Primer UBC 350

On agrose gel, total 111 RAPD loci amplified with group II genotypes of which were found polymorphic (90%). It also amplified one unique loci in all genotypes and 4 unique loci among resistant and susceptible inbred line. One unique loci of size <0.5 kb for CM 500 and 4 unique loci of 1.9 kb for Amar and >0.5 kb for CM 600 and <0.5 kb for CM 600 and <0.5 kb for CM 500. On agrose gel this primer amplified total 6 RAPD loci with group II genotypes, of which 5 were found polymorphic 8.3 per cent. This primer amplified unique loci of <0.5 kb for CA00106. This primer amplified total 4 RAPD loci, with 4 polymorphic loci (100%) in case of susceptible genotypes and 5 RAPD loci, with 2 polymorphic loci (40%) with one unique loci in resistant inbred line. This primer amplified total of 8 RAPD loci with group III genotypes of which 6 were found polymorphic (75%), this primer also amplified 2 unique loci among all genotypes and three unique loci in between susceptible and resistant inbred line. The unique loci with size of 2.0 kb in case of CA00310 and >0.8 in case of CA8124. Unique loci with three resistant genotypes were 2.0 kb for CML 465 and 0.8 kb for CA03124 and 0.5 kb for CA03124. This primer also amplified total 8 RAPD loci for susceptible genotypes of which 7 were found polymorphic (87.5%) with no unique loci. With resistant genotypes amplified total 6 RAPD loci out of which 4 were polymorphic (66.6%) and three loci were polymorphic. This primer also amplified total 7 RAPD loci group IV with four polymorphic loci (57.1%). It

also amplified three unique loci among all inbred studied and 7 unique loci among resistant and susceptible inbred line. Three unique loci, with size of >0.5 kb for CA03142, >0.5 CA03142, <0.5 551. This primer amplified total 4 unique loci among resistant and susceptible genotypes of which three unique loci of size 0.9 kb for CA 14501, <0.9 for CA14501, <0.5 kb for CA14519 all three were susceptible genotypes and one unique loci of size <0.5 kb for 561. The total number of RAPD loci, polymorphic loci and unique loci amplified by this primer are given in Table 28 to 35 and 41 to 44.

4.3.3.11 Primer UBC 386

On agarose gel this primer amplified total 7 RAPD loci with Group I out of which 6 were found polymorphic (85%). It also amplified a unique loci among all genotypes, and three unique loci among susceptible and resistant genotypes. One unique loci among all genotypes of 1.5 kb size for CM 121 and three unique loci of size 1.5 kb for CM 121, and 0.5 kb size for cm 210 and >0.5 kb size for CM 500. On agarose gel this primer amplified total 10 RAPD loci with group II genotypes with 8 polymorphic loci (80%), and it also amplified two unique loci among all genotypes and two unique loci among resistant and susceptible inbred line. The unique loci among all genotypes of size <0.5 kb for CA14509 and <0.5 kb for CA00106 and unique loci among resistant and susceptible genotypes of size 1.9 kb for CA03147 and 1.9 kb for CA00106 and <0.5 for CA14509. This primer UBC 386 also amplified total 9 RAPD loci with group III genotypes out of which 8 were found polymorphic (88.8%). It also amplified one unique loci among all genotypes and three unique loci among resistant and susceptible inbred line. One unique loci among all genotypes, of size <0.5 kb for CA03106 and three unique loci among resistant and susceptible genotypes of size <0.5 kb for CA03131, <0.5 kb for CA03106 and 0.5 kb for CML 465. This primer also amplified total of 10 RAPD loci with group IV inbred out of which 6 were found polymorphic (60%) and it also gave four unique loci among all genotypes and 6

among all resistant and susceptible genotypes. The four unique loci among all genotypes were of size <0.5 kb for 561, and <0.5 kb for CA00104 and <0.5 for CA00104, <0.5 for CA14519. Unique loci among resistant and susceptible line of 0.5 kb for CA00104 and <0.5 for CA14519 both were susceptible and four genotypes with unique loci of 0.8 kb for 556 and >0.5 kb for 561 and <0.5 kb or 561 and <0.5 kb for 556. The total number of RAPD loci, polymorphic loci and unique loci in all genotypes and unique loci in resistant and susceptible genotypes amplified by primer UBC 386 in Table 28 to 35 and 41 to 44.

4.6 Genetic variation based on RAPD markers

Associations among the maize genotypes revealed by unweighted pair group method with Arithmetic mean (UPGMA) cluster analysis are presented. The dendrogram reading separated the inbred cluster line (Table 45).

The result described in group wise (I, II, III and IV). In case of group first the similarity coefficient ranged from a minimum of 0.26 to maximum of 0.68. All fifteen genotype divided into cluster I, II and III. Cluster I further divided into two minor clusters (I) and (II). Minor cluster (I) contain three accessions, or genotypes 13, 14 and 15. The maize genotypes 13 and 14 showed similarity of 61 per cent and genotypes 14 and 5 related with similarity of 53 per cent. Cluster two divided into two minor cluster I and II. Minor cluster I, retain genotypes 5 and 9 with similarity of 57 per cent and 6 and 7 with similarity of 52 per cent. Cluster III, contain three minor cluster I, II and III, minor cluster I, and 2 showed similarity of about 68 per cent and genotypes 2 and 3 showed similarity of about 63 per cent. Cluster I and II showed similarity of 45 per cent and cluster II and III showed similarity of about 49 per cent.

In case of second group the similarity coefficient ranged from minimum of 1.5 per cent to maximum of 64.1 per cent. All eighteen genotype was divided into four clusters. Cluster I, retains two maize genotypes 8-18 with similarity of 25 per cent, cluster two contain, 9 maize inbred line. genotypes showed

Table 45 : Similarity coefficient of group I maize genotypes

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1														
2	0.686	1													
3	0.636	0.633	1												
4	0.588	0.589	0.632	1											
5	0.268	0.339	0.339	0.298	1										
6	0.333	0.424	0.397	0.386	0.5	1									
7	0.356	0.443	0.438	0.361	0.404	0.529	1								
8	0.339	0.381	0.422	0.39	0.469	0.453	0.421	1							
9	0.345	0.483	0.406	0.373	0.578	0.52	0.455	0.491	1						
10	0.456	0.542	0.508	0.365	0.357	0.5	0.466	0.5	0.509	1					
11	0.362	0.381	0.444	0.367	0.5	0.571	0.397	0.6	0.549	0.527	1				
12	0.357	0.474	0.397	0.339	0.5	0.51	0.444	0.481	0.583	0.473	0.604	1			
13	0.407	0.542	0.462	0.344	0.333	0.473	0.417	0.474	0.456	0.571	0.556	0.558	1		
14	0.362	0.426	0.492	0.367	0.385	0.453	0.397	0.455	0.463	0.527	0.6	0.51	0.615	1	
15	0.328	0.328	0.453	0.377	0.396	0.436	0.339	0.439	0.473	0.458	0.547	0.491	0.536	0.64	1

similarity of 52 per cent with genotypes 17. Genotypes 11 showed similarity of 57 per cent. Inbred line 16 showed similarity of 45 per cent genotypes 12 showed similarity of 64 per cent genotypes 15. Genotypes 13 showed similarity of 5 per cent with inbred 13. Cluster III contain three genotypes 5, 6 and 7. Inbred line 5 showed similarity of 57 per cent with inbred 6 and genotypes 6 showed similarity of 40 per cent with 7. Cluster IV contains four genotypes 1, 2, 3 and 4. Inbred line 1 and 2 related with 53 per cent of similarity and four with similarity of 54 per cent (Table 46).

Genotypes of group III, so similarity coefficient ranged from 0.15 to 0.58 per cent. Dendrogram divided into four clusters. Cluster I contain three genotypes 10, 18 and 11. Inbred line 10 showed similarity of 48.1 which genotypes 18 and 18 showed similarity of 41 per cent in cluster II genotypes 8 showed 46 per cent of similarity (Table 47). Genotypes 12 showed similarity of 51 per cent with genotypes 13, and genotypes 17 showed similarity of 0.5 per cent and genotypes 16 showed similarity of 48 per cent with genotypes 15. In cluster III, genotypes five showed similarity of 4 per cent with genotypes 9 and genotypes seven showed similarity of 4 per cent with genotypes six. In cluster IV, genotypes 1 showed similarity of 40 per cent with 2 and genotypes three showed similarity of 47 per cent with inbred four.

Cluster analysis of group IV genotypes showed minimum similarity coefficient ranged from 14.8 to 68.8, maximum of 68 per cent. Dendrogram divided into four clusters (Table 48). Cluster I, showed that genotypes 16 showed 61 per cent similarity with genotypes 17 and genotypes 18 showed 66 per cent similarity with genotypes 16. Cluster III showed genotypes 9 showed 59 per cent of similarity with genotypes 10 and genotypes 10 showed 55 per cent. Similarity with genotypes twelve and genotypes twelve showed similarity of 58 per cent with 14 and genotypes 14 showed 48 per cent. Similarity with genotypes eleven and genotypes 13 showed 57 per cent similarity with genotypes fifteen. In cluster II, genotypes 3 showed 48 per cent similarity with genotypes four and genotypes

Table 46 : Similarity coefficient of group II maize genotypes

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	
A1	1																		
A2	0.535	1																	
A3	0.522	0.489	1																
A4	0.457	0.523	0.543	1															
A5	0.313	0.286	0.314	0.422	1														
A6	0.294	0.245	0.346	0.367	0.575	1													
A7	0.275	0.275	0.278	0.347	0.409	0.512	1												
A8	0.193	0.236	0.22	0.255	0.3	0.36	0.367	1											
A9	0.24	0.265	0.294	0.313	0.439	0.409	0.419	0.362	1										
A10	0.226	0.275	0.302	0.269	0.409	0.444	0.422	0.396	0.525	1									
A11	0.327	0.383	0.353	0.347	0.442	0.477	0.391	0.314	0.419	0.422	1								
A12	0.255	0.333	0.333	0.3	0.452	0.488	0.432	0.435	0.429	0.465	0.615	1							
A13	0.271	0.22	0.327	0.265	0.415	0.488	0.395	0.37	0.462	0.5	0.5	0.553	1						
A14	0.265	0.216	0.269	0.286	0.405	0.442	0.386	0.488	0.415	0.356	0.525	0.538	0.541	1					
A15	0.26	0.313	0.264	0.255	0.364	0.432	0.348	0.383	0.439	0.409	0.512	0.649	0.568	0.513	1				
A16	0.298	0.298	0.226	0.24	0.487	0.452	0.333	0.4	0.462	0.429	0.579	0.475	0.514	0.541	0.526	1			
A17	0.192	0.265	0.222	0.235	0.283	0.378	0.356	0.422	0.526	0.419	0.564	0.5	0.541	0.45	0.513	0.5	1		
A18	0.152	0.178	0.118	0.102	0.282	0.262	0.268	0.25	0.324	0.3	0.405	0.417	0.455	0.324	0.563	0.5	0.441	1	

Table 47 : Similarity coefficient of group III maize genotypes

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	
A1	1																		
A2	0.409	1																	
A3	0.463	0.455	1																
A4	0.333	0.362	0.476	1															
A5	0.244	0.304	0.234	0.289	1														
A6	0.167	0.262	0.275	0.244	0.306	1													
A7	0.159	0.17	0.262	0.205	0.4	0.4	1												
A8	0.178	0.295	0.222	0.25	0.308	0.257	0.278	1											
A9	0.195	0.178	0.214	0.214	0.306	0.379	0.313	0.467	1										
A10	0.273	0.277	0.289	0.349	0.317	0.237	0.225	0.378	0.306	1									
A11	0.222	0.18	0.239	0.213	0.293	0.15	0.2	0.25	0.15	0.472	1								
A12	0.214	0.222	0.205	0.233	0.256	0.273	0.189	0.484	0.4	0.441	0.371	1							
A13	0.152	0.267	0.17	0.25	0.342	0.294	0.179	0.455	0.294	0.417	0.389	0.586	1						
A14	0.213	0.326	0.157	0.204	0.341	0.297	0.136	0.368	0.333	0.41	0.421	0.515	0.486	1					
A15	0.159	0.279	0.178	0.262	0.361	0.313	0.158	0.353	0.355	0.256	0.263	0.375	0.394	0.471	1				
A16	0.178	0.188	0.17	0.222	0.244	0.189	0.211	0.371	0.294	0.378	0.316	0.438	0.412	0.444	0.394	1			
A17	0.19	0.2	0.182	0.238	0.297	0.206	0.194	0.452	0.323	0.263	0.382	0.536	0.5	0.485	0.483	0.5	1		
A18	0.186	0.17	0.205	0.262	0.4	0.2	0.294	0.353	0.355	0.485	0.412	0.419	0.394	0.351	0.257	0.394	0.387	1	

Table 48 : Similarity coefficient of group IV maize genotypes

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	
A1	1																		
A2	0.46	1																	
A3	0.302	0.373	1																
A4	0.264	0.478	0.488	1															
A5	0.265	0.313	0.513	0.462	1														
A6	0.241	0.259	0.422	0.378	0.462	1													
A7	0.26	0.28	0.463	0.415	0.559	0.45	1												
A8	0.203	0.241	0.36	0.32	0.488	0.61	0.442	1											
A9	0.172	0.232	0.477	0.34	0.415	0.465	0.553	0.523	1										
A10	0.2	0.241	0.4	0.326	0.4	0.488	0.462	0.548	0.59	1									
A11	0.148	0.203	0.314	0.25	0.395	0.477	0.356	0.568	0.535	0.524	1								
A12	0.154	0.245	0.425	0.41	0.471	0.41	0.457	0.405	0.556	0.688	0.487	1							
A13	0.161	0.2	0.348	0.304	0.41	0.429	0.4	0.561	0.525	0.553	0.575	0.559	1						
A14	0.222	0.241	0.34	0.326	0.366	0.419	0.39	0.444	0.476	0.667	0.488	0.588	0.513	1					
A15	0.235	0.231	0.429	0.289	0.514	0.381	0.459	0.476	0.513	0.541	0.525	0.5	0.6	0.5	1				
A16	0.26	0.255	0.463	0.381	0.432	0.381	0.459	0.378	0.475	0.541	0.419	0.545	0.436	0.583	0.543	1			
A17	0.245	0.314	0.37	0.386	0.366	0.452	0.425	0.444	0.512	0.463	0.422	0.459	0.513	0.622	0.5	0.629	1		
A18	0.255	0.25	0.386	0.311	0.421	0.439	0.486	0.4	0.463	0.45	0.409	0.405	0.357	0.487	0.571	0.667	0.611	1	

five showed similarity of 55 per cent with genotypes seven. Genotypes 6 showed similarity of 61 per cent to genotypes 8.

4.7 SDS-PAGE analysis of maize genotypes

In the electrophoretic study of the present investigation, the protein were extracted and then separated on the basis of size and electric charge in a gel matrix, positioned in an electric field. This separation of proteins gave a specific protein band pattern, characteristics of the given genotype under investigation. The results of SDS-PAGE analysis obtained in all the genotypes are given below. Association among the maize genotypes revealed by unweighted pair group method with arithmetic (UPGMA) cluster analysis are presented. The dendrogram readily separated in inbred line into clusters (Table 49 to 55).

Maize genotypes used in SDS-PAGE analysis grouped into seven group and result are also given groupwise. Dendrogram divided all the maize genotypes into three clusters I, II, III, IV. In cluster I, contains maize genotypes, genotypes 9 and 11 showed similarity of about 90 per cent. And maize genotypes 12 showed similarity of 100 per cent with genotypes thirteen and genotypes thirteen showed similarity of 87 per cent with genotypes ten. In cluster II, genotypes four showed absolute similarity with genotypes five and genotypes five also showed the 100 per cent. Similarity with genotypes six. Genotypes six showed 89 per cent of similarity with seven, genotypes seven showed the 88 per cent similarity with genotypes eight. In cluster one, genotypes one showed the absolute similarity.

The II group maize genotype, showed the minimum similarity coefficient 68 per cent to maximum of 100 per cent. Dendrogram divided into three cluster. Cluster one contain inbred line two with 88 per cent similarity with genotypes three. Cluster tow contain, genotypes six with similarity of 100 per cent genotypes eight and genotypes eight also showed similarity of 100 per cent with the genotypes nine. Inbred line nine, then and eleven also showed 100 per cent

PROTEIN**GROUP-I**

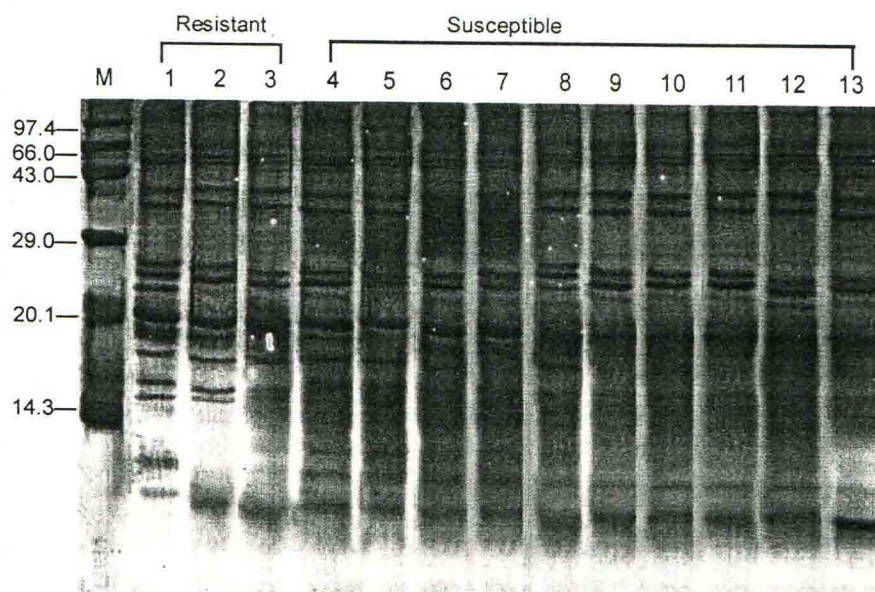
MARKER(medium range)

1. CM104
2. CM105
3. CM500
4. CM121
5. CM115
6. CM119
7. CM120
8. CM123
9. CM125
10. CM400
11. CM209
12. CM210
13. CM111

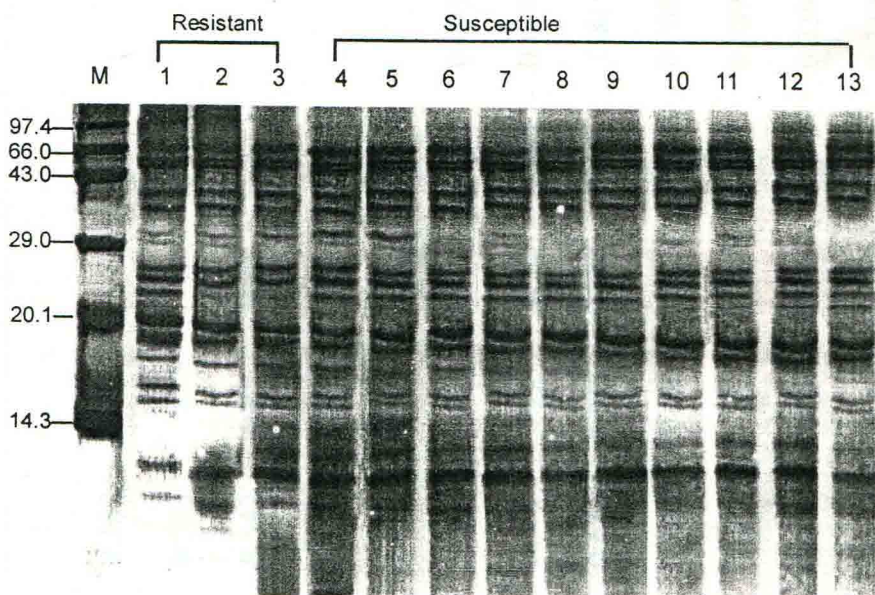
GROUP-II

Marker (medium range)

1. CM600
2. CA00106
3. C49704
4. Amar
5. CA03106
6. CA003134
7. CA00370
8. CA34507
9. CA00102
10. CA00396
11. CA00334
12. CA34506
13. CA03131



SDS-PAGE- PROFILE (Group-I)



SDS-PAGE- PROFILE (Group-II)

Table 49 : Similarity coefficient based on UPGMA Jaccard's coefficient similarity matrix of group I

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13
A1	1												
A2	0.583	1											
A3	0.583	1	1										
A4	0.542	0.85	0.85	1									
A5	0.542	0.85	0.85	1	1								
A6	0.542	0.85	0.85	1	1	1							
A7	0.542	0.762	0.762	0.895	0.895	0.895	1						
A8	0.591	0.75	0.75	0.889	0.889	0.889	0.889	1					
A9	0.625	0.773	0.773	0.81	0.81	0.81	0.727	0.8	1				
A10	0.556	0.75	0.75	0.708	0.708	0.708	0.708	0.625	0.792	1			
A11	0.64	0.783	0.783	0.739	0.739	0.739	0.739	0.727	0.909	0.875	1		
A12	0.64	0.783	0.783	0.739	0.739	0.739	0.739	0.727	0.909	0.875	1	1	
A13	0.64	0.783	0.783	0.739	0.739	0.739	0.739	0.727	0.909	0.875	1	1	1

Table 50 : Similarity coefficient based on UPGMA Jaccard's coefficient similarity matrix of group II

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13
B1	1												
B2	0.833	1											
B3	0.737	0.882	1										
B4	0.833	0.778	0.882	1									
B5	0.889	0.833	0.833	0.941	1								
B6	0.778	0.722	0.824	0.938	0.882	1							
B7	0.889	0.833	0.833	0.941	1.000	0.882	1						
B8	0.778	0.722	0.824	0.938	0.882	1.000	0.882	1					
B9	0.778	0.722	0.824	0.938	0.882	1.000	0.882	1	1				
B10	0.833	0.778	0.778	0.882	0.941	0.938	0.941	0.938	0.938	1			
B11	0.833	0.778	0.778	0.882	0.941	0.938	0.941	0.938	0.938	1.000	1		
B12	0.833	0.684	0.778	0.882	0.833	0.938	0.833	0.938	0.938	0.882	0.882	1	
B13	0.833	0.684	0.778	0.882	0.833	0.938	0.833	0.938	0.938	0.882	0.882	1.000	1

Table 51 : Similarity coefficient based on UPGMA Jaccard's coefficient similarity matrix of group III

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13
C1	1												
C2	0.950	1											
C3	0.792	0.826	1										
C4	0.833	0.792	0.958	1									
C5	0.68	0.64	0.8	0.84	1								
C6	0.8	0.76	0.92	0.96	0.88	1							
C7	0.76	0.72	0.88	0.92	0.84	0.96	1						
C8	0.792	0.75	0.917	0.958	0.8	0.92	0.958	1					
C9	0.792	0.75	0.917	0.958	0.8	0.92	0.958	1.000	1				
C10	0.792	0.75	0.917	0.958	0.8	0.92	0.958	1.000	1.000	1			
C11	0.792	0.75	0.917	0.958	0.8	0.92	0.958	1.000	1.000	1.000	1		
C12	0.792	0.75	0.917	0.958	0.8	0.92	0.958	1.000	1.000	1.000	1.000	1	
C13	0.792	0.75	0.917	0.958	0.8	0.92	0.958	1.000	1.000	1.000	1.000	1.000	1

Table 52 : Similarity coefficient based on UPGMA Jaccard's coefficient similarity matrix of group IV

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13
D1	1												
D2	0.6	1											
D3	0.5	0.792	1										
D4	0.5	0.792	1.000	1									
D5	0.5	0.792	1.000	1.000	1								
D6	0.5	0.792	1.000	1.000	1.000	1							
D7	0.5	0.792	1.000	1.000	1.000	1.000	1						
D8	0.5	0.792	1.000	1.000	1.000	1.000	1.000	1					
D9	0.5	0.792	1.000	1.000	1.000	1.000	1.000	1.000	1				
D10	0.5	0.792	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1			
D11	0.5	0.792	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1		
D12	0.5	0.792	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1	
D13	0.5	0.792	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1

Table 53 : Similarity coefficient based on UPGMA Jaccard's coefficient similarity matrix of group V

	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13
E1	1												
E2	0.600	1											
E3	0.640	0.955	1										
E4	0.640	0.955	1	1									
E5	0.577	0.720	0.760	0.760	1								
E6	0.640	0.720	0.760	0.760	0.692	1							
E7	0.640	0.720	0.760	0.760	0.692	1.000	1						
E8	0.640	0.720	0.760	0.760	0.692	1.000	1.000	1					
E9	0.640	0.720	0.760	0.760	0.692	1.000	1.000	1.000	1				
E10	0.640	0.720	0.760	0.760	0.692	1.000	1.000	1.000	1.000	1			
E11	0.680	0.760	0.800	0.800	0.731	0.957	0.957	0.957	0.957	0.957	1		
E12	0.640	0.720	0.760	0.760	0.692	1.000	1.000	1.000	1.000	1.000	0.957	1	
E13	0.667	0.680	0.720	0.720	0.654	0.955	0.955	0.955	0.955	0.955	0.913	0.955	1

Table 54 : Similarity coefficient based on UPGMA Jaccard's coefficient similarity matrix of group VI

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
F1	1												
F2	0.750	1											
F3	0.750	1.000	1										
F4	0.750	1.000	1.000	1									
F5	0.688	0.938	0.938	0.938	1								
F6	0.688	0.938	0.938	0.938	1.000	1							
F7	0.688	0.938	0.938	0.938	1.000	1.000	1						
F8	0.750	1.000	1.000	1.000	0.938	0.938	0.938	1					
F9	0.688	0.938	0.938	0.938	1.000	1.000	1.000	0.938	1				
F10	0.688	0.938	0.938	0.938	1.000	1.000	1.000	0.938	1.000	1			
F11	0.688	0.938	0.938	0.938	1.000	1.000	1.000	0.938	1.000	1.000	1		
F12	0.688	0.938	0.938	0.938	1.000	1.000	1.000	0.938	1.000	1.000	1.000	1	
F13	0.688	0.938	0.938	0.938	1.000	1.000	1.000	0.938	1.000	1.000	1.000	1.000	1

Table 55 : Similarity coefficient based on UPGMA Jaccard's coefficient similarity matrix of group VII

	G1	G2	G3	G4	G5	G6	G7	G8
G1	1							
G2	0.813	1						
G3	0.632	0.75	1					
G4	0.789	0.632	0.833	1				
G5	0.789	0.632	0.833	1.000	1			
G6	0.842	0.684	0.789	0.947	0.947	1		
G7	0.842	0.684	0.789	0.947	0.947	1.000	1	
G8	0.842	0.684	0.789	0.947	0.947	1.000	1.000	1

similarity. Similarity twelve and 13 were also 100 per cent. Similar in cluster I, the inbred one showed 83 per cent similarity with inbred four, and inbred five showed 100 per cent similarity with genotypes seven.

In group III, the dendrogram divided into two cluster. Cluster one contain eleven inbred line, genotypes five showed 0.8 per cent similarity with genotypes thirteen. Genotypes 8, 9, 10, 11, 12 and 13 showed 100 per cent similarity to each other. Inbred line four showed 96 per cent similarity with the genotypes six. Genotypes three showed 95 per cent similarity with genotypes four. In cluster I, genotypes showed 95 per cent similarity with genotypes two. In group IV, genotypes divided into two cluster, cluster one contain genotypes 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13 all genotypes showed 100 per cent. Similarity to each other and genotypes 1 showed 95 per cent similarity with genotypes two.

In group V, maize genotypes, the dendrogram divided into the three cluster, cluster one contain genotypes 6, 7, 8, 9, 10, 11, 12 and 13, all showed 100 per cent similarity. Cluster II contain inbred line, five which showed 76 per cent similarity with genotypes four and, inbred four showed 100 per cent similarity with inbred three and inbred tow showed 100 per cent similarity with three. And genotypes one showed 0.6 per cent similarity with genotypes two.

In group VI, maize genotypes divided into tow cluster, by denedrogram, all the genotypes showed 100 per cent similarity with each other. In case of group VII, genotypes divided into two clusters. Inbred line 3 showed 100 per cent similarity with genotypes one. In cluster II, genotypes 4, 5, 6, 7 and 8 showed hundred per cent similarity and genotypes one showed 78 per cent similarity with genotypes four.

A perusal of result summarized for group I maize genotypes in Table 56. revealed that the in zone I all the genotypes showed similar banding pattern, except, genotypes 5, 6, 7 which showed thick band of >66.0 which is absent in other inbred line, and all three genotypes were susceptible. In zone I, genotypes

11, 12 and 13 also showed similar with thin band which were absent in all the other inbred line, and all three genotypes were susceptible. In zone II, all the genotypes showed similar banding pattern, band three thin band absent in genotypes five which was also susceptible inbred line. In zone III two thick band present in case of the resistant genotypes 1 and 2 but in other genotypes these thick bands were absent. In zone four, two thick band <14.3 kd which were present in all susceptible genotypes but absent in resistant genotypes I and II. In zone IV, one thick band of <14.3 kb present in resistant genotypes which was absent in all other inbred line. Total number of band present in group I genotypes given in Table 56.

In case of Group II maize inbred line. The total number of band present or absent given in Table 57. In group II, in zone 1, one thin band which present in 2 and 3 genotypes absent in all the other maize inbred line. The genotypes of 2 and 3 lane were resistant, in zone II, one thin band of size <43.0 kb present in genotypes 1 and 2, were absent in all the other maize genotypes and genotypes 1 and 2 were resistant. In zone II, one thick band of size 22.1 kb present only in resistant genotypes 1, which was resistant absent in all other inbred line. In zone four a thin band of size less than 20.1 kd present in all the three resistant genotypes and absent in other maize inbred line. One band of size greater than 14.3 kd absent in two genotypes but 1 and 2, but present in all the other inbred line and zymogram of all genotypes.

The results of group III maize genotypes summarized in Table 58. In zone I in maize genotypes two thick band of size <66.0 kd present in resistant genotypes of lane 1, which was absent in all the other maize inbred line. In zone two one thick band present in all the genotypes except in, resistant genotypes of lane 1. In zone III, one band of size <14.3 kd present in all the genotypes but absent in resistance genotypes 1. In zone IV one band of size <14.3 present in resistant genotypes but absent in all the other inbred line.

GROUP-III

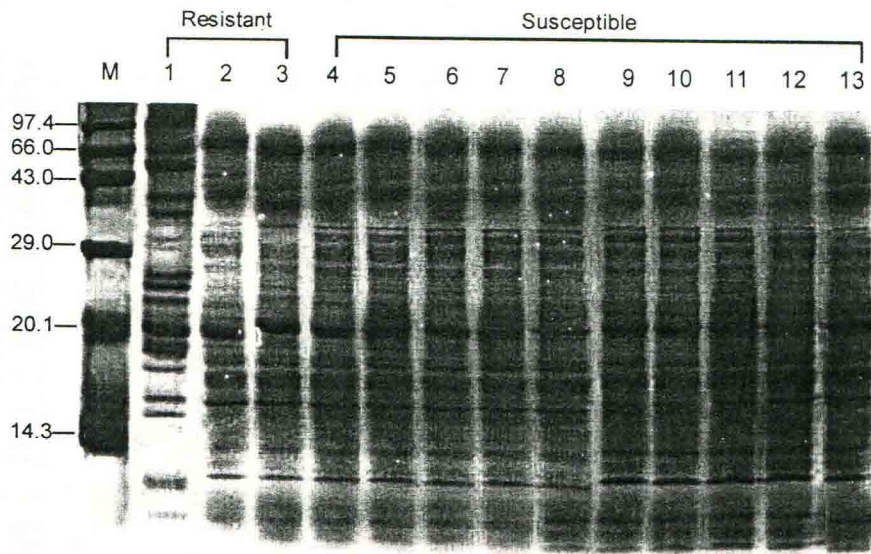
Marker (medium range)

1. CA003147
2. CA14509
3. CA141518
4. CA34516
5. CA03149
6. CA14510
7. CA00332
8. A00334
9. CML425
10. CML430
11. CA03124
12. CA00378
13. CML421

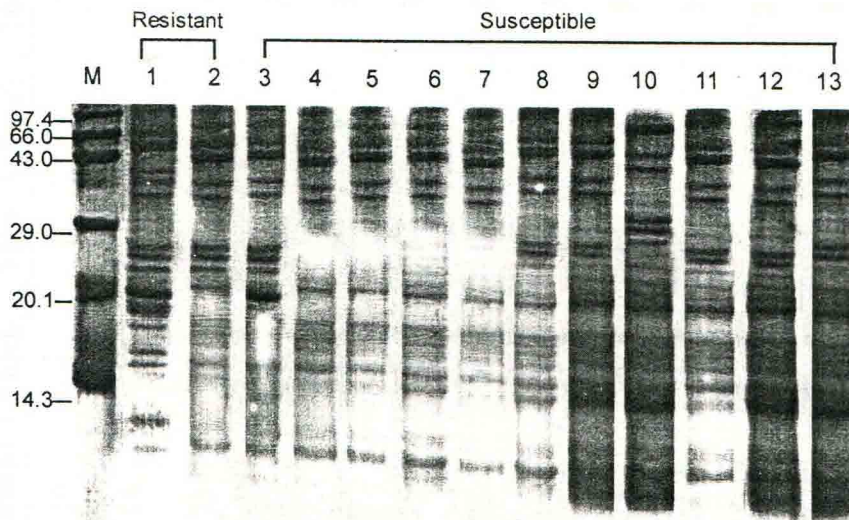
GROUP-IV

Marker (medium range)

1. CA00310
2. CA03124
3. CA14711
4. Pop. 147-F#161-3-2-B-1-B
5. CA14519
6. CA00104
7. CA03142
8. CA14527
9. CA03130
10. CA14527
11. CA03130
12. CA14527
- 13.



SDS-PAGE- PROFILE (Group-III)



SDS-PAGE- PROFILE (Group-IV)

Table 56 : Number of protein bands with group I genotypes

Generation	Total no. of bands	Zone 1			Zone 2			Zone 3			Zone 4		
		Thick	Thin	Total	Thick	Thin	Total	Thick	Thin	Total	Thick	Thin	Total
1.	19	5	1	6	3	4	7	4	0	4	1	1	2
2.	19	5	1	6	3	4	7	4	0	4	0	2	2
3.	25	5	2	7	3	4	7	1	3	4	0	6	6
4.	25	5	2	7	3	5	8	2	2	4	0	6	6
5.	23	6	2	8	3	2	5	1	3	4	0	6	6
6.	25	6	1	7	3	5	8	1	4	5	0	6	6
7.	25	6	1	7	3	5	8	1	3	4	0	6	6
8.	23	5	1	6	3	5	8	1	2	3	0	6	6
9.	23	5	1	6	5	3	8	0	3	3	0	6	6
10.	23	5	1	6	3	5	8	0	3	3	0	6	6
11.	23	5	2	7	2	5	7	0	3	3	0	6	6
12.	24	5	2	7	3	5	8	0	3	3	0	6	6
13.	24	5	2	7	3	5	8	0	3	3	0	6	6

Table 57 : Number of protein bands with group II genotypes

Generation	Total no. of bands	Zone 1			Zone 2			Zone 3			Zone 4		
		Thick	Thin	Total	Thick	Thin	Total	Thick	Thin	Total	Thick	Thin	Total
1.	17	5	0	5	0	2	2	5	0	5	1	4	5
2.	17	5	1	6	0	2	2	4	0	4	1	4	5
3.	17	5	1	6	0	1	1	4	0	4	1	5	6
4.	16	5	0	5	0	1	1	5	0	5	1	4	5
5.	17	5	0	5	0	2	2	5	0	5	1	4	5
6.	15	5	0	5	0	0	0	5	0	5	1	4	5
7.	17	5	0	5	0	2	2	5	0	5	1	4	5
8.	15	5	0	5	0	0	0	5	0	5	1	4	5
9.	16	5	0	5	0	1	1	5	0	5	1	4	5
10.	17	5	0	5	0	2	2	5	0	5	1	4	5
11.	17	5	0	5	0	2	2	5	0	5	1	4	5
12.	16	5	0	5	0	1	1	5	0	5	1	4	5
13.	16	5	0	5	0	1	1	5	0	5	1	4	5

Table 58 : Number of protein bands with group III genotypes

Generation	Total no. of bands	Zone 1		Zone 2		Zone 3		Zone 4			
		Thick	Thin	Thick	Thin	Thick	Thin	Thick	Thin		
1.	19	4	1	4	4	2	2	4	2	0	2
2.	24	1	3	3	6	2	4	6	2	3	5
3.	23	1	3	3	6	2	4	6	2	2	4
4.	23	1	3	3	5	2	4	6	2	2	4
5.	23	1	3	3	5	2	4	6	2	2	4
6.	23	1	3	3	5	2	4	6	2	2	4
7.	23	1	3	3	5	2	4	6	2	2	4
8.	23	1	3	3	5	2	4	6	2	2	4
9.	23	1	3	3	5	2	4	6	2	2	4
10.	23	1	3	3	5	2	4	6	2	2	4
11.	23	1	3	3	5	2	4	6	2	2	4
12.	23	1	3	3	5	2	4	6	2	2	4
13.	23	1	3	3	5	2	4	6	2	2	4

Table 58 : Number of protein bands with group III genotypes

Generation	Total no. of bands	Zone 1		Zone 2		Zone 3		Zone 4		
		Thick	Thin	Thick	Thin	Thick	Thin	Thick	Thin	
1.	19	4	1	5	4	4	2	2	0	2
2.	24	1	3	4	3	6	2	2	3	5
3.	23	1	3	4	3	6	2	2	2	4
4.	23	1	3	4	3	5	2	2	2	4
5.	23	1	3	4	3	5	2	2	2	4
6.	23	1	3	4	3	5	2	2	2	4
7.	23	1	3	4	3	5	2	2	2	4
8.	23	1	3	4	3	5	2	2	2	4
9.	23	1	3	4	3	5	2	2	2	4
10.	23	1	3	4	3	5	2	2	2	4
11.	23	1	3	4	3	5	2	2	2	4
12.	23	1	3	4	3	5	2	2	2	4
13.	23	1	3	4	3	5	2	2	2	4

In group IV maize genotypes, zymogram revealed that one band of size <29.0 kb present in all the other maize genotypes but absent in, genotypes 10, which was a susceptible inbred line. In zone II, two thick band present in three resistant maize genotypes of size >20.1 kd present in other genotypes in very thin from in zone III, two bands of size <20.1 kd present in maize genotypes of lane 1, and it was absent in all the other genotypes. In zone IV one band of size <14.3 kb present in lane 1 absent in all the other maize genotypes. One band of size <14.3 kb present in all the other maize genotypes but absent in genotypes first. The result of total number of band present in group IV genotypes given in (Table 59).

The results of SDS-PAGE analysis of group V maize genotypes given in Table 60. The zymogram of all genotypes revealed that two bands of size 66.0 kd and >29.0 kd present in all the maize inbred line, but absent in maize genotypes 1 and in maize genotypes 13. One thin band of size <29.0 kd present in resistant genotypes 1, but absent other genotype. One thin band of size >20.1 kb present in three genotypes but absent in all the other genotypes. In zone II, two thin band of size <20.1 kd present in the resistant genotypes of lane 1, and it was absent in all the other maize genotypes. In zone IV, one band of size <14.0 kd present in all genotypes but absent in genotypes 1, 2, 3 and 4, two thick band of size <14.3 kd present in four genotypes, 1, 2, 3 and 4 but absent in all the other maize inbred line.

The result of SDS-PAGE analysis of group VI genotypes given in Table 61. In group VI the genotypes of moderately and highly susceptible group which showed disease reaction in between (2.0 to 4.0 kb). All the genotypes of this group in zone I, revealed two common bands of size > 43.0 kd, which were present in all the genotypes. In zone second two common band of size 29.0 kd and 25.0 kd size, represented all the susceptible genotypes. In zone III, one band of size 20.1 kd present in all the susceptible inbred line.

In group VII, maize genotypes total number of band present, given in Table 62. The zymogram of all the maize genotypes revealed that two bands of

Table 59 : Number of protein bands with group IV genotypes

Generation	Total no. of bands	Zone 1		Zone 2		Zone 3		Zone 4		
		Thick	Thin	Thick	Thin	Thick	Thin	Thick	Thin	
1.	17	3	2	4	6	5	0	2	0	2
2.	18	3	3	3	4	2	4	6	1	2
3.	19	3	3	2	4	2	4	6	1	3
4.	17	3	3	0	3	2	4	6	1	2
5.	17	3	3	0	3	2	4	6	1	2
6.	18	3	3	0	3	2	4	6	0	3
7.	19	4	2	0	4	2	4	6	1	3
8.	16	3	2	0	2	2	4	6	1	3
9.	20	3	2	0	5	1	5	6	1	4
10.	21	3	1	2	8	1	5	6	1	4
11.	14	3	1	2	8	2	4	6	1	4
12.	22	3	2	2	8	2	3	5	1	4
13.	23	3	2	2	8	2	4	6	1	4

Table 60 : Number of protein bands with group V genotypes

Generation	Total no. of bands	Zone 1		Zone 2		Zone 3		Zone 4			
		Thick	Thin	Thick	Thin	Thick	Thin	Thick	Thin		
1.	13	4	2	3	2	5	2	2	2	1	3
2.	21	4	2	0	7	7	2	3	2	1	3
3.	22	4	2	1	7	8	2	3	2	1	3
4.	21	4	2	1	6	7	2	3	2	1	3
5.	23	4	2	1	6	7	2	3	0	5	5
6.	19	4	2	1	6	7	2	3	0	4	4
7.	22	4	2	1	6	7	2	3	0	4	4
8.	22	4	2	1	6	7	2	3	0	4	4
9.	22	4	2	1	6	7	2	3	0	4	4
10.	22	4	2	1	6	7	2	3	0	4	4
11.	23	4	2	3	5	8	2	3	0	4	4
12.	21	4	2	1	4	5	2	3	0	4	4
13.	17	4	2	1	1	2	2	3	0	4	4

GROUP-V

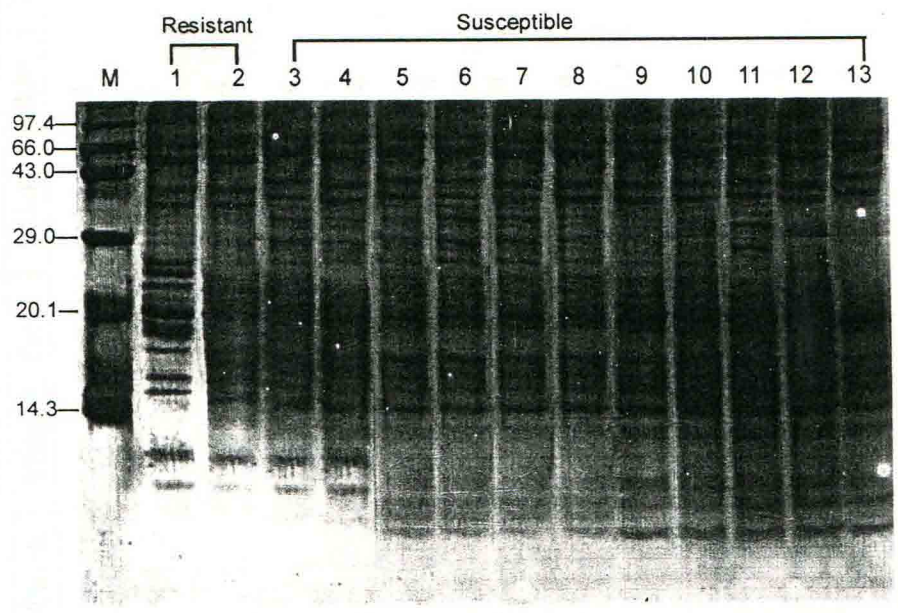
Marker (medium range)

1. 556
2. 561
3. CML413
4. CA14501
5. CA03116
6. CA14709
7. CA14524
8. CA14520
9. CML428
10. CA00334
11. 550
12. 551
13. 552

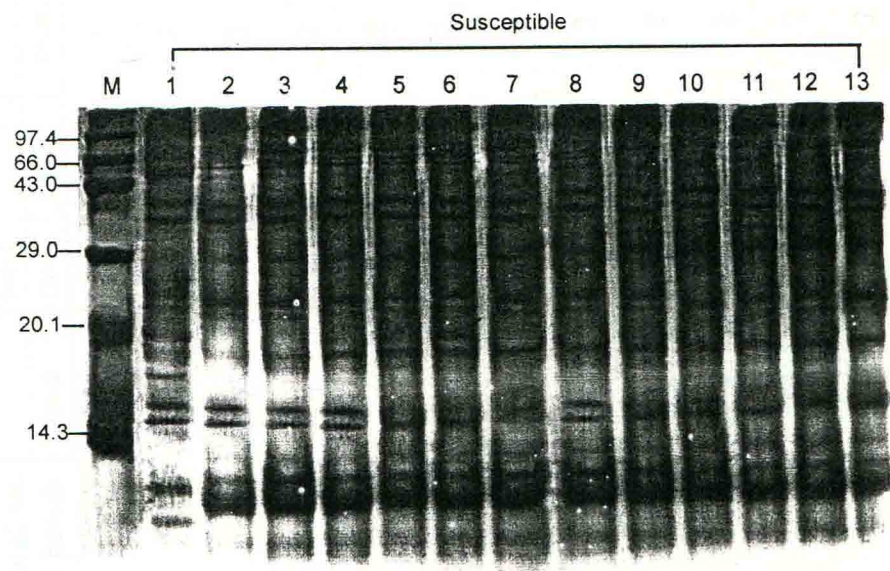
GROUP-VI

Marker (medium range)

1. Surya
2. D-995
3. D-994
4. 553
5. 554
6. 556
7. 557
8. 558
9. 559
10. 560
11. 561
12. 562
13. 563



SDS-PAGE- PROFILE (Group-V)



SDS-PAGE- PROFILE (Group-VI)

Table 61 : Number of protein bands with group VI genotypes

Generation	Total no. of bands	Zone 1		Zone 2		Zone 3		Zone 4			
		Thick	Thin	Thick	Thin	Thick	Thin	Thick	Thin		
1.	2	0	2	1	1	2	1	3	1	3	4
2.	2	3	5	2	0	2	1	3	1	4	5
3.	2	3	5	2	0	2	1	3	1	4	5
4.	2	3	5	2	0	2	1	3	1	4	5
5.	2	3	5	2	0	2	1	2	1	4	5
6.	2	3	5	2	0	2	1	2	1	4	5
7.	2	3	5	2	0	2	1	2	1	4	5
8.	2	3	5	2	0	2	1	3	1	4	5
9.	2	3	5	2	0	2	1	2	1	4	5
10.	2	3	5	2	0	2	1	2	1	4	5
11.	2	3	5	2	0	2	1	2	1	4	5
12.	2	3	5	2	0	2	1	2	1	4	5
13.	2	3	5	2	0	2	1	2	1	4	5

Table 62 : Number of protein bands with group VII genotypes

Generation	Total no. of bands	Zone 1		Zone 2		Zone 3		Zone 4	
		Thick	Thin	Thick	Thin	Thick	Thin	Thick	Thin
1.	18	2	1	2	2	4	4	5	2
2.	14	2	1	2	2	4	0	3	1
3.	17	2	3	2	2	4	0	3	1
4.	20	2	3	2	2	4	0	3	4
5.	20	2	3	2	2	4	0	3	4
6.	20	2	3	2	2	4	0	3	4
7.	20	2	3	2	2	4	0	3	4

I	43.0 kd	II	29.0 kd	III	20.1 kd	IV

size <43.0 kd present in genotypes 1 and 2 absent in all the other maize genotypes. One band of thin range present in all the maize genotypes but absent in 1 and 2 genotypes. Two thick band of size greater than 43.0 kd present in all the genotypes in zone I. In zone II two thick and two thin band of size <43.0 kd and greater than 29.0 kd present in all the genotypes. In zone III, four thick band present in all the maize genotypes. In zone IV, two thick band of size 20.1 kd present in genotypes 1, absent in thick from in all the genotypes but present in thin from in all the genotypes. Three thick and one thin band of size <20.1 kd present in all maize genotypes and zyamogram of all genotypes.

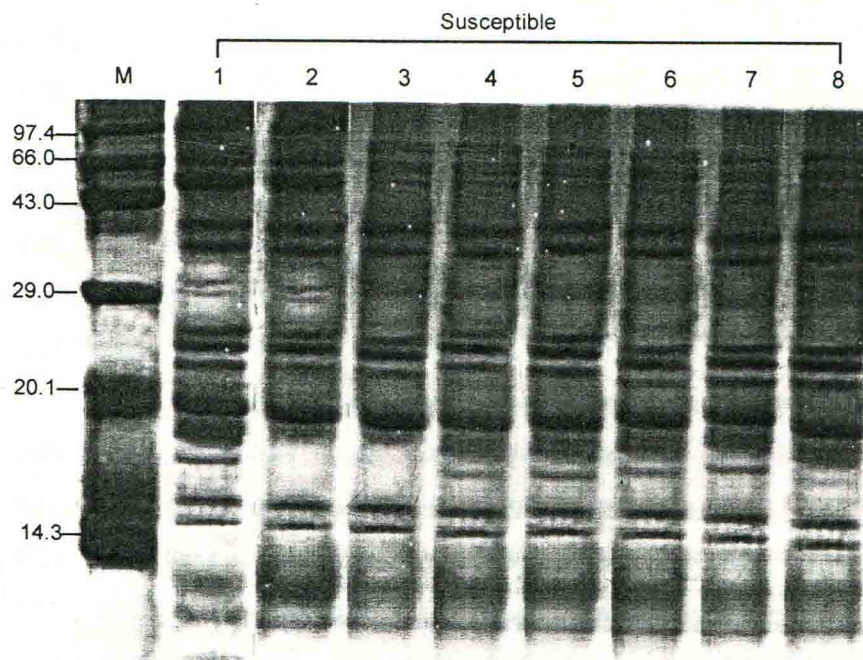
4.8 Relative mobility (Rf value)

Protein, when denatured by heating in presence of excess of SDS and 2-mercaptoethanol binds with 3DS in constant weight ratio as it they have essentially identical charge density. This complete migrate by all the polypeptides after staining was measured for the top of the resolving gel. The distance migrated was expressed as Fe value which was calculated by dividing the distance between origin and tracking dye. Each Rf value thus represented the mobility of a specific polypeptide in protein mixture. The Rf value calculated of each polypeptide band of different inbred were calculated given in Table 63 to 69.

GROUP-VII

Marker (medium range)

1. Kanchan
2. Amar
3. 565
4. 566
5. 567
6. 558
7. 569
8. 570



SDS-PAGE- PROFILE (Group-VII)

Table 66: Rf-value of protein bands observed in group IV genotypes

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13
1.	0.05	-	-	-	-	-	-	-	-	-	-	-	-
2.	-	0.10	0.10	0.10	0.10	0.10	0.10	-	-	0.10	-	-	-
3.	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	-	-	0.16	0.16
4.	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
5.	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	-	0.23	0.23	0.23
6.	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	-	0.25	0.25	0.25
7.	0.31	-	-	-	-	-	-	-	0.31	0.31	0.31	0.31	0.31
8.	0.33	-	-	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33
9.	-	-	-	-	-	-	-	-	-	0.35	0.35	0.35	0.35
10.	0.36	0.36	0.36	-	-	-	-	-	0.36	-	-	-	-
11.	0.38	0.38	0.38	-	-	-	-	-	0.38	0.38	0.38	0.38	0.38
12.	-	-	-	-	-	-	-	-	-	0.40	0.40	0.40	0.40
13.	0.41	0.41	0.41	-	-	-	0.41	-	-	0.41	0.41	0.41	0.41
14.	-	-	-	0.45	0.45	0.45	0.45	-	-	0.45	0.45	0.45	0.45
15.	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
16.	0.51	-	-	-	-	-	-	-	-	-	-	-	-
17.	0.53	-	-	-	-	-	-	-	-	-	-	-	-
18.	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58
19.	-	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
20.	-	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61
21.	-	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63
22.	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
23.	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
24.	0.85	-	-	-	-	-	-	-	-	-	-	-	-
25.	-	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
26.	-	-	-	-	-	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
27.	0.91	0.91	0.91	0.91	0.91	-	0.91	0.91	0.91	0.91	0.91	0.91	0.91
28.	-	1.00	1.00	-	-	1.00	-	-	1.00	1.00	1.00	1.00	1.00

Discussion

Maize is one of the world's leading cereal crops with an average productivity of 4.25 tonnes per hectare (FAO, 2002). In India the low productivity has increased during the last decade is due to several factors. The major factors responsible for bring down the yield of maize, disease are probably the most important. BLSB was earlier reported as a minor disease of maize (Payak and Renfro, 1966). The importance of this disease was only a curiosity in India until the 1970's when an epidemic occurred in warm and humid foothill areas in the Himalayas and the adjacent plains. This disease caused an estimated loss of 24-32 per cent in different cultivars depending on disease severity levels (Lal *et al.*, 1980).

For stabilizing yield of any crop, the availability of resistant varieties against major diseases is of paramount importance. As for as resistance breeding is convened, dependable source(s) of resistance is vital. In order to workout the morphological and molecular variation in BLSB resistant and susceptible genotypes the present investigation was under taken. For investigating morphological variation, different morphological character were studied under epiphytocal condition in field as well as glasshouse in condition; the morphological factors like plant height, cob placement and incubation period, blighting shape and blighting size, blighting colour, tassel texture and tassel colour and tasseling day and silking day were correlated with the severity of BLSB. For investigation the molecular variation the random amplified polymorphic DNA and SDS-PAGE were taken as a molecular markers. Experimental material comprises eighty six maize genotypes, which were obtained from CIMMYT office located at New Delhi, National sources, and Department of Genetics and Plant Breeding, G.B. Pant University of Agriculture

and Technology, Pantnagar. These genotypes were separated in two group resistant and susceptible, through field, glasshouse and laboratory evaluation technique.

5.1 Morphological marker or character

Morphological marker, plant height, cob placement and incubation period, blighting shape and blighting size and blighting colour, tassel texture and tasseling day and silking day were taken for present investigation, in relation to severity of banded leaf and sheath blight of maize, both in resistant and susceptible genotypes. But there is no reference, about the relationship between the morphological markers and severity of BLSB. So that in present investigation these character were taken as a morphological variation, in both resistant and susceptible genotypes. Plant height was measured in all the replication of experimental material of all the three group, 44 CIMMYT lines, 14 CM lines and 26 genotypes of Pantnagar. Mean height (cm) was taken for analysis and correlated with disease reaction. The results obtained from field experiment categorized into three groups on the basis of the disease reaction, resistant, susceptible and intermediate. The result obtained from the field experiment, represent that all the maize genotypes, CA001060, CA049Y04, CA031147, CA14509, CA14518 and CA00310 (CML 465), CA93124 show the mean disease score between (1.0 to 2.0). So all the inbred were resistant, when the mean data of plant height compared in all resistant genotypes, the average height in between 160 to 175. In seven resistant genotypes, four genotypes represent the height above 175 cm. So we can say that the height is one factor responsible for the disease resistance in case of susceptible genotypes. Total of 38 genotypes after excluding the resistant genotypes of which 14 genotypes showing the mean plant height in between 130 to 160 cm. So this prove that the low plant height may increase the severity of the disease. Incase of susceptible genotypes CML 428 the mean height was found 138 cm and the field disease rating score 4.5.

taken for present investigation was the cob placement and the cob placement related with the plant height. After the experimental result it was proved that longer the plant height, higher the cob placement. In all the resistance genotypes, CA00106, CA049Y04, CA03147, CA14518, CA00310 (CML 465), CA03124, CA03124 and CM 104, CM 104, CM 500, CM 600 and 556, 561, the average cob placement height which obtained through field experiment 60 cm to 80 cm.

The average cob placement height was same of genotypes under glasshouse condition, were 40 to 60 cm in comparison to susceptible genotypes, which represent the average cob placement height 45 to 60 cm in field condition. The average cob placement height under glasshouse condition were 35 to 50 cm. From the experimental result it is proved that with the plant height the cob placement height also increases. In case of resistant genotypes CM 600 the plant height 176 cm in field condition and the cob placement height was 72.3 cm. In glasshouse condition the plant of CM 600 was 165 cm and the cob placement height 61.6 cm. In susceptible genotypes CM 400 the average mean plant height in field conditions was 159.3 cm and average cob placement height 66.0 cm. In glass house condition the average mean height of CM 400 was 152.0 cm and cob placement height was 43.3 cm. So from the experimental result, it is clear that the average cob placement height increases with the increase of plant height. But there was no literally report about the, correlation between the cob placement height and disease severity in the banded leaf and sheath blight. But it could be explained that if the cob placement height is more, for reaching the diseases to cob will take more time, and for early maturing genotypes, the crop harvest upto reaching the diseases at threshold level.

So the cob placement could be second morphological character, for identification of resistant and susceptible maize genotypes or it may be helpful for the study of morphological variation among the maize genotypes in relation to banded leaf and sheath blight.

The third character which is taken for the present investigation was the incubation period. The incubation period recorded in all maize genotypes in field as well as in glass house condition, revealed that resistant maize genotypes, CA 00106, CA049Y04, CA03147, CA14509, CA14518, CA03106, CA00310, CML 4651, CA03124 and CM 104, CM 105, CM 500, CM 600 and in 561 and 556 the average incubation period were 72 hrs. While in case of susceptible genotypes the incubation period in field condition were in between 24 to 48 hours. In glasshouse condition also the average incubation period of resistant genotypes, CA00106, CA049Y04, CA03147, CA14509, CA14518, CA00310 (CML 465) and CA03124, CM104, CM105, CM500, CM600, in between 48 to 72 hrs in susceptible genotypes the incubation period in glasshouse condition vary between 24 to 48 hrs. From the experimental result, it is clear that the incubation period, in resistant genotypes is higher than in susceptible genotypes in both glasshouse and field condition, in resistant genotypes the pathogen produced symptom latter than in susceptible genotypes. So it helped in reduction of disease severity in resistant plant. So incubation period also played an important role in differentiating resistant and susceptible genotypes.

Fourth important character which taken for present investigation for differentiating resistant and susceptible genotypes or for study the morphological variation in maize genotypes. The average spot size in resistant genotypes CA00106, CA049Y904, CA 03147, CA14509, CA14518, CA00310 (CML 465), CA03124, CM104, CM105, CM500, CM600, 561, in between 1.0 to 1.5 cm. In case of susceptible genotypes in all three group CIMMYT, CM, Pantnagar genotypes, the average mean spot size were in between 1.0 to 2.6 cm. In glasshouse condition, the average mean spot size in resistant genotypes, were in between 0.6 cm to 1.5 cm. In susceptible genotypes the average spot size in glasshouse condition vary in between 1.0 to 2.0 cm. So from the experimental result, it was clear that in resistant plant the spot size or blighting size was less in

comparison to the susceptible genotypes. In resistant genotypes, it might be possible that the host plant restrict the pathogen to increase in size, as a result the spot size were smaller in resistant genotypes as compare to susceptible genotypes. And susceptible genotypes, they were unable to restrict the pathogen spread in host surface and as a result the spot size were larger in susceptible genotypes. So spot size also related to disease severity in host plant. So spot size may also becomes an important tool for identification of resistant and susceptible genotypes and an important character for study the morphological variation in maize genotypes.

Tassel texture also taken as a morphological character, for differentiating the resistant and susceptible genotypes, tassel texture of resistant genotypes CA 00106, CA049Y05, CA03147, CA14509, CA03124, CM104, CM105, CM500, CM600 all represent dense tassel texture only CA00310 (CML 429) show lax tassel texture, and two genotypes 556, 561 show medium texture in tassel condition. And all genotypes represent same tassel texture under the glasshouse condition. It is possible that the tassel texture may be associated with the resistant and susceptible genotypes. It may play important role in study of morphological variation in maize genotypes.

Tassel colour, was also taken as a morphological marker, for study the morphological variation in maize genotypes and the relationship between the tassel colour and disease reaction of resistant and susceptible maize genotypes. From the experimental result, it clear that all the resistant genotypes show purple tassel colour in both in the field condition as well as in glasshouse condition. So the tassel colour might be associated with the disease reaction, and it can be used for the differentiation of the resistant and susceptible genotypes.

Silk colour was also taken as morphological character for study of the morphological variation in resistant and susceptible maize genotypes. There was no clear differentiation, of resistant and susceptible genotypes by the silk colour.

Blighting shape was also taken as a morphological character for study morphological variation among resistant and susceptible genotypes. Blighting shape of all the resistant maize genotypes, CA00106, CA049Y04, CA03147, CA14509, CA0031 (CML465) CA03124, CM104, CM105, CM500, CM600 all show rounded blighting shape. In comparison to the susceptible maize genotypes which show banded pattern of blighting shape. Round blighting shape restrict the pathogen spread in host surface and latter it covered by the round black/brown line. It is possible that for this reason, the severity of banded leaf and sheath blight in resistant maize genotypes less in comparison to the susceptible maize genotypes. In most susceptible genotypes represent, blighting shape in banded pattern; the banded pattern help in increase the blighting in whole host surface, and it increases the severity of disease in maize genotypes. So blighting shape can be used for the identification of the morphological variation among all the resistant and susceptible maize genotypes.

Blighting colour of all maize genotypes which represent disease reaction (1.0 to 2.0) CA00106, CA03147, CA14509, CM104, CM105, CM500, CM600 show yellow blighting colour which is surrounded by the brown ring at the latter stages. The yellow colour on maturation of the plant turn to pale white and which restrict the further growth of the pathogen, and ultimately reduced the disease severity of the disease banded leaf and sheath blight. While in case of the moderately susceptible and resistant genotypes most of which show the yellow and water soaked blighting colour. The yellow water soaked blight colour helped in the spread of the pathogen and it increased the severity of the disease. So the blighting colour could be helpful for the differentiation of the resistant and susceptible genotypes.

Day for tasseling was also taken as a morphological character for study the morphological variation among the resistant and susceptible maize genotypes. Day of tasseling in resistant genotypes, CA00106, CA049Y04, CA03147, CA14509, CA14518CA00310, CML465, CA03125, CM104, CM600

and CM104 showed average tasseling day in between (50 to 55) and in moderately susceptible genotypes show day to tasseling between (40 to 50) day. And in highly susceptible genotypes the day of tasseling was in between (40 to 50) day. In glasshouse condition the date of tasseling vary in between (45 to 50 day). In moderately susceptible genotypes day to tassel was in between 40 to 45. Maize genotypes which were highly susceptible show tasseling day in between 40 to 45 day. The tasseling could play an important role in the identification of the resistant and susceptible maize genotypes. Days to tasseling affect the disease severity in (BLSB) by the inoculation date of pathogen. Because for creation of the artificial epiphytotic in field condition, the inoculation done before onset of the tasseling. So tasseling date represents stage of susceptibility of plant. If the tasseling time in the genotypes is more, the infection start latter and before reaching the pathogen in cob, the plant reach at maturity stage or harvesting stage. So it reduces the severity of the disease. In case of the susceptible genotypes the tasseling day are less, and susceptible stage come earlier and the disease severity in susceptible genotypes increases rapidly.

Days to silk also taken as a morphological character for the study the morphological variation in resistant and susceptible genotypes. All the resistant genotypes show the days to silk in between (50 to 55 day). Moderately susceptible maize genotypes also show the days to silk in between (50 to 55 day). In glasshouse condition also the silking day in resistant and susceptible genotypes was in between (50 to 55) day. Days to silk gave not much variation among the resistant and susceptible genotypes.

5.2 RAPD marker analysis for disease reaction

The RAPD pattern among resistant and susceptible genotypes showed high polymorphism in group I, maize inbred line, the highest number of RAPD loci (12) was obtained with primer UBC 572 on agrose gel. A part from polymorphic loci several unique loci was detected which could held in

identification and differentiation of resistant and susceptible genotype among resistant group genotypes with primer 12SS10T4, CM 500, CM 105, CM 104 gave unique loci of size >1.5 kb, >0.5 kb and >0.5 kb and unique loci in susceptible genotypes CM 115 with size of <0.5 kb for CM 121, a susceptible genotypes. Primer UBC 535 give 2 unique loci of size 1.9 kb and 1.3 kb with susceptible genotypes CM 209, CM 121, UBC 535 primer give 3 unique loci in resistant genotypes CM 105, CM 500, CM 104 of size >0.8 kb, <0.5 kb and <0.5 kb. Primer UBC 337 give unique loci of size >2.0 kb, 2.0 kb, <2.0 and <0.5 kb with genotypes Amar, CM 115, CM 111 and CM 209. Primer UBC 552 give unique loci of size 1.3, <1.3 and >0.9 kb with resistant genotypes, CM 105, CM 105 and CM 500. Primer UBC 600 give one unique loci of size 1.0 kb with susceptible genotypes CM 120 and one unique loci of size <0.5 kb with resistant genotypes CM 500. Primer UBC 572 give three unique loci of size 1.9 kb, 1.9 kb >1.5 kb with resistant genotypes CM 120 and CM 11. This primer also give unique loci of size <1.5 kb, <1.5 kb, < 1.3 kb, < 1.5 kb with CM 600, CM 104, CM 105 resistant genotypes. Primer UBC 350 give unique loci of 1.9 kb size with susceptible genotype Amar and also give 3 unique loci with resistant genotypes, CM 600 of size of >0.5 kb and 1.3 and <0.5 kb with genotypes CM 500 and 600. Primer UBC 386 give two unique bands of 1.5 kb, <0.5 kb with susceptible genotypes, CM 121 and CM 210 and one unique loci with resistant genotypes >0.5 kb with CM 500. In group II genotypes maximum number of RAPD loci found with primer 12S10T4 on agrose gel among susceptible genotypes primer 12S10T4 gave six unique loco of size 1.3 kb, <1.3 kb, >0.9 kb, > 0.5 kb, <0.5 kb, CML 471, CML 425, CML 429, CM 469, CML 428 with resistant genotypes, it gave unique loci of size >0.9 kb, >0.5 kb, with CA03147, CA00106. Primer UBC 18, two unique loci of size 1.3 kb, >0.8 kb, CML 433, CML 421 with susceptible genotypes and four unique loci of size >0.5, <0.5, <0.5, <0.5 kb with CA049Y04, CA03147 and CA14509. Primer UBC 535 gave one unique loci of size 2.0 kb with CM 469. It gives flour unique loci with

resistant genotypes of size 1.5, 1.3, <0.5, CA03147, CA03147, Ca14509, and UBC 552. Primer gives unique loci of size 2.0 kb with CML 431 susceptible genotypes. It give three unique loci with resistant genotypes of size >2.0, 1.9, <0.5 kb with CA14509, CA03147 and CA03147. Primer UBC 600 give a unique loci of size with resistant genotypes 428 of UBC 600 gives a unique loci of size with resistant genotypes 428 of size >0.5 kb. It also give a unique loci of size >0.9 kb with CA049Y04. Primer UBC 532 give unique loci of size 0.8 kb with CML 433. Primer UBC 350 give one unique loci of size <0.5 kb with Ca00106, UBC 538 give 3 unique loci with resistant genotypes CA03147, CA00106, Ca14509 of size 1.9 kb, <0.5 kb, <0.5 kb. Group III genotypes also give unique loci with some genotypes. Primer 1210T4 give unique loci of size 1.5 kb with susceptible genotypes CML 428. It also amplified 4 unique loci of size <1.3, 1.3, 0.8, >0.5 kb with CML 475, CA14518, CML 465, CA03124 resistant genotypes. Primer UBC 18 give one unique loci of size of 0.8 kb with CA03124 and two unique loci of size <0.5 and 1.3 kb with CA14518 and CML 475 resistant genotypes.

Primer UBC 337 gives four unique loci of size 2.0, 1.9, 1.5, 1.3 kb with susceptible genotypes, CA003134, CA003134, CML 428, CA00344 and with resistant genotype. It gives unique loci of <0.5, <0.5, <0.5, <0.5 kb with resistant genotypes, CML 475, CML 475, CA03124 and CML 475. Primer UBC 552 give one unique loci of size 1.5 kb with CA00396 susceptible genotypes, and three unique loci of 1.9 kb, <1.9, <0.5 kb with Ca00310 (CML 465), CA00310 (CML 465) and CML 475. Primer UBC 600 give three unique loci with resistant genotypes, CA00334, CA00334, CA00310 (CML 465). Primer UBC 532 give unique loci with susceptible genotypes CA031149, CA03124 of size >0.9; >0.9 and two unique loci with resistant genotypes, CA14518, Ca14520 of size >0.9 and <0.5 kb respectively. Primer UBC 534 give two unique loci of size >0.9 and <0.9 with susceptible genotypes. Primer UBC 350 amplified three unique loco of size 2.0 and 0.8 kb with CML 465 and CA03124 and 0.5 kb band with CA03124.

Primer UBC 386 amplified two unique band of size <0.5 kb and <0.5 kb with susceptible genotypes, CA03131 and CA03106 and one unique loci in resistant genotypes CML 465 of size 0.5 kb. Group IV genotypes give unique loci, Primer 12S10T04 give tow unique loci of size 1.5 to <1.3 kb with resistant genotypes 561 and 556 and primer UBC 18 give one unique primer of size <0.5 kb with inbred 561. The primer 535 give unique loci with susceptible genotypes 565 with >0.9 kb. Primer UBC 337 give unique loci of <0.5 and <0.5 kb with CA00104 and CA14517 and three loci of size 0.8, >0.5 , <0.5 kb with genotypes 556, 561 and 556. Primer UBC 552 give three unique loci with resistant genotypes 561, 556 and 556 with size >0.9 , <0.5 and 0.5 kb, UBC 600 give three unique loci with susceptible inbred with size of >0.9 , <0.5 , <0.5 kb for CA14519, CA14519, 558 and three unique loci with resistant genotypes 561, 556 and 556 with size of >0.9 kb, <0.5 kb and <0.5 kb. Primer UBC 532 give one unique loci of size >1.5 kb with 569 and five loci with size of >0.9 kb, <0.9 , <0.5 , <0.5 kb with genotypes 556, 561, 566, 551, 561. Primer 8, UBC 572 amplified tow unique loci of size >0.5 kb with CA14527 and <0.5 kb size loci with inbred CA00104. Two unique loci also amplified with resistant genotypes, >0.5 kb with 556 and <0.5 kb with 556. Primer UBC 534 give three unique loci of size 1.3 kb, <1.3 kb, <0.9 kb with 550, CA00104, 532 and three unique loci of size 0.5, 0.5, <0.5 kb with resistant genotypes 56, 556 and 556. Primer UBC 350 give three unique loci with susceptible genotypes CA14501, CA14501, Ca14519 of size 0.9 kb, <0.9 kb, <0.5 kb and single unique loci of <0.5 kb 566. Primer UBC 38 give two unique loci of size 0.5 kb and <0.5 kb with susceptible genotypes, CA00104, CA14519 and 4 unique loci of 0.8, >0.5 , <0.5 , <0.5 kb with resistant genotypes, 556, 561, 561 and 556.

Primer, 12SS10T4, UBC 18, UBC 535, UBC 572, UBC 534, UBC 350, UBC 386, distinct RAPD pattern for all group I, maize genotypes primer 12S10T04, UBC 18, UBC 535, UBC 337, 552 and UBC 600 gave a distinct RAPD pattern with ail genotypes of group II maize genotypes with distinct

unique loci. In group III, all the primer give, equal distinct pattern in all genotypes with distinct unique loci. In group IV also all primer gave equal number of unique band in all genotypes from these unique loci genotypes studied could be discriminated but since large number of unique loci were present helpful in inbred differentiation. It was not possible to discriminate the bands for disease resistance and susceptibility.

Similarity coefficient also plays an important role in differentiation of resistant and susceptible genotypes. Resistant genotypes of (group I) 1 and 2 depicted the similarity of 68 per cent and resistant inbred 2 and 3 depicted the similarity of 68 per cent and resistant inbred 2 and 3 depicted the similarity of 68 per cent, and all the resistant inbred come in cluster III. Susceptible inbred come in cluster I and cluster II. The similarity coefficient between cluster II and III was 49 per cent. So from the dendedrogram it was clear that the resistant inbred differ from the susceptible.

Resistant genotypes of (group II) in dendedrogram come into cluster IV in genotypes 1, 2, 3 and 4; inbred 1 and 2 related with 53 per cent. Cluster IV and cluster III, showed similarity of 45 per cent showed from the similarity coefficient and dendedrogram it was clear that the resistant genotypes differ from the susceptible lines.

Resistant genotypes of group (III) also come into the same cluster in the dendedrogram and genotypes 1st show similarity of 40 per cent with genotypes second and inbred second show 45 per cent of similarity. Susceptible genotypes come under the cluster III, II and I. Resistant genotypes of group (II) come in cluster IV and inbred first so, 46 per cent similarity with inbred second. So dendedrogram differentiate resistant and susceptible genotypes clearly.

5.3 The SDS-PAGE marker analysis for disease reaction

The SDS-PAGE pattern among resistant and susceptible genotypes did not showed much differentiation among the resistant and susceptible genotypes,

in group I genotypes, SDS-PAGE analysis give, no clear differentiation among the resistant and susceptible genotypes. In two resistant genotypes 1, 2, two their band present in which were in susceptible genotypes in as thick from but present in thin from, and two band which were present in all the susceptible genotypes were absent in two resistant genotypes. In group II genotypes also there were not much differentiation among the resistant and susceptible genotypes. In zone first one band of size greater than 43.0 kb found in resistant genotypes and which were absent in susceptible genotypes, this band might be responsible for the resistant in the genotypes. One band of <43.0 kb present in resistant genotypes but absent in susceptible one. So this band of size <43.0 kd might be involved in disease resistant. One thick band also present in resistant genotypes 1 which were absent in all the other susceptible genotypes, might be evolved in resistant in this line. One thick band present in all the susceptible genotypes but absent in resistant genotypes. In group III genotypes, also there were not much differentiation among the resistant and susceptible genotypes. In resistant genotypes 1, one thick band of size less than 66.0 kb present, which were absent in all other genotypes. This may also be involved in conferring resistant to this genotypes. In group V maize genotypes two thick bands of size <20.1 kd present in resistant genotypes in zone III and one single thick band of size <14.3 kb also present in resistant genotypes one, but it was absent in all the other maize genotypes. In group V maize genotypes, also two thin band of size less than 20.1 kd present in resistant genotypes which absent in all the other susceptible genotypes. In group VI and VII all the genotypes were come in category of moderately susceptible and all these genotypes showed similar pattern.

Association among maize genotypes revealed by unweighted pair group method with arithmetic mean (UPGMA) in group I maize genotypes, all resistant maize genotypes come in cluster III and all show similarity upto 85 per cent. In group II maize genotypes all the three resistant plant come in two cluster, 2 and 3 resistant inbred come in I cluster and one resistant inbred 1 come in cluster 3.

Inbred 2 and 3 show 88 per cent similarity and resistant genotypes 1 show 73 per cent of similarity.

In group III, resistant genotypes one and two show similarity of 95 per cent and with genotypes three they show similarity of 82 per cent. In group IV both resistant genotypes 1 and 2 show similarity of 0.6 and they fall in different clusters. In group V two clusters and show similarity of only 0.6 per cent. Group VI and VII contain only susceptible inbred and they show 100 per cent similarity in group VI, showed from SDS-PAGE analysis it is clear that the resistant plant show similarity upto 95 per cent in each other. While the susceptible plant showed similarity o upto 100 per cent. SDS-PAGE analysis helpful for finding the differentiation of resistant and susceptible genotypes.

*Summary
and
Conclusion*

The present investigation entitled “Morphological and molecular variation in maize (*Zea mays* L.) with special reference to banded leaf and sheath blight” was undertaken with the objective to study the morphological, molecular variation in maize, through morphological and molecular character which help in identification of resistant and susceptible genotypes in maize against the disease banded leaf and sheath blight. The study was conducted at the Crop Research Centre (CRC), Department of Plant Pathology and Molecular Biology and Genetic Engineering Department, G.B. Pant University of Agriculture and Technology, Pantnagar. The experimental material consisted of 86 maize genotypes (Table 2 and 3). The genotypes were evaluated under field condition during year 2003. The experimental material also evaluated under the glasshouse condition and through laboratory method and was planted under epiphytotic condition in randomized complete block design with three replications. All the genotypes were planted in pots with three replications. Inoculations in field and glasshouse condition were done by inserting two infected sorghum grains between the leaf sheath and stem on lower third/fourth internode, above the ground level just before the onset of tassel emergence stage of the crop. In laboratory, leaf pieces were inoculated by placing single infected grain culture on the mid rib of adaxial surface and incubated at $28\pm 1^{\circ}\text{C}$ for two days. The observations were recorded in field after second day of inoculation and 48 hrs after infection in the laboratory. Plants with score 1.0 to 2.0 were finally scored as resistant and 2.0 to 4.0, moderately susceptible and (4.0 to 5.0) as highly susceptible, scoring was done on the individual plant basis. All the resistant and susceptible genotypes by taking into four groups used for detection of

polymorphic DNA. In RAPD analysis of anionic detergent (SDS), genomic DNA extracted from leaf obtained from seedling. The total protein of all the inbred lines also extracted from leaf obtained from seedling.

The morphological characters, i.e. plant height, cob placement and incubation period, spot size, blighting colour, blighting shape, tasseling texture, tassel colour, tasseling date and silking date were also recorded in all the replications. The data were subjected to appropriate analysis. The results obtained are summarized as follow.

1. From the field, glasshouse and laboratory experiment, it was found that the genotypes, CA00106, CA049Y04, CA03147, CA14509, CA14518 and CA00310, CA03124, CM 104 and CM 105, CM 500, CM 600, 556, 561 represent the mean disease reaction in between 1.0 to 2.0 and they were grouped as resistant. Out of 86 genotypes used for the present investigation.
2. The plant height, morphological character, which used for differentiation or study of morphological variation in all resistant and susceptible genotypes. It was found that the all resistant genotypes which showed the disease reaction in between (1.0 to 2.0), CA00106, CA049Y04, CA03147, CA14509, CA14518 and CA00310, CA03124 and CM 104 and CM 105, CM 500, 600, exhibited average plant height (130 to 160 cm in field condition and 75 to 150 cm in glasshouse conditions) in comparison to susceptible genotypes. So, from present investigation it may be concluded that plant height may affect the disease severity.
3. The cob placement was also used for the study of morphological variation in resistant and susceptible genotypes. It was found that all the resistant genotypes CA00106, CA049Y04, CA03147, CA14509, CA14518 and CA00310, CA03124 and CM 104 and CM 500, depicted the average mean height for cob placement height (60-80 cm) in field conditions and

average mean height (40-60 cm) under glasshouse conditions, in comparison to susceptible genotypes which represent average mean height (45-70 cm) in field (30-50 cm) and in glasshouse conditions.

4. Incubation period was also taken as a morphological character for differentiation of resistant and susceptible genotypes. In the resistant genotypes, CA00106, CA049Y04, Ca03147, CA14509, CA14518 and CA00310, CA03124 and CM 104 and CM 500, depicted that average mean incubation period of 72 hrs in field and glasshouse conditions in comparison to susceptible genotypes which depicted the average mean incubation period of 24 to 48 hrs.
5. Spot size was another morphological character taken the study of morphological variation among resistant and susceptible genotypes. The resistant genotypes CA00106, CA049Y04, CA03147, Ca14509, CA14518 and CA00319, CA03124 and CM 104 and CM 105, CM 500, CM 600, 561, 556 and all genotypes showed the average spot size of 1.0 to 1.5 cm in field as well as glasshouse condition; in comparison to the susceptible genotypes which show average spot size in between (0.6 to 1.6 cm).
6. Blighting shape, also taken as morphological markers for differentiation of resistant and susceptible genotypes. The blighting shape of all resistant genotypes CM 104, CM 105, CM 500, CM 600, 556, 561, CA00106, CA049Y04, CA03147, CA14509, CA14518, CA00310 and CA03124 are depicted the round blight shape in both field and glasshouse condition. In comparison to all susceptible inbred lien of which majority of them showed banded blighting shape in both field and glasshouse condition.
7. Blighting colour, also caused for differentiation of resistant and susceptible genotypes. The blighting colour in all resistant genotypes depicted by the yellow colour spot which on later stages covered by the

brown black ring, in both field as well as in glasshouse condition. In comparison to susceptible genotypes which showed water soaked yellow colour spot.

8. Tassel texture used as a morphological character in preset investigation which indicated there was no direct relationship between tassel texture and severity of banded leaf and sheath blight, but it was found that majority of resistant genotypes, CM 104, CM 104 and CM 500, CM 600, CM 00106 and CA049Y04, CA03147, CA14509, CA14518 and CA00310, CA03124 depicted the disease tassel texture expect few resistant genotypes which showed lax and medium tassel texture in both glasshouse and field conditions. In comparison with susceptible genotypes.
9. Tassel colour, tassel anther glume and glume base colour was also taken as a morphological character for study the morphological variation. It was found that the resistant group materials CM 104, CM 105, CM 500, CM 600 and CA00106, CA049Y04, CA03147, CA14509, CA14518 and CA00310, CA03124, depicted the purple tassel colour in both field and glasshouse conditions in comparison to susceptible genotypes which depicted the green and yellow tassel colour, though there was no direct relation between in tassel colour and severity of BLSB.
10. Days to tassel date, also taken as a morphological character for differentiation of the resistant and susceptible genotypes, the majority of resistant genotypes, CM 104, CM 105, CM 500, CM 600 and CA00106, CA049Y04, CA03147, CA1450, CA14518 and CA00310, CA03124, the mean average days to tassel was 50 to 50 days in field condition and the mean average tasseling day in between (45-50) in glasshouse in comparison to susceptible genotypes which show the average mean days to tassel (40-50) in field and (40-45) in glasshouse condition.

11. In group I (maize genotypes), primer 12S510T4, UBC 18, UBC 535, UBC 572, UBC 534, UBC 350 gave a distinct RAPD pattern for all 15 inbred lines of group I. UBC 535 which amplified three loci of size 1.9, 0.5, <0.5 in CM 210, CM 121 and CM 104, same primer amplified loci of <0.8 kb in resistant genotypes, but the same loci were absent in susceptible genotypes.
12. UBC 572 amplified loci of 1.3 kb in resistant genotype CA049Y04, but the same loci were absent in susceptible.
13. UBC 552 amplified three unique loci in resistant genotypes 561, 556 of size >0.9, <0.5 but same loci were absent in susceptible genotype.
14. UBC 18 primer amplified a unique loci of 0.5 kb in susceptible genotype 565 of size <0.5, which was absent in resistant genotype.
15. The primer UBC 600 amplified one unique loci of 0.8 kb in group II resistant genotypes which was absent in susceptible genotypes.
16. UBC 532 primer amplified one unique loci of 0.8 kb in resistant genotypes (group II) but it was absent in susceptible group. Primer UBC 572 amplified two loci of size one 1.3 kb and other >1.3 kb in resistant genotypes but these both loci were absent in susceptible genotypes. Primer UBC 350 amplified one unique loci of <0.5 kb in susceptible genotypes which was absent in resistant genotypes, primer 386 amplified three loci of size 1.9, <0.5, <0.5, <0.5 kb when these were absent in susceptible genotypes.
17. Primer UBC 535 amplified a unique loci of <0.5 kb in resistant genotypes which were absent in susceptible genotypes of group III. Primer UBC 600 amplified 3 loci in group III resistant genotypes of <0.5, 1.5, 0.8 kb, but these loci were absent in susceptible genotypes. Primer UBC 534 amplified two unique loci of size >0.9, >0.9 kb in resistant genotypes,

which were absent in resistant genotypes. Primer UBC 350 amplified 3 loci of size 2.0, 0.8 and 0.5 kb in resistant genotypes which were absent in susceptible genotypes.

18. Primer 12S510T4 amplified 2 loci of size 1.5 and <1.3 kb in resistant genotypes (group IV), which were absent in susceptible genotypes. Primer UBC 18 amplified one unique loci of size <0.5 kb in resistant genotypes which were absent in susceptible genotypes. Primer UBC 535 amplified unique loci of 0.9 kb in susceptible genotypes, but which were absent in resistant genotypes. UBC 552 gave three loci of size >0.9, <0.5 and <0.5 kb in resistant genotypes, which were absent in susceptible genotypes.
19. In Group I genotypes there was not much differentiation among resistant and susceptible genotypes. Two thick bands of size <14.3 kd present in resistant genotypes, which were present in thin form in susceptible in bred line. Two bands of size (<14.3 kd) present in all the susceptible line but absent in resistant genotypes, this protein band might be responsible for susceptibility in lines.
20. In group II genotypes one bands of size <43.0 kd present in all the resistant genotypes but absent in susceptible genotypes. One thick bands of size (14.3 kd) present in all susceptible genotypes but absent in all the resistant genotypes.
21. In group III genotypes, two band of size <66.0 kd present in resistant inbred genotypes one showed a thick band but absent in susceptible genotypes. One thick band of size <14.3 kd present all thin susceptible genotypes but absent in resistant inbred line 1.
22. In group IV genotypes, three resistant depicted two thin bands of size less than (20 kd) present only in resistant genotypes, which were absent in susceptible genotypes. So, this band might be evolves in conferring resistant to these genotypes.

23. Group VI and VII all genotypes of susceptible in nature, and they gave many bands which were present in all the susceptible genotypes. These common band might be responsible for their high susceptibility in comparison to resistant genotypes.

From the present investigation it is concluded that all the resistant maize genotype represent the morphological variation in plant height, cob placement and incubation period, spot size, days to tassel and days to silking. They also represent variation in molecular level. So, these resistant maize genotypes can be utilized breeding resistant maize genotypes.

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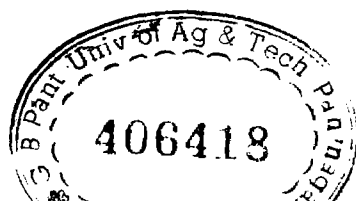
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Appendices

APPENDIX-I

Name and source of chemical used

Sl. No.	Chemical	Source
1.	Agarose	Genei
2.	Assay buffer for PCR	Genei
3.	Bromophenol blue	Sigma
4.	Chloroform	Qualigen
5.	DNA size marker	Genei
6.	dNTPs mix	Genei
7.	Ethidium bromide	Sigma
8.	Glacial acetic acid	Sigma
9.	HCl (absolute)	SRL
10.	Isomayl alcohol	SRL
11.	Isopropanol	Sigma
12.	Liquid nitrogen	Indian make
13.	Na ₂ EDTA 2H ₂ O	SRL
14.	NaCl	Merck
15.	NaOH	Himedia
16.	Potassium acetate	Himedia
17.	RAPD primer	Genei
18.	RNase	Bohringer
19.	SDS (sodium lauryl sulphate)	Himedia
20.	Sodium acetate	Qualigen
21.	Sucrose	Himedia
22.	Taq. DNA polymerase	Genei
23.	Tris (hydroxy methyl amino methane)	Himedia

APPENDIX-II

Glasswares and instruments

Sl. No.	Chemical
1.	BOD incubator
2.	Camera with red filter (Canon)
3.	Constant temperature water bath (LKB, USA)
4.	Cooling microfuge (Remi)
5.	Deep freezer (Blue Star)
6.	Digital balance (Adair Dutt and Co.)
7.	Dry bath (Genei, India)
8.	Electrical balance (Premisa, India)
9.	Electrophoresis apparatus (Biotech)
10.	Eppendorf tubes, centrifuge tubes and tips (Tarson)
11.	Glassware (Corning and Borosil, India)
12.	Heating mantle (Sunvik, UK)
13.	Laminar airflow system (Clean Air, India)
14.	Magnetic Steiner (Atlantis)
15.	Microfuge (Servall, USA)
16.	Micropipettes (Tarson, India and Microlit)
17.	Oakridge tubes (Tarson, India)
18.	pH meter (Systronics, India)
19.	Power pack (Genei, Hoefer, Biotech)
20.	Refrigerated high speed centrifuge RC-5C (Sorvall)
21.	Refrigerator (Videocon and Godrej)
22.	Thermocycler (Biometra)
23.	Ultraviolet transilluminator (Fotodyne)
24.	Vertical slab gel apparatus (Genei)

APPENDIX-III

Abbreviations used

AFLP	:	Amplified fragment length polymorphism
AP-PCR	:	Arbitrary primed polymerase chain reaction
BSA	:	Bulked segregant analysis
DNA	:	Deoxyribonucleic acid
dNTPs	:	Deoxy-nucleotide triphosphate
EDTA	:	Ethylene diamine tetra acetic acid
MP-PCR	:	Micro-satellite primed polymerase chain reaction
NILS	:	Near isogenic lines
PCR	:	Polymerase chain reaction
RAPD	:	Random amplified polymorphic DNA
RAMPO	:	Random amplified micro-satellite polymorphism
RFLP	:	Restriction fragment length polymorphism
RNase	:	Ribo-nuclease
SCARs	:	Sequence characterized amplified regions
SDS	:	Sodium dodecyl sulphate
SSCP	:	Single strand conformation polymorphism
SSR	:	Single sequence repeat
SSRP	:	Single sequence repeat polymorphism
TAE	:	Tris acetic EDTA
TE buffer	:	Tris EDTA buffer
UPGMA	:	Unweighted pair group method with arithmetical means

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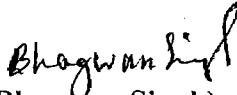
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ABSTRACT

One of the main deterrent to high grain yield in maize is its susceptibility to several diseases. Banded leaf sheath blight (BLSB) caused by (*Rhizoctonia solani* f.sp. *sasakii*) has become a serious threat to its cultivation in the north western plains of India. The pathogen spreads from the basal sheath to the developing ear under favourable environmental conditions. The developing ear is completely damaged and arise up prematurely with cracking of husk leaves. The main objective of the present investigation, to differentiate the resistant and susceptible maize genotype through the morphological and molecular markers. The morphological character used for present investigation, were plant height, cob placement and incubation period, spot size, tassel colour and tassel texture, tasseling day, silking day, blighting shape and blighting size. Molecular markers used for present investigation were, random amplified polymorphic DNA (RAPD) and SDS-PAGE. Total 85 genotype used for present investigation. The evaluation of these genotype was done under artificial epiphytotic condition as well as in glasshouse and by laboratory method. By this evaluation all these genotype categorized into resistant, susceptible groups. Twelve genotype were found resistant, which depicted the disease reaction in between (1.0 to 2.0). CA00106, CA049Y04, CA03147, CA14509, CA14518, CA00310 (CML 465), CA03124, CM 104, CM 1.05, CM 500, CM 600, 556, 561 and rest of genotype categorized into susceptible groups. These resistant and susceptible genotype used for the, molecular characterization. Plant height which used for differentiation of resistant and susceptible genotype. It was found that all the resistant genotypes showed average plant height (160 to 180 cm) in field condition and (140 to 160 cm) in glasshouse condition, which were greater than susceptible genotypes. The cob placement height also affect the disease severity in maize genotypes. All the resistant maize genotype depicted the average cob placement height (160 to 175), in field and (145 to 165) in glasshouse condition. It was found that higher the cob placement height low the disease severity. Incubation period was also higher as 72 hrs in resistant plant, in comparison to susceptible genotypes in which average incubation period was between (24 to 48 hrs). The spot size in resistant genotype also less (1.0 to 1.5 cm) in comparison to susceptible genotypes, in which the average mean size was 1.5 to 2.5 cm. The blighting shape in resistant genotypes was round in shape in comparison to susceptible genotype. Blighting colour in resistant genotype was yellow and latter stage, it was covered by the brown dark ring. Susceptible inbred lien depicted water soaked pale yellow colour spot. The data of tasseling in resistant plant was found late in comparison the susceptible genotypes. Tassel colour in majority of resistant plant was found purple in colour, RAPD pattern between resistant and susceptible groups showed many unique loci, in all the genotype. Primer UBC 337 gave four unique loci of size (>2.0, 2.0, <2.0 and <2.0) in Amar CM 115, CM 111 and CM 209 in susceptible in bred line which were absent in resistant inbreds. Primer UBC 535 gave a unique loci of <0.5 kb with CML 465 resistant genotypes. Same loci absent in susceptible inbred, primer 12S510T4 gave 2 unique loci of size 1.5 and <1.3 kb which were present in resistant inbred 561 and 556. Same loci absent in susceptible genotype SDS-PAGE also helpful in differentiation of resistant and susceptible genotypes, resistant genotypes CM 600, CA00106 and CA049Y04 gave two bands of size <43.0 kb which where absent in susceptible genotypes. Though morphological and molecular marker both help in identification of resistant and susceptible genotypes, which play further role in breeding for disease resistant genotypes.


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