GENOTYPIC AND SEASONAL INFLUENCE ON LEAFSPOT DISEASE IN AMARANTH

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Genotypic and seasonal influence on leaf spot disease in amaranth"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Genotypic and seasonal influence on leaf spot disease in amaranth"** is a record of research work done independently by Smt. K. Krishnakumary, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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INTRODUCTION

1. INTRODUCTION

Consumption of herbs is as old as human race itself. Green leafy vegetables represent an excellent component of the habitual diet in the tropical countries making it richer in minerals, vitamins, proteins and also a good source of roughage. Majority of the Indian population are vegetarian, but per capita intake of vegetables is estimated to be only about 210 g against the requirement of about 285 g. Among them 80 g leafy vegetables are required for a balanced diet, hence very rightly leafy vegetables are known to be protective food (Pandey, 1993).

Amaranthus constitutes a single major group of leafy vegetables and its cultivation is more widespread in southern India. It occupies a unique place with regard to easiness to culture, high suitability for both home gardens and commercial cultivation, faster growth rate and highly favourable response to added fertilizers and organic manure. (Grubben and Vanslotten, 1981). Because of its low production cost and high productivity per unit area, amaranth is considered to be the cheapest leafy vegetable in the market and therefore it could be rightly described as a "poor man's vegetable". Every 100 g of edible portion of *Amaranthus tricolor* contain 4 g protein, 2.7 g minerals, 397 mg calcium, 349 mg iron, 341 mg potassium, 9200 I.U. vitamin A, 99 mg vitamin C, 247 mg magnesium, 83 mg phosphorous, 230 mg sodium, 0.03 mg thiamin, 88 mg chlorine, 1.2 mg niacin and 0.3 mg riboflavin (National Institute of Nutrition, 1991).

In India high priority is bestowed to eradicate xerophthalmia which is one of the serious diseases caused due to nutritional deficiency

particularly vitamin A. According to an estimate by World Health Organisation, half a million children go blind every year and several millions more exhibit other symptoms of vitamin A deficiency. The malnutrition due to vitamin A deficiency can be alleviated by including adequate quantities of amaranth in the diet.

Amaranth, a multipurpose plant is grown for vegetable, grain and ornamental purposes. Conventionally, six species namely, *Amaranthus tricolor*, *A. spinosus*, *A. dubius*, *A. viridis* and *A. blitum* are considered as vegetable types. The species *A. viridis* and *A. spinosus* are weeds in many parts of India, though they are used as a delicious and much relished leaf vegetable in rural Kerala. *A. tricolor* is the principal vegetable species consumed worldwide, particularly in the south east Asian countries (Prakash and Pal, 1991). *A. hypochondriacus*, *A. caudatus* and *A. cruentus* are the important grain species. Amaranth seeds also contain essential aminoacids such as lysine (5%), methionine (3.2%), tryptophan (4.4%), and are rich in protein (17%) (Sanft, 1979 ; Zhao and Ma, 1987). It is to be noted that approximately 192 million children under the age of five suffer from acute or chronic protein energy malnutrition and anemia. Mixing amaranth seeds with the cereal based diet will augment the protein quality and quantity in a vegetarian diet and thereby reducing the protein energy malnutrition in the developing countries (Thomas and Krishnamurthy, 1988).

Despite high degree of nutritive value, the main constraint to their nutritional exploitation is the presence of some anti-nutritive factors like oxalates and nitrates in the leaves (Cheeke and Bronson, 1980 ; Gupta and Wagle, 1988). The oxalate levels in foods are of concern, as free oxalates bind essential dietary divalent minerals, primarily

calcium and make them nutritionally unavailable. The calcium oxalates thus formed may accumulate resulting in oxal urea or kidney stone. The present levels of oxalates and nitrates however do not pose a nutritional problem under normal conditions of amaranth consumption.

A realistic estimate of the extent of amaranth cultivation is not available, but it finds a place in every kitchen garden. Commercial growing of amaranth has also been taken up in small pockets in Kerala. The warm humid tropical climate is so congenial for its cultivation and a fresh leaf yield as high as 30-50 tonnes per hectare over a period of four months can be realized from this crop (Sirohi and Sivakami, 1995). This may be the highest yield per unit of land per unit of time that can be obtained from any such leafy vegetable. Under Kerala conditions, harvesting by repeated cutting is preferred to a single harvest by uprooting as it provides several crops from a single planting, hence a more efficient resource utilisation. It is well suited for home gardens and assures seed availability and flowering.

Among the various biotic and abiotic factors which limit the production of amaranth, vulnerability to leaf spot diseases is the most serious. Cultivation of amaranth suffered a set back in the recent past due to severe outbreak of leaf spot diseases especially in the red varieties. Incidence of leaf spot diseases is very severe in Kerala during rainy season, making the farmers reluctant to take up it's cultivation. Even total loss in yield was observed when plants were infected in the seeding stage (Plate I).

The conventional plant protection measures for the control of diseases are inefficient and undesirable from the point of view of human health

diseases is the use of leaf spot resistant varieties. In this backdrop, the present study was taken up with the following objectives.

1. Collection, maintenance and evaluation/characterisation of amaranth accessions against leaf spot disease , assessment of genetic divergence and grouping of accessions.

2. Isolation and identification of the pathogens associated with leaf spot disease

3. Seasonal influence on leaf spot disease and yield in amaranth and correlation studies.

4. Studies on biochemical bases of resistance to leaf spot

5. Biochemical cataloguing of amaranth accessions into different classes using enzyme patterns

Plate 1. A field view of amaranth plants infected with leaf spot disease

Review of literature

2. REVIEW OF LITERATURE

Amaranth constitutes a single major group of leafy vegetables having great potential for combating under and malnutrition in dietary, which has attained commercial significance in southern India. It is the most important popular leaf vegetable grown throughout the length and breadth of Kerala. The literature on various aspects of the crop production is reviewed here under.

2.1. Yield and yield attributes

2.1.1. Growth and yield

Van Eijinatten (1970) reported that in tropical areas, the production of green leaves from some amaranth species could reach 12 – 17 t ha $^{-1}$ in rainy season. Mugerwa and Bwabye (1974) investigated the productivity of a number of tropical grasses and legumes and commented that none of these yielded as much dry matter in a period of two months as amaranth (9000 Kg ha $^{-1}$). They suggested that because of its rapid growth, it would be possible to take two crops of amaranth in a growing season.

Mohideen and Shanmughasubramanian (1974) found that green yield of amaranth had a significant positive correlation with the number of leaves, weight of leaves, leaf length, leaf breadth, length of stem, weight of stem and diameter of stem.

Effect of transplanting on growth attributes and seed yield was studied by Mohideen and Rajagopal (1975) with two amaranth varieties, viz. A-62 (*A. hypochondriacus*) and A-3 (*A. dubius*) of short and medium duration respectively. Results indicated that for medium duration varieties transplanting would be a good practice to get higher seed yield in seed production programmes.

Mohideen and Muthukrishnan (1979) observed highly significant positive genotypic and phenotypic correlations between yield of greens on one hand and stem weight, leaf weight, stem diameter, leaf length, plant height and leaf breadth on the other. The leaf / stem ratio had a highly significant negative correlation with yield of greens.

Effect of age of seedling and population density was studied by Sulekha (1980) and she reported that transplanting 15-20 days old seedling at low-density plantings of 20 x 10cm spacing was better considering yield and quality parameters.

High heritability estimates associated with high genetic advance were obtained for weight of stem, leaf / stem ratio, yield of greens and weight of leaves indicating the usefulness of phenotypic selection, in a study involving 25 accessions (Devadas *et al.* 1989).

Pan and Sirohi (1992) reported a positive correlation of characters namely, number of clippings, length of lamina, duration of harvest and days to flowering with total yield which indicated that selection for these traits in positive direction could bring substantial improvement in the vegetable yield of *A. tricolor.*

Vijayakumar *et al.* (1982) conducted studies on growth and development of certain types of amaranth namely *A. tristis*, *A. tricolor*, *A. dubius* and *A. blitum* and observed that the plant height was significantly associated with the yield of greens (Stem and leaves) at all stages of growth. Krishnankutty (1983) observed that all growth parameters and yield of amaranth decreased under shaded condition and found it as a shade sensitive plant. Hilman and Abidin (1987) reported that the plant growth and yield of four amaranth cultivars increased with increase in the rate of nitrogen application.

A trial by Singh *et al.* (1985) on *A. tristis* with N at 20-60 kg ha^{-1} showed that the maximum plant height and optimum leaf- stem ratio resulted in the highest green yield with the highest dose of nitrogen applied in split dose i.e., one half as a basal dressing and other half as a top dressing after eight weeks of sowing.

Based on a field trial on *A. tricolor* with four levels of nitrogen (0, 30,70 and 110 Kg ha $^{-1}$) , Subhan (1989) reported that the plant height, leaf area, and fresh weight increased with increasing nitrogen application. He observed that yield also was the highest with a split application of 110 Kg ha^{-1}.

Pan *et al.* (1991) observed high heritability with high genetic advance for weight of stem and yield of greens in *A. tricolor* indicating great scope for improvement of these traits by selection.

Varalakshmi and Reddy (1994) reported that green yield of vegetable amaranth showed highly significant positive correlation with plant height, leaf length, leaf weight and stem weight.

Sukumar (1997) reported that NPK levels and cutting had a significant impact on various growth, yield and quality characters of *A. tricolor*. The plant height and green yield increased with increasing level of NPK and the extent of increase was more with respect to nitrogen application.

2.1.2. Pruning and yield

According to Enyi (1965) and Grubben (1976), cutting significantly delayed bolting and increased seed yield in amaranth. In the study to find out the clipping response of two species of amaranth, Mohideen and Rajagopal (1974) reported that the cultivar 'Arakeerai' (*A. tricolor* var. *tristis*) responded favourably for cutting, registering an yield of 11, 736 Kg ha^{-1} as compared to ' Sirukeerai' (*A. blitum)* with an yield of 8680 Kg ha^{-1}.

Mohideen *et al.* (1982) identified an amaranth variety Co-3 which yields 31 t ha^{-1} in ten harvests taken at weekly intervals for seven weeks starting twenty days after sowing.

Devadas *et al.* (1986) reported that in amaranth bolting can be delayed by cutting and thus vegetative phase can be prolonged. This study also indicated that red amaranth bolted late compared to green amaranth.

Olufolaji and Tayo (1989) compared two harvesting methods in amaranth and reported that pruning was superior to uprooting with respect to total number of leaves, branches and total fresh weight yield.

Bansal *et al.* (1993) studied the manipulation of source-sink in relation to productivity of amaranth through pruning treatments. Results indicated that pruning of 25 per cent leaves at pre-flowering remarkably suppressed grain yield. On the other hand, 25 per cent pruning of leaves at post flowering proved to be the best treatment from multiple use crop model in amaranth var. Annapoorna yielding 13.39 Q ha^{-1} of grain yield along with 45.7 Q ha^{-1} of fresh green leaves for vegetable yield.

Devadas *et al.* (1993) compared growth and yield parameters of 12 red and 11 green amaranths and observed that red types have broader and longer leaves, fewer branches, took longer to bolt and were taller at bolting than green types.

2.1.3. Season and yield

Yield per plant was observed to be highly influenced by the environment (Prasad *et al.* 1980). The investigations of Sreerangaswami *et al.* (1980) also brought out the existence of strong "Genotype x Environment interactions" in the diverse genetic populations of amaranth.

According to Hackett and Carolance (1982) the favourable temperature range for amaranth is 26-30° C and the upper limit of day and night temperatures are 45°C and 30°c respectively.

Mohideen *et al.* (1982) made a critical study in 75 germplasm of vegetable amaranth (*A. tricolor*) using different genetic parameters for two seasons. The range, mean and coefficient of variation for different

characters were generally high and showed a decrease in monsoon as compared to summer season.

The two season trials in A. tricolor with nitrogen at 50-200 Kg ha^{-1} and phosphorous at 50-100Kg ha^{-1} showed that the yields were the highest (11.6 t ha^{-1}) in the summer crop receiving the highest nitrogen rate as compared to the autumn crop which yielded 10.6 t ha^{-1} at the same nitrogen rate (Ramachandra and Thimmaraju, 1983)

Evaluation of four grain amaranth species during three sowing dates were done by Santos (1989) and the results revealed that as day length shortened, the yield decreased. Higher yields were obtained in the early sowing (August 25th) and the lowest yields recorded in the late sowing (September 14th) indicating that photoperiod is a determining factor for growth and production of amaranth. As days shortened, flowering occurred at an earlier date.

Sirohi and Sivakami (1995) compared the performance of different varieties of amaranth, viz. Pusa keerthi, Co-2, Pusa kiran and Badichaulai and found that Pusakeerthi was better for cultivation in summer season (51 t ha^{-1}) and Pusa kiran was the highest yielder in Kharif (35 t ha^{-1}).

2.2. Leaf spot diseases in amaranth

2.2.1. Etiology and symptomatology

In India, leaf spot disease caused by *Alternaria* sp was reported in

amaranth by Solankure and Rao (1973). Kumar and Rao (1976) reported occurrence of a leaf spot disease of Amaranth species (*A. viridis*) during rainy season and winter. Disease symptoms were the appearance of small brown to black necrotic lesions on leaves and the pathogen was identified as *Colletotrichum* sp., a typical isolate belonging to *Colletotrichum dematium.*

Tari and Mlasani (1994) described a leaf blight incited by *Choanephora cucurbitarum* and a leaf spot incited by *Alternaria amaranthi* on cultivated amaranth, *Amaranthus cruentus* in Tanzania.

Suharban *et al.* (1994) reported a leaf spot disease of *Amaranthus gangeticus* Linn. in Kerala caused by *Colletotrichum gloeosporioides* (Penz.) Sacc. The disease was characterized by the prevalence of a number of small whitish spots on leaves which coalesce together resulting in blighting of leaves.. When the disease advanced, the spots increased in size covering whole leaf area leading to complete drying. The incidence is more in older leaves.

Kamalanayar *et al.* (1996) reported a new foliar blight on *Amaranthus tricolor* Linn. caused by *Rizoctonia solani.* The disease was characterized by light cream coloured spots on the foliage which rapidly spread causing extensive damage leading to economic losses.

2.2.2. Weather and disease

Siddaramaiah *et al.* (1978) reported higher incidence of disease in rainy season due to the presence of optimal conditions necessary for disease development. Role of weather on leaf spot disease (*Alternaria*

alternata) development in brinjal was studied by Dingar and Mohit Singh (1986) and their results indicated that disease development reached a peak during the last week of October and first week of November when mean temperature was 24 –26°C and RH was 47-52 %. Scanty rains and long dry spell affected disease development more adversely than intermittent rainfall.

Sukumar and Ramalingham (1989) related higher incidence of leaf spot during rainy season to its dependence on rainy splash for the dispersal of its spores.

Adebitan (1994) reported that a temperature of 15-30° C was suitable for *Colletotrichum truncatum* to grow on PDA, 28° C being optimum for growth and 25°C for sporulation. He also observed that growth of the fungus was more in complete darkness, but the sporulation was severely affected.

Rahman *et al.* (1994) observed that temperature has a significant role in the mycelial growth and acervuli development of *Colletotrichum* sp. on culture medium and the fungus grew faster at 30°C with high acervuli formation on PDA + bean extract medium.

Dang *et al.* (1995) reported that the development and progress of a disease under natural condition is influenced by prevailing environmental factors, the type of host cultivars and availability of pathogen inoculum.

Gokulapalan and Reghunath (1995) reported that the leaf spot disease in amaranth is more pronounced in rainy season and in summer

splash irrigation resulted in the spread of fungal spores to neighbouring plants.

Kamalanayar *et al.* (1996) reported that foliar blight of *A. tricolor* was severe during the post monsoon period of 1994 (August-September) and all stages of the crop were found to be susceptible to the disease.

2.2.3. Effect of toxin on disease development

Many fungi which cause leaf spot diseases are also reported to produce toxins. Production of toxins of *Colletotrichum* spp. has been studied by various investigators. Lin (1948) reported the production of toxic metabolite by *G cingulata.*
Goodman (1960) reported that the toxin produced by *Colletotrichum fiscum* caused spotting of tomato foliage and this toxin affected plants which were not infected by the pathogen.

Narain and Das (1970) reported the production of toxins by *C. capsici* causing anthracnose of chillies.

Nair and Ramakrishnan (1973) conducted detailed studies on the toxins produced by *C. capsici* causing leaf spot disease of turmeric. In treatment with exo and endo toxin solutions, visible alterations in the inoculated area of turmeric leaves were noticed within four hours of inoculation.

Varma (1991) studied the effect of exo and endo toxins of *C. gloeosporioides* causing leaf spot of *Plumbago indica* and observed that endotoxic metabolites produced symptoms much earlier than that

produced by exotoxic metabolites. Richard's medium was found the best for toxin production and maximum toxic activity was observed at 20th day of incubation at room temperature.

Sharma and Sharma (1996) reported production of toxin by *C. gloeosporioides* causing citrus dieback and toxin production in vitro took place in Richard's solution after 22 days.

2.2.4. Host resistance

The use of resistant varieties is a simple and economic means of evading plant diseases. Many workers have studied the host resistance against *Colletotrichum* sp. in cowpea and other vegetables. The Physiology and biochemistry of plant pathogen interaction was studied by Misaghi (1982). However, in the case of amaranth, not much information is available on this aspect

Oladiran and Oso (1983) observed that the severity of brown blotch of cowpea caused by *C. truncatum* and *C. capsici* increased with age of the pods except VITA-1 which did not show any disease symptoms.

Sohi and Rawal (1983) screened 141 cowpea varieties against *C. lindemuthianum* and 21 varieties were found to be resistant to this pathogen.

Out of the 27 cultivars of *Vigna radiata* screened for resistance to *C. dematium* and *C. lindemuthianum*, none had shown resistance in 1985, but Pusa 109 was highly resistant and MC 353 resistant during 1986. Some cultivars showing susceptible or moderately

resistant reactions in 1985 had different reactions in the following year (Thakur and Khare, 1989).

Twusami *et al.* (1989) evaluated 62 IITA and local cowpea lines under natural infection for fungal diseases and found that 30 lines were apparently resistant to multiple diseases, 19 lines moderately susceptible and 13 lines susceptible to more than one disease. When seedlings of 44 lines showing resistance to multiple diseases were inoculated with *C. truncatum*, 16 were resistant, 15 moderately resistant and 13 susceptible.

Rego *et al.* (1995) reported the effect of plant age on disease reaction by *Colletotrichum arbiculare* in watermelon and melon. They observed that the cotyledons of water melon were more susceptible than the true leaves, while the true leaves of melon were more susceptible than the cotyledons.

Gokulapalan and Reghunath (1995) reported that red amaranth was highly susceptible to leaf spot whereas green varieties exhibited varying degree of tolerance. Hence, growing of green amaranth in combination with red amaranth to have a mixed stand of resistant and susceptible types was suggested to manage leaf spot disease to a certain extent.

2.3. Biochemical basis of resistance

Plants have their own defence mechanism against all microorganisms. Mahadevan (1973) reported that resistance to parasitic microorganisms like bacteria, fungi and virus is not only due to

structural barriers like thick epidermis, leaf hairs and thick cuticle, but also due to sugar content, osmotic pressure, pH and other factors. In some cases the pathogen overcomes these defence barriers with offensive chemicals and causes disease. A wide range of chemicals especially phenolics, phytotoalexins and host enzymes show antifungal and antiviral activities in plant disease resistance.

2.3.1. Phenolics

Phenolics and its protective role against diseases in vegetables were reported by many scientists (Kuc, 1964 and Patil *et al.* 1964 in potato ; Rajan , 1985 and Sadankumar, 1995 in tomato ; Gangappa, 1986 and Gopinath and Madalageri, 1986 in brinjal ; Markose , 1996 in chilli and Paul, 1998 in solanaceous crops). Catechol, procatechuic acids, phenols, flavanoids and tomatin are the main pre-infectional inhibitors present in plants (Stoessel, 1969 ; Langecake *et al.*1972).

Hampton and Fulton (1959) reported that toxic quinones are commonly formed from dihydroxic phenolic compounds. Among the different phenolics, orthodihydroxy phenolics (OD-phenolics) are known to be highly toxic and play a major role in disease resistance (Mahadevan, 1966).

Phenolics play a key role in fusarium wilt resistance in tomato. Tomato plants inoculated with *Fusarium oxyporum, F. lycopersici* synthesized increased amounts both of total and orthodyhydroxy phenols. These compounds were synthesized rapidly in resistant than in susceptible plants (Matta *et al.* 1967).

Phenolics are easily oxidised by polyphenol oxidase and peroxidase to highly reactive quinones which are effective inhibitors of sulphydryl enzymes thereby preventing the metabolic activities of host and parasitic cells (Mahadevan, 1970).

Sridhar and Ou (1974) reported increase in total phenol content after infection. The importance of phenolic compounds in host-parasitic interaction is characterized by oxidation-reduction reactions and resistance expressed by oxidation of phenols to quinone which are more toxic to microorganisms (Sempio *et al.* 1975).

Hunter (1978) reported that Orthodihydroxy phenolic compounds such as caffeic acid, chlorogenic acid, orthoquinones and tannins can strongly inhibit the activities of extracellular enzymes produced by microorganisms ,in addition to growth inhibition.

Arora (1983) observed decrease in the total phenols in mung beans (*Vigna radiata*) infected with *Rizoctonia solani.* In some instances, total phenol was found to increase after infection (Sharma *et al.* 1983).

Phenolics in high concentrations are toxic to plant cells themselves (Tepper and Anderson, 1984). Hence, phenolics will normally be present in small quantities only in plants and these quantities may not be sufficient to suppress the development of pathogens.

Correlation of total phenol content to resistant/susceptibility to bacterial wilt was studied by some workers. In his studies, Sitaramaiah *et al.* (1984)) was unable to correlate the total phenol content to resistant/susceptibility to bacterial wilt in brinjal.

Rajan (1985) observed a negative correlation between resistance and total phenol content in tomato and inferred that lower levels of phenolics in roots of resistant variety may be due to the increased rate of oxidation of phenolics. However Geetha (1989) was unable to correlate the total phenol content to resistant/susceptibility to bacterial wilt in brinjal.

A positive association between OD-phenol content in the roots and bacterial wilt resistance was reported by some workers. The resistant lines had higher OD-phenol content compared to the susceptible lines in tomato and brinjal (Rajan, 1985 and Gangappa, 1986).

Increase in total phenol after infection was reported by Bansal *et al.* (1986) and Luthra *et al.* (1988). Park *et al.* (1988) could observe no difference in total phenol content between healthy and infected fruits of *Capsicum annum.*

Bajaj (1988) reported that chlorogenic acid and caffeic acid are the most important phenolic compounds involved in disease resistance mechanisms.

Vidyasekharan (1990) reported that in many plants pathogen interactions, the synthesis of phenolics is activated after infection and the high amount of phenolics synthesized rapidly suppress the pathogen development.

Changes in the level of total phenols was determined in healthy and Alternaria leaf blight susceptible leaves of *Brassica* species (Gupta *et al.,* 1995). Results indicated an initial increase in the level of total phenols reaching maximum at 60 days after sowing followed by a

decline with the age of the plant. Tolerant species registered considerable higher amount of total phenols compared to susceptible ones at all stages of plant development. Their amount however, decreased after infection in all the species, but this depletion was more pronounced in susceptible species.

Chowdhury (1995) reported higher quantity of total phenol and OD-phenol in the post infection period in groundnut plants by *Puccinia arachidis*. Sadankumar (1995) and Markose (1996) reported a positive association between OD-phenol content in the roots and bacterial wilt resistance in tomato and brinjal respectively.

Milter *et al.* (1997) studied the biochemical changes in chickpea accessions and found higher amount of total phenol in resistant types than the susceptible accessions to gray mould caused by *Botrytis cinerea*. They also reported a decrease in phenol in the accessions after inoculation by *B. cinerea*.

2.3.2. Host enzymes and disease resistance

Increased peroxidase activity in resistant potato plants was reported by Fehrmann and Diamond (1967).

Germplasm of any crop can be characterized through electrophoresis (Peiru and Brewbaker, 1973). Host enzymes like peroxidase and polyphenol oxidase play an important role in disease resistance. These enzymes are responsible for synthesis of quinones from phenolics. Quinones are highly bactericidal and fungitoxic (Rama and Dunleavy,1975). Hence, an increased activity of these enzymes might play a role in disease resistance. Obukowicz and Kennedy (1981)

observed the importance of polyphenol oxidase enzyme in resistance against *P. solanacearum* in tobacco. Park *et al.* (1988) reported no difference in peroxidase activity between healthy and infected fruits of *Capsicum annum.* Oh (1988) found that in soybean, the peroxidase activity markedly increased on infection with soybean necrotic virus (Smv-N) and was higher in the susceptible variety than in resistant line.

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

Increased peroxidase activity in resistant chilli was reported by Singh and Singh (1989). Reuveni *et al.* (1990) observed a high correlation ($P<0.05$) between peroxidase activity in the leaves of melon and resistance to *Pseudomaonas cubensis* suggesting that this rapid assay was possible in preliminary selection of melons resistant to fungus.

Ahmed *et al.* (1994) observed high polyphenol activity in okra varieties resistant to YVMV than in susceptible varieties. In groundnut, Duan *et al.* (1994) found that there was no significant difference in pre-inoculation polyphenol oxidase activity between the bacterial wilt resistant and susceptible varieties, but the differences were significant after inoculation.

Chowdhury (1995) reported higher peroxidase and polyphenol oxidase activity in infected groundnut plants by *Puccinia arachidis.*

Specific activities of polyphenol oxidase, peroxidase and catalase were determined in healthy and *Alternaria* blight susceptible leaves of *Brassica* species by Gupta *et al.* (1995). They observed high specific activity of polyphenol oxidase in tolerant species while that of peroxidase remained low when compared with susceptible species. In response to infection, the activity of both the enzymes increased, comparatively at a much faster rate in the susceptible species. Catalase activity was considerably higher at initial stages of plant growth in all the species, which dropped markedly at later stages.

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

Pritamkalia (1998) studied the enzymic association of powdery mildew resistance in garden pea and reported an increased activity of peroxidase and polyphenol oxidase in the resistant accessions in the pre-infectional stage than in the susceptible genotype. In post-infectional stage, a marked increase in peroxidase activity was observed in both resistant and susceptible accessions. Post-infectional increase in polyphenol oxidase activity was observed in both resistant and susceptible accessions except a few which either exhibited no change or decrease in post- infectional PPO activity.

2.3.3. Nutrient content and disease resistance

The requirement of L- ascorbic acid in various metabolic pathways and its biosynthesis in plants have been discussed by Isherwood and Mapson (1962) and they reported that plants resistant to various pathogens have been shown to have a higher ascorbic acid content than susceptible ones.

Gangawane and Datar (1978) observed that infection by the pathogen *Alternaria solani* invariably reduced the ascorbic acid content in the leaves of susceptible varieties of tomato whereas resistant germplasm showed much higher ascorbic acid content.

Sharma and Chowfla (1991) reported that in *A. caudatus,* infection by amaranthus mosaic virus decreased the chlorophyll a and b content and reducing and nonreducing sugars.

Biochemical and physiological changes in chilli leaves inoculated with *Alternaria solani* was studied by Veeramohan *et al.* (1994) and they reported that inoculation with the pathogen resulted in a decrease in the rate of photosynthesis and in amounts of chlorophyll, reducing and nonreducing sugars.

The changes in biochemical contents of vitamin C, chlorophyll and total carotenoids in the cucumber mosaic virus infected leaves/seeds of *Amaranthus* and *Chenopodium* species were studied by Dhan-Prakash *et al.* (1995) and reported that vitamin C (37-79%) carotenoids (19-52%) and chlorophyll (5-45%) were lower in all the tested species.

2.3.4. Variation in nutrient composition in amaranth

Grubben (1976) found variation in different species of Amaranth for the ascorbic acid content which ranged from 325-1250 mg in 100 g of dry matter. Variations in the content of ascorbic acid (12-120 mg), potassium (0.41-0.58 %) and calcium (105-506mg/ 100g of fresh matter) were reported by Joel Elias (1977) in different varieties of amaranth.

Stobart *et al.* (1980) reported that potassium applied as KCl or KNO_3 enhanced chlorophyll production in seedlings of *A. caudatus*.

Mohideen *et al.* (1982) reported that an amaranth variety Co-3 contains 35.9 mg ascorbic acid per gram.

Influence of N and K_2O on carotene, ascorbic acid and chlorophyll content of amaranth was studied by Subbiah and Ramanathan (1982) and reported that N increased plant crude protein, carotene and chlorophyll contents, but decreased the ascorbic acid content. K had no marked effect on carotene, ascorbic acid and chlorophyll levels, but increased the crude protein content in late harvested crops.

Vijayakumar and Shanmughavelu (1985) reported the nutritive value of seven types of amaranth which ranged from 32.9 –44.2 mg/ 100 g for ascorbic acid, 9.9 – 10.9 mg for carotene content, 16.5 –21.9 % for crude fiber, 12.5 – 14.5% of protein and 2.3 – 2.5% for calcium content.

Prakash *et al.* (1993) studied vitamin C content in the leaves of 62 varieties of amaranth and reported a variation between 62 and 288

mg/100g for grain and vegetable type amaranth. They observed that the vegetable types contained less vitamin C than the grain type. The highest amount of vitamin C were found in middle aged leaves at 13-21 position, while lowest amount was in the youngest leaves.

Kononkov *et al.* (1995) studied the content of chlorophyll, carotenoids and beta-cyanin in leaves of different species of *Amaranthus* and reported highest values in *A. tricolor* accessions (Chlorophyll a+b – 14.61 mg/g, carotenoid content – 4.95 mg/g, amaranthin – 34.2 mg/g).

2.3.5. Antinutritional factors

2.3.5.1. Oxalates and nitrates

Foods with high oxalic acid are reported to cause various disorders and even death (Jeghers and Murphy, 1945 ; James, 1968 ; Singh and Sharma, 1968 ; and Singh *et al.* 1971 and Dhanprakash and Pal, 1991).

Sreevastava and Krishnan (1959) observed a total oxalate content of 12.78% in the leaves of *A. gangeticus* on dry weight basis and also reported that young leaves contained a larger proportion of water soluble oxalates compared to mature and old leaves.

The presence of considerable quantities of oxalic acid and nitrate in amaranth lead to a belief that the recommendation of large quantities of leafy vegetables in human nutrition is harmful (Sadik,1971). One of the biological functions generally attributed to oxalic acid is that it

locks the cations thus causing a physiological deficiency of these nutrients.

According to Deutsch (1977), "genotype x environmental interactions " appeared large for oxalates and calcium contents and also oxalates became more of a problem when plants are grown under stress. He also opined that healthy adults need not be concerned about the presence of these compounds as the leafy greens make up only a fraction of the daily food intake . One would need a daily intake of more than 100g of fresh green to raise the nitrate and oxalate levels. Studies on the consumption of leafy vegetables revealed a daily intake of 5 g in Latin America, 11 g in central America and 21 g in Africa (Grubben, 1976). This intake is definitely non-consequential as compared to 100g required for causing nutritional defect due to the higher nitrate and oxalate levels.

Marderosian *et al.* (1980) analysed different species and accessions of amaranth for oxalate content and reported that mean oxalate levels were about 0.75 per cent (fresh weight) for leaves and 0.06 per cent for stems.

Devadas (1982) reported a variation. from 0.94 – 1.29 per cent for oxalate in 25 accessions of vegetable amaranth and 0.55-0.94 per cent of nitrate. Hill and Rawatte (1982) recorded *in A. retroflexus* 5.36 per cent oxalic acid in the leaves and 2.66 per cent in the stems on dry weight basis.

Sanni (1983) reported that *A. hybridus* contained a nitrate level of 1675 ppm on dry weight basis which exceeded the safe limit of

500ppm recommended by WHO. No appreciable loss of nitrate was observed after 48 hours of cold storage.

George (1986) reported high level of oxalates in summer in all the 19 accessions studied (6.17 –12.63%) than in kharif (4.43 –10.4%). Mallika (1987) reported a variation from 3.6 – 5.1 % for oxalate in eight amaranth species. She also observed differences in the two sections of the family *Amaranthus*. Members of the section *Amaranthus* in general are characterized by lower content of oxalate and nitrate than section *Blitopsis*. But *A. spinosus* contains the highest amount of both these factors.

Meena *et al.* (1987) estimated the soluble oxalate content in *A. tricolor* as 5.2 % and Ca : oxalate ratio as 0.6. Figueroa (1989) reported that red amaranth types had a higher concentration of anti-nutritional substances and this could be the reason for lower protein quality in red types. George *et al.* (1989) observed that all green entries had low oxalate contents, being the lowest in Co-1 (3.04%) whereas red and green-red entries with high protein and beta-carotene had high oxalate contents.

George (1986) reported that among the five high oxalate amaranth entries, four were red coloured and among the accessions Co-2 had the highest oxalate content and the lowest content was observed in Co-1. All red and green-red types with high protein and β-carotene also had high oxalates.

Vityakon and Standal (1989) estimated the total oxalate content in *A. tricolor* and a high amount of 91g kg^{-1} of dry matter was recorded. They isolated potassium and magnesium oxalate which are soluble in

boiling water and an associated insoluble residue which was predominantly calcium oxalate.

Prakash and Pal (1990) reported an increase in the oxalate levels with leaf position (1.28 – 3% in four week and 1.4 – 3.3 in eight week old plants) whereas the change in nitrate (0.35 – 0.49 %) was not significant and virtually remained unchanged with leaf position.

Thamburaj *et al.* (1994) reported variation in the oxalate content from 0.82 to 1.92 % in 41 types of amaranth. It was observed that red colour types had higher oxalate content as compared to green colour types.

Lin *et al.* (1995) observed that amaranth accessions with low nitrate content were characterised by light leaf colour, short leaf stalk, wide leaf blade and small plant spread.

Sukumar and Rajan (1998) observed that oxalate content in *A. tricolor* was higher in plants grown under high temperature and dry conditions and also noticed that oxalate content was lower under double cut system than that from first cut.

2.3.5.2. Causes of anti nutritional factors build-up

The relationship between nutrient supply and oxalate accumulation need much attention. According to Grutz (1956) cations promote oxalic acid formation while anions inhibit it. High P_2O_5 application obstructs oxalic acid formation so that the promoting effect of Ca cannot take effect. Oxalic acid formation was also reduced by N applied as ammonium nitrate.

The relationship between nutrient supply and nitrate accumulation has been well documented (Commoner, 1970). The amount and source applied and the time and the method of application govern the effects of nitrogen fertilizers on nitrate accumulation in vegetables. The usual effect is that increasing the level of nitrogen nutrition increases the nitrate concentration in vegetables (Barker and Maynard, 1971 ; Peek *et al.* 1971 ; Schmidt *et al.* 1971 ; Trevino and Murray, 1975).

According to Singh (1974) the form in which nitrogen is applied influences the oxalate synthesis differently and this influence may be specific to individual plant species.

The degree of accumulation of nitrate is controlled by the genetic potential of the plant and also by the nitrate content of soil and plant growing environment (Maynard and Barker ,1979 and Palma Vityakon, 1986). Nitrate accumulation increased with a higher rate of nitrogenous fertilizer application, use of nitrate form of fertilizers (KNO_3, NH_4NO_3), pre plant broadcast application of N- fertilizers and condition of water stress.

Singh *et al.* (1985) observed that the highest leaf oxalic acid content was in *A. tristis* applied with N at 0-60 kg ha^{-1} in split dose i.e., one half as a basal dressing and the other half as a top dressing after eight weeks from sowing.

A study conducted by Sukumar (1997) indicated that application of nitrogen and phosphorous brought out significant decrease in the oxalate content from 7.3% to 4.84% upto the highest level tried (N 150 Kg ha^{-1} and P 100 Kg ha^{-1}). But potassium application increased the

oxalate content significantly from 5.7% to 6.4%. A clear-cut increase in nitrate accumulation from 0.17% to 0.74 % was noticed with increasing level of nitrogen. Nitrate accumulation was not significantly affected by P levels whereas potassium significantly increased the nitrate content from 0.17 % to 0.67 %.

2.4. Isozyme pattern and disease resistance

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

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

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

manipulating quantitative variation. Gabriel and Ellinghoe (1982) noted genotypic differences in banding pattern of esterase in barley cultivars showing various levels of resistance to powdery mildew. Out of eight bands, differences were exhibited in EST 4, EST 5 and EST 6 (Hwang *et al.*1982). Based on these differences they classified the cultivars into highly resistant, moderately resistant and highly susceptible. Hulupi *et al.* (1988) studied the role of isozymes against resistance to leaf rust in coffee. Based on PPO isozyme bands they classified the accessions into three groups, same as to their resistance to leaf rust.

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

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

Ming and Xian (1988) conducted isozymes analysis of PRX and EST through PAGE in different organs of II watermelon varieties differing in resistance to fusarium wilt disease. There were some differences in PRX and EST isozymes spectrum in stems and leaves and the varieties differing in FWR. Therefore isozymes method could be used to screen watermelon varieties for FWR. Oh (1988) conducted studies on the electrophoretic pattern of peroxidase in resistant as well as susceptible soybean varieties, in early period of infection by soybean necrotic virus (SMV-N). He found that the electrophoretic isozyme pattern was

changed by the infection in the susceptible variety while no new isozyme bands were observed in the incompatible host parasite combination.

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

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

Pavlov (1989) used peroxidase isozyme spectra for identification of remote hybrids in tomato.

Yu and Wang (1990) reported that the seedling of susceptible watermelon cultivars to fusarium wilt disease had one or two isozyme bands more than those of the resistant cultivars and hence this factor could be used for early screening against disease.

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

Indian tomato cultivars were distinguished into diferent groups based on the protein banding pattern by Chakrabarthi *et al.* (1992) ; Henn *et al.* (1992) and Mather *et al.* (1993).

Studies by Wagih (1992) revealed that when infected with tobacco necrosis necro virus, the affected cucumber seedlings showed an additional major isozyme band of amylase (Rf 0.7), with respect to the healthy plants.

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

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

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

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

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

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

Markose (1996) observed that the number of bands of peroxidase in roots was two in Ujwala (resistant to bacterial wilt) while it was three in Pusa Jwala (susceptible). In leaves, Ujwala had only one band while it was two in Pusa Jwala. In case of esterase enzyme, there were three medium thick bands in Ujwala whereas Pusa Jwala had only one feeble band. Inoculation had no effect in the banding pattern for both the enzymes.

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

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

Fan *et al.* (1996) reported an increased content of total phenol, polyphenol oxidase and perodase isoenzymes in leaves of *Venturia nashicola* resistant pear c.v. Yali. They also reported an additional band of peroxidase isoenzyme in the fast band area of the resistant cultivar.

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

Morales *et al.* (1997) studied the ability of grapevine peroxidase isoenzymes B5 to oxidase transresveratrol. They suggested that this isoenzyme enables a specific metabolic function, which act as a marker of disease resistance in grapevine leaves and shoots for Gamay Rough grape berries.

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

Materials and Methods

3. MATERIALS AND METHODS

The present study was undertaken from October 1996 to April 1998 in the Vegetable Research Farm of the Department of Olericulture, College of Horticulture, Vellanikkara, Thrissur. The location is situated in an altitude of 22.25m, 10°32'N latitude and 76°16'E longitude with a typical humid tropical climate. The soil type is deep, well drained sandy loam with pH 5.1.

The details of the materials used and the methods followed in this study are presented under the following heads:

1. Collection, maintenance and evaluation of amaranth accessions for the incidence of leaf spot diseases, assessment of genetic divergence and grouping of accessions.

2. Isolation and identification of the pathogen(s) associated with leaf spot diseases.

3. Seasonal influence on leaf spot incidence and yield in amaranth and correlation studies.

4. Studies on biochemical basis of resistance to leaf spot disease.

5. Biochemical cataloguing of accessions into different classes using enzyme patterns.

3.1. Evaluation of amaranth accessions for resistance to leaf spot diseases

3.1.1. Materials

The experimental materials included 168 accessions of amaranth.

These accessions consisted of released varieties, promising lines, local and exotic collections belonging to different *Amaranth* species, viz. *A. tricolor*, *A. dubius*, *A. viridis*, *A. spinosus* and *A. hypochondriacus*. The list of these accessions are given in Table 1. Amaranth accessions collected from different sources were genetically catalogued based on the descriptor developed for amaranth by IBPGR (Table 2).

3.1.2. Design and layout

The experimental materials were planted in a Randomised Block Design with two replicates. Field evaluation of 168 amaranth accessions was conducted during October 1996 to March 1997. Planting was done in rows at a spacing of 15cm between plants. Out of the 20 plants per replicate, 10 plants were used for recording vegetable yield by periodic harvest (4 cuts) and the remaining plants were used for recording disease incidence. The crop was raised as per the Package of Practices Recommendations for crops (KAU,1996). Three weeks old seedlings were transplanted in the main field. A susceptible check, Kannara local was planted as border row around the entire experimental plot to provide sufficient inoculum for leaf spot disease.

3.1.3. Observations on morphological characters

(a) Plant height

Five plants were selected randomly in each row on the 30th day after transplanting and at flowering and their height was recorded.

Table 1. Amaranth accessions used in the study

Sl. No.	Name of accessions.	Source
1	A-149	KHDP, Vellanikkara
2	A-150	AICVIP, Vellanikkara
3	A-151	AICVIP, Vellanikkara
4	A-152	AICVIP, Vellanikkara
5	A-153	AICVIP, Vellanikkara
6	A-154	AICVIP, Vellanikkara
7	A-155	AICVIP, Vellanikkara
8	A-156	AICVIP, Vellanikkara
9	A-4	Dept. Olericulture, Vellanikkara
10	A-157	AICVIP, Vellanikkara
11	A-158	AICVIP, Vellanikkara
12	A-159	AICVIP, Vellanikkara
13	A-160	AICVIP, Vellanikkara
14	A-161	AICVIP, Vellanikkara
15	A-162	KHDP, Vellanikkara
16	A-163	AICVIP, Vellanikkara
17	A-164	AICVIP, Vellanikkara
18	A-165	NBPGR Regional Stn, PKV, Akola
19	A-166	KHDP, Vellayani
20	A-167	Muthalamada
21	A-168	Bihar
22	A-169	Bihar
23	A-6	Dept. Olericulture, Vellanikkara
24	A-170	KHDP , Vellayani
25	A-171	AICVIP, Vellanikkara
26	A-3	Dept. Olericulture, Vellanikkara/TNAU Coimbatore
27	A-172	AICVIP, Vellanikkara
28	A-173	Bihar
29	A-174	Bihar
30	A-175	KHDP, Vellayani
31	A-176	Bihar
32	A-177	Kannur
33	A-178	AICVIP, Vellanikkara
34	A-179	KHDP, Vellayani
35	A-180	Kannur
36	A-181	AICVIP, Vellanikkara
37	A-182	Ratanshi Velji Shah, Bombay
38	A-183	AICVIP, Vellanikkara
39	A-184	Ratanshi Velji Shah, Bombay
40	A-185	Thrissur local
41	A-186	Mala
42	A-187	Alpara

43	A-188	Peechi
44	A-189	COA, Vellayani
45	A-190	KHDP, Vellanikkara
46	A-191	Coimbatore
47	A-192	AICVIP, Vellanikkara
48	A-193	KHDP, Vellayani
49	A-194	Kuttiadi
50	A-195	Kuttiadi
51	A-196	TNAU, Coimbatore
52	A-197	Calicut
53	A-198	Calicut
54	A-199	NBPGR,PKV, Akola
55	A-200	Ratanshi Velji Shah, Bombay
56	A-201	USDA, Origin Bolivia
57	A-202	Bihar
58	A-203	AICVIP, Vellanikkara
59	A-204	AICVIP, Vellanikkara
60	A-205	AICVIP, Vellanikkara
61	A-206	AICVIP, Vellanikkara
62	A-207	Kuttiyadi
63	A-208	NBPGR,PKV, Akola
64	A209	NBPGR,PKV, Akola
65	A-210	IIHR, Bangalore
66	A-211	USDA, Origin, Jamaica
67	A-212	Moorkanikkara
68	A-213	Calicut
69	A-214	Kalamasseri
70	A-215	Kollam
71	A-216	AICVIP, Vellanikkara
72	A-217	Vellanikkara
73	A-218	Patna
74	A-219	Kaduthuruthy
75	A-220	Muthalamada
76	A-221	Moorkanikkara
77	A-222	Marayamangalam
78	A-223	Thrithala
79	A-224	KHDP, Vellayani
80	A-225	KHDP, Vellayani
81	A-226	Panniyur
82	A-227	Balussery
83	A-228	NBPGR,PKV, Akola
84	A-229	AICVIP, Vellanikkara
85	A-230	Nelliampathy
86	A-231	Nelliampathy
87	A-232	AVRDC, Taiwan
88	A-233	AICVIP, Vellanikkara
89	A-234	AVRDC, Taiwan
90	A-235	Kodungallur

91	A-236	Ambalavayal
92	A-237	Ratanshi Velji Shah, Bombay
93	A-238	Bihar
94	A-239	Ernakulam
95	A-240	Calicut
96	A-241	Kriachira
97	A-242	Goa
98	A-243	Angamali
99	A-244	Thamarassery
100	A-245	Goa
101	A-246	Aluva
102	A-247	Puthenchira
103	A-248	Perumbavur
104	A-249	Thrissur
105	A-250	Thiruvalla
106	A-251	Calicut
107	A-252	Puthukkad
108	A-253	Vellanikkara
109	A-254	Mattannoor
110	A-255	Calicut
111	A-256	Calicut
112	A-257	Koyilandy
113	A-258	Kannur
114	A-259	Kannur
115	A-260	Kanjangad
116	A-261	Kannur
117	A-262	Pilicode
118	A-263	Pilicode
119	A-264	Pilicode
120	A-265	Panniyur
121	A-266	Puttur
122	A_267	Kanjangad
123	A-268	IARI, Delhi
124	A-269	Pazhakkappilly
125	A-270	Ambalavayal
126	A-271	Bihar
127	A-272	Bihar
128	A-273	Trivandrum
129	A-274	Trivandrum
130	A-275	Trivandrum
131	A-276	Trivandrum
132	A-277	Trivandrum
133	A-278	Trivandrum
134	A-279	Trivandrum
135	A-280	Trivandrum
136	A-281	Trivandrum
137	A-282	Trivandrum
138	A-283	USDA, Origin : Florida

139	A-284	Trivandrum
140	A-285	Trivandrum
141	A-286	Trivandrum
142	A-287	Ernakulam
143	A-288	Ernakulam
144	A-289	Kollam
145	A-290	Kollam
146	A-291	Aluva
147	A-292	Perumbavoor
148	A-293	Angamali
149	A-294	Calicut
150	A-295	Balussery
151	A-296	Calicut
152	A-297	Calicut
153	A-298	Kasergode
154	A-299	Trivandrum
155	A-300	Kollam
156	A-301	Kannara
157	A-302	Puthur
158	A-303	Chalakkudy
159	A-304	Palghat
160	A-305	Palghat
161	A-306	Palghat
162	A-307	Kollam
163	A-308	Trivandrum
164	A-309	Palghat
165	A-310	Kannur
166	A-311	Kannur
167	A-312	Kannur
168	A-313	Kannara

Table 2 Amaranth descriptor list (IBPGR)

1. Plant characters

1.1 Growth habit
1. Erect
2. Prostrate

1.2 Stem pigmentation
1. Green
2. Purple or pink
3. Others

1.3 Stem pubescence
1. None
2. Low
3. Conspicuous

1.4 Petiole pigmentation
1. Green
2. Purple or pink
3. Others

1.5 Leaf pigmentation
1. Entire lamina purple or pink
2. Basal area pigmented
3. Central spot
4. Two stripes (V- shaped)
5. One stripe (V- shaped)
6. Margin and vein pigmented
7. Palegreen or chlorotic stripe on normal green
8. Normal green
9. Dark green
10. Others

1.6 Leaf pubescence
1. None
2. Low
3. Conspicuous

1.7 Leaf shape
1. Lanceolate
2. Elliptical
3. Cuneate

4. Obovate
5. Ovatainate
6. Rhombic
7. Oval

1.8 Leaf margin

1. Entire
2. Crenate
3. Undulate
4. Others

1.9 Prominence of leaf veins
1. Smooth
2. Rugose

2. Inflorescence characters

2.1 Terminal inflorescence – laterals
1. Present
2. Absent

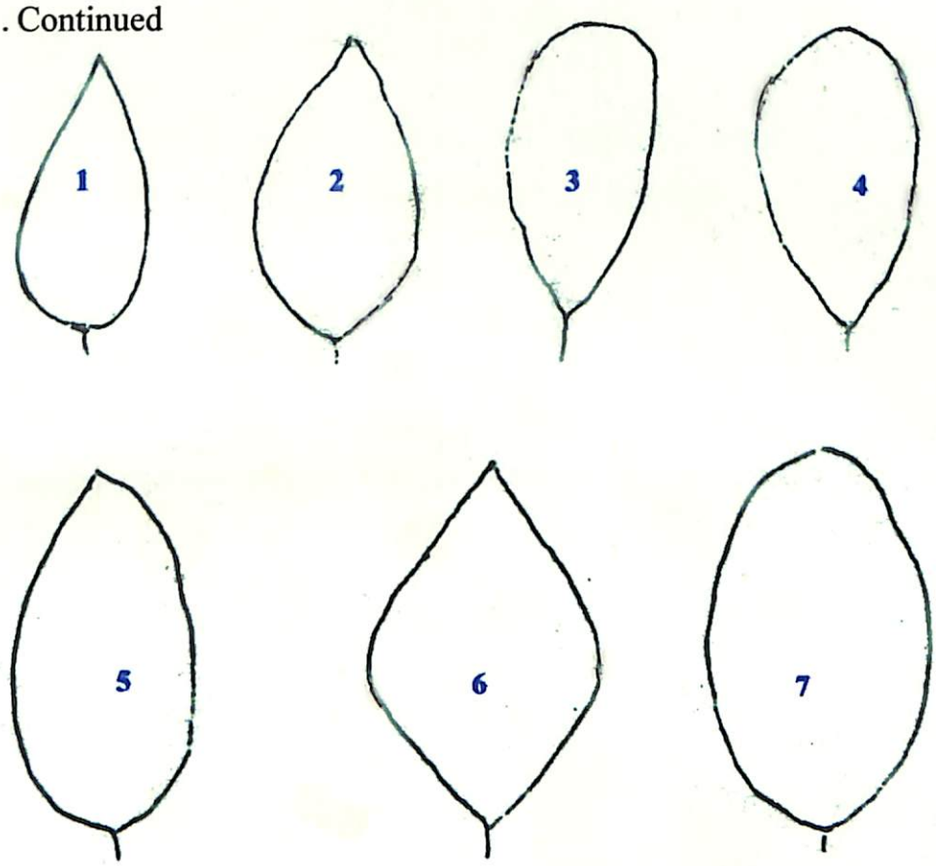
2.2 Terminal inflorescence shape
1. Spike
2. Panicle with short branches
3. Panicle with long branches
4. Club – shaped at tips
5. Others

2.3 Terminal inflorescence latitude
1. Erect
2. Drooping

2.4 Terminal inflorescence colour
1. Yellow
2. Green
3. Pink
4. Red
5. Others

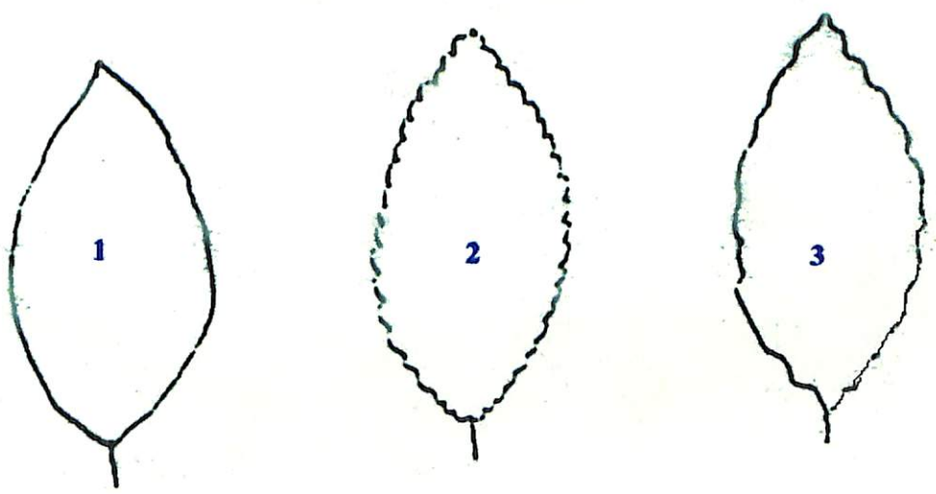
2.5 Presence of axillary inflorescence
1. Absent
2. Present

Table 2 . Continued



1.7. Leaf shape

1.Lanceolate 2.Elliptical 3.Cuneate 4.Obovate 5.Ovatainate 6.Rhombic 7.Oval



1.8 Leaf margin

1.Entire 2.Crenate 3.Undulate

(b) Girth of stem

Girth of the stem was taken at harvest after 30 days of transplanting from five randomly selected plants.

(c) Length and width of leaf

The fifth leaf from the terminal bud was harvested from five randomly selected plants on the 30th day of transplanting and the length was measured. The width of the leaf was measured at the region of maximum width.

(d) Branches per plant

The number of branches per plant was counted at the time of flowering

(e) Length of lateral branches

The length of first lateral branch from the base of the plant and length of terminal lateral branch was recorded at the time of flowering from five randomly selected plants.

(f) Days to 50% bolting

From the plants kept unharvested, days to 50% bolting was recorded.

(g) Inflorescence length and density index

Length of terminal inflorescence and auxillary inflorescence, if present, were recorded from the selected plants. Number of flowers

in the 5 cm basal portion of inflorescence were recorded as inflorescence density index.

(h) Yield

From the five plants selected for observation, vegetable yield per plant was recorded at each harvest. First harvesting was done on the 30th day of transplanting and then at an interval of ten days. The weight of greens per plant from different harvests constitute total vegetable yield per plant.

(i) Leaf-stem ratio

Both leaf yield and stem yield were recorded separately to calculate the leaf-stem ratio

(j) Other traits

Qualitative observations like colour, shape and pubescence of leaf and stem; colour, shape and latitude of the inflorescence and growth habit of the plant were recorded.

3.1.4. Symptomatology, incidence and intensity of leaf spot diseases

Observations on symptoms, disease incidence and intensity/severity in the main field were recorded at fortnightly intervals after transplanting. Five plants per genotype from one replicate were selected for scoring the disease after tagging the plants serially. Five leaves starting from the top 3rd to 7th leaf towards the base were recorded each time.

The percentage disease incidence was calculated by using the formula,

$$\text{Percentage disease incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

Disease severity was rated as per the scoring system given below

Disease grade	Percentage of leaf area infected
0	Nil
1	<10
2	10-25
3	26-50
4	51-75
5	>75

Based on the percentage of plant area infected, disease severity/intensity was calculated using the following formula (Wheeler, 1969).

$$\text{\% disease severity/intensity (PDI)} = \frac{\text{Sum of all numerical rating} \times 100}{\text{Total number of leaves taken for observation} \times \text{Maximum disease grade}}$$

Based on percentage disease severity, the accessions were grouped into five categories as suggested by Rajkumar *et al.* (1995) which are as follows:

Disease severity (%)	**Category**
0	Immune
1-10	Highly resistant
10.1-25.0	Moderately resistant
25.1-50.0	Moderately susceptible
>50	Highly susceptible

From percentage disease incidence and percentage disease severity value, coefficient of disease index (CODEX) was calculated as per Datar and Mayee (1981):

$$\text{CODEX} = \frac{\text{\% disease incidence x \% disease severity}}{100}$$

3.1.5. Statistical analysis

The data related to each character were analysed by applying the analysis of variance (ANOVA) techniques as given by Panse and Sukhatme (1978). Non heirarchical eucledian cluster analysis was done as per the method suggested by Spark (1973)

3.2. Isolation and identification of the pathogens associated with leaf spot diseases

3.2.1. Collection, isolation and identification of pathogen

Amaranth leaves infected with different leaf spot diseases were collected during October 1996 from the experimental field at Vellanikkara and were brought to the laboratory, washed under tap water and dried with blotting paper. Isolation of the pathogen was done using standard techniques. Leaves were cut into small pieces of 5 mm size and then surface sterilised with 0.1 per cent of mercuric chloride for 15 seconds and washed with three changes of sterile distilled water. The leaf bits were then transferred aseptically to sterile petridishes containing potato dextrose agar (PDA). Petri dishes were incubated at room temperature (25 ± 2°C) and examined daily for the growth of the pathogen. Fungi developed on PDA were isolated and identified (Riker and Riker, 1936).

The identification of the fungi was done based on the morphological characters described by IMI No.4343 and Mordue, 1971.

3.2.2. Pathogenicity of fungi associated with leaf spot disease

The pathogenicity of the isolated fungi was studied in pot culture by artificial inoculation under laboratory condition using the same host plant. For the aritificial inoculation, 20 days old seedlings were inoculated with 10ml of inoculum having concentration of 27 x 10^4 mycelial bits for *R. solani* and !0^6 spores/ml in case of *C. capsici* using an atomiser. Inoculated plants were observed daily for the disease appearance. Seedlings inoculated with sterile water served as control. Pathogen was reisolated from the infected plants and then compared with original culture.

3.2.3. Screening of accessions for resistance to leaf spot disease by artificial inoculation of the pathogen(s)

Based on the results from the first field experiment, ten amaranth accessions from five different categories (based on disease severity) were selected. Seeds of these ten selected accessions were sown in earthen pots of 20cm diameter. Three pots were maintained for each genotype and kept under natural conditions in iron-framed cages covered with muslin cloth. After germination, two plants were retained in each pot. These seedlings were inoculated two times, ten days and 20 days after sowing with 10ml of the inoculum of either of the pathogens as described above using an atomiser. Infected leaves were also retained in the pot to provide sufficient inoculum for the infection. Inoculated plants were covered with moistened polythene bags to provide humidity for disease development. Observation was taken daily and the disease development and the symptoms produced were noted.

3.3. Seasonal influence on leaf spot incidence and yield in amaranth

The amaranth accessions, two each selected from the five classes obtained from the first experiment based on disease severity were used as the material for this study. These accessions were raised in the field at monthly interval for one year starting from May 1997 to find out the seasonal effect on the incidence of leaf spot disease. Three weeks old seedlings were transplanted in the plots of 1.5 x 1.5m size with a spacing of 30 cm between rows and 15cm between plants. The field experiment was laid out in a Randomised Block Design with three replications. There were 50 plants per replication and all the cultural operations were done as per the Package of Practices Recommendations (KAU, 1996).

Observations on disease incidence and disease severity were worked out using standard score chart at fortnightly intervals. Plant growth and yield characters like plant height, branches per plant, leaf length, leaf width, days to 50 percent bolting and total vegetable yield were recorded. The data were analysed statistically as per Panse and Sukhatme (1978) for each month and pooled analysis of all the seasons were also carried out.

Meteorological data for the period was also recorded. The daily data on different weather parameters, viz. maximum and minimum temperatures, morning and evening relative humidity and rainfall were collected from the Principal Agrometeorological Station of the College of Horticulture, Vellanikkara for the crop period from May 1997 to April 1998. The five-day mean values of the weather parameters were taken into account to determine the effect of weather elements on the leaf spot disease, growth and yield of amaranth.

3.4. Biochemical basis of leaf spot resistance

The following biochemical factors were estimated using leaf samples from second harvest for selected ten accessions belonging to five different classes during summer (March 1998) and rainy seasons (July 1997). The estimations were carried out in the laboratories of Departments of Olericulture and Biochemistry, College of Horticulture, Vellanikkara. The following biochemical factors were studied.

1. Total phenols
2. O.D. Phenols
3. Chlorophyll 'a' and 'b' and anthocyanin
4. Anti-nutrient factors like oxalates and nitrates
5. Ascorbic acid
6. Isozyme banding pattern
   (a). Peroxidase isoenzyme (PRX)
   (b) Polyphenol oxidase isoenzyme (PPO)

3.4.1. Estimation of total phenols

3.4.1.1. Sample preparation

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

One gram of plant tissue (leaf) was homogenized in a mortar and pestle with 10 ml methanol. The homogenized material was centrifuged at 3000 rpm for ten minutes. The supernatant was collected in a separate test tube. The sediments were reground in a mortar and pestle with 5 ml methanol, centrifuged as above and pooled together to form a volume of 15 ml.

3.4.1.2. Estimation

Total phenols were estimated by Folin Ciocalteau method (Madhavan and Sridhar, 1982). The intensity of colour was red at 650nm in spetronic 20® spectrophotometer. The total phenol content was calculated from a standard curve of catechol and was expressed as mg/gm of sample.

3.4.2 Estimation of O.D. phenol

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

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

3.4.3. Chlorophyll and anthocyanin pigments

Chlorophyll "a" and "b" contents were estimated as per Sadasivan and Manickam, 1992. Anthocyanin was estimated by the method followed by George (1986). The leaf used for estimating pigments

was fixed as the 9th leaf from the bottom as suggested by Devadas (1982).

3.4.4. Oxalates and nitrates

Plant samples were collected from the field, rinsed in tap water and dried to a constant weight at a temperature of 60-70°C. The dried plant materials were ground to pass through 0.5 mm mesh sieve. Oxalate content of the dried plant sample was assayed by calorimetric method and nitrate content by distillation technique (Marderosian *et al.* 1980).

3.4.5. Ascorbic acid

Ascorbic acid content was estimated as per the procedure suggested by Sadasivan and Manickam (1992).

3.4.6. Isozyme analysis

All the ten accessions used in the above biochemical assay were taken for isozyme analysis. Polyphenol oxidase and peroxidase isoenzymes were analysed during summer (March 1998) and rainy season (July 1997).

3.4.6.1. Electrophoresis

Polyacrylamide gel electrophoresis (PAGE) using vertical slab gel was carried out for preparing zymograms of peroxidase and polyphenol oxidase isoenzymes. Acrylamide monomeres were polymerised with N-N methylene bis acrylamide [CH_2 (NH CONH = $CH_2)_2$ bis} to obtain the gel. Freshly prepared ammonium per

sulphate acted as catalyst and N,N,N', N' – tetramethyl ethylene diamine (TEMED) as chain initiator.

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

3.4.6.2. Peroxidase isoenzyme

Sample preparation

Leaf samples were cut with sharp scissors and collected in refrigerated chests. Then these samples were washed thoroughly with distilled water for three times and wiped with filter paper to absorb moisture.

The following extraction buffer was used for peroxidase enzyme extract preparation

Tris	21.1995 g
Citric acid	2.62675 g
Ascorbic acid	0.52839 g
Cystiene HCl	0.52689 g
Distilled water	500 ml
Sucrose	17% (fresh)
pH 7.0	

Homogenisation and centrifugation

Leaf samples of each accession (1g) were chopped into small pieces under pre-chilled extraction buffer (1 ml) in a pre-chilled mortar and pestle. To this 17 per cent sucrose was added at the time of

extraction. The mortar and pestle were placed in a tray containing ice in order to maintain the grinding temperature at 4°C.

The homogenized material was centrifuged at 20,000 rpm for 15 minutes in Kobota® 6900 refrigerated centrifuge at 4°C. The supernatant was used as enzyme source for peroxidase isoenzyme electrophoresis.

Gel preparation

The following stock solutions were prepared.

1. Solution A

Tris	19.8g
Temed	0.23 ml
1N HCl	24 ml
Distilled water	100 ml
Adjust the pH to 9	

2. Solution B

Acrylamide	30.0g
Bisacrylamide	0.9g
Distilled water	100 ml

3. Solution C

Ammonium per sulphate	0.14g
Distilled water	100 ml
Prepared freshly	

The polyacrylamide gel used for resolving the isoenzymes consisted of 7.5% separation gel. The slab gel unit 'Biochem' is used for the study. The size of the slab gel was 16cm x 14cm x 0.01cm. Solutions A and B were stored in amber coloured bottles at 0-4°C. Solution C was prepared fresh for each run. Stock A, B and C were

pipetted out in the ratio 1:1: 2, mixed well and the solution was gently injected by a syringe in between glass plates kept in the polymerisation stand. Care was taken to avoid bubbles in the gel while pouring the gel mixtures. Immediately inserted a comb for making wells and allowed to polymerise. After polymerisation, the comb was removed for electrophoretic run.

Electrophoretic run

The following solutions were prepared

1. Electrode buffer

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

2. Tracer dye

Bromophenol blue	0.0002%

After polymerisation , the gels were transferred to electrophoretic apparatus. The upper and lower tanks were filled with pre-chilled electrode buffer. 20 µl samples were applied to each well with the help of transfer pipette of E.Merck®. The above operation was carried out at 4°C. The upper tank was connected to cathode and lower one to anode. Bromophenol blue was added to the upper tank as tracer dye. The enzyme extracts were subjected to electrophoresis under the alkaline system of Davis (1994).

The run was carried out at 4°C till the tracer dye (Bromophenol blue) reached the anode end of the gel column. Cooling system was used to circulate cool water at 4°C as a mean of heat dissipation and also to prevent the enzyme from denaturation. The current was adjusted at 20 mA per slab. It took 4 hours for completion of the run. After completion of the run, the gel was transferred to the staining solutions

Staining gel for peroxidase

The staining solution is composed of the following:

Benzidine	0.208 g
Acetic acid	18 ml
H_2O_2	2 ml
Distilled water	80 ml

Fresh stain was prepared each time. Acetic acid and benzidine were mixed, heated to boil, cooled and filtered. Hydrogen peroxide was added at the time of staining. The gel was immersed in staining solution till blue bands appeared and destained in 7% acetic acid. As the bands faded on standing for long time, photographs were taken immediately.

3.4.6.3. Polyphenol oxidase isoenzyme

All the steps mentioned below were carried out at 4°C. The plant samples were prepared in the same way as done for peroxidase enzyme.

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

.0.05 M Tris-HCl

0.1% Ascorbic acid

0.1% Cystein-HCl

0.002% Magnesium chloride

17% Sucrose (prepare fresh)

pH 8.0

Stored at 4°C

Homogenization and centrifugation

This part of the experiment was carried out at 4°C. Leaf samples 1 g each were chopped into small pieces. To this 2 ml of extraction buffer containing 17 % sucrose was added at the time of extraction. The sample was homogenized by grinding well with a pre-chilled mortar and pestle placed in a tray containing ice.

The slurry was centrifuged at 20,000 rpm for 20 min at 4°C in a Kobato® 6900 make refrigerated centrifuge. The supernatant was used as enzyme source for polyphenol oxidase isoenzyme analysis.

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

Staining gel for polyphenol oxidase

The staining solution composed of the following chemicals:

0.1 M Potassium phosphate buffer (p^H 7.0)	200ml
P-Phenylene diamine	0.2g
Catechol	600mg

Equilibrated the gel for 30-60 minutes in the staining solution until yellow bands appeared. The bands were fixed using destaining solution. Photographs were taken and zymograms were drawn.

Results

4. RESULTS

Results of the investigations are presented under the following heads :

4.1 Evaluation of amaranth accessions for resistance to leaf spot disease, genetic cataloguing and grouping of accessions

4.2. Isolation and identification of pathogens associated with leaf spot diseases

4.3 Seasonal influence on leaf spot incidence and yield in Amaranth and correlation studies

4.4 Biochemical bases of resistance to leaf spot

4.5 Biochemical cataloguing of accessions using enzyme patterns

4.1. Evaluation of amaranth accessions for resistance to leaf spot disease, genetic cataloguing and grouping of germplasm

4.1.1. Evaluation of amaranth accessions for resistance to leaf spot disease

One hundred and sixty eight amaranth accessions belonging to vegetable and grain types were screened under natural conditions against the leaf spot disease. This preliminary screening was done during October – November, 1996 when the environmental conditions were highly favourable for the disease development. The results of the experiment are presented in Table 3. It was found that the accessions differed significantly for the disease reaction. Out of the 168 entries, no infection was noticed in 14 entries and

Table 3 Reaction of amaranth accessions to leaf spot disease at different growth stages of the crop

Sl. No	Accessions	15 days after transplanting			30 days after transplanting			45 days after transplanting		
		Disease incidence %	Disease severity %	CODEX	Disease incidence %	Disease severity %	CODEX	Disease incidence %	Disease severity %	CODEX
1	2	3	4	5	6	7	8	9	10	11
1	A-149	0	0	0	0	0	00	18.815	12.625	2.437
2	A-150	0	0	0	0	0	0	13.515	9.760	1.351
3	A-151	0	0	0	22.515	17.655	4.516	61.500	56.665	36.372
4	A-152	0	0	0	13.775	10.365	1.691	100.00	65.370	65.370
5	A0153	0	0	0	16.830	10.370	1.794	94.155	63.830	60.206
6	A0154	0	0	0	18.785	11.535	2.183	100.00	76.685	76.685
7	A-155	0	0	0	15.060	10.390	1.585	87.960	51.625	45.228
8	A-156	5.335	4.600	0.255	14.415	9.325	1.372	82.860	52.860	43.643
9	A-4	14.475	9.650	1.404	54.630	37.845	20.813	67.360	28.810	19.083
10	A-157	12.980	8.710	1.153	58.030	40.310	23.226	92.02	41.685	38.305
11	A-158	28.845	22.275	6.440	69.560	34.345	24.176	100.00	67.890	67.890
12	A-159	28.835	20.085	5.776	59.560	39.110	23.213	80.915	60.885	49.389
13	A-160	59.785	43.320	29.950	90.615	57.700	52.319	98.115	59.110	58.085
14	A-161	45.830	41.155	18.924	74.325	56.320	42.215	96.19	61.430	59.282
15	A-162	15.275	9.710	1.498	39.775	29.840	11.894	50.26	38.305	19.449
16	A-163	14.065	8.050	1.130	31.360	25.735	8.144	44.58	35.560	15.877
17	A-164	50.115	23.210	11.762	88.275	48.160	42.536	100.00	74.00	74.0
18	A-165	31.715	18.125	5.947	35.285	19.295	6.768	50.825	29.485	15.048
19	A-166	0	0	0	38.520	20.870	8.070	56.85	43.450	24.886
20	A-167	45.535	22.135	10.143	59.545	34.775	20.830	83.835	53.950	45.254

Table 3. Continued

1	2	3	4	5	6	7	8	9	10	11
21	A-168	20.715	11.150	2.325	38.470	19.175	7.432	50.250	27.260	13.738
22	A-169	23.505	10.365	2.4446	33.990	10.670	3.636	40.560	21.820	8.831
23	A-6	16.305	9.960	1.620	58.565	23.770	13.881	95.945	69.985	67.164
24	A-170	11.890	6.520	0.771	49.505	19.420	9.640	65.045	23.985	15.630
25	A-171	0	0	0	20.855	7.560	1.645	68.025	36.230	24.576
26	A-3	0	0	0	0	0	0	8.09	6.810	0.548
27	A-172	0	0	0	0	0	0	13.345	5.480	0.785
28	A-173	0	0	0	0	0	0	27.295	15.890	4.403
29	A-174	0	0	0	69.025	25.485	17.458	100.00	83.070	83.070
30	A-175	13.785	9.230	1.261	39.510	22.615	8.881	69.085	42.465	29.234
31	A-176	0	0	0	19.045	9.285	1.737	36.600	21.225	7.709
32	A-177	0	0	0	0	0	0	0	0	0
33	A-178	0	0	0	10.705	6.915	0.727	23.880	18.195	4.44
34	A-179	19.885	9.420	1.844	24.010	19.785	4.768	54.015	42.390	23.272
35	A-180	-	-	-	13.810	7.010	0.989	21.085	9.740	2.067
36	A-181	14.190	9.440	1.373	21.050	13.515	2.817	74.05	47.490	35.318
37	A-182	59.310	29.790	17.845	86.360	61.835	53.470	100.00	81.965	81.965
38	A-183	0	0	0	16.985	9.920	1.674	20.99	15.425	3.201
39	A-184	0	0	0	20.475	12.450	2.568	37.485	26.820	10.129
40	A-185	0	0	0	0	0	0	13.770	5.305	0.778
41	A-186	0	0	0	0	0	0	5.315	2.310	0.132
42	A-187	32.015	23.165	7.423	41.990	38.195	16.086	66.29	47.565	31.577
43	A-188	36.270	21.800	7.857	66.965	40.460	27.180	88.260	54.090	47.820
44	A-189	46.995	25.960	12.347	68.835	50.690	35.017	96.155	76.035	73.631
45	A—190	23.925	18.225	4.453	73.870	47.435	35.028	96.080	60.445	58.144
46	A-191	35.385	20.715	7.375	55.510	31.200	17.394	94.060	44.690	42.115

Table 3. Continued

1	2	3	4	5	6	7	8	9	10	11
47	A-192	30.690	19.740	6.050	83.045	62.260	51.748	100.00	69.885	69.885
48	A-193	17.640	10.675	1.912	30.27	16.605	9.618	46.110	25.565	11.754
49	A-194	0	0	0	0	0	0	17.335	8.415	1.431
50	A-195	34.660	21.665	7.457	87.690	63.435	55.650	100.00	85.015	85.015
51	A-196	40.505	21.395	8.607	69.090	46.810	32.390	100.00	82.805	82.805
52	A-197	50.640	26.215	13.293	90.470	73.380	66.399	100.00	82.985	82.985
53	A-198	30.290	21.275	6.385	87.600	72.540	63.365	95.835	72.960	70.311
54	A-199	22.795	14.750	3.419	25.000	17.320	4.368	33.050	23.720	7.955
55	A-200	52.085	21.395	11.019	66.800	31.910	21.575	91.445	59.055	54.298
56	A-201	0	0	0	10.480	56.75	0.599	19.310	13.805	2.780
57	A-202	18.785	10.810	2.038	20.865	16.335	3.364	50.490	31.295	14.499
58	A-203	0	0	0	20.990	10.950	2.315	29.035	11.485	3.356
59	A-204	0	0	0	0	0	0	0	0	0
60	A-205	0	0	0	0	0	0	0	0	0
61	A-206	0	0	0	0	0	0	0	0	0
62	A-207	0	0	0	0	0	0	15.990	8.975	1.458
63	A-208	55.875	40.295	22.646	81.115	70.415	57.200	100.00	86.490	86.490
64	A209	46.865	40.195	18.882	71.545	51.015	37.334	100.00	76.790	76.790
65	A-210	0	0	0	27.790	14.300	4.005	41.35	22.125	9.14
66	A-211	0	0	0	0	0	0	0	0	0
67	A-212	61.015	50.750	30.993	79.525	64.865	51.461	95.835	68.880	65.738
68	A-213	59.540	51.950	30.839	81.065	66.825	54.253	100.00	74.920	74.920
69	A-214	70.835	55.275	39.345	87.010	68.940	60.160	100.00	79.725	79.725
70	A-215	68.015	60.440	41.231	81.085	68.295	55.308	100.00	80.420	80.420
71	A-216	0	0	0	0	0	0	25.335	12.365	3.199
72	A-217	0	0	0	0	0	0	0	0	0

1	2	3	4	5	6	7	8	9	10	11
73	A-218	52.310	28.420	15.192	72.590	44.440	32.103	91.010	60.425	55.016
74	A-219	66.010	60.410	40.053	78.845	59.675	47.661	100.00	73.085	73.085
75	A-220	71.870	50.285	36.337	84.070	57.995	49.321	100.00	73.280	73.280
76	A-221	67.375	57.285	38.200	79.135	62.540	49.397	100.00	71.865	71.865
77	A-222	64.745	48.885	31.768	88.365	64.670	56.874	100.00	70.415	70.415
78	A-223	72.815	50.290	36.990	86.850	62.870	54.473	100.00	75.840	75.840
79	A-224	0	0	0	0	0	0	17.015	7.730	1.443
80	A-225	41.060	20.965	8.066	58.370	22.38	13.06	61.865	24.485	15.14
81	A-226	0	0	0	0	0	0	0	0	0
82	A-227	0	0	0	0	0	0	0	0	0
83	A-228	0	0	0	0	0	0	12.310	6.86	0.929
84	A-229	28.270	12.785	3.559	32.335	15.285	5.059	46.035	24.765	11.384
85	A-230	0	0	0	0	0	0	0	0	0
86	A-231	0	0	0	0	0	0	0	0	0
87	A-232	0	0	0	0	0	0	0	0	0
88	A-233	0	0	0	0	0	0	0	0	0
89	A-234	37.535	30.270	11.263	59.710	44.410	26.730	100.00	69.97	69.970
90	A-235	0	0	0	0	0	0	10.315	5.65	0.558
91	A-236	0	0	0	0	0	0	9.165	5.215	0.472
92	A-237	0	0	0	0	0	0	19.135	10.545	2.071
93	A-238	0	0	0	0	0	0	21.015	16.470	3.440
94	A-239	47.185	23.790	11.103	57.335	33.495	18.873	76.835	43.320	33.575
95	A-240	41.035	25.285	10.976	71.070	29.135	20.726	86.180	51.660	45.771
96	A-241	30.335	11.495	3.465	35.870	21.195	7.701	52.370	29.495	15.764
97	A-242	31.605	17.350	5.505	51.035	20.285	10.524	74.545	39.665	29.918
98	A-243	20.880	10.540	2.222	40.460	17.240	7.066	66.535	36.605	24.134

Table 3. Continued

1	2	3	4	5	6	7	8	9	10	11
99	A-244	68.870	31.315	21.428	75.535	50.290	38.333	100.00	78.465	78.465
100	A-245	32.335	15.385	5.095	40.860	23.795	9.789	67.495	29.515	20.069
101	A-246	60.860	48.885	29.001	68.865	53.475	38.992	100.00	76.515	76.515
102	A-247	66.860	35.195	23.643	77.365	38.790	30.186	100.00	55.920	55.920
103	A-248	52.050	32.965	17.313	69.065	43.015	29.820	94.180	62.285	59.246
104	A-249	50.840	30.285	15.428	67.370	38.585	26.151	100.00	69.08	69.08
105	A-250	58.370	30.440	17.713	67.370	33.865	22.892	88.670	51.825	46.087
106	A-251	15.685	11.135	1.791	40.350	30.175	12.136	57.875	39.335	22.659
107	A-252	40.995	25.260	10.313	58.310	22.675	13.044	84.375	43.860	36.988
108	A-253	0	0	0	0	0	0	8.400	2.750	0.227
109	A-254	55.465	25.290	13.825	78.865	47.285	37.113	100.00	66.270	66.270
110	A-255	0	0	0	0	0	0	8.345	5.860	0.368
111	A-256	0	0	0	0	0	0	13.835	10.285	1.474
112	A-257	52.345	43.897	23.402	82.365	58.905	48.621	100.00	75.485	75.485
113	A-258	67.355	38.390	25.658	87.340	65.495	57.550	100.00	70.890	70.890
114	A-259	48.870	38.885	19.423	77.335	53.485	41.620	92.855	63.025	58.211
115	A-260	52.350	30.285	15.647	79.245	60.385	47.902	89.710	63.995	57.248
116	A-261	51.865	32.265	16.344	78.870	54.025	42.727	96.155	63.280	60.965
117	A-262	66.835	50.235	33.667	82.335	68.285	55.981	100.00	82.315	82.315
118	A-263	55.575	45.285	24.659	77.335	64.055	49.570	100.00	73.820	73.820
119	A-264	48.875	43.785	21.958	70.870	46.70	33.617	100.00	69.270	69.270
120	A-265	52.335	35.765	19.456	80.865	58.285	47.041	100.00	65.285	65.285
121	A-266	15.220	9.785	1.506	40.870	26.285	10.712	58.87	36.705	21.766
122	A_267	64.025	48.775	31.287	76.870	67.815	52.154	100.00	74.330	74.330
123	A-268	60.870	41.410	25.119	72.375	52.00	37.527	100.00	67.030	67.030

Table 3. Continued

1	2	3	4	5	6	7	8	9	10	11
149	A-294	41.010	27.785	15.157	79.010	65.300	51.761	100.00	83.240	83.240
150	A-295	66.835	28.895	19.344	87.035	77.740	68.017	100.00	88.240	88.240
151	A-296	36.535	21.730	11.318	58.835	40.215	23.310	83.165	47.740	38.877
152	A-297	0	0	0	31.035	12.740	4.181	70.865	42.620	30.415
153	A-298	0	0	0	25.545	10.715	3.117	58.875	37.770	22.321
154	A-299	0	0	0	42.545	29.365	12.515	52.335	34.365	19.364
155	A-300	11.220	3.295	0.370	68.720	54.410	37.517	90.685	63.695	58.468
156	A-301	27.370	13.550	3.867	78.840	66.295	52.337	100.00	68.610	68.610
157	A-302	30.770	10.160	3.397	70.540	35.315	24.870	100.00	82.785	82.785
158	A-303	63.710	38.385	24.564	88.810	73.165	65.049	100.00	85.285	85.285
159	A-304	34.660	17.785	6.292	78.735	69.030	54.795	100.00	77.900	77.900
160	A-305	22.810	8.985	2.313	82.310	65.385	53.798	100.00	71.240	71.240
161	A-306	71.045	41.995	29.522	88.535	70.765	62.501	100.00	77.795	77.795
162	A-307	23.600	7.120	1.834	72.535	52.995	38.361	100.00	77.865	77.865
163	A-308	40.870	18.915	8.044	81.010	68.960	55.951	100.00	79.485	79.485
164	A-309	31.010	12.625	3.948	84.710	68.785	58.447	100.00	74.785	74.785
165	A-310	70.865	40.165	29.316	82.035	67.705	55.792	100.00	76.915	76.915
166	A-311	27.060	14.445	4.190	73.810	63.885	47.300	100.00	77.955	77.955
167	A-312	46.870	23.715	11.052	74.590	55.690	41.799	100.00	78.200	78.200
168	A-313	53.815	20.265	11.080	82.845	60.270	50.090	100.00	68.175	68.175
CD										
-05%		15.62	11.68	8.78	16.1	11.34	11.40	16.55	14.96	17.85
-01%		20.50	15.32	11.53	21.12	14.88	14.92	21.67	19.59	23.42

they were found to be immune to the disease. Of the 14 immune accessions, 12 were green and 2 were greenish red types (Table 5). A total of 15 entries were identified as resistant to leaf spot disease in which the disease severity was less than 10 per cent. In all these varieties disease incidence was also comparatively low ranging from 5.6 to 21 per cent (Table. 6). Among the resistant types, A-186 recorded the lowest disease incidence (5.3%), disease severity (2.3%) and CODEX value (0.13%). All the resistant accessions except A-180 showed late infection only at the stage of 45 days after transplanting (Table 6). Resistant accessions belonged to red type (1), green (12) and reddish green (2) which are listed in Table 5.

Among the accessions evaluated, 19 showed moderate resistant reaction (10-25% disease severity) and they belonged to all colour groups except reddish green. But 13 of them were green types. In the case of A-237 disease severity and CODEX values were low and were 10.5 per cent and 2.07 per cent respectively (Table. 4).

Out of the 34 moderately susceptible accessions, 24 belonged to the green types and their disease severity ranged between 25 to 50 percentage.

Eighty six accessions were found highly susceptible (DS >50%) which included red (28), green (31) purple (10), greenish red (9) and reddish green (8) (Table 5). The popular varieties like Kannara local (A-6) and Arun (A-189) were highly susceptible to the disease and showed 95.9 and 96.15 per cent disease incidence and 69.9 per cent and 76.03 per cent disease severity respectively.

Out of the 14 immune accessions, 11 belonged to *Amaranthus hypochondriacus* species, one to *A. viridis* (A-211) and two to *A.*

Table 4 Evaluation of amaranth accessions for resistance against leaf spot

Sl. No	Accessions	Colour	Disease incidence (%)	Disease severity (%)		Total (DS) %	CODEX (%)	Disease resistance	Yield per Plant (g)
				R. solani	*C. capsici*				
1	2	3	4	5	6	7	8	9	10
1	A-149	G	18.815	0	12.625	12.625	2.437	MR	312.650
2	A-150	G	13.515	1.610	8.150	9.760	1.351	R	285.500
3	A-151	RG	61.500	12.315	44.350	56.665	36.372	HS	192.900
4	A-152	RG	100.00	11.160	54.210	65.370	65.370	HS	98.300
5	A-153	RG	94.155	8.420	55.430	63.850	60.206	HS	103.00
6	A-154	R	100.00	76.685	0	76.685	76.685	HS	305.55
7	A-155	G	87.960	0	51.625	51.625	45.228	HS	148.00
8	A-156	G	82.860	0	52.620	52.620	43.643	HS	155.65
9	A-4	G	67.360	0	28.810	28.810	19.083	MS	290.350
10	A-157	G	92.020	0	41.685	41.685	38.305	MS	380.450
11	A-158	R	100.00	67.890	0	67.890	67.890	HS	165.250
12	A-159	R	80.915	60.885	0	60.885	49.389	HS	138.250
13	A-160	GR	98.155	8.010	51.100	59.110	58.085	HS	495.300
14	A-161	GR	96.19	13.220	48.210	61.430	59.282	HS	460.350
15	A-162	G	50.26	0	38.305	38.305	19.449	MS	66.350
16	A-163	G	44.58	0	35.560	35.560	15.877	MS	41.250
17	A-164	G	100.00	0	74.00	74.00	74.00	HS	180.250
18	A-165	RG	50.825	3.212	26.273	29.485	15.048	MS	181.250
19	A-166	LG	56.85	0	43.450	43.450	24.886	MS	950.250

Table 4. Continued

1	2	3	4	5	6	7	8	9	10
20	A-167	GR	83.835	0	53.950	53.950	45.254	HS	360.250
21	A-168	G	50.250	0	27.260	27.260	13.738	MS	445.150
22	A-169	R	40.560	21.820	0	21.820	8.831	MR	275.350
23	A-6	R	95.945	69.985	0	69.985	67.164	HS	1005.300
24	A-170	P	65.045	23.986	0	23.985	15.630	MR	610.700
25	A-171	GR	68.025	16.110	20.120	36.230	24.576	MS	405.250
26	A-3	G	8.090	0	6.810	6.810	0.548	R	1500.250
27	A-172	G	13.345	0	5.480	5.480	0.785	R	1415.400
28	A-173	G	27.295	0	15.890	15.890	4.403	MR	250.400
29	A-174	G	100.00	0	83.070	83.070	83.070	HS	775.400
30	A-175	R	69.085	42.465	0	42.465	29.234	MS	740.250
31	A-176	GR	36.600	3.121	18.104	21.225	7.709	MR	165.350
32	A-177	GR	0	0	0	0	0	I	912.850
33	A-178	G	23.880	0	18.195	18.195	4.44	MR	495.150
34	A-179	R	54.015	42.390	0	42.390	23.272	MS	710.600
35	A-180	R	21.085	9.740	0	9.740	2.067	R	205.400
36	A-181	G	74.05	0	47.490	47.490	35.318	MS	645.400
37	A-182	R	100.00	81.965	0	81.965	81.965	HS	830.300
38	A-183	G	20.99	0	15.425	15.425	3.201	MR	412.900
39	A-184	G	37.485	0	26.820	26.820	10.129	MS	580.150
40	A-185	G	13.770	0	5.305	5.305	0.778	R	1410.300
41	A-186	G	5.315	0	2.310	2.310	0.132	R	1187.750

Table 4. Continued

1	2	3	4	5	6	7	8	9	10
42	A-187	R	66.290	47.565	0	47.565	31.577	MS	1035.250
43	A-188	R	88.260	54.090	0	54.090	47.820	HS	1012.650
44	A-189	R	96.155	76.035	0	76.035	73.633	HS	942.850
45	A-190	P	96.080	60.445	0	60.445	58.144	HS	947.650
46	A-191	G	94.060	0	44.690	44.690	42.115	MS	1100.100
47	A-192	G	100.00	0	69.885	69.885	69.885	HS	980.050
48	A-193	G	46.110	0	25.565	25.565	11.754	MS	1010.150
49	A-194	RG	17.335	8.415	0	8.415	1.431	R	837.650
50	A-195	RG	100.00	85.015	0	85.015	85.015	HS	780.300
51	A-196	G	100.00	0	82.805	82.805	82.805	HS	150.150
52	A-197	RG	100.00	82.985	0	82.985	82.985	HS	1025.300
53	A-198	RG	95.835	5.320	67.640	72.960	70.311	HS	1000.150
54	A-199	G	33.050	0	23.720	23.720	7.955	MR	218.300
55	A-200	G	91.445	0	59.055	59.055	54.298	HS	413.500
56	A-201	G	19.310	2.502	11.303	13.805	2.780	MR	81.100
57	A-202	G	50.490	0	31.295	31.295	14.499	MS	94.650
58	A-203	G	29.035	0	11.485	11.485	3.356	MR	513.750
59	A-204	G	0	0	0	0	0	I	1116.150
60	A-205	G	0	0	0	0	0	I	1001.250

Table 4. Continued

1	2	3	4	5	6	7	8	9	10
61	A-206	G	0	0	0	0	0	I	923.300
62	A-207	RG	15.990	8.975	0	8.975	1.458	R	523.750
63	A-208	G	100.00	11.270	75.220	86.490	86.480	HS	347.250
64	A209	G	100.00	16.280	60.510	76.790	76.790	HS	1110.150
65	A-210	LG	41.35	0	22.125	22.125	9.14	MR	624.050
66	A-211	G	0	0	0	0	0	I	426.300
67	A-212	R	95.835	68.880	0	68.880	65.738	HS	482.850
68	A-213	R	100.00	74.920	0	74.920	74.920	HS	931.00
69	A-214	R	100.00	79.725	0	79.725	79.725	HS	256.150
70	A-215	P	100.00	80.420	0	80.420	80.420	HS	608.650
71	A-216	G	25.335	0	12.365	12.365	3.199	MR	383.350
72	A-217	G	0	0	0	0	0	I	545.400
73	A-218	R	91.010	60.425	0	60.425	55.016	HS	367.950
74	A-219	R	100.00	73.085	0	73.085	73.085	HS	546.150
75	A-220	RG	100.00	73.280	0	73.280	73.280	HS	661.990
76	A-221	R	100.00	71.865	0	71.865	71.865	HS	585.550
77	A-222	R	100.00	70.415	0	70.415	70.415	HS	327.700
78	A-223	R	100.00	75.840	0	75.840	75.840	HS	420.650
79	A-224	G	17.015	0	7.730	7.730	1.443	R	438.00

Table 4. Continued

1	2	3	4	5	6	7	8	9	10
80	A-225	LG	61.865	0	24.485	24.485	15.14	MR	1173.00
81	A-226	GR	0	0	0	0	0	I	351.050
82	A-227	G	0	0	0	0	0	I	1390.400
83	A-228	G	12.310	0	6.86	6.86	0.929	R	181.650
84	A-229	G	46.035	0	24.765	24.765	11.384	MR	359.500
85	A-230	G	0	0	0	0	0	I	913.250
86	A-231	G	0	0	0	0	0	I	865.700
87	A-232	G	0	0	0	0	0	I	180.700
88	A-233	G	0	0	0	0		I	990.500
89	A-234	G	100.00	12.430	57.540	69.970	69.970	HS	570.300
90	A-235	G	10.315	0	5.65	5.650	0.558	R	910.550
91	A-236	G	9.165	0	5.215	5.215	0.472	R	669.850
92	A-237	G	19.135	0	10.545	10.545	2.071	MR	158.050
93	A-238	R	21.015	16.470	0	16.470	3.440	MR	280.500
94	A-239	G	76.835	8.120	35.200	43.320	33.575	MS	340.350
95	A-240	G	86.180	11.220	40.440	51.660	45.770	HS	561.650

Table 4. Continued

1	2	3	4	5	6	7	8	9	10
96	A-241	G	52.370	0	29.495	29.495	15.764	MS	75.700
97	A-242	G	74.545	0	39.665	39.665	29.918	MS	234.550
98	A-243	G	66.535	0	36.605	36.605	24.134	MS	229.250
99	A-244	G	100.00	16.235	62.230	78.465	78.465	HS	340.00
100	A-245	G	67.495	0	29.515	29.515	20.069	MS	241.000
101	A-246	R	100.00	76.515	0	76.515	76.515	HS	316.950
102	A-247	R	100.00	55.920	0	55.920	55.920	HS	487.950
103	A-248	R	94.180	62.285	0	62.285	59.246	HS	659.950
104	A-249	R	100.00	69.08	0	69.08	69.08	HS	290.500
105	A-250	G	88.670	9.610	42.215	51.825	46.087	HS	165.200
106	A-251	G	57.875	0	39.335	39.335	22.659	MS	253.850
107	A-252	G	84.375	4.230	39.630	43.860	36.988	MS	240.500
108	A-253	G	8.400	0	2.750	2.750	0.227	R	210.250
109	A-254	G	100.00	0	66.270	66.270	66.270	HS	165.750
110	A-255	G	8.345	0	5.860	5.860	0.368	R	118.200
111	A-256	G	13.835	0	10.285	10.285	1.474	MR	167.750
112	A-257	G	100.00	18.225	57.260	75.485	75.485	HS	237.750
113	A-258	R	100.00	70.890	0	70.890	70.890	HS	297.350
114	A-259	R	92.855	63.025	0	63.025	58.211	HS	432.150
115	A-260	R	89.710	63.995	0	63.995	57.248	HS	287.850

Table 4. Continued

1	2	3	4	5	6	7	8	9	10
116	A-261	R	96.155	63.280	0	63.280	60.965	HS	264.750
117	A-262	R	100.00	82.315	0	82.315	82.315	HS	387.200
118	A-263	R	100.00	73.820	0	73.820	73.820	HS	273.750
119	A-264	G	100.00	0	69.270	69.270	69.270	HS	190.650
120	A-265	G	100.00		65.285	65.285	65.285	HS	141.500
121	A-266	R	58.87	36.705	0	36.705	21.766	MS	396.500
122	A_267	G	100.00	0	74.330	74.330	74.330	HS	113.200
123	A-268	R	100.00	67.030	0	67.030	67.030	HS	482.000
124	A-269	G	77.045	0	49.515	49.515	39.043	MS	227.750
125	A_270	G	5.650	0	3.250	3.250	0.154	R	524.700
126	A-271	G	20.460	0	15.385	15.385	3.167	MR	489.750
127	A-272	G	39.360	0	28.490	28.490	11.153	MS	206.050
128	A-273	G	24.370	0	16.295	16.295	4.053	MR	1000.00
129	A-274	G	88.835	9.253	53.532	62.785	55.704	HS	300.500
130	A-275	G	60.875	0	33.860	33.860	20.586	MS	365.500
131	A-276	G	38.210	0	26.985	26.985	10.332	MS	82.800
132	A-277	G	100.00	15.450	66.550	81.00	81.00	HS	390.850
133	A-278	R	100.00	76.125	0	76.125	76.125	HS	417.100
134	A-279	RG	100.00	85.645	0	85.645	85.645	HS	395.850
135	A-280	G	90.850	0	68.715	68.715	61.184	HS	310.250

Table 4 Continued

1	2	3	4	5	6	7	8	9	10
136	A-281	G	100.00	12.310	64.405	76.715	76.715	HS	411.250
137	A-282	G	100.00	0	68.715	68.715	68.715	HS	955.550
138	A-283	P	81.185	65.500	0	65.500	56.845	HS	462.850
139	A-284	G	0	0	0	0	0	I	1487.900
140	A-285	G	0	0	0	0	0	I	1240.650
141	A-286	G	100.00	0	62.740	62.740	62.740	HS	158.200
142	A-287	GR	100.00	0	70.115	70.115	70.115	HS	163.00
143	A-288	G	75.77	0	50.170	50.170	38.006	HS	262.950
144	A-289	G	52.265	0	38.270	38.270	20.209	MS	180.500
145	A-290	P	81.185	65.270	0	65.270	57.710	HS	280.150
146	A-291	G	75.535	0	45.510	45.510	34.875	MS	680.500
147	A-292	G	90.605	0	52.675	52.675	47.699	HS	263.500
148	A-293	GR	100.00	9.210	78.520	87.730	87.730	HS	419.950
149	A-294	GR	100.00	0	83.240	83.240	83.240	HS	270.150
150	A-295	G	100.00	0	88.240	88.240	88.240	HS	70.150
151	A-296	P	83.165	47.740	0	47.740	38.877	MS	208.00
152	A-297	G	70.865	0	42.620	42.620	30.415	MS	187.850
153	A-298	RG	58.875	37.770	0	37.770	22.321	MS	580.400
154	A-299	LG	52.335	0	34.365	34.365	19.364	MS	990.5000

Table 4 Continued

155	A-300	G	90.685	0	63.695	63.695	58.468	HS	700.750
156	A-301	R	100.00	68.610	0	68.610	68.610	HS	1027.750
157	A-302	GR	100.00	0	82.785	82.785	82.785	HS	180.650
158	A-303	P	100.00	85.285	0	85.285	85.285	HS	488.200
159	A-304	P	100.00	77.900	0	77.900	77.900	HS	287.750
160	A-305	G	100.00	0	71.240	71.240	71.240	HS	355.500
161	A-306	GR	100.00	0	77.795	77.795	77.795	HS	425.300
162	A-307	G	100.00	0	77.865	77.865	77.865	HS	867.850
163	A-308	P	100.00	79.485	0	79.485	79.485	HS	565.650
164	A-309	G	100.00	0	74.785	74.785	74.785	HS	385.250
165	A-310	P	100.00	76.915	0	76.915	76.915	HS	865.350
166	A-311	GR	100.00	10.750	67.205	77.955	77.955	HS	402.900
167	A-312	P	100.00	78.200	0	78.200	78.200	HS	628.000
168	A-313	P	100.00	68.175	0	68.175	68.175	HS	549.550

G- Green ; LG- Light green ; R – red ; GR- Greenish red ; RG- Reddish green ; P purple HR – Highly resistant ; MS- Moderately resistant ; R- Resistant ; HS- Highly susceptible ; MS- Moderately susceptible ; I- Immune

Table 5 Classification of amaranth accessions based on colour and leaf spot disease reaction

Category	Colour type						
	Red	Green	Light green	Purple	Greenish red	Reddish green	Total numbers
Immune	Nil	A-204, ,A-205, A-206, A-211, A-217, A-227, A-230, A-231, A-232, A-233, A-284, A-285	Nil	Nil	A-177 A-226	Nil	14 (8.33%)
Resistant	A-180	A-150, A-3, A-172, A-185. A-186, A-224, A-228, A-235, A-236, A-253, A-255, A_270	Nil	Nil	Nil	A-194 A-207	15 (8.93%)
Moderately resistant	A-169, A-238	A-149, A-173, A-178, A-183, A-199, A-201, A-203, A-216, A-229, A-237, A-256, A-271, A-273,	A_210 A-225	A-170	A-176	Nil	19 (11.31%)
Moderately susceptible	A-175, A-179 A-187, A-266	A-4, A-157, A-162, A-163, A-168, A-181, A-184, A-191, A-193, A-202, A-239, A-241, A-242, A-243, A-245,A-251, A-252, A-269, A-272, A-275, A-276, A-289, A-291,A-297,	A-166 A-299	A-296	A-171	A-165 A-298	34 (20.2%)

Table 5. Continued

Category	Colour type						
	Red	Green	Light green	Purple	Greenish red	Reddish green	Total numbers
Highly susceptible	A-154, A-158, A-159, A-6 A-182, A-188. A-189, A-212 A-213, A-214, A-218, A-219 A-221, A-222, A-223, A-246 A-247, A-248, A-249, A-258 A-259, A-260, A-261, A-262 A-263, A-268, A-278, A-301	A-155, A-156, A-164, A-174, A-192, A-196, A-200, A-208, A209, A-234, A-240, A-244, A-250, A-254, A-257, A-264, A-265, A_267, A-274, A-277, A-280, A-281, A-282, A-286, A-288, A-292, A-295, A-300, A-305, A-307, A-309	Nil	A-190, A-215 A-283, A-290 A-303, A-304 A-308, A-310 A-312, A-313	A-160, A-161 A-167 A-287 A-293 A-294 A-302 A-306 A-311	A-151 A-152 A-153 A-195 A-197 A-198 A-220 A-279	86 (51. 2%)
Total	35 (20.83%)	92 (54.76%)	4 (2.38%)	12 (7.14%)	13 (7.74%)	12 (7.74%)	168

Table 6 Disease incidence, disease severity and CODEX value in fifteen resistant amaranth accessions

Accessons	Disease incidence %	Disease severity %	CODEX	Days after transplanting at which disease noticed
A-150	13.51	9.76	1.35	45
A-3	8.09	6.81	0.55	45
A-172	13.35	5.48	0.79	45
A-180	21.08	9.74	2.07	30
A-185	13.77	5.31	0.78	45
A-186	5.32	2.31	0.13	45
A-194	17.34	8.42	1.43	45
A-207	15.99	8.98	1.46	45
A-224	17.02	7.73	1.44	45
A-228	12.31	6.86	0.93	45
A-235	10.31	5.65	0.56	45
A-236	9.17	5.221	0.47	45
A-253	8.4	2.75	0.23	45
A-255	8.34	5.86	0.36	45
A-270	5.65	3.25	0.15	45

spinosus (A-217 and A-232) (Table.7). Temporal pattern of infection in 168 amaranth accessions is presented in Table 8. Infection was noticed at an early stage of 15 days after transplanting in 110 accessions (65.5%), whereas in 24 accessions (14.3%) infection was noticed after 30 days of transplanting and in 20 accessions (11.9%) infection occurred considerably at a very late stage (about 45 days after transplanting). However, no infection was noticed in 14 types (8.3%) throughout its growth period or until the harvest period was completed.

Table 7 Amaranth accessions immune to infection

Species	Accessions
A. hypochondriacus	**A-177, A-204, A-205, A-206, A-226, A-227, A-230, A-231, A-233, A-284, A-285**
A. viridis	**A-211**
A. spinosus	**A-217, A-232**

Table 8. Temporal pattern of infection in amaranth accessions

Days after transplanting	No. of accessions infected	% of accessions infected
15	**110**	**65.48**
30	**24**	**14.29**
45	**20**	**11.90**
No infection	**14**	**8.33**

Classification of amaranth accessions based on yield is furnished in Table 9. The yield ranged from 138 g (A-159) to 1005g (A-6) per plant in the red type. About 57.5 per cent of the red types belonged to the yield group of 250-500 g. The highest yield was obtained

Table 9 Classification of amaranth accessions based on colour and yield

Yield/plant (g)	Accessions					
	Red	Green	Light green	Purple	Greenish red	Reddish green
< 250	A-158, A-159, A-180	A-155, A-156, A-162, A-163, A-164, A-196, A-199, A-201, A-202, A-228, A-232, A-237, A-241, A-242, A-297, A-243, A-245, A-250, A-252, A-253, A-254, A-255, A-256, A-257, A-264, A-265, A_267, A-269, A-272, A-276, A-286, A-289, A-295, A 297	Nil	A-296	A-176, A-287, A-302	A-151 A-152 A-153, A-165
250-500	A-154, A-169, A-212, A-214, A-218, A-222, A-223, A-238, A-246, A-247, A-249, A-258, A-259, A-260, A-261, A-262, A-263, A-266, A-268, A-278	A-149, A-150, A-4, A-157, A-168, A-173, A-178, A-183, A-200, A-208, A-211, A-216, A-224, A-229, A-239, A-244, A-251, A-271, A-274, A-275. A-277, A-280, A-281, A-288, A-292, A-305, A-309	Nil	A-283, A-290, A- 303, A-304	A-160, A-161 A-167, A-171 A-226, A-293 A-294, A-306 A-311	A-279
500-750	A-175, A-179 A-219, A-221 A-248	A-181, A-184, A-203, A-217, A-234, A-236, A-240, A-270, A-291,A-300	A-210	A-170, A- 215, A- 308, A-312, A-313	Nil	A-207 A-220 A-298
750 –1000	A-182, A-189, A-213	A-174, A-192, A-206, A-230, A-231 A-233, A-235, A-273, A-282, A-307	A-166, A-299	A-190, A-310	A-177	A-194, A-195
1000-1250	A-6, A-187, A-188 A-301	A-186, A-191, A-193, A-204, A-205, A209, A-285,	A-225	Nil	Nil	A-197 A-198
1250-1500	Nil	A-3, A-172, A-185, A-227, A-284	Nil	Nil	Nil	Nil

from the green amaranth accessions, viz. A-3, A-172, A-185, A-227 and A-284. Out of the 92 green types, 33 numbers (35.8%) gave the yield of less than 250 g. About 5.43 per cent of green types yielded more than 1250 g. and the highest yield was from A-3 (1500 g) per plant.

All the light green types were moderate yielders giving 624 – 1173 g/plant. Out of the 12 purple types , 42 per cent gave a yield of 500-750 g. A-190 and A-310 were the highest yielders in this group giving 947 g and 865 g respectively.

All the greenish red type (except A-177 having an yield of 912 g) were low yielders, (less than 500 g). The yield of reddish green types varied from 98 g in A-152 to 1025 g in A-197 (Table 4).

4.1.2. Genetic cataloguing of amaranth germplasm

Amaranth germplasm were genetically catalogued based on the IBPGR descriptor as mentioned in Table 2. Morphological characters like growth habit, leaf characters, stem characters and inflorescence characters were recorded and presented in Table 10. The growth belonged to prostrate or erect. Out of the 168 accessions studied, 97 per cent belonged to erect types (Table 11). Leaf pigmentation varied from green, greenish red, red reddish green to purple. Among the accessions 54 per cent had green leaves, 20 per cent had red and 7 per cent each had greenish red, reddish green and purple leaves. Only 2 per cent had a leaf pigmentation of light green.

Leaf shape of amaranth accessions ranged from elliptical, lanceolate, cuneate, rhombic, oval to ovatainate. Most of the

Table 10 Morphological descriptions of the amaranth accessions studied

Sl. No	Accessions	Terminal inflorescence characters				Axillary inflorescence	Leaf characters					Stem characters			Growth habit
		Laterals	Shape	latitude	colour		Pubescence	Pigmentation	Shape	Prominance of vein	Margin	Petiol pigmentation	Stem Pigmentation	Pube-scence	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	A-149	A	Spike	D	G	P	Nil	G	Elliptical	Smooth	Undulate	R	G	Nil	Erect
2	A-150	A	Spike	D	G	P	Nil	G	Elliptical	Smooth	Undulate	R	G	Nil	Erect
3	A-151	A	Spike	D	R	P	Nil	RG	Elliptical	Smooth	Undulate	R	R	Nil	Erect
4	A-152	A	Spike	D	G	P	Nil	RG	Elliptical	Rugose	Undulate	R	R	Nil	Erect
5	A-153	A	Spike	D	G	P	Nil	RG	Elliptical	Rugose	Undulate	R	R	Nil	Erect
6	A-154	A	Spike	D	R	P	Nil	R	Elliptical	Smooth	Undulate	R	R	Nil	Erect
7	A-155	A	Spike	SD	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	Nil	Erect
8	A-156	A	Spike	SD	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	Nil	Erect
9	A-4	A	Spike	S	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	G	Nil	Erect
10	A-157	A	Spike	S	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	G	Nil	Erect
11	A-158	A	Spike	D	R	P	Nil	R	Cuneate	Smooth	Undulate	R	R	Nil	Erect
12	A-159	A	Spike	D	R	P	Nil	R	Cuneate	Smooth	Undulate	R	R	Nil	Erect
13	A-160	A	Spike	D	R	P	Nil	GR	Lanceolate	Smooth	Undulate	R	R	Nil	Erect
14	A-161	A	Spike	D	R	P	Nil	GR	Lanceolate	Smooth	Undulate	R	R	Nil	Erect
15	A-162	A	Spike	SD	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	G	Nil	Prostrate
16	A-163	A	Spike	S	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	G	Nil	Prostrate
17	A-164	A	Spike	S	G	P	Nil	G	Rhombic	Smooth	Undulate	G	G	Nil	Erect
18	A-165	A	Spike	S	R	P	Nil	RG	Elliptcal	Smooth	Undulate	R	RG	Nil	Erect
19	A-166	A	Spike	SD	LG	P	Nil	LG	Lanceolate	Smooth	Undulate	G	G	Nil	Erect
20	A-167	A	Spike	SD	R	P	Nil	GR	Elliptical	Smooth	Undulate	R	R	NIL	Erect
21	A-168	A	Spike	SD	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	NIL	Erect
22	A-169	A	Spike	SD	R	P	Nil	R	Rhombic	Smooth	Undulate	R	R	NIL	Erect
23	A-6	A	Spike	SD	R	P	Nil	R	Elliptical	Smooth	Undulate	R	R	NIL	Erect
24	A-170	A	Spike	SD	R	P	Nil	P	Ovatainate	Rugose	Undulate	P	R	NIL	Erect

Table 10 continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
25	A-171	A	Spike	SD	LR	P	Nil	GR	Elliptical	Rugose	Undulate	LR	R	NIL	Erect
26	A-3	P	Panicle	E	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	NIL	Erect
27	A-172	P	Panicle	E	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	NIL	Erect
28	A-173	A	Spike	E	G	P	Nil	G	Oval	Smooth	Entire	R	R	NIL	Prostrate
29	A-174	A	Spike	E	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	G	NIL	Erect
30	A-175	A	Spike	E	R	P	Nil	R	Oval	Smooth	Undulate	R	R	NIL	Erect
31	A-176	A	Spike	E	G	P	Nil	GR	Elliptical	Smooth	Undulate	G	R	NIL	Prostrate
32	A-177	P	Panicle	E	R	P	Nil	GR	Elliptical	P	Undulate	GR	GR	NIL	Erect
33	A-178	A	Spike	E	R	P	Nil	G	Elliptical	Smooth	Undulate	R	LR	NIL	Erect
34	A-179	A	Spike	E	R	P	Nil	R	Oval	Smooth	Undulate	R	R	NIL	Erect
35	A-180	A	Spike	E	R	P	Nil	R	Elliptical	Smooth	Undulate	R	R	NIL	Erect
36	A-181	A	Spike	E	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	NIL	Erect
37	A-182	A	Spike	E	R	P	Nil	R	Lanceolate	Smooth	Undulate	R	R	NIL	Erect
38	A-183	A	Spike	E	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	NIL	Erect
39	A-184	A	Spike	E	G	P	Nil	G	Ovatainate	Smooth	Undulate	G	G	NIL	Erect
40	A-185	P	Panicle	SD	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	NIL	Erect
41	A-186	P	Panicle	SD	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	NIL	Erect
42	A-187	A	Spike	E	R	P	Nil	R	Elliptical	Smooth	Undulate	R	R	NIL	Erect
43	A-188	A	Spike	E	R	P	Nil	R	Elliptical	Smooth	Undulate	R	R	NIL	Erect
44	A-189	A	Spike	E	R	P	Nil	R	Elliptical	Smooth	Undulate	R	R	NIL	Erect
45	A-190	A	Spike	E	R	P	Nil	P	Elliptical	Smooth	Undulate	R	R	NIL	Erect
46	A-191	A	Spike	E	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	NIL	Erect
47	A-192	A	Spike	E	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	NIL	Erect
48	A-193	A	Spike	E	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	NIL	Erect
49	A-194	A	Spike	E	R	P	Nil	RG	Lanceolate	Smooth	Undulate	R	R	NIL	Erect
50	A-195	A	Spike	E	R	P	Nil	RG	Lanceolate	Smooth	Undulate	R	R	NIL	Erect
51	A-196	A	Spike	E	R	P	Nil	G	Elliptical	Smooth	Undulate	Pink	Pink	NIL	Erect
52	A-197	A	Spike	E	R	P	Nil	RG	Lanceolate	Smooth	Undulate	R	R	NIL	Erect
53	A-198	A	Spike	E	R	P	Nil	RG	Lanceolate	Smooth	Undulate	R	R	NIL	Erect
54	A-199	A	Spike	E	G	P	Nil	G	Ovatainate	Smooth	Entire	G	G	NIL	Erect
55	A-200	A	Spike	E	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	NIL	Erect

Table 10 continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
56	A-201	A	Spike	SD	G	P	Nil	G	Lanceolate	Smooth	Entire	G	G	NIL	Erect
57	A-202	A	Spike	E	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	NIL	Erect
58	A-203	A	Spike	SD	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	LR	NIL	Erect
59	A-204	P	Panicle	E	G	P	Nil	G	Lanceolate	Smooth	Entire	G	G	NIL	Erect
60	A-205	P	Panicle	E	G	P	Nil	G	Lanceolate	Smooth	Entire	G	G	NIL	Erect
61	A-206	P	Panicle	E	G	A	Nil	G	Elliptical	Smooth	Entire	G	G	NIL	Erect
62	A-207	A	Spike	E	R	P	Nil	RG	Lanceolate	Smooth	Undulate	R	R	NIL	Erect
63	A-208	A	Spike	E	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	G	NIL	Erect
64	A209	A	Spike	E	G	P	Nil	LG	Lanceolate	Smooth	Undulate	G	G	NIL	Erect
65	A-210	A	Spike	E	G	P	Nil	LG	Ovatainate	Smooth	Undulate	G	G	NIL	Erect
66	A-211	P	Panicle	SD	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	NIL	Erect
67	A-212	A	Spike	E	R	P	Nil	R	Lanceolate	Smooth	Undulate	R	R	NIL	Erect
68	A-213	A	Spike	E	R	P	Nil	R	Lanceolate	Smooth	Undulate	R	R	NIL	Erect
69	A-214	A	Spike	E	R	P	Nil	R	Elliptical	Smooth	Undulate	R	R	NIL	Erect
70	A-215	A	Spike	E	P	P	Nil	P	Lanceolate	Smooth	Undulate	P	P	NIL	Erect
71	A-216	P	Panicle	SD	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	NIL	Erect
72	A-217	A	Spike	SD	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	G	Spiny	Erect
73	A-218	A	Spike	E	R	P	Nil	R	Elliptical	Smooth	Undulate	R	R	N	Erect
74	A-219	A	Spike	E	R	P	Nil	R	Elliptical	Smooth	Undulate	R	R	N	Erect
75	A-220	A	Spike	E	R	P	Nil	RG	Ovatainate	Smooth	Undulate	R	R	N	Erect
76	A-221	A	Spike	E	R	P	Nil	R	Elliptical	Smooth	Undulate	R	R	N	Erect
77	A-222	A	Spike	E	R	P	Nil	R	Ovatainate	Smooth	Undulate	R	R	N	Erect
78	A-223	A	Spike	E	R	P	Nil	R	Elliptical	Smooth	Undulate	P	P	N	Erect
79	A-224	P	Panicle	E	G	P	Nil	G	Cuneate	Smooth	Entire	G	G	N	Erect
80	A-225	A	Spike	E	G	P	Nil	LG	Elliptical	Smooth	Undulate	G	G	N	Erect
81	A-226	P	Panicle	E	G	A	Nil	GR	Lanceolate	Smooth	Entire	R	G	N	Erect
82	A-227	P	Panicle	E	G	A	Nil	G	Elliptical	Smooth	Entire	G	G	N	Erect
83	A-228	A	Spike	E	G	P	Nil	G	Elliptical	Smooth	Undulate	P	P	N	Erect
84	A-229	P	Panicle	E	P	P	Nil	G	Lanceolate	Smooth	Undulate	P	P	N	Erect
85	A-230	P	Panicle	SD	R	A	Nil	G	Lanceolate	Smooth	Undulate	P	R	N	Erect
86	A-231	P	Panicle	SD	B	A	Nil	G	Lanceolate	Smooth	Undulate	G	G	N	Erect
87	A-232	P	Panicle	E	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	G	Spiny	Erect

Table 10 continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
88	A-233	P	Panicle	E	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	N	Erect
89	A-234	A	Spike	E	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	N	Erect
90	A-235	P	Panicle	E	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	N	Erect
91	A-236	P	Panicle	E	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	N	Erect
92	A-237	A	Spike	E	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	G	N	Erect
93	A-238	A	Spike	E	R	P	Nil	R	Lanceolate	Smooth	Undulate	G	G	N	Erect
94	A-239	A	Spike	E	G	P	Nil	G	Lanceolate	Smooth	Undulate	R	R	N	Erect
95	A-240	A	Spike	E	G	P	Nil	G	Lanceolate	Smooth	Undulate	R	G	N	Erect
96	A-241	A	Spike	E	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	G	N	Erect
97	A-242	A	Spike	E	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	G	N	Erect
98	A-243	A	Spike	E	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	G	N	Erect
99	A-244	A	Spike	E	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	G	N	Erect
100	A-245	A	Spike	E	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	N	Erect
101	A-246	A	Spike	E	R	P	Nil	R	Lanceolate	Smooth	Undulate	R	R	N	Erect
102	A-247	A	Spike	E	R	P	Nil	R	Lanceolate	Smooth	Undulate	R	R	N	Erect
103	A-248	A	Spike	E	R	P	Nil	R	Lanceolate	Smooth	Undulate	R	R	N	Erect
104	A-249	A	Spike	E	R	P	Nil	R	Lanceolate	Smooth	Undulate	R	R	N	Erect
105	A-250	A	Spike	E	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	G	N	Erect
106	A-251	A	Spike	E	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	G	N	Erect
107	A-252	A	Spike	E	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	G	N	Erect
108	A-253	A	Spike	E	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	G	N	Erect
109	A-254	A	Spike	E	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	N	Erect
110	A-255	A	Spike	E	G	P	Nil	G	Lanceolate	Smooth	Entire	G	G	N	Erect
111	A-256	A	Spike	E	G	P	Nil	G	Lanceolate	Smooth	Entire	G	G	N	Erect
112	A-257	A	Spike	E	G	P	Nil	G	Elliptical	Smooth	Entire	G	G	N	Erect
113	A-258	A	Spike	E	R	P	Nil	R	Lanceolate	Smooth	Entire	R	R	N	Erect
114	A-259	A	Spike	E	R	P	Nil	R	Elliptical	Smooth	Entire	R	R	N	Erect
115	A-260	A	Spike	E	R	P	Nil	R	Lanceolate	Smooth	Entire	R	R	N	Erect
116	A-261	A	Spike	E	R	P	Nil	R	Elliptical	Smooth	Entire	R	R	N	Erect
117	A-262	A	Spike	E	R	P	Nil	R	Elliptical	Smooth	Entire	R	R	N	Erect
118	A-263	A	Spike	E	R	P	Nil	R	Elliptical	Smooth	Entire	R	R	N	Erect
119	A-264	A	Spike	E	G	P	Nil	G	Lanceolate	Smooth	Entire	G	G	N	Erect

Table 10 continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
120	A-265	A	Spike	E	G	P	Nil	G	Elliptical	Smooth	Entire	G	G	N	Erect
121	A-266	A	Spike	E	R	P	Nil	R	Lanceolate	Smooth	Undulate	R	R	N	Erect
122	A_267	A	Spike	E	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	G	N	Erect
123	A-268	A	Spike	E	R	P	Nil	R	Lanceolate	Smooth	Undulate	R	R	N	Erect
124	A-269	A	Spike	E	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	N	Erect
125	A_270	P	Panicle	E	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	G	N	Erect
126	A-271	A	Spike	E	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	G	N	Erect
127	A-272	A	Spike	E	G	P	Nil	G	Lanceolate	Smooth	Undulate	G	G	N	Erect
128	A-273	P	Panicle	E	G	P	Nil	G	Elliptical	Rugose	Undulate	G	G	N	Erect
129	A-274	A	Spike	E	G	P	Nil	G	Lanceolate	Rugose	Undulate	G	G	N	Erect
130	A-275	A	Spike	E	G	P	Nil	G	Elliptical	Smooth	Entire	G	G	N	Erect
131	A-276	A	Spike	E	G	P	Nil	G	Elliptical	Rugose	Crenate	G	G	N	Erect
132	A-277	A	Spike	E	G	P	Nil	G	Elliptical	Rugose	Crenate	G	G	N	Erect
133	A-278	A	Spike	E	R	P	Nil	R	Elliptical	Rugose	Crenate	R	R	Nil	Erect
134	A-279	A	Spike	E	R	P	Nil	RG	Elliptical	Smooth	Entire	R	R	Nil	Erect
135	A-280	A	Spike	E	G	P	Nil	G	Elliptical	Smooth	Entire	G	G	Nil	Erect
136	A-281	A	Spike	E	G	P	Nil	G	Elliptical	Rugose	Undulate	G	G	Nil	Erect
137	A-282	A	Spike	E	G	P	Nil	G	Elliptical	Rugose	Undulate	G	G	Nil	Erect
138	A-283	A	Spike	E	R	P	Nil	P	Elliptical	Rugose	Entire	P	P	Nil	Erect
139	A-284	P	Panicle	E	G	P	Nil	G	Lanceolate	Rugose	Entire	G	G	LOW	Erect
140	A-285	P	Panicle	E	G	P	Nil	G	Lanceolate	Rugose	Undulate	G	G	LOW	Erect
141	A-286	A	Spike	E	G	P	Nil	G	Lanceolate	Smooth	Entire	G	G	Nil	Erect
142	A-287	A	Spike	E	G	P	Nil	GR	Lanceolate	Rugose	Entire	G	G	Nil	Erect
143	A-288	A	Spike	E	G	P	Nil	G	Lanceolate	Rugose	Undulate	R	GR	Nil	Erect
144	A-289	A	Spike	E	G	P	Nil	G	Elliptical	Smooth	Entire	G	G	Nil	Erect
145	A-290	A	Spike	E	R	P	Nil	P	Elliptical	Rugose	Undulate	R	R	Nil	Erect
146	A-291	A	Spike	E	G	P	Nil	G	Rhombic	Rugose	Undulate	G	G	Nil	Erect
147	A-292	A	Spike	SD	R	P	Nil	G	Elliptical	Rugose	Undulate	R	R	Nil	Erect
148	A-293	A	Spike	E	R	P	Nil	GR	Lanceolate	Rugose	Undulate	R	R	Nil	Erect
149	A-294	A	Spike	E	R	P	Nil	GR	Lanceolate	Rugose	Undulate	R	GR	Nil	Erect
150	A-295	A	Spike	E	G	P	Nil	G	Oval	Smooth	Undulate	R	R	Nil	Erect
151	A-296	A	Spike	E	R	P	Nil	P	Elliptical	Rugose	Undulate	P	P	Nil	Erect

Table 10 continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
152	A-297	A	Spike	E	G	P	Nil	G	Elliptical	Smooth	Undulate	G	G	Nil	Erect
153	A-298	A	Spike	E	R	P	Nil	RG	Elliptical	Rugose	Undulate	P	R	Nil	Erect
154	A-299	A	Spike	E	LG	P	Nil	LG	Elliptical	Rugose	Undulate	G	G	Nil	Erect
155	A-300	A	Spike	E	G	P	Nil	G	Lanceolate	Rugose	Undulate	G	G	Nil	Erect
156	A-301	A	Spike	E	R	P	Nil	R	Elliptical	Rugose	Undulate	R	R	Nil	Erect
157	A-302	A	Spike	E	G	P	Nil	GR	Lanceolate	Rugose	Undulate	G	G	Nil	Erect
158	A-303	A	Spike	E	R	P	Nil	P	Elliptical	Rugose	Undulate	P	P	Nil	Erect
159	A-304	A	Spike	E	R	P	Nil	P	Elliptical	Rugose	Undulate	P	R	Nil	Erect
160	A-305	A	Spike	E	G	P	Nil	G	Elliptical	Rugose	Undulate	G	G	Nil	Erect
161	A-306	A	Spike	E	R	P	Nil	GR	Lanceolate	Rugose	Undulate	R	R	Nil	Erect
162	A-307	A	Spike	E	G	P	Nil	G	Elliptical	Rugose	Undulate	G	G	Nil	Erect
163	A-308	A	Spike	E	R	P	Nil	P	Elliptical	Rugose	Undulate	P	P	Nil	Erect
164	A-309	A	Spike	E	G	P	Nil	G	Lanceolate	Rugose	Undulate	G	G	Nil	Erect
165	A-310	A	Spike	E	R	P	Nil	P	Lanceolate	Rugose	Undulate	P	P	Nil	Erect
166	A-311	A	Spike	E	R	P	Nil	GR	Lanceolate	Rugose	Undulate	R	R	Nil	Erect
167	A-312	A	Spike	E	R	P	Nil	P	Elliptical	Rugose	Undulate	P	P	Nil	Erect
168	A-313	A	Spike	E	R	P	Nil	P	Lanceolate	Rugose	Undulate	P	P	Nil	Erect

Abbreviations : Terminal inflorescence characters - Laterals , A- absent, P – present ; Latitude, D-drooping , SD- Semidrooping, E- erect : Axillary inflorescence – A-Absent ; P- Present Colour : G, green ; LG-Light green ; GR- greenish red ; P – Purple ; B – brown ; R- red, RG – Reddish Green ; LR – Light Red

Table 11 Summary of morphological descriptions of the amaranth accessions

Sl. No.	Characters	Accessions (%)
1	Leaf pigmentation	
	Green	54.76
	Light green	2.38
	Greenish red	7.74
	Red	20.83
	Reddish green	7.14
	Purple	7.14
2	Stem pigmentation	
	Green	52.98
	Red	36.31
	Reddish green	0.60
	Greenish red	1.79
	Light red	1.19
	Pink	0.60
	Purple	6.55
3	Growth habit	
	Erect	97.62
	Prostrate	2.38
4	Leaf shape	
	Elliptical	48.21
	Lanceolate	42.26
	Cuneate	1.79
	Rhombic	1.79
	Oval	2.38
	Ovatainate	3.58
5	Inflorescence shape	
	Spike	85.71
	Panicle	14.29

accessions had an elliptical lamina (48 %) followed by lanceolate (42%) and rarely cuneate and rhombic (2 % each).

The stem pigmentation of the 168 accessions came under seven classes including green, red, reddish green, greenish red, light red, pink and purple. Most of the accessions (52%) had green stem followed by red (36%) and they rarely had reddish green and pink stem (0.6% each).

The shape of the inflorescence of different accessions was either spike or panicle. Eighty six per cent had spike inflorescence and the rest (14%) with panicle inflorescence.

4.1.3. Assessment of genetic divergence and grouping of accessions

D^2 statistics is a valuable tool for obtaining quantitative estimates of divergence between biological populations. Based on Eucledian distance cluster analysis the total 168 amaranth accessions were grouped into 14 clusters (Table 12). The different clusters and their variable means and ranges are presented in table 13.

The clusters I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, XII1, and XIV comprised of 9, 11, 18, 2, 3, 3, 16, 24, 14, 24, 8, 5, 12 and 19 accessions respectively. Among the 14 clusters, cluster numbers VIII and X had maximum number of accessions (24 each) followed by cluster number XIV and III with 19 and 18 accessions respectively. Clusters V and VI consisted of three accessions each and cluster IV had only two accessions (Table 12).

Mean value of plant height at 30^{th} day (65.6 cm) and at flowering (117.14 cm) was maximum in cluster XII which contained

Table 12 Clustering pattern in 168 accessions of amaranthus

Cluster Number	Number of accessions in each cluster	Accessions
I	9	A.244, A.252, A.261, A.264, A.265, A.267, A.271, A.272, A.275
II	11	A.149, A.150, A.164, A.169, A.176, A.203, A.211, A.245, A.257, A.259, A.292
III	18	A.196, A.199, A.202, A.208, A.216, A.217, A.218, A.224, A.228, A.232, A.237, A.238, A.239, A.242, A.250, A.251, A.253, A.254
IV	2	A.284, A.285
V	3	A.173, A.258, A.276
Vi	3	A.153, A.162, A.163
VII	16	A.207, A.209, A.210, A.213, A.214, A.219, A.220, A.221, A.222, A.223, A.225, A.234, A.236, A.240, A.248, A.249
VIII	24	A.229, A.268, A.274, A.277, A.278, A.279, A.283, A.288, A.290, A.291, A.294, A.298, A.299, A.300, A.302, A.303, A.305, A.306, A.307, A.308, A.309, A.310, A.311, A.312
IX	14	A.174, A.180, A.201, A.202, A.241, A.246, A247, A.256, A.260, A.262, A.266, A.269, A.281, A.293
X	24	A.166, A.6, A.170, A.3, A.172, A.175, A.177, A.179, A.181, A.182, A.184, A.187, A.188, A.189, A.190, A.191, A.192, A.193, A.194, A.195, A.197, A.198, A.301, A.313
XI	8	A.206, A.226, A.227, A.230, A.231, A.233, A.235, A.270
XII	5	A.185, A.186, A.204, A.205, A.273
XIII	12	A.243, A.255, A.263, A.280, A.282, A.286, A.287, A.289, A.295, A.296, A.297, A.304
XIV	19	A.151, A.152, A.154, A.155, A.156, A.4, A.157, A.158, A.159, A.160, A.161, A.165, A.167, A.168, A.171, A.178, A.183, A.212, A.215

accessions A-185, A-186, A-204, A-205 and A-273 (Table 13). There were accessions without any branches as revealed from the range value of cluster XI (Range 0-7.5 and mean 1.98). The accessions with no branches were A-226, A-227, A-230 and A-231. Mean value for number of branches was maximum (15.29) in cluster X which constituted 24 accessions. Range and mean of leaf length was the highest in cluster XII (Range 17.25-29.15 and mean 21.00) followed by cluster 10 (Range 13.20 – 28.90 and mean 19.12) and was the lowest in cluster III (range 4.75 – 12.75 and mean 7.86). Leaf width was maximum in cluster X (Range 7.30 – 14.00 and mean 10.30) and minimum in cluster V (Range 2.65 – 4.15 and mean 3.48). The cluster IV which consisted two accessions namely A-284 and A-285, which flowered very late (mean 66.50 days) followed by cluster XII with five accessions (mean 56.09). Early flowering was observed in cluster XIII which had 12 accessions namely A-243, A-255, A-263, A-280, A-282, A-286, A-287, A-289, A-295, A-296, A-297 and A-304. The range and mean value for flowering in cluster XIII was 23.50 to 39.50 and 35.83 respectively.

The cluster IV recorded maximum mean values for length of terminal inflorescence (29.55) followed by cluster VI (28.18) and the values were minimum in cluster VII (8.90) which contained 16 accessions. Axillary inflorescence was absent in certain accessions in cluster XI (A-206, A-226, A-227, A-230 and A-231) as revealed from the range value 0-5.65 and mean 1.54. Mean value of the length of inflorescence was the highest in cluster VI (17.15) followed by cluster XIV (12.34).

The mean value for leaf stem ratio was the highest in cluster VII (3.50) which contained 16 accessions followed by cluster 1 (3.35) which contained nine accessions. The lowest mean value for this

Table 13 Range and mean of variables in 168 amaranth accessions in different clusters

Cluster Number	Height at 30th day (cm)		Height at flowering (cm)		Number of branches		Stem girth (cm)		Length of basal lateral branches (cm)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
I	8.30 - 23.50	14.84	23.80 - 47.35	35.59	2.65 - 7.15	4.12	2.30 - 4.10	3.39	7.45 - 19.10	12.58
II	19.55 - 30.15	24.68	41.85 - 65.20	53.88	4.15 - 12.50	7.12	2.70 - 5.65	3.90	15.90 - 45.00	31.03
III	10.25 - 41.50	23.62	24.30 - 76.90	49.10	1.65 - 11.20	7.15	2.15 - 5.65	3.62	7.60 - 19.15	13.06
IV	44.85 - 60.05	52.45	84.60 - 102.10	93.35	3.95 - 4.05	4.00	3.65 - 6.00	4.82	3.60 - 6.65	5.12
V	9.80 - 20.65	14.27	20.25 - 37.20	29.02	3.75 - 6.65	5.03	2.60 - 4.10	3.47	8.00 - 17.25	11.82
VI	9.55 - 22.35	13.90	34.25 - 41.85	38.25	4.05 - 6.90	5.20	2.60 - 7.00	4.12	28.40 - 35.10	32.07
VIII	19.15 - 48.95	33.02	43.65 - 91.35	67.04	1.25 - 13.00	8.07	2.95 - 6.90	5.01	9.70 - 24.50	14.61
VIII	30.55 - 63.95	44.60	65.00 - 113.00	88.70	5.90 - 15.50	12.47	1.80 - 4.75	3.53	5.80 - 37.50	22.71
IX	15.20 - 39.45	26.36	33.15 - 73.60	53.86	3.05 - 9.65	5.95	3.65 - 7.65	4.83	8.75 - 35.80	19.71
X	26.15 - 64.65	49.94	61.50 - 123.40	94.44	5.90 - 20.75	15.29	3.95 - 7.85	5.47	24.65 - 68.25	45.35
XI	31.50 - 68.95	47.96	67.60 - 131.35	94.89	0 - 7.5	1.98	6.70 - 10.20	8.26	0 - 20.25	3.74
XII	51.15 - 86.30	65.66	102.50 - 133.00	117.14	8.80 - 14.15	11.93	10.95 - 13.85	12.31	7.80 - 38.80	20.17
XIII	27.45 - 52.45	39.32	54.80 - 90.50	74.75	8.65 - 15.50	12.29	2.30 - 6.15	3.75	15.90 - 50.05	27.32
XIV	31.30 - 57.15	43.26	65.85 - 95.95	83.98	6.20 - 15.65	11.47	3.45 - 9.25	5.60	21.95 - 57.05	40.05

Table 13 Continued

Cluster No	Length of top lateral branches (cm)		Leaf length (cm)		Leaf width (cm)		Days to bolt		Terminal inflorescence length (cm)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
I	3.00 - 10.30	6.57	8.35 - 12.25	10.31	3.80 - 7.40	5.61	35.50 - 46.00	38.83	8.55 - 20.95	13.07
II	8.70 - 13.75	11.31	7.85 - 15.30	11.60	3.00 - 9.50	5.75	35.00 - 45.50	39.73	10.65 - 20.25	15.99
III	3.25 - 8.20	5.12	4.75 - 12.75	7.86	1.75 - 8.15	3.57	31.50 - 51.50	39.53	6.00 - 14.25	9.12
IV	2.15 - 5.80	3.95	16.00 - 18.10	17.05	6.05 - 7.20	6.62	63.00 - 70.00	66.50	29.30 - 29.80	29.55
V	2.85 - 7.70	4.73	6.40 - 11.15	8.40	2.65 - 4.15	3.48	41.00 - 44.00	42.67	18.10 - 30.70	22.65
VI	23.10 - 25.40	24.58	15.20 - 16.05	15.63	4.50 - 7.60	5.90	39.50 - 43.50	41.17	24.60 - 30.30	28.18
VIII	3.65 - 12.95	6.43	9.70 - 22.70	15.84	4.55 - 10.20	6.98	38.00 - 50.00	44.28	5.65 - 13.05	8.90
VIII	2.90 - 10.00	6.85	10.25 - 21.35	16.03	4.35 - 9.00	6.46	34.00 - 49.00	41.83	12.95 - 27.35	18.15
IX	6.75 - 15.20	9.05	10.50 - 16.30	13.45	3.40 - 11.20	6.78	35.00 - 48.00	41.18	6.05 - 20.50	13.44
X	4.95 - 26.45	10.61	13.20 - 28.90	19.12	7.30 - 14.00	10.30	41.00 - 60.50	50.85	13.85 - 40.25	19.34
XI	0 - 9.75	1.69	13.45 - 23.20	17.15	4.50 - 8.75	6.31	45.50 - 62.50	54.50	11.25 - 25.50	17.38
XII	2.15 - 14.75	7.95	17.25 - 29.15	21.00	6.85 - 10.70	8.89	54.00 - 63.50	56.90	17.40 - 33.55	25.48
XIII	5.90 - 16.85	10.14	5.50 - 14.60	10.70	2.75 - 6.75	4.50	23.50 - 39.50	35.83	13.25 - 23.00	18.23
XIV	6.70 - 15.85	10.11	11.80 - 24.00	18.35	4.65 - 12.95	8.57	36.00 - 49.00	43.21	15.05 - 26.55	21.72

Table 13 Continued

Cluster No	Axillary inflorescence length (cm)		Inflorescence density index		Leaf/Stem ratio		Yield/plant (g)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
I	2.65 - 9.95	6.68	3.00 - 5.60	3.98	2.25 - 4.75	3.35	113.20 - 489.75	261.32
II	0 - 10.65	7.70	3.05 - 5.15	4.02	0.70 - 2.40	1.66	165.30 - 513.70	303.05
III	1.70 - 7.20	4.61	2.65 - 5.00	3.59	0.90 - 2.50	1.45	94.60 - 545.40	261.99
IV	9.00 - 12.45	10.73	4.00 - 4.30	4.15	2.25 - 2.35	2.30	1240.65 - 1487.90	1364.27
V	10.50 - 18.10	13.42	3.65 - 4.65	4.32	0.85 - 1.00	0.93	82.80 - 297.40	210.18
VI	12.00 - 20.30	17.15	4.10 - 5.15	4.47	0.90 - 2.50	1.45	41.30 - 103.00	70.20
VIII	2.25 - 9.60	4.84	3.00 - 5.65	3.69	2.40 - 4.45	3.50	256.15 - 1173.00	619.50
VIII	4.15 - 14.55	9.20	3.25 - 5.00	4.05	1.45 - 4.15	2.65	180.65 - 700.75	489.10
IX	4.10 - 12.45	7.85	4.65 - 6.65	5.51	1.50 - 4.35	2.90	75.70 - 775.40	332.50
X	7.65 - 14.00	9.96	4.20 - 7.25	5.64	1.30 - 4.75	2.88	549.50 - 1500.25	922.95
XI	0 - 5.65	1.54	2.65 - 4.15	3.50	1.05 - 2.45	1.91	351.05 - 1390.40	858.68
XII	4.00 - 17.65	9.15	3.05 - 6.65	4.55	1.55 - 2.20	2.00	1000.00 - 1410.30	1143.09
XIII	8.15 - 16.55	11.67	3.40 - 4.15	3.74	1.50 - 3.30	2.46	70.15 - 955.50	261.87
XIV	7.65 - 15.75	12.34	3.65 - 6.65	4.71	0.65 - 3.35	2.02	98.30 - 608.65	327.46

character was recorded in cluster V (0.93) which consisted of three accessions namely A-173, A-258 and A-276.

Cluster IV which included accessions A-284 and A-285 recorded the highest values for yield (Range 1240-1487 and mean 1364.27) followed by cluster XII (Range 1000-1410 and mean 1143.09) with five accessions namely A-185, A-186, A-204, A-205 and A-273. Cluster VI which included three accessions namely, A-153, A-162 and A-163 had the lowest mean value for yield (70.2).

Crossing among divergent parents is likely to yield heterotic hybrids. In the present study, inter cluster distance among the fourteen cluster was studied and presented in Table 14. Analysis of intercluster distance revealed that the genetic divergence was maximum between cluster VI and cluster XII (8.74) followed by cluster VI and cluster XI (8.56). The intercluster distance between cluster VIII and cluster XIII was low (2.14) suggesting less genetic divergence among them compared to other clusters.

4.2. Isolation and identification of the pathogen associated with leaf spot disease.

Investigations on various aspects of leaf spot disease of amaranth such as symptomatology, etiology and pathogenicity were studied and the results are presented below.

4.2.1. Symptomatology

Two types of leaf spot symptoms were observed (Plate II).

Table 14 Non heirarchial eucledian cluster analysis (Spark, 1973)

Cluster No	Distance between cluster centroids													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0.00													
2	2.78	0.00												
3	2.64	2.61	0.00											
4	7.07	6.59	7.23	0.00										
5	3.65	3.30	3.45	6.73	0.00									
6	6.08	4.57	6.73	8.20	5.23	0.00								
7	2.92	3.37	3.51	5.82	5.30	6.99	0.00							
8	4.01	3.02	4.05	5.30	4.79	6.09	2.78	0.00						
9	2.39	2.36	3.40	6.32	4.02	5.43	2.65	3.08	0.00					
10	6.44	5.12	6.78	5.42	7.06	6.86	4.73	3.58	4.65	0.00				
11	5.72	5.47	5.32	4.24	6.40	8.56	4.02	4.72	5.16	5.88	0.00			
12	8.41	7.27	8.29	4.89	8.45	8.74	6.40	5.79	6.90	4.60	4.75	0.00		
13	3.67	2.35	3.57	6.62	4.03	5.14	3.72	2.14	3.22	4.89	5.98	7.01	0.00	
14	5.12	3.36	5.32	5.77	5.16	5.04	4.22	2.44	3.52	2.83	5.53	5.23	3.06	0.00

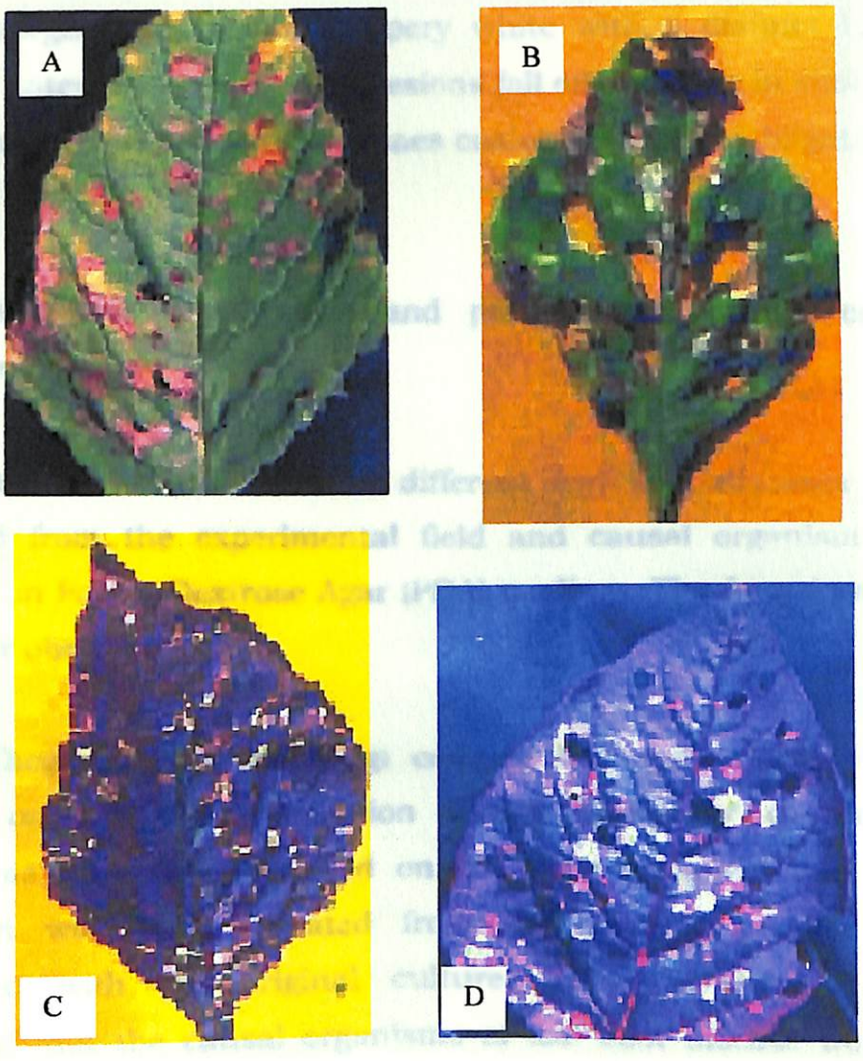


Plate II Symptoms

A – Initial symptom of *C. capsici*
B - Shot hole symptom of *C. capsici*
C- Initial symptom of *R. solani*
D - Shot hole symptom of *R. solani*

Type 1: Appearance of dirty white grey spot on the leaves. The spots increase in size and number, covering the entire foliage.

Type 2: Appearance of small chlorotic spots which later turns brown, surrounded by an yellow halo. As the disease advances, the spot enlarges and becomes papery white with a distinct brown margin. Later the centre of the lesions fall off resulting in shot hole symptoms. These lesions sometimes coalesce leading to blighting of leaves.

4.2.2. Isolation, identification and pathogenicity of the causal organisms

Amaranth leaves infected with different leaf spot diseases were collected from the experimental field and causal organism was isolated on Potato Dextrose Agar (PDA) medium. The fungal growth was later observed.

The pathogenicity of the fungi causing leaf spot diseases was proved on artificial inoculation under laboratory conditions. Symptoms were first observed on 3-4 days of inoculation. The pathogen was again isolated from the infected portion and compared with the original culture. The pathogenicity test indicated that the causal organisms of leaf spot disease were *R. solani* Kuhn and *C. capsici* (Syd.) Butler and Bisby. *R. solani* was found to be associated with producing type 1 symptom and *C. capsici* producing type 2 symptom.

4.2.3. Morphological characters of leaf spot pathogens

The pathogens associated with leaf spot disease were identified as

R. solani and *C. capsici* based on morphological characters (IMI No. 4343 and Mordue, 1971) and they are described below.

Rhizoctonia solani on PDA at room temperature produced colourless hyphae which later turn yellowish and then deep brown. The cells of hyphae at advancing age of the colony are 6.8µm wide and upto 150µm long. The mycelia covered a 90mm petridish within 48 h. The hyphal, branches developed at right angles (Plate III).

Colletotrichum capsici colonies on PDA at room temperature were dense, white at first which turned grey rapidly. Conidia were hyaline, fusiform, gradually tapering toward each end and measuring 21.36 – 24.92 x 3.56 µ in size (Plate III).

4.2.4. Classification of amaranth accessions based on infection

Classification of amaranth accessions based on infection by pathogen is presented in Table 15 and Fig. 1). Among the red types, all the 35 accessions were infected by *Rhizoctonia solani* Kuhn only. Out of 92 green types, 66 accessions were infected by *Colletotrichum capsici* (Syd.) Butler and Bisby and 14 accessions infected by both *R. solani* and *C. capsici.* The four light green types were found to be susceptible to *C. capsici* and all the 12 purple were infected by *R. solani.* Out of the 13 greenish red types, five were attacked by C. capsici and six by both fungi. Among the 12 reddish green types, seven were infected by *R. solani* and five by both fungi. Out of the 168 types studied, 12 green accessions and two greenish red types were immune to both fungi.

Accessions which showed vulnerability to both the fungi were studied in detail. Infected plant parts collected from these revealed

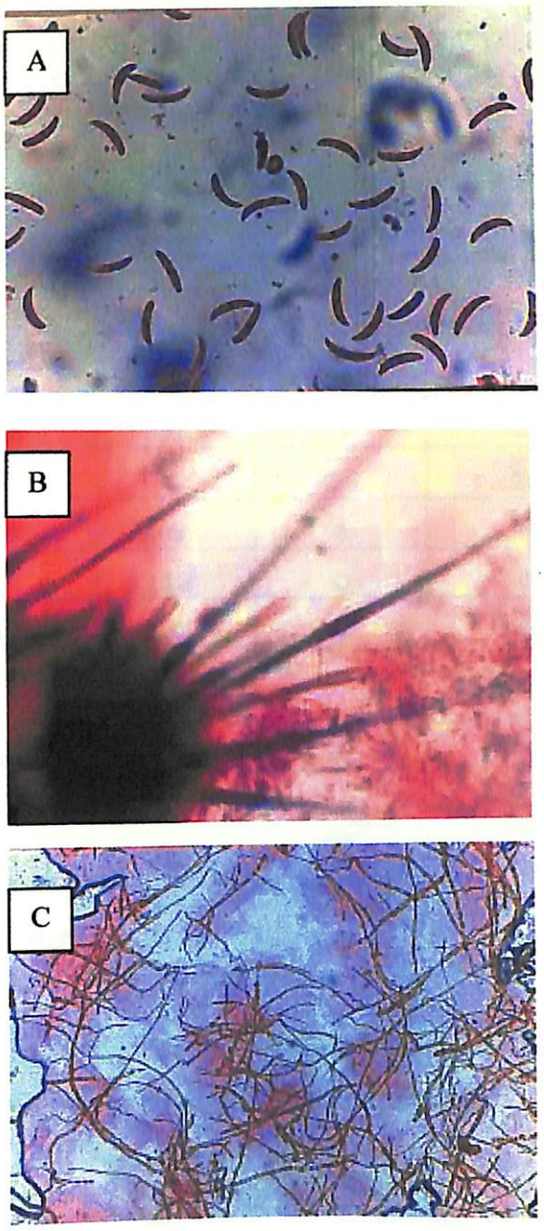


Plate III. Morphological characters of the pathogens

A - Conidia of *C. capsici*
B - Acervuli of *C. capsici* in host tissue
C - Mycelium of *R. solani*

that *C. capsici* is the major pathogen and *R. solani* is of minor importance (Table 16). These fungi were identified based on the morphological characters mentioned under 4.2.3.

Table 15 Classification of amaranth accessions based on infection by pathogens

Colour group	Number of Accessions coming under each class	No. of accessions infected by			No infection
		R. solani	*C. capsici*	Both fungi	
Red	35	35	0	0	0
Green	92	0	66	14	12
Light green	4	0	4	0	0
Purple	12	12	0	0	0
Greenish red	13	0	5	6	2
Reddish green	12	7	0	5	0
Total	168	54	75	25	14

4.2.5. Screening of amaranth accessions against leaf spot disease by artificial inoculation of the pathogen

Ten amaranth accessions (two from each of the five disease classes) which showed good performance with regard to disease resistance and yield in field experiments were selected and screened for resistance/susceptibility to disease by artificial inoculation of the pathogens (Plate IV – VI). The data on disease incidence and disease severity developed after 15, 30 and 45 days of inoculation were recorded. The reaction of amaranth accessions to leaf spot disease is given in the Table 17.

The accessions A-227 and A-204 did not show any symptoms on artificial inoculation indicating that these are immune to leaf spot

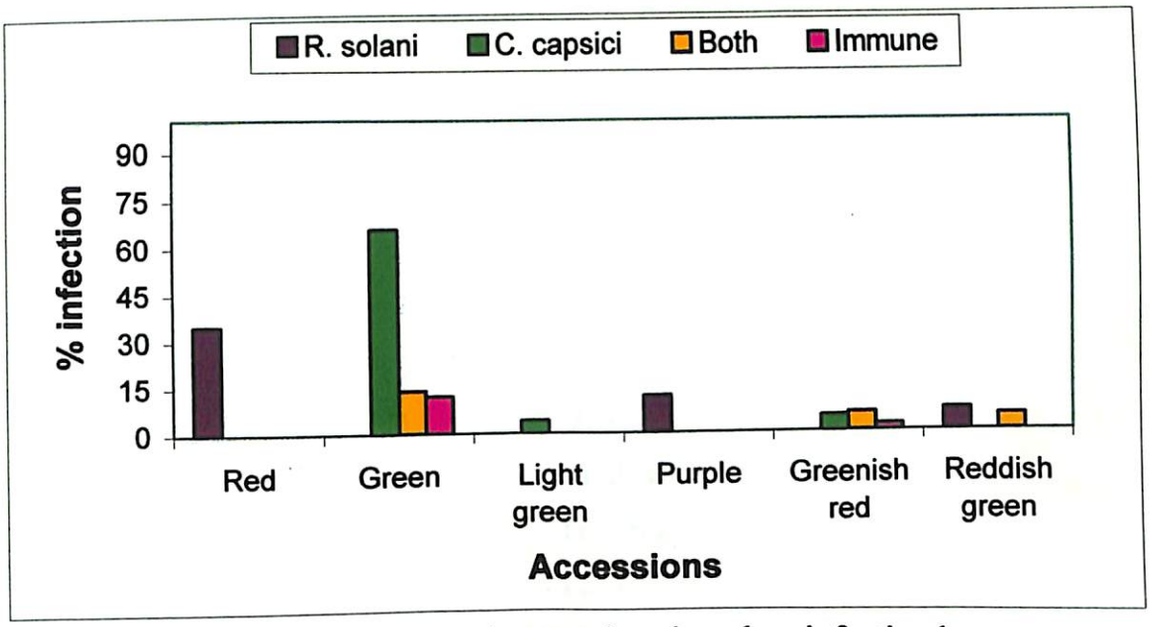


Fig. 1. Classification of amaranth accessions based on infection by pathogens

Table 16 Disease severity in twenty five amaranth accessions infected by *R. solani* and *C. capsici*

Sl. No	Accessions	Disease severity (%)	
		R. solani	*C. capsici*
1	A150	1.610	8.150
2	A151	12.315	44.350
3	A152	11.160	54.210
4	A153	8.420	55.430
5	A160	8.010	51.100
6	A161	13.220	48.210
7	A165	3.212	26.273
8	A171	16.110	20.120
9	A176	3.121	18.104
10	A198	5.320	67.640
11	A201	2.502	11.303
12	A208	11.270	75.220
13	A209	16.280	60.510
14	A234	12.430	57.540
15	A239	8.120	35.200
16	A240	11.220	40.440
17	A244	16.235	62.230
18	A250	9.610	42.215
19	A252	4.230	39.630
20	A257	18.225	57.260
21	A274	9.253	53.532
22	A277	15.450	66.550
23	A281	12.310	64.405
24	A293	9.210	78.520
25	A311	10.750	67.205

Plate IV Amaranth accessions selected for studying seasonal influence on disease

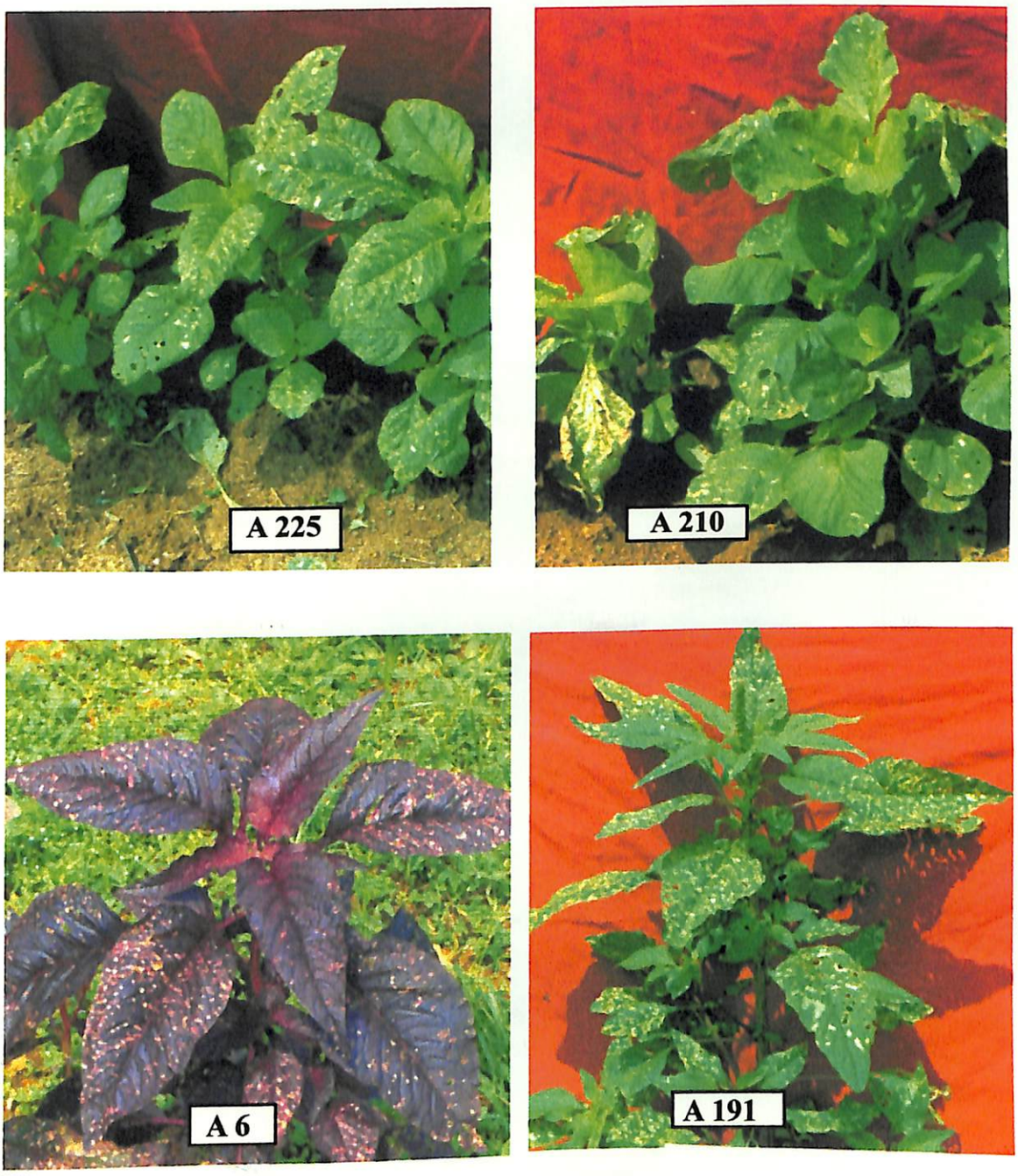


Plate V. Amaranth accessions selected for studying seasonal influence on disease

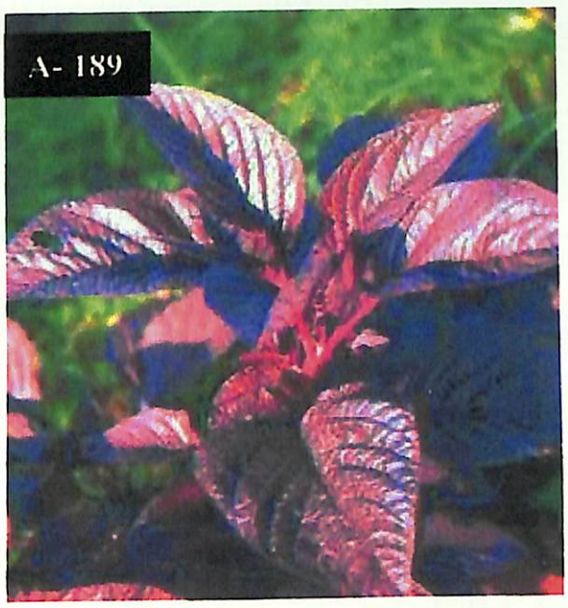


Plate VI Amaranth accessions selected for studying seasonal influence on disease

Table 17. Reaction of amaranth accessions to leaf spot disease by artificial inoculation of the pathogen

Sl. No	Accessions	15 days after inoculation			30 days after inoculation			45 days after inoculation			Category
		Disease incidence %	Disease severity %	CODEX	Disease incidence %	Disease severity %	CODEX	Disease incidence %	Disease severity %	CODEX	
1	A-227	0	0	0	0	0	0	0	0	0	I
2	A-204	0	0	0	0	0	0	0	0	0	I
3	A-3	0	0	0	0	0	0	3.15	1.71	0.054	R
4	A-194	0	0	0	0	0	-	15.21	9.82	1.49	R
5	A-225	19.31	13.82	2.67	23.82	18.11	4.31	33.12	23.71	7.85	M.R
6	A-210	26.89	16.38	4.40	43.34	18.34	7.95	63.34	22.67	14.36	M.R
7	A-6	25.23	26.67	6.73	41.67	36.68	15.28	54.17	38.34	20.77	M.S
8	A-191	31.67	38.34	12.14	52.38	39.67	20.78	83.34	42.35	35.29	M.S
9	A-189	65.73	51.83	34.07	86.56	62.63	54.21	92.68	68.75	63.72	H.S
10	A-182	71.25	50.16	35.74	89.96	66.73	60.03	100	72.65	72.65	H.S.

disease. Two accessions, A-3 and A-194 showed resistant reaction in which disease severity ranged from 1.71 to 9.82 per cent on artificial inoculation . A-225 and A-210 showed disease severity of 23.7 and 22.67 per cent and they are grouped under the class moderately resistant. A-6 and A-191 are came under the class moderately susceptible since the disease severity percentage varied between 38.34 and 42.35 per cent. A-189 and A-182 showed a disease severity of 68.75 and 72.65 per cent and hence grouped under the category of highly susceptible.

The same type of symptoms as described under 4.2.3 were observed on artificial inoculation also.

4.3. Crop and weather relationship in amaranth

4.3.1. Seasonal influence on leaf spot disease in amaranth

Ten selected lines belonging to different disease infection classes with respect to leaf spot were evaluated for the disease response for one year at monthly plantings from May 1997 to April 1998 . Observations made on the disease incidence (DI) and disease severity (DS) at 15, 30 and 45 days after transplanting (DAT) are presented in Table 18-20. Four accessions namely, A-227, A-204, A-3 and A-194 were not included in pooled analysis as observations were zero in all months for A-227 and A-204 and zero in certain months in A-3 and A-194.

All the accessions were free from disease during January and February 1998. In May 1997, March and April 1998 planting, the crop was free from disease in the early stages of growth (fifteen days after transplanting) and the disease appeared only at 30 days

Table 18. Seasonal influence on leaf spot disease during the period 1997 to 1998 (15 days after transplanting)

Genotypes		May	June	July	August	September	October	November	December	January	February	March	April	Pooled Mean
A.227	DI %	0	0	0	0	0	0	0	0	0	0	0	0	0
	DS %	0	0	0	0	0	0	0	0	0	0	0	0	0
A.204	DI %	0	0	0	0	0	0	0	0	0	0	0	0	0
	DS %	0	0	0	0	0	0	0	0	0	0	0	0	0
A.3	DI %	0	2.73 D	6.17 E	0	0	0	0	0	0	0	0	0	0
	DS %	0	0.53 E	2.10 E	0	0	0	0	0	0	0	0	0	0
A.194	DI %	0	9.53 D	18.20 D	11.47 D	23.10 F	7.87 E	14.07 E	9.47 D	0	0	0	0	0
	DS %	0	4.20 DE	6.27 E	5.37 D	7.67 G	4.30 E	3.70 E	2.80 E	0	0	0	0	0
A.225	DI %	0	12.77 f	32.20 e	40.60 e	36.57 e	27.73 e	8.37 e	16.83 e	0	0	0	0	25.01 f
	DS %	0	9.33 c	18.47 c	22.43 c	15.77 c	12.23 cd	6.40 c	8.27 bc	0	0	0	0	13.27 d
A.210	DI %	0	26.57 e	28.20 f	44.37 d	33.83 f	21.67 f	28.20 d	12.00 f	0	0	0	0	27.83 e
	DS %	0	12.23 c	14.20 c	21.43 c	10.40 c	7.87 c	12.40 bc	6.30 c	0	0	0	0	12.12 d
A.6	DI %	0	50.80 d	65.33 d	81.10 c	64.73 c	35.07 d	38.03 c	28.50 c	0	0	0	0	51.94 d
	DS %	0	27.87 b	36.40 b	44.33 b	35.77 b	16.50 bc	17.70 b	10.20 abc	0	0	0	0	27.25 c
A.191	DI %	0	60.73 c	72.80 c	80.03 c	58.87 d	43.20 c	28.57 d	24.03 d	0	0	0	0	52.60 c
	DS %	0	32.30 b	39.97 b	42.33 b	41.00 b	22.33 b	14.77 bc	14.53 abc	0	0	0	0	29.60 c
A.189	DI %	0	75.07 b	77.87 b	84.87 b	83.60 b	56.70 b	59.03 b	31.20 b	0	0	0	0	66.90 b
	DS %	0	46.00 a	45.37 b	59.27 a	51.10 a	32.40 a	36.23 a	16.77 ab	0	0	0	0	41.02 b
A.182	DI %	0	82.37 a	82.13 a	91.83 a	92.43 a	64.83 a	67.73 a	32.90 a	0	0	0	0	73.46 a
	DS %	0	55.10 a	57.70 a	62.80 a	54.47 a	38.73 a	41.70 a	20.20 a	0	0	0	0	47.24 a

Superscripts in capital letters indicate individual analysed data
Superscripts in small letters indicate pooled analysis data

Table 19. Seasonal influence on leaf spot disease during the period 1997 to 1998 (30 days after transplanting)

Genotypes		May	June	July	August	September	October	November	December	January	February	March	April	Pooled Mean
A.227	DI %	0	0	0	0	0	0	0	0	0	0	0	0	0
	DS %	0	0	0	0	0	0	0	0	0	0	0	0	0
A.204	DI %	0	0	0	0	0	0	0	0	0	0	0	0	0
	DS %	0	0	0	0	0	0	0	0	0	0	0	0	0
A.3	DI %	0	7.53 E	10.30 E	4.13 E	0	0	0	0	0	0	0	0	0
	DS %	0	2.30 D	4.20 F	0.93 E	0	0	0	0	0	0	0	0	0
A.194	DI %	3.27 C	18.50 E	29.97 D	19.37 D	23.50 D	10.30 E	18.07 E	0	0	0	0	0	0
	DS %	0.23 D	6.13 D	12.83 E	8.47 D	8.20 G	4.20 F	4.43 F	0	0	0	0	0	0
A.225	DI %	4.87 a	36.43 d	48.70 c	43.17 b	37.87 c	31.37 c	14.17 d	0	0	0	8.17 a	2.17 a	25.21 c
	DS %	2.10 a	18.60 c	26.33 c	24.40 c	18.77 d	15.33 cd	10.03 d	0	0	0	1.90 a	0.10 a	13.06 c
A.210	DI %	5.47 a	52.33 d	44.23 c	44.13 b	40.90 c	25.57 c	18.93 d	0	0	0	6.03 a	6.43 a	27.11 c
	DS %	0.83 a	22.60 c	24.20 c	22.83 c	14.60 d	9.47 d	8.33 d	0	0	0	2.13 a	2.20 a	11.91 c
A.6	DI %	7.87 a	71.07 c	72.83 b	88.00 a	68.43 b	43.63 bc	42.03 c	0	0	0	11.27 a	18.20 a	47.04 b
	DS %	3.10 a	42.27 b	44.53 b	47.23 b	39.33 c	24.87 c	21.00 cd	0	0	0	3.77 a	6.33 a	25.83 b
A.191	DI %	9.23 a	75.87 bc	84.87 ab	91.97 a	66.40 b	51.50 ab	51.43 bc	0	0	0	7.93 a	10.03 a	49.91 b
	DS %	5.40 a	44.30 b	48.50 b	49.27 b	44.23 bc	28.03 bc	25.17 bc	0	0	0	4.77 a	6.47 a	28.46 b
A.189	DI %	6.23 d	95.67 a	100.00 a	100.00 a	92.10 a	63.40 a	62.93 ab	0	0	0	12.00 a	9.13 a	60.16 a
	DS %	2.80 a	62.37 a	66.50 a	64.90 a	55.90 ab	47.77 a	38.10 b	0	0	0	6.43 a	7.20 a	39.11 a
A.182	DI %	10.37 a	92.60 ab	100.00 a	100.00 a	100.00 a	69.47 a	76.03 a	0	0	0	17.93 a	15.47 a	64.65 a
	DS %	6.30 a	63.80 a	74.00 a	68.53 a	61.00 a	41.93 ab	52.77 a	0	0	0	7.20 a	7.73 a	42.59 a

Superscripts in capital letters indicate individual analysed data
Superscripts in small letters indicate pooled analysis data

Table 20. Seasonal influence on leaf spot disease during the period 1997 to 1998 (45 days after transplanting)

Genotypes		May	June	July	August	September	October	November	December	January	February	March	April	Pooled Mean
A.227	DI %	0	0	0	0	0	0	0	0	0	0	0	0	0
	DS %	0	0	0	0	0	0	0	0	0	0	0	0	0
A.204	DI %	0	0	0	0	0	0	0	0	0	0	0	0	0
	DS %	0	0	0	0	0	0	0	0	0	0	0	0	0
A.3	DI %	3.13 D	10.80 D	12.60 D	8.77 D	2.43 E	2.03 E	0	0	0	0	0	0	0
	DS %	0.70 E	3.93 G	5.70 F	1.73 E	0.50 E	0.03 E	0	0	0	0	0	0	0
A.194	DI %	9.33 D	24.30 D	39.30 C	26.57 C	26.03 D	16.00 D	19.87 E	0	0	0	0	0	0
	DS %	7.40 DE	9.47 F	19.37 E	11.63 D	9.40 D	6.17 D	5.20 F	0	0	0	0	0	0
A.225	DI %	22.63 b	31.43 c	64.43 b	46.40 b	42.77 c	38.07 d	22.40 d	0	0	0	11.03 a	8.67 a	31.98 c
	DS %	11.23 b	22.57 c	35.03 c	27.97 c	24.23 c	18.10 bc	11.53 d	0	0	0	3.40 a	3.90 a	17.55 c
A.210	DI %	34.80 b	62.57 b	64.50 b	48.30 b	46.87 c	40.60 d	16.87 d	0	0	0	9.87 a	8.37 a	36.97 c
	DS %	17.07 b	28.03 c	29.10 c	25.53 c	21.13 c	14.60 c	9.37 d	0	0	0	2.63 a	4.50 a	16.89 c
A.6	DI %	32.93 b	91.77 a	100.00 a	100.00 a	70.47 b	55.03 cd	33.07 cd	0	0	0	19.37 a	15.57 a	57.58 b
	DS %	18.27 b	56.80 b	62.40 b	51.37 b	47.03 b	28.40 bc	21.70 cd	0	0	0	6.57 a	6.10 a	33.18 b
A.191	DI %	38.13 b	85.70 a	100.00 a	100.00 a	67.37 b	60.77 bc	42.20 bc	0	0	0	19.60 a	16.03 a	59.53 b
	DS %	23.00 b	52.90 b	69.13 ab	54.53 b	45.93 b	32.37 b	26.10 c	0	0	0	9.00 a	9.03 a	35.78 b
A.189	DI %	70.87 a	100.00 a	100.00 a	100.00 a	100.00 a	84.07 a	66.57 ab	0	0	0	19.97 a	15.97 a	73.05 a
	DS %	48.90 a	75.43 a	72.73 ab	69.40 a	60.87 a	52.87 a	44.20 b	0	0	0	10.70 a	9.63 a	4.94 a
A.182	DI %	61.67 a	100.00 a	100.00 a	100.00 a	100.00 a	79.23 ab	80.03 a	0	0	0	25.83 a	16.27 a	73.67 a
	DS %	43.43 a	76.83 a	81.40 a	72.47 a	63.77 a	57.03 a	58.03 a	0	0	0	12.03 a	8.20 a	52.58 a

Superscripts in capital letters indicate individual analysed data
Superscripts in small letters indicate pooled analysis data

after transplanting. During the period from June1997 - December 1997, the disease was observed to affect the crop at the early stage itself immediately after transplanting. In December planted crop, the disease was prevalent only in the early stage of the crop (15 days after transplanting) and symptoms disappeared towards the later stages.

The disease incidence and severity increased with the age of the plant reaching the maximum at 45th day after transplanting. The disease incidence of the genotype A-3 increased from 2.73 (15 DAT) to 10.8 (45 DAT) in June and similarly disease severity increased from 0.53 to 3.93 per cent. In A-182, DI and DS in June increased from 82.3 and 55.1 to 100 and 76 per cent respectively. In March and April planted crops also disease appeared 30 days after transplanting. A similar increasing trend was noticed for DI and DS from 30 days after transplanting to 45 days after transplanting.

Among the 10 accessions studied, six namely A.225, A.210, A.6, A.191, A.189 and A.182 were found to be affected by the disease in all the ten months of the year except January and February 1998. No disease symptoms were noticed in two accessions (A.227 and A.204) throughout the year and they were found to be immune to the disease. The genotype A-3 was free from disease from November to April months; infection was noticed at 15 days after transplanting in June and July; at 30 days after transplanting in August and at 45 days after transplanting in May, September and October planted crop. The Disease severity of this genotype varied from 0.03 to 5.70 per cent in different months with the maximum value at 45 days after transplanting in July. Since DS is less than 10 per cent, this genotype appears to be resistant to this disease in all the months.

Genotype A.194 was found to be free of disease for plantings from January to April 1998. The maximum DS value for this genotype in July and August crops was 19.37 per cent and 11.63 per cent respectively bringing it under the class "moderately resistant". In the remaining months, it appears to come under the "class resistant".

Among the six accessions in which disease was present in all the ten months of the year except January and February 1998. Maximum DI and DS at 45 DAT was for the accessions A.189 and A.182 and minimum for A.225 and A.210. Disease incidence and severity of the genotype A.225 was maximum in July (DI 64.4 % and DS 35.03 %) followed by August (DI 46.4 % and DS 27.97 %) and minimum in March and April.

Accessions showed differential response to the changing environmental conditions. A.225 is found to fall in the class 'Resistant' in the months of March and April (DI 8.67 to11.03 % and DS 3.40 to 3.90), 'Moderately resistant' in May, June, September, October and November and 'Moderately susceptible' in July and August.

Genotype A.210 was found to be resistant to the disease in March and April 1998; moderately resistant in May, September, October and November 1997 and moderately susceptible in June, July and August 1997.

In the genotype A.6, the highest value of DI (100 %) was recorded in July and August and it was highly susceptible to the disease in June, July and August 1997; moderately susceptible in September and October 1997; moderately resistant in May and November 1997 and resistant in March and April 1998.

The genotype A.191 belonged to resistant class in March and April planted crop, moderately resistant in May crop, moderately susceptible in September and October crop and highly susceptible in June, July and August.

The two accessions A.189 and A.182, were resistant in April; moderately resistant in March crop; moderately susceptible in May crop A-182 was highly susceptible in the rest of months namely June, July, August, September, October and November. However, A.189 was found moderately susceptible in November and highly susceptible during June-October.

4.3.2. Influence of climatic factors on leaf spot infection

The climatic factors like maximum and minimum temperature, relative humidity and rainfall during the cropping periods were recorded to relate its influence on disease development and severity (Table 21).

In 1998, when disease was absent in January and February, the monthly maximum temperature was 33.2°C and 39.5°C respectively During the same months relative humidity was 63.1 and 68.4 per cent respectively and the rain record was zero. In the case of crops planted in March and April 1998, when the disease was absent upto 15 days after transplanting and disease developed at later stages, the monthly maximum temperature recorded were 36.3°C and 36.6°C respectively. For the same months, relative humidity values were 66.6 and 67.8 per cent respectively and the rain recorded were 11 mm and 61.4 mm respectively. For these months rain was received in the last weeks only. The December

crop in which the disease disappeared towards the later stage, received a total of 66.7 mm rain in the first two weeks and no rain in the last two weeks.

The disease incidence was the maximum (100%) in July and August for two accessions (A-6 and A-191) and for A-189 and A-182, cent percent disease incidence was observed during the period from June to September (Table 20, Fig 2, Fig. 3 and Fig. 4).

Maximum disease severity was recorded in July for all the accessions and found that the maximum temperature, humidity and rain fall during this period were 28.8°C, 89.6 per cent and 891.2mm respectively. Minimum DS was observed in March and April crop when the monthly maximum temperature was 36.3°C and 36..6°C , Relative humidity, 66.6 per cent and 67.8 per cent and rain 11mm and 61.4mm respectively (Table 21, Fig. 5, Fig 6 and Fig 7)

The role of meteorological parameters like maximum and minimum temperatures, RH and total rainfall on leaf spot disease development was examined in detail. Correlation coefficients between disease incidence, disease severity and CODEX and weather elements in overlapping period prior to observation of diseases were worked out. Period in which the weather elements significantly influenced the leaf spot disease occurrence at different stages of growth of amaranth were identified and the corresponding correlation coefficients are presented in table 22-26.

Leaf spot disease in amaranthus was negatively correlated with maximum and minimum temperatures whereas relative humidity and total rainfall were positively correlated with the disease. Maximum temperature during the period from 6-15 days after

Table 21. Weather data during the period from May 1997 to April 1998

Period		Temperature (°C)				Relative humidity (%)		Rain fall (mm)	
		Weekly		Monthly					
		maximum	minimum	maximum	minimum	Weekly	Monthly	Weekly	Monthly
May-97	1st week	34.8	24.3	34.4	24.4	70.5	72.2	15.4	87.0
	2nd week	34.6	24.8			75.5		19.6	
	3rd week	33.7	24.7			72.5		0.0	
	4th week	34.7	23.8			69.5		28.0	
	5th week	34.0	24.9			73.0		24.0	
Jun-97	1st week	33.7	23.2	30.7	22.8	74.0	83.5	109.4	750.8
	2nd week	31.1	23.2			80.0		50.0	
	3rd week	31.3	23.2			85.0		99.2	
	4th week	26.6	21.8			95.0		492.2	
Jul-97	1st week	27.7	22.1	28.8	22.5	88.0	89.6	239.5	891.2
	2nd week	28.8	22.3			93.0		192.9	
	3rd week	29.8	22.8			86.0		207.0	
	4th week	29.0	22.7			91.5		252.1	
Aug-97	1st week	29.1	23.3	28.9	22.9	86.5	86.1	143.6	671.4
	2nd week	27.7	21.9			89.0		280.5	
	3rd week	29.6	23.1			83.5		24.3	
	4th week	29.6	22.9			87.0		151.8	
	5th week	28.9	23.4			84.5		71.2	

Table 21 Continued

Period		Temperature (oC)				Relative humidity (%)		Rain fall (mm)	
		Weekly		Monthly					
		maximum	minimum	maximum	minimum	Weekly	Monthly	Weekly	Monthly
Sep-97	1st week	30.4	23.0	30.5	23.3	83.0	82.5	5.4	162.8
	2nd week	31.4	23.1			79.0		55.2	
	3rd week	29.5	22.7			86.5		76.1	
	4th week	30.8	24.3			81.5		26.1	
Oct-97	1st week	31.7	24.7	32.2	23.6	78.5	76.2	13.2	225.0
	2nd week	33.3	23.1			72.5		84.3	
	3rd week	32.4	23.6			74.0		53.1	
	4th week	31.7	23.5			78.0		28.9	
	5th week	31.7	23.1			78.0		45.5	
Nov-97	1st week	31.0	23.6	31.6	23.7	78.0	77.6	74.6	181.0
	2nd week	32.3	22.8			77.5		30.4	
	3rd week	31.2	24.5			79.5		74.4	
	4th week	31.9	23.7			75.5		1.6	
Dec-97	1st week	30.6	24.4	31.7	24	73.5	72.1	23.1	66.7
	2nd week	32.1	23.9			74.0		43.6	
	3rd week	32.1	24.9			74.0		0.0	
	4th week	32.1	22.7			67.0		0.0	

Table 21 Continued

Period		Temperature (oC)				Relative humidity (%)		Rain fall (mm)	
		Weekly		Monthly					
		maximum	minimum	maximum	minimum	Weekly	Monthly	Weekly	Monthly
Jan-98	1st week	31.6	24.2	33.2	23.6	59.5	63.1	0.0	0.0
	2nd week	32.0	23.3			61.0		0.0	
	3rd week	33.7	22.1			69.5		0.0	
	4th week	34.2	24.3			65.0		0.0	
	5th week	34.6	24.3			60.5		0.0	
Feb-98	1st week	34.8	23.3	39.5	23.7	66.5	68.4	0.0	0.0
	2nd week	34.4	23.4			64.0		0.0	
	3rd week	33.4	23.6			73.5		0.0	
	4th week	35.3	24.3			69.5		0.0	
Mar-98	1st week	35.9	23.6	36.3	23.5	69.0	66.6	0.0	11.0
	2nd week	35.5	23.8			64.5		0.0	
	3rd week	37.5	23.7			64.0		0.0	
	4th week	36.2	22.9			69.0		11.0	
Apr-98	1st week	37.5	25.1	36.6	25.7	61.5	67.8	0.0	61.4
	2nd week	36.4	26.1			69.0		0.0	
	3rd week	36.6	26.8			69.0		4.2	
	4th week	35.9	24.6			71.5		57.2	

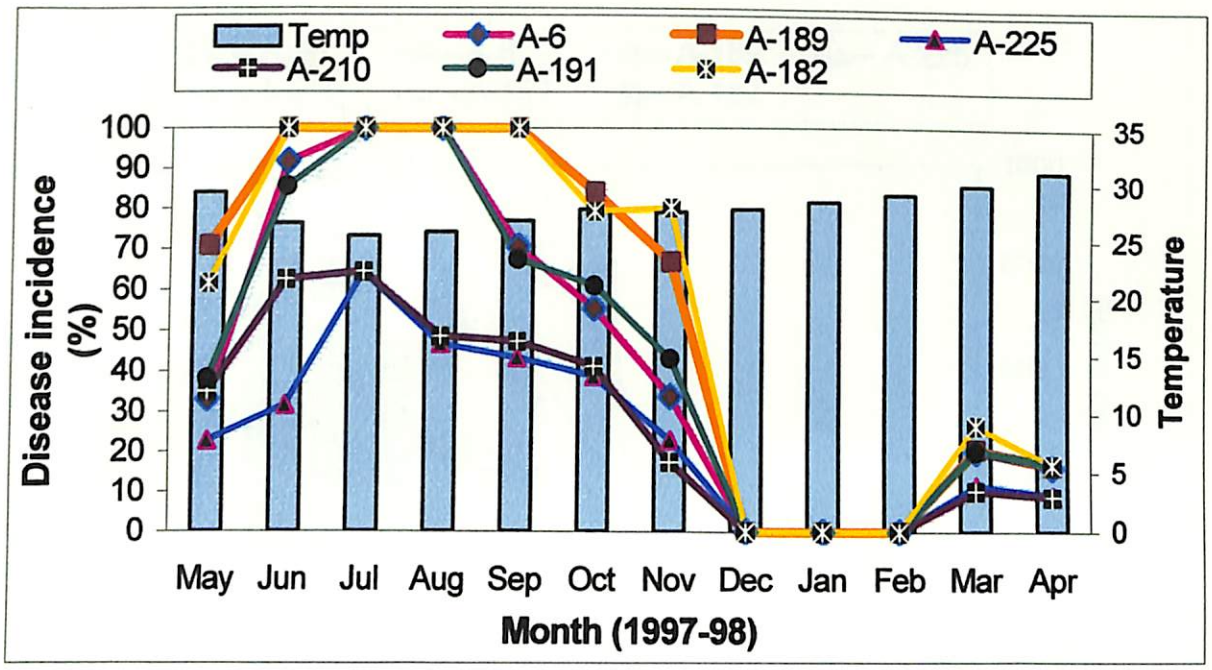


Fig. 2 Influence of monthly maximum temperature on disease incidence in six amaranth accessions

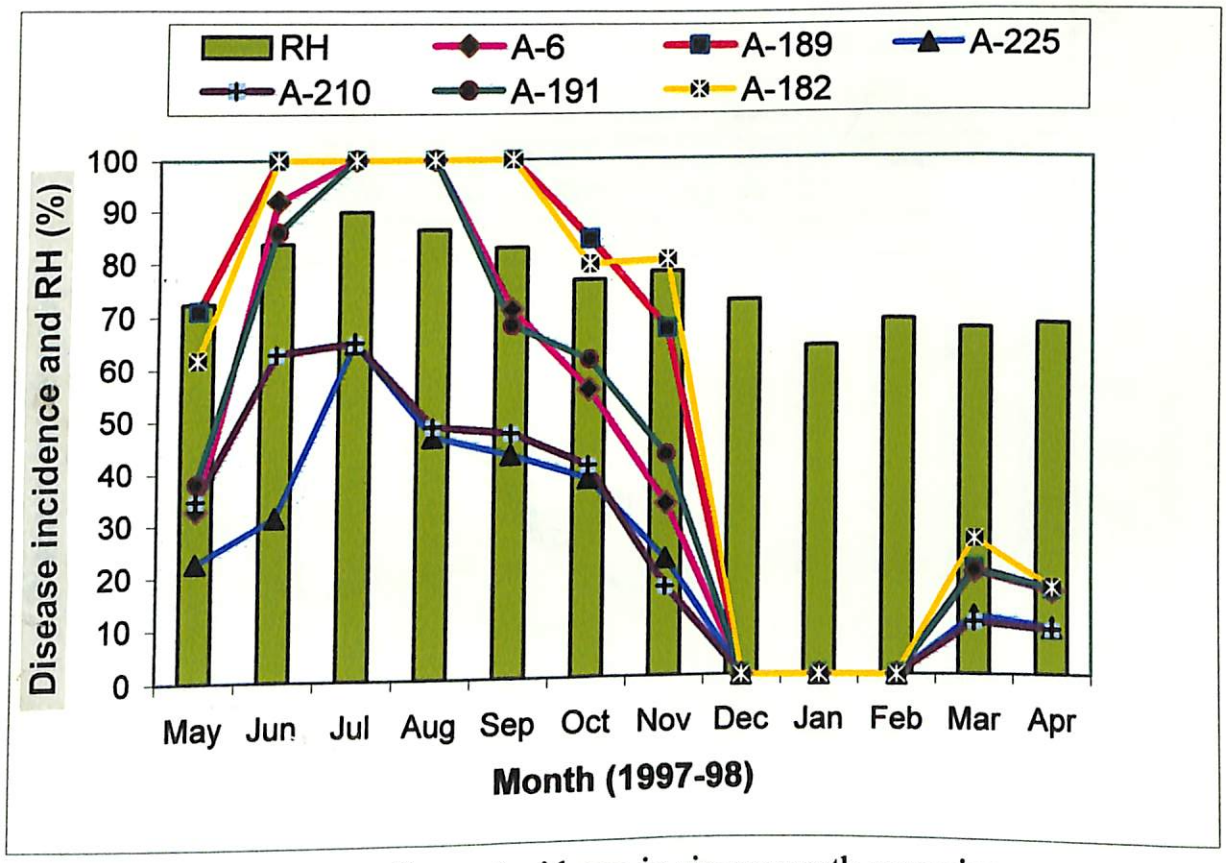


Fig.3 Influence of RH on disease incidence in six amaranth accessions

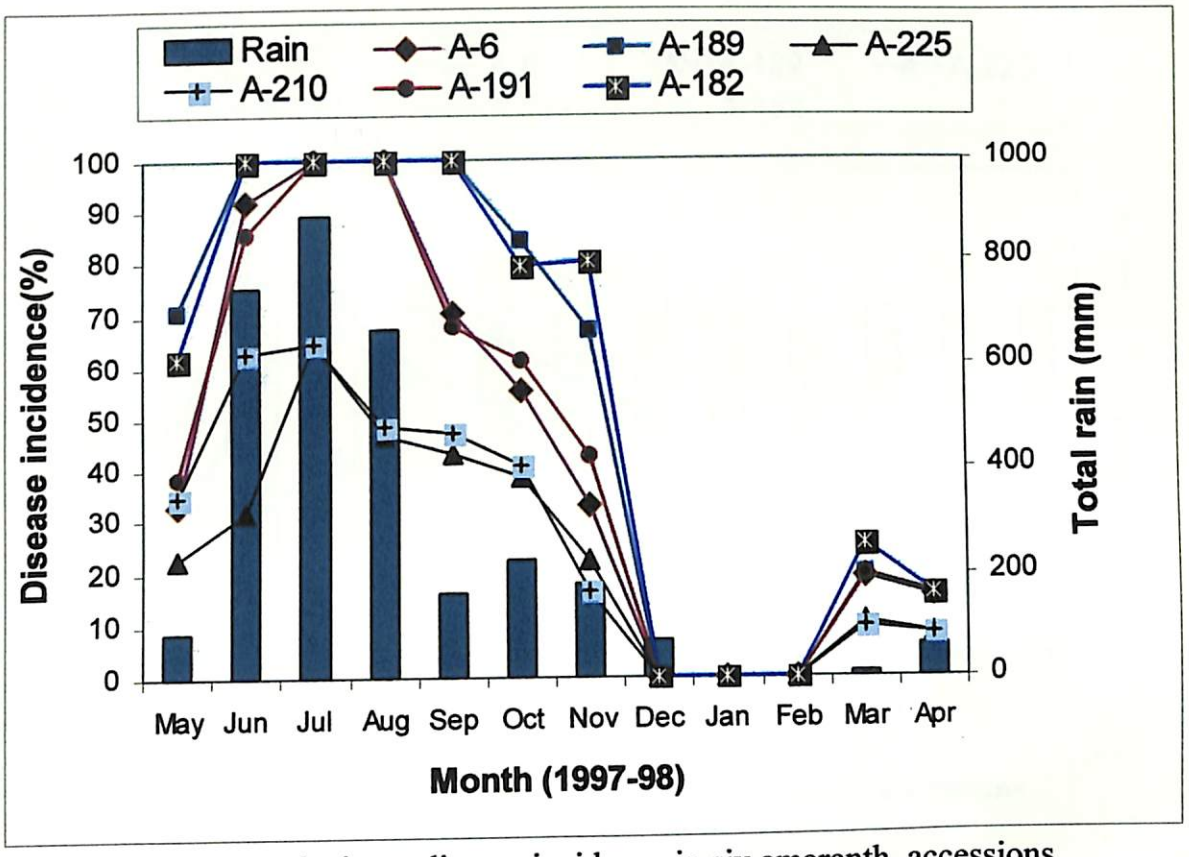


Fig. 4. Influence of rain on disease incidence in six amaranth accessions

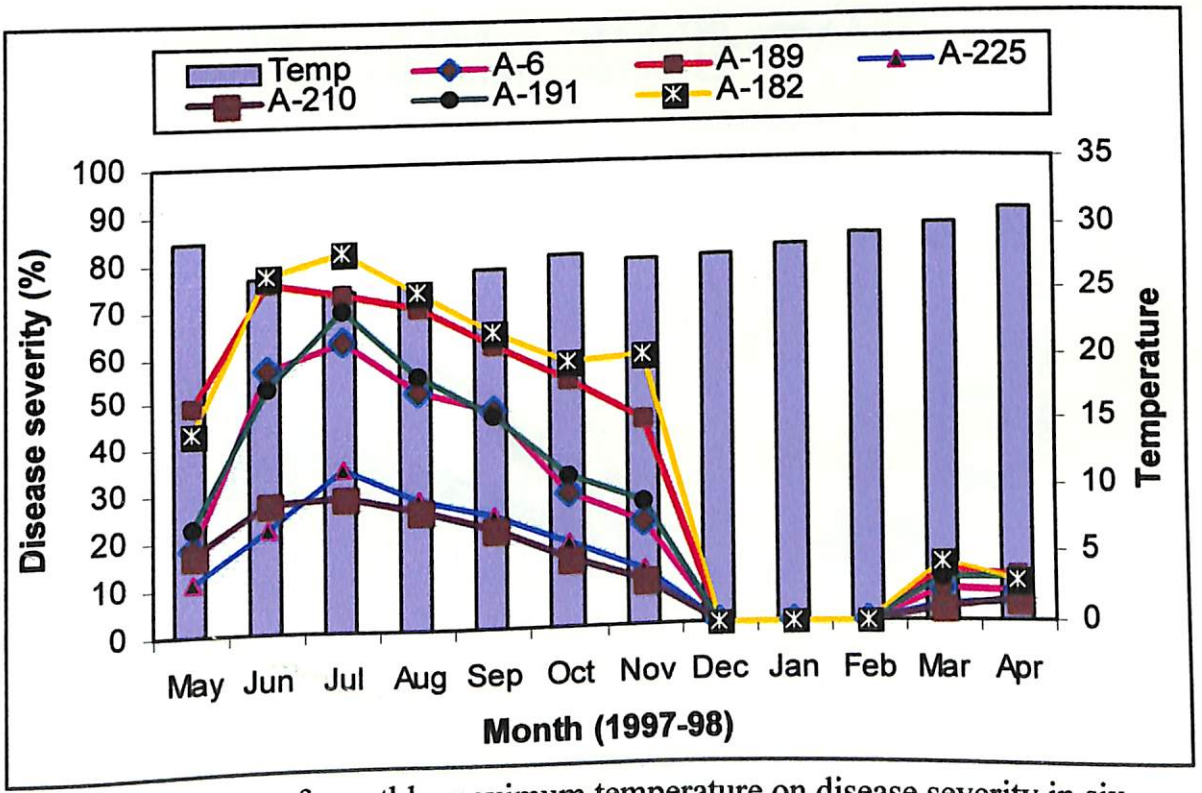


Fig. 5. Influence of monthly maximum temperature on disease severity in six amaranth accessions

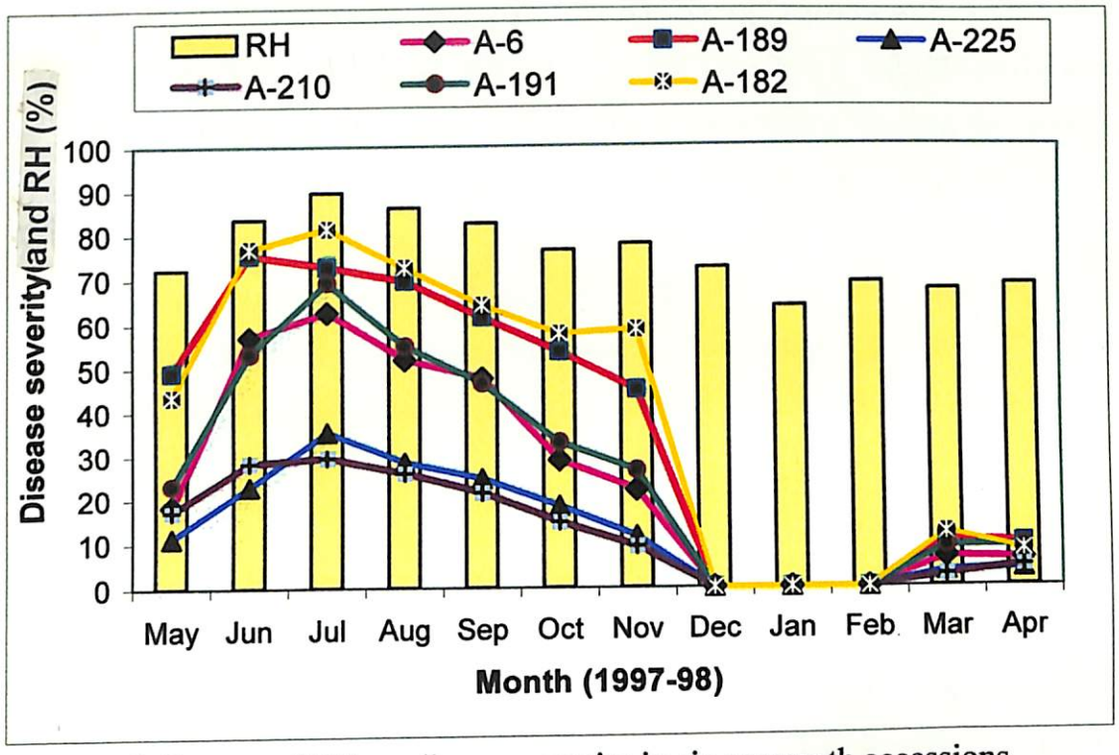


Fig.. 6. Influence of RH on disease severity in six amaranth accessions

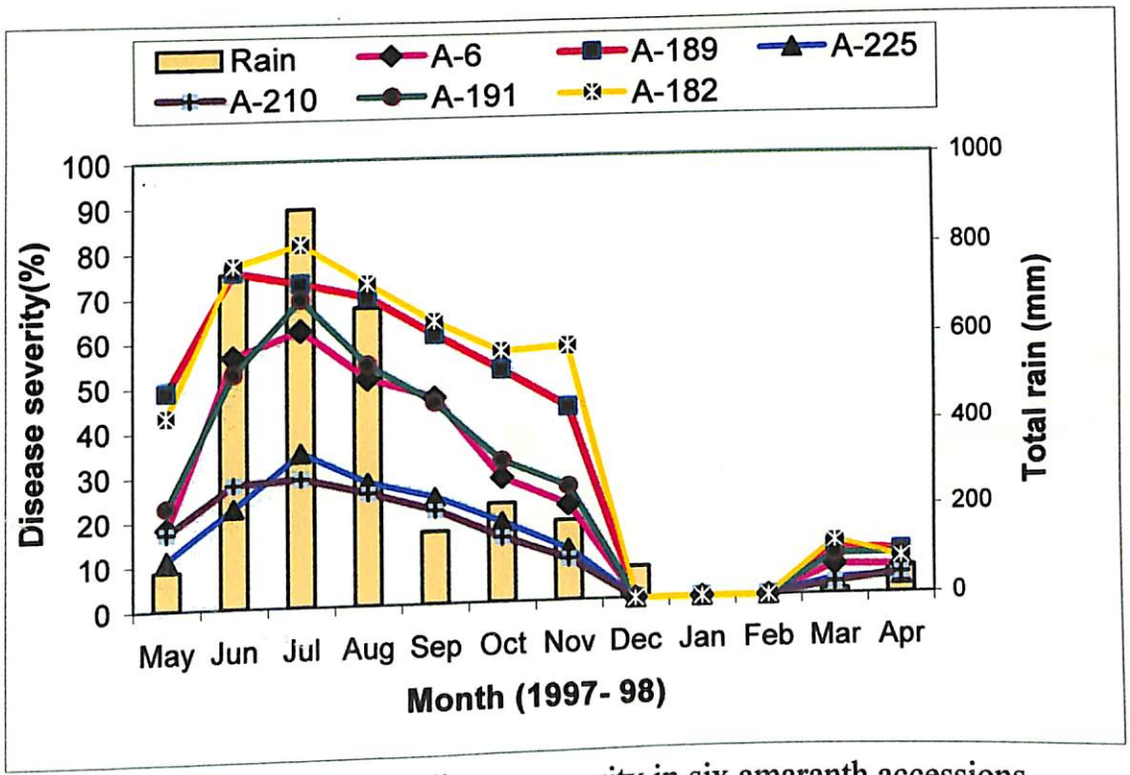


Fig. 7. Influence of rain on disease severity in six amaranth accessions

transplanting was negatively correlated with leaf spot disease in seven accessions except in A.3 in which no significant correlation was found. DI, DS and CODEX at 30 DAT was significantly and negatively correlated with maximum temperature during 6-30 days in 5 accessions (A.194, A. 225, A. 191, A. 189 and A. 182), 11-30 days in two accessions (A. 210 and A.6) and only during 16-20 days in A.3. Maximum temperature during 6-45 days significantly influenced leaf spot disease in two accessions (A. 194 and A. 225), 11-45 days in A.6 and A. 191 and 16-45 days in A.3, A.210, A.189 and A.182 (Table 22).

Leaf spot disease occurrence at 15 days after transplanting was significantly and negatively influenced by the minimum temperature during 11-15 days after transplanting in seven accessions except A.3 (Table 23). In A.194, A.225 and A.210, the disease severity at 30 days after transplanting was influenced by the minimum temperatures during 6-15 days and 21-30 days whereas in A.6, A.191, A.189 and A.182 the disease severity was influenced by the minimum temperatures during 11-15 days and 21-30 days. Disease severity at 45 days after transplanting was influenced by the minimum temperatures during 6-15, 21-30 and 41-45 days for A.194, A.225, A.210, A.6 and A.191 whereas in A.189 and A.182 the minimum temperatures during 11-15, 21-30 and 41-45 days influence disease severity. In A.3, minimum temperatures during 6-15 and 41-45 days after transplanting influenced disease severity.

Morning relative humidity during 6-15 days after transplanting influenced significantly and positively the disease severity in six accessions except A.3 and .194 (Table 24). In A.3, no significant correlation was noticed whereas in A.194 morning relative humidity during 11-15 days influenced disease severity significantly.

Table 22. Correlation coefficients between maximum temperature in identified period and leaf spot disease at different stages of amaranth

Accessions	Maximum temperature								
	15 DAT			30 DAT			45 DAT		
	DI	DS	CODEX	DI	DS	CODEX	DI	DS	CODEX
A.3	NS	NS	NS	-0.777 **(16-20)	-0.747 **(16-20)	-0.709 **(16-20)	-0.781 **(16-45)	-0.736 **(16-45)	-0.719 **(31-45)
A.194	-0.780 **(6-15)	-0.796 **(6-15)	-0.664 *(6-15)	-0.844 **(6-30)	-0.834 **(6-30)	-0.742 **(6-30)	-0.850 **(6-45)	-0.820 **(6-45)	-0.756 **(6-45)
A.225	-0.824 **(6-15)	-0.859 **(6-15)	-0.808 **(6-15)	-0.798 **(6-30)	-0.837 **(6-30)	-0.798 **(6-30)	-0.754 **(6-45)	-0.794 **(6-45)	-0.763 **(6-45)
A.210	-0.834 **(6-15)	-0.846 **(6-15)	-0.805 **(6-15)	-0.793 **(11-30)	-0.788 **(11-30)	-0.760 **(11-30)	-0.765 **(16-45)	-0.773 **(16-45)	-0.796 **(16-45)
A.6	-0.854 **(6-15)	-0.825 **(6-15)	-0.807 **(6-15)	-0.796 **(11-30)	-0.826 **(11-30)	-0.821 **(11-30)	-0.772 **(11-45)	-0.798 **(11-45)	-0.795 **(11-45)
A.191	-0.798 **(6-15)	-0.800 **(6-15)	-0.763 **(6-15)	-0.824 **(6-30)	-0.830 **(6-30)	-0.832 **(6-30)	-0.797 **(11-45)	-0.805 **(11-45)	-0.820 **(11-35)
A.189	-0.793 **(6-15)	-0.803 **(6-15)	-0.773 **(6-15)	-0.830 **(6-30)	-0.824 **(6-30)	-0.848 **(6-30)	-0.747 **(16-45)	-0.787 **(16-45)	-0.812 **(16-45)
A.182	-0.784 **(6-15)	-0.793 **(6-15)	-0.776 **(6-15)	-0.808 **(6-30)	-0.825 **(6-30)	-0.858 **(6-30)	-0.750 **(16-45)	-0.817 **(16-45)	-0.844 **(16-45)

Figures in paranthesis shows the period in days identified to have influence on disease

*** significant at 5 % level**

**** significant at 1 % level**

Table 23. Correlation coefficients between minimum temperature in identified period and leaf spot disease at different stages of amaranth

Axccessions	Minimum temperature								
	15 DAT			30 DAT			45 DAT		
	DI	DS	CODEX	DI	DS	CODEX	DI	DS	CODEX
A.3	NS	NS	NS	NS	NS	NS	-0.619 *(6-15)	-0.720 **(6-15)	-0.763 **(41-45)
							-0.794 **(26-45)	-0.781 **(41-45)	
A.194	-0.706 **(11-15)	-0.741 **(11-15)	-0.601 *(11-15)	-0.714 **(6-15)	-0.730 **(6-15)	-0.672 *(6-15)	-0.725 **(6-15)	-0.697 *(6-15)	-0.648 *(6-15)
				-0.754 **(21-30)	-0.775 **(21-30)	-0.677 *(21-30)	-0.763 **(21-30)	-0.696 *(21-30)	-0.647 *(21-25)
							-0.731 **(41-45)	-0.757 **(41-45)	-0.705 *(41-45)
A.225	-0.742 **(11-15)	-0.797 **(11-15)	-0.734 **(11-15)	-0.715 **(11-15)	-0.739 **(6-15)	-0.720 **(6-15)	-0.697 *(6-15)	-0.718 **(6-15)	-0.689 *(6-15)
				-0.790 **(21-30)	-0.814 **(21-30)	-0.767 **(21-30)	-0.714 **(26-30)	-0.736 **(21-30)	-0.714 **(26-30)
							-0.611 *(41-45)	-0.665 *(41-45)	-0.665 *(41-45)
A.210	-0.774 **(11-15)	-0.801 **(11-15)	-0.749 **(11-15)	-0.678 *(11-15)	-0.715 **(6-15)	-0.673 *(6-15)	-0.632 *(11-15)	-0.662 *(6-15)	-0.636 *(6-15)
				-0.728 **(21-30)	-0.753 **(21-30)	-0.704 **(21-30)	-0.654 *(26-30)	-0.648 *(21-30)	-0.653 *(21-30)
							-0.688 *(41-45)	-0.693 *(41-45)	-0.769 **(41-45)
A.6	-0.810 **(11-15)	-0.793 **(11-15)	-0.768 **(11-15)	-0.714 **(11-15)	-0.715 **(11-15)	-0.733 **(11-15)	-0.712 **(11-15)	-0.704 **(6-15)	-0.702 *(6-15)
				-0.737 **(21-30)	-0.762 **(21-30)	-0.758 **(21-30)	-0.725 **(21-30)	-0.725 **(21-30)	-0.726 **(21-30)
							-0.660 *(41-45)	-0.694 *(41-45)	-0.729 **(41-45)
A.191	-0.787 **(11-15)	-0.779 **(11-15)	-0.759 **(11-15)	-0.729 **(11-15)	-0.718 **(11-15)	-0.742 **(11-15)	-0.721 **(11-15)	-0.714 **(6-15)	-0.725 **(6-15)
				-0.782 **(21-30)	-0.764 **(21-30)	-0.779 **(21-30)	-0.736 **(21-30)	-0.721 **(21-30)	-0.745 **(21-30)
							-0.669 *(41-45)	-0.692 *(41-45)	-0.742 **(41-45)
A.189	-0.764 **(11-15	-0.771 **(11-15	-0.755 **(11-15	-0.712 **(11-15)	-0.699 *(11-15)	-0.725 **(11-15)	-0.657 *(11-15)	-0.663 *(11-15)	-0.688 **(11-15)
				-0.780 **(21-30)	-0.784 **(21-30)	-0.789 **(21-30)	-0.677 *(21-30)	-0.680 *(21-30)	-0.728 **(21-30)
							-0.616 *(41-45)	-0.681 *(41-45)	-0.719 **(41-45)
A.182	-0.752 **(11-15)	-0.772 **(11-15)	-0.754 **(11-15)	-0.701 *(11-15)	-0.717 **(11-15)	-0.738 **(11-15)	-0.675 *(11-15)	-0.684 *(11-15)	-0.714 **(11-15)
				-0.759 **(21-30)	-0.773 **(21-30)	-0.787 **(21-30)	-0.694 *(21-30)	-0.717 **(21-30)	-0.764 **(21-30)
							-0.610 *(41-45)	-0.695 *(41-45)	-0.729 **(41-45)

Figures in paranthesis shows the period in days identified to have influence on disease
*** Significant at 5 % level**
**** Significant at 1 % level**

Table 24. Correlation coefficients between morning relative humidity in identified period and leaf spot disease at different stages of amaranth

Accessions	Morning relative humidity								
	15 DAT			30 DAT			45 DAT		
	DI	DS	CODEX	DI	DS	CODEX	DI	DS	CODEX
A.3	NS	NS	NS	0.809 **(16-20)	0.750 **(16-20)	0.692 *(16-20)	0.843 **(16-20)	0.778 **(16-20)	0.739 **(16-20)
							0.701 *(26-45)	0.648 *(31-45)	0.608 *(31-45)
A.194	0.638 *(11-15)	0.709 **(11-15)	0.598 *(11-15)	0.779 **(6-30)	0.751 **(6-30)	0.655 *(6-30)	0.663 **(6-35)	0.710 **(6-45)	0.634 *(6-45)
							0.710 **(41-45)		
A.225	0.753 **(11-15)	0.772 **(6-15)	0.751 **(6-15)	0.730 **(6-30)	0.762 **(6-30)	0.715 **(6-30)	0.667 *(6-20)	0.731 **(6-45)	0.643 *(6-45)
							0.697 *(26-45)		
A.210	0.699 *(6-15)	0.697 *(6-15)	0.667 *(6-15)	0.779 **(6-30)	0.763 **(6-30)	0.718 **(6-30)	0.679 *(6-20)	0.764 **(6-45)	0.735 **(6-45)
							0.745 **(26-45)		
A.6	0.744 **(6-15)	0.745 **(6-15)	0.735 **(6-15)	0.811 **(6-30)	0.806 **(6-30)	0.771 **(6-30)	0.779 **(6-20)	0.751 **(6-45)	0.717 **(6-45)
							0.748 **(26-45)		
A.191	0.743 **(6-15)	0.743 **(6-15)	0.738 **(6-15)	0.803 **(6-30)	0.806 **(6-30)	0.776 **(6-30)	0.733 **(6-20)	0.759 **(6-45)	0.718 **(6-45)
							0.750 **(26-45)		
A.189	0.700 **(6-15)	0.704 *(6-15)	0.706 *(6-15)	0.799 **(6-30)	0.794 **(6-30)	0.787 **(6-30)	0.778 **(6-15)	0.742 **(6-45)	0.732 **(6-45)
							0.717 **(26-45)		
A.182	0.689 *(6-15)	0.703 *(6-15)	0.704 *(6-15)	0.797 **(6-30)	0.805 **(6-30)	0.804 **(6-30)	0.766 **(6-15)	0.733 **(6-45)	0.730 **(6-45)
							0.717 **(26-45)		

Figures in paranthesis shows the period in days identified to have influence on disease
*** Significant at 5 % level**
**** Significant at 1 % level**

Leafspot disease at 30 days after transplanting was influenced significantly by the morning relative humidity during 6-30 days in all accessions except A.3. Relative humidity during 6-45 days influenced disease severity at 45 days after transplanting in all accessions except A.3.

Disease severity at 15 DAT was significantly correlated with evening relative humidity during 1-15 days in all accessions except A.3 (Table 25). At 30 DAT, disease severity was correlated with the relative humidity during 1-30 days in all accessions except A.3. Relative humidity in the evening during 6-45 days influenced disease severity in A.194, A.225, A.210, A.189 and A182, relative humidity during 1-45 days influenced disease severity in A.6 and A.191 whereas relative humidity alone during 16-45 days influenced disease severity in A-3.

Rainfall during 6-15 days influenced disease severity at 15 days after transplanting, in six accessions except A-3 and A-194 (Table 26). Disease severity at 30 days after transplanting was significantly and positively influenced by the rainfall during 1-30 days in all the accessions. Rainfall during 1-45 days influenced disease severity in A-3, A-210, A-6, A-191, A-189 and A-182 whereas in A-194 and A-225, disease severity was influenced by the rainfall during 1-30 and 41-45 days.

4.3.3. Seasonal influence on yield contributing characters

4.3.3.1. Plant height

The accessions differed significantly for the plant height at 30th day of planting in different months (Table 27). The highest average

Table 25. Correlation coefficients between evening relative humidity in identified period and leaf spot disease at different stages of amaranth

Accessions	Evening relative humidity								
	15 DAT			30 DAT			45 DAT		
	DI	DS	CODEX	DI	DS	CODEX	DI	DS	CODEX
A.3	NS	NS	NS	0.863 **(16-30)	0.628 *(16-30)	0.580 *(16-30)	0.850 **(16-45)	0.762 **(16-45)	0.722 **(16-45)
A.194	0.858 **(6-15)	0.863 **(1-15)	0.763 **(6-15)	0.875 **(1-30)	0.875 **(1-30)	0.772 **(1-30)	0.869 **(1-45)	0.846 **(6-45)	0.725 **(6-45)
A.225	0.842 **(6-15)	0.887 **(1-15)	0.826 **(6-15)	0.897 **(1-30)	0.911 **(1-30)	0.868 **(1-30)	0.818 **(6-45)	0.867 **(6-45)	0.751 **(6-45)
A.210	0.857 **(1-15)	0.865 **(1-15)	0.800 **(1-15)	0.918 **(1-30)	0.900 **(1-30)	0.869 **(1-30)	0.891 **(6-45)	0.904 **(6-45)	0.877 **(6-45)
A.6	0.896 **(1-15)	0.877 **(1-15)	0.843 **(1-15)	0.898 **(1-30)	0.923 **(1-30)	0.879 **(1-30)	0.888 **(1-45)	0.889 **(1-45)	0.852 **(1-45)
A.191	0.856 **(1-15)	0.862 **(1-15)	0.826 **(1-15)	0.913 **(1-30)	0.921 **(1-30)	0.897 **(1-30)	0.907 **(1-45)	0.896 **(1-45)	0.861 **(1-35)
A.189	0.851 **(1-15	0.849 **(1-15)	0.828 **(1-15)	0.918 **(1-30)	0.930 **(1-30)	0.927 **(1-30)	0.872 **(6-45)	0.912 **(6-45)	0.905 **(6-45)
A.182	0.840 **(1-15)	0.853 **(1-15)	0.830 **(1-15)	0.896 **(1-30)	0.906 **(1-30)	0.910 **(1-30)	0.867 **(6-45)	0.914 **(6-45)	0.911 **(6-45)

Figures in paranthesis shows the period in days identified to have influence on disease
*** Significant at 5 % level**
**** Significant at 1 % level**

Table 26. Correlation coefficients between rainfall in identified period and leaf spot disease at different stages of amaranth

Accessions	Rainfall								
	15 DAT			30 DAT			45 DAT		
	DI	DS	CODEX	DI	DS	CODEX	DI	DS	CODEX
A.3	0.892 **(1-10)	0.881 **(1-10)	0.862 **(1-10)	0.886 **(1-30)	0.870 **(1-30)	0.840 **(1-30)	0.781 **(1-45)	0.898 **(1-45)	0.928 **(1-45)
A.194	NS	NS	NS	0.899 **(1-30)	0.936 **(1-30)	0.914 **(1-30)	0.917 **(1-30)	0.896 **(1-30)	0.918 **(1-30)
							0.726 **(41-45)	0.767 *(41-45)	0.825 **(41-45)
A.225	0.639 *(11-15)	0.746 **(6-15)	0.768 **(6-15)	0.884 **(1-30)	0.908 **(1-30)	0.927 **(1-30)	0.872 **(1-30)	0.903 **(1-30)	0.912 **(1-30)
							0.674 *(41-45)	0.678 *(41-45)	0.738 **(41-45)
A.210	0.688 *(11-15)	0.812 **(6-15)	0.878 **(6-15)	0.871 **(1-30)	0.937 **(1-30)	0.893 **(1-30)	0.697 *(1-45)	0.661 *(1-45)	0.766 **(1-45)
A.6	0.716 **(6-15)	0.705 *(6-15)	0.791 **(6-15)	0.880 **(6-30)	0.896 **(1-30)	0.873 **(6-30)	0.638 *(1-45)	0.686 *(1-45)	0.703 *(1-45)
A.191	0.688 *(1-15)	0.627 *(6-15)	0.721 **(6-15)	0.903 **(1-30)	0.891 **(1-30)	0.909 **(1-30)	0.647 *(1-45)	0.710 **(1-45)	0.732 **(1-45)
A.189	0.585 *(6-15)	0.636 *(6-15)	0.655 *(6-15)	0.881 **(1-30)	0.890 **(1-30)	0.909 **(1-30)	0.765 **(6-30)	0.626 *(1-45)	0.634 *(1-45)
A.182	0.600 *(6-10)	0.623 *(6-15)	0.636 *(6-15)	0.858 **(1-30)	0.900 **(1-30)	0.909 **(1-30)	0.784 **(6-30)	0.657 *(1-45)	0.668 *(1-45)

Figures in paranthesis shows the period in days identified to have influence on disease
*** Significant at 5 % level**
**** Significant at 1 % level**

plant height (59.65 cm) was observed in Aug 1997 planting and the lowest in February and March 1998 planting (32.61 cm and 31.38 cm respectively). On the whole A-3 recorded the highest average plant height (68.33cm) followed by A-227 (53.46 cm) and A-204 (52.51 cm). The lowest plant height at 30th day was recorded in A-225 (23.96 cm).

August planting resulted in maximum plant height in all the accessions and it ranged from 34.90 cm (A-225) to 94.8 cm (A-3).

4.3.3.2. Stem girth

The stem girth was maximum in March 1998 planting (7.01 cm) followed by February planting (6.84 cm) and minimum in July 1997 planting (3.87 cm) (Table 28). Among the accessions, A-204 recorded the highest value for stem girth (10.15 cm) followed by A-3 (8.35 cm) and the lowest in A-182 (2.6 cm). Maximum stem girth observed in March crop ranged from 4.30 cm (A-182) to 12.37 cm (A-3).

4.3.3.3. Plant height at flowering

The average plant height at flowering was maximum (103.3 cm) was observed in August 1997 planting and minimum (60.69 cm) in March 1998 planting (Table 29). Among the accessions, A-3 recorded the highest average plant height (118.63 cm) and the lowest by A-225 (50.39 cm). Maximum plant height was observed in all accessions except A-189 in August which ranged from 65.53 cm (A-225) to 159.43 cm (A-3).

4.3.3.4. Branches per plant

Mean number of branches per plant at flowering stage was presented in table 30. Genotype A-227 is a non branching type (0)

Table 27 Seasonal influence on plant height at 30th day in selected amaranth accessions

Accessions	Plant height (cm)												
	May	June	July	August	September	October	November	December	January	February	March	April	Mean
A.227	51.33 bc	42.37 ab	43.97 b	48.37 ab	47.87 b	50.33 b	46.33 b	51.60 b	53.70 b	58.60 b	74.73 b	72.30 b	53.46 b
A.204	57.97 b	40.47 b	42.63 b	45.80 ab	48.73 b	56.57 b	48.97 b	54.00 b	51.50 b	53.83 b	68.53 bc	61.10 c	52.50 b
A.3	69.37 a	52.73 ab	57.60 a	55.93 a	62.23 a	67.80 a	61.83 a	69.20 a	69.30 a	73.20 a	94.80 a	86.00 a	68.33 a
A.194	53.37 bc	28.37 cd	30.37 c	41.40 b	44.43 b	45.70 b	43.43 b	46.60 b	49.67 bc	54.63 b	62.53 cd	57.49 cd	46.50 c
A.225	22.50 f	17.90 d	15.23 d	17.87 d	22.73 c	19.40 c	22.77 c	27.53 c	27.60 e	28.40 c	34.90 e	30.73 e	23.96 f
A.210	31.90 ef	23.13 cd	20.90 cd	28.80 c	22.60 c	27.93 c	26.57 c	33.93 c	33.83 de	31.83 c	40.07 e	36.90 e	29.87 e
A.6	47.47 bcd	32.90 bc	29.20 c	36.80 bc	42.83 b	44.77 b	38.33 b	48.07 b	39.70 cd	49.67 b	56.90 d	52.90 cd	43.29 cd
A.191	48.20 bc	32.27 bc	28.13 c	36.87 bc	42.87 b	44.70 b	46.27 b	48.27 b	48.40 bc	52.43 b	57.33 d	56.43 cd	45.16 c
A.189	36.60 de	27.20 cd	23.00 cd	38.13 bc	44.10 b	46.10 b	41.93 b	47.30 b	38.57 cde	47.23 b	50.80 d	48.40 d	40.78 d
A.182	43.27 cd	28.77 cd	22.80 cd	36.77 bc	37.93 b	44.83 b	48.67 b	50.97 b	47.63 bc	51.80 b	55.90 d	52.50 cd	43.49 cd
Mean	46.20 d	32.61 h	31.38 h	38.67 g	41.63 fg	44.81 def	42.51 ef	47.75 cd	45.96 de	50.16 c	59.65 a	55.48 b	

Table 28 Seasonal influence on stem girth in selected amaranth accessions

Accessions	Stem girth (cm)												
	May	June	July	August	September	October	November	December	January	February	March	April	Mean
A.227	6.80 b	6.10 a	4.97 c	6.13 b	5.67 bc	7.13 b	5.87 c	6.37 b	7.93 b	8.57 b	9.00 b	8.53 b	6.92 c
A.204	10.80 a	7.13 a	9.07 a	7.47 ab	10.37 a	10.30 a	9.73 a	8.17 a	11.67 a	13.20 a	12.17 a	11.73 a	10.15 a
A.3	8.10 b	6.90 a	6.50 b	8.27 a	7.70 b	7.17 b	7.70 b	6.33 b	7.67 b	10.70 b	12.37 a	10.77 ab	8.35 b
A.194	3.30 cd	3.13 b	2.47 ef	2.67 cd	2.47 d	1.60 d	1.87 e	3.27 cd	3.80 c	5.20 c	5.00 c	5.27 c	3.34 de
A.225	2.50 cde	2.33 bc	2.80 def	2.53 cd	2.97 d	4.13 c	3.33 de	3.93 cd	3.37 c	6.00 c	5.70 c	5.37 c	3.75 de
A.210	1.77 de	2.67 bc	1.87 fg	2.97 cd	3.67 cd	4.27 c	3.37 de	3.60 cd	3.63 c	4.97 c	4.10 c	3.80 c	3.39 de
A.6	4.00 c	2.60 bc	3.77 d	4.33 c	3.87 cd	5.03 c	4.67 cd	4.50 c	4.33 c	6.20 c	6.83 bc	5.60 c	4.64 d
A.191	2.70 cde	3.77 b	3.43 de	3.20 cd	3.67 cd	4.53 c	4.43 cd	3.40 cd	3.43 c	4.80 c	5.17 c	5.70 c	4.02 d
A.189	2.40 cde	3.10 b	2.47 ef	2.67 cd	2.33 d	3.00 cd	2.77 de	3.40 cd	3.53 c	4.43 c	5.47 c	5.07 c	3.39 de
A.182	1.17 e	1.33 c	1.33 g	1.50 d	2.33 d	2.87 cd	3.13 de	2.37 d	2.80 c	4.33 c	4.30 c	4.47 c	2.60 e
Mean	4.36 bc	3.91 cd	3.87 d	4.17 c	4.50 bc	5.00 b	4.69 bc	4.53 bc	5.22 b	6.84 a	7.01 a	6.63 ab	

Table 29. Seasonal influence on plant height at flowering in selected amaranth accessions

Accessions	Plant height (cm)												
	May	June	July	August	September	October	November	December	January	February	March	April	Mean
A.227	75.20 bc	79.93 bc	93.13 b	118.60 b	102.27 b	92.13 b	84.80 b	79.90 b	71.97 c	72.70 b	67.97 b	70.60 b	84.10 b
A.204	76.33 bc	82.46 bc	87.00 b	108.43 bc	97.00 b	88.13 b	83.57 b	76.33 b	75.90 bc	67.90 bc	66.23 b	69.33 b	81.55 bc
A.3	114.47 a	124.93 a	125.17 a	159.43 a	142.73 a	121.83 a	114.53 a	111.10 a	115.53 a	99.00 a	93.47 a	101.30 a	118.63 a
A.194	67.47 c	73.53 c	87.93 b	102.00 bcd	94.90 b	85.40 b	84.10 b	80.23 b	71.67 c	67.27 bc	60.43 bc	64.10 b	78.26 cd
A.225	50.93 d	54.07 d	58.03 c	65.53 e	57.47 c	56.90 c	50.10 c	45.90 c	42.37 d	39.40 d	36.23 d	47.73 c	50.39 f
A.210	47.60 d	53.93 d	63.30 c	71.87 e	66.00 c	61.93 c	56.00 c	50.07 c	46.17 d	46.43 d	42.40 d	43.93 c	54.14 e
A.6	68.93 bc	73.43 c	91.07 b	100.93 cd	97.97 b	92.17 b	84.43 b	80.57 b	70.47 c	64.40 bc	57.90 bc	60.83 b	78.59 cd
A.191	78.66 b	84.23 b	93.00 b	109.80 bc	101.13 b	92.07 b	85.50 b	77.87 b	86.27 b	68.03 bc	65.07 bc	71.53 b	84.43 b
A.189	69.32 bc	76.03 bc	85.77 b	90.93 d	92.23 b	81.90 b	87.53 b	77.70 b	65.23 c	59.20 c	54.43 c	62.07 b	75.20 d
A.182	75.07 bc	85.53 b	93.23 b	105.43 bcd	97.77 b	94.53 b	87.27 b	78.93 b	84.00 b	72.30 b	62.80 bc	68.33 b	83.77 b
Mean	72.40 f	78.81 de	87.76 c	103.30 a	94.95 b	86.70 c	81.78 d	75.86 ef	72.96 f	65.66 g	60.69 h	65.98 g	

Table 30. Seasonal influence on branch number at flowering in selected amaranth accessions

Accessions	Branch number												
	May	June	July	August	September	October	November	December	January	February	March	April	Mean
A.227	-	-	-	-	-	-	-	-	-	-	-	-	-
A.204	4.13 g	2.37 e	3.17 c	5.43 d	7.27 f	6.37 d	5.33 d	7.33 f	7.20 e	8.07 c	9.20 g	9.37 d	6.27 f
A.3	7.23 ef	5.13 cd	4.07 c	7.13 cd	8.07 ef	9.27 c	11.23 bc	10.30 e	10.20 d	12.13 b	12.37 f	11.63 c	9.06 e
A.194	6.20 fg	4.17 d	6.13 b	8.13 bc	7.70 f	9.57 c	10.10 c	12.30 cd	11.83 cd	10.07 bc	13.40 ef	11.47 c	9.26 e
A.225	9.27 de	6.20 bc	7.23 b	8.17 bc	9.13 de	11.23 c	10.30 c	11.70 d	12.47 cd	11.43 b	14.70 cde	12.10 c	10.33 d
A.210	12.47 ab	8.07 a	9.23 a	10.13 b	10.17 cd	9.17 c	12.17 b	14.20 b	16.73 b	16.23 a	18.20 ab	16.63 ab	12.78 b
A.6	14.53 a	7.20 ab	9.13 a	13.17 a	16.23 a	18.40 a	12.50 b	18.70 a	19.23 a	16.67 a	20.17 a	18.13 a	15.34 a
A.191	11.97 bc	6.23 bc	7.30 b	9.07 bc	11.57 b	14.13 b	12.30 b	10.43 e	11.57 cd	12.23 b	16.33 bcd	15.47 b	11.55 c
A.189	10.17 cd	6.13 bc	7.27 b	10.30 b	12.17 b	14.23 b	16.27 a	13.27 bc	12.30 cd	10.13 bc	16.53 bc	12.23 c	11.75 c
A.182	9.00 de	5.20 cd	7.23 b	9.33 bc	11.17 bc	10.33 c	12.30 b	11.33 de	12.23 cd	10.43 b	14.27 def	11.40 c	10.35 d
Mean	9.44 g	5.63 I	6.75 h	8.99 g	10.39 f	11.41 e	11.39 e	12.17 cd	12.64 bc	11.93 de	15.02 a	13.16 b	

and in the remaining nine accessions, the maximum number of branches were observed during March planting (15.02) and the minimum during June planting (5.63). The genotype A-6 recorded the highest mean number (15.34) and the lowest by A-204 (6.27). Maximum branches were observed in March crop which ranged from 9.20 (A-204) to 20.17 (A-6).

4.3.3.5 Length of the leaf

All the 10 accessions differed significantly for their leaf length and the maximum overall mean length of leaf was observed in March crop (20.08 cm) and the minimum in June crop (8.83 cm) (Table 31). The genotype A-204 recorded the highest mean value for leaf length (23.90 cm) and the lowest by A-210 (9.55 cm). Leaf length in March crop ranged from 13.37 cm (A-210) to 30.33 cm (A-204).

4.3.3.6 Width of the leaf

On the whole the maximum leaf width (9.99 cm) was observed in March 1998 planting and the minimum (3.95 cm) in June 1997 planting. Similarly, the highest mean leaf width was observed in accessions A-225 (9.10 cm), A-6 (9.28 cm) and A-189 (9.28 cm) and the lowest in A-227 (3.49 cm). Leaf width in March crop ranged from 6.23 cm (A-227) to 13.33 cm (A-6). Accessions A-225, A-191 and A-189 were on par with A-6 for this character in March planting (Table 32).

4.3.3.7 Days to bolt

Among different months the maximum duration for flowering was observed in March planting (65 days) and the shortest was observed in November planting (48 days) (Table 33). Among the

Table 31. Seasonal influence on leaf length in selected amaranth accessions

Accessions	Leaf length (cm)												
	May	June	July	August	September	October	November	December	January	February	March	April	Mean
A.227	16.20 b	10.43 b	12.40 b	14.43 b	18.23 b	16.23 b	12.20 cd	14.47 b	12.50 de	17.30 bc	21.33 b	18.43 bc	15.35 c
A.204	22.30 a	16.27 a	18.37 a	18.33 a	24.60 a	28.37 a	26.63 a	27.43 a	22.27 a	25.40 a	30.33 a	26.50 a	23.90 a
A.3	16.50 b	9.37 bc	11.73 bc	14.50 b	16.43 b	17.67 b	19.43 b	14.43 b	18.23 b	18.40 b	21.53 b	19.37 b	16.47 b
A.194	13.47 bc	7.37 cd	9.40 cd	11.40 cd	11.63 c	10.27 cde	9.53 d	10.43 d	11.40 ef	14.60 cd	18.20 c	15.40 d	11.92 ef
A.225	12.40 c	6.40 cd	7.50 d	9.30 d	11.40 c	7.63 e	10.23 d	9.43 d	11.30 ef	13.47 d	16.37 c	14.40 de	10.82 g
A.210	9.37 d	5.70 d	6.47 d	8.60 d	10.47 c	10.30 cde	9.60 d	8.73 d	9.40 f	10.27 e	13.37 d	12.37 e	9.55 h
A.6	15.37 b	9.37 bc	12.27 b	10.23 cd	12.30 c	11.40 cd	13.53 c	13.30 bc	14.60 cd	17.30 bc	21.43 b	18.70 bc	14.15 d
A.191	14.40 bc	7.53 bcd	8.50 d	10.43 cd	9.60 c	11.60 c	12.27 cd	10.80 cd	14.40 cd	16.73 bc	18.33 c	16.40 cd	12.58 e
A.189	16.40 b	9.40 bc	11.50 bc	12.30 bc	10.40 c	11.33 cd	13.40 c	11.30 cd	15.80 bc	18.30 b	22.30 b	19.33 b	14.31 d
A.182	13.93 bc	6.50 cd	8.40 d	10.40 cd	12.53 c	8.53 de	11.43 cd	9.50 d	12.60 de	14.60 cd	17.63 c	14.47 de	11.71 f
Mean	15.03 d	8.83 I	10.65 h	11.99 g	13.76 ef	13.33 f	13.83 ef	12.98 f	14.25 de	16.64 c	20.08 a	17.54 b	

Table 32. Seasonal influence on leaf width in selected amaranth accessions

Accessions	Leaf width (cm)												
	May	June	July	August	September	October	November	December	January	February	March	April	Mean
A.227	3.40 c	1.53 d	2.13 e	2.27 e	2.50 d	3.50 d	3.23 e	3.23 d	4.40 d	4.30 d	6.23 c	5.20 c	3.49 e
A.204	6.20 b	3.30 c	4.27 cd	4.20 d	5.40 b	5.23 bc	7.20 ab	6.27 b	5.30 cd	7.30 c	8.43 b	7.27 b	5.86 c
A.3	7.30 b	3.43 c	4.33 cd	5.40 cd	5.40 b	4.50 cd	4.23 de	5.53 bc	6.50 c	7.37 c	9.30 b	8.40 b	5.98 c
A.194	6.33 b	3.30 c	3.57 d	4.40 d	3.50 cd	3.20 d	5.23 cd	4.53 cd	5.43 cd	6.43 c	8.20 b	7.17 b	5.11 d
A.225	9.20 a	5.40 ab	6.37 ab	8.50 a	9.20 a	6.60 b	7.20 ab	11.30 a	10.50 a	10.30 ab	12.23 a	12.37 a	9.10 a
A.210	6.37 b	3.20 c	4.40 cd	6.40 bc	5.53 b	4.50 cd	4.37 de	5.30 bc	6.60 c	7.30 c	8.37 b	7.37 b	5.81 c
A.6	10.23 a	5.37 ab	6.50 ab	7.43 ab	8.63 a	8.20 a	8.23 a	10.27 a	9.20 ab	11.50 a	13.33 a	12.43 a	9.28 a
A.191	9.37 a	4.27 bc	5.40 bc	7.50 ab	8.60 a	6.43 b	7.30 ab	10.23 a	8.50 b	9.40 b	12.30 a	11.40 a	8.39 b
A.189	9.30 a	6.30 ab	7.43 a	7.30 ab	9.17 a	8.40 a	7.30 ab	10.60 a	9.40 ab	11.53 a	13.10 a	11.47 a	9.28 a
A.182	6.23 b	3.37 c	4.47 cd	5.23 cd	4.33 bc	5.43 bc	6.40 bc	4.53 cd	5.50 cd	6.60 c	8.37 b	8.47 b	5.74 c
Mean	7.39 d	3.95 h	4.89 g	5.86 ef	6.23 e	5.60 f	6.07 e	7.18 d	7.13 d	8.20 c	9.99 a	9.15 b	

Table 33. Seasonal influence on days to bolt in selected amaranth accessions

Accessions	Days to bolt												
	May	June	July	August	September	October	November	December	January	February	March	April	Mean
A.227	72.00 b	70.00 a	68.00 a	76.00 a	80.00 b	77.00 b	78.00 a	81.00 a	75.00 b	80.00 b	89.00 a	85.00 a	77.58 b
A.204	84.00 a	74.00 a	75.00 a	76.00 a	87.00 a	88.00 a	72.00 b	77.00 a	84.00 a	86.00 a	92.00 a	90.00 a	81.83 a
A.3	53.00 cd	49.00 c	44.00 cd	45.00 d	54.00 c	52.00 c	48.00 cd	49.00 bc	51.00 de	50.00 ef	59.00 de	52.00 de	50.50 e
A.194	55.00 cd	50.67 bc	48.67 bc	55.67 b	51.33 c	50.33 cd	44.33 cde	47.67 bcd	57.67 c	59.67 d	65.67 c	62.00 c	54.06 d
A.225	46.33 ef	46.00 c	42.33 de	44.33 d	42.00 e	43.00 ef	36.33 gh	39.00 ef	43.33 fg	48.00 ef	52.33 fg	50.33 de	44.44 g
A.210	47.00 ef	46.00 c	43.67 cd	46.00 cd	44.33 de	42.00 ef	38.00 fg	43.00 de	47.00 efg	48.00 ef	50.33 g	49.33 de	45.39 g
A.6	58.00 c	56.00 b	51.00 b	52.00 b	50.00 c	52.00 c	49.00 c	52.67 b	59.00 c	67.00 c	72.33 b	69.00 b	57.33 c
A.191	50.00 de	49.00 c	46.00 bcd	45.00 d	44.00 de	46.33 de	40.33 efg	44.00 cde	48.00 ef	53.00 e	56.00 ef	54.00 d	47.97 f
A.189	58.00 c	56.00 b	47.00 bcd	51.00 bc	49.00 cd	46.00 de	43.00 def	48.00 bcd	56.00 cd	59.00 d	64.00 cd	61.67 c	53.22 d
A.182	42.00 f	39.00 d	38.00 e	39.00 e	36.00 f	40.00 f	32.00 h	36.00 f	42.00 g	45.00 f	49.00 g	47.00 e	40.42 h
Mean	56.53 d	53.57 e	50.07 g	53.00 ef	53.77 e	53.67 e	48.10 h	51.73 f	56.30 d	59.57 c	64.97 a	62.03 b	

accessions, maximum duration for flowering was taken by A-204 (82 days) and minimum by A-182 (40 days).

The March crop took more number of days in all the accessions which ranged from 49 (A-182) to 92 (A-204), whereas November planted crops took less number of days 32 (A-182) to 72 days (A-204) to flower.

4.3.3. 8 Leaf stem ratio

The maximum overall mean leaf stem ratio was observed in March 1998 planting (1.99) and the minimum in June and July 1997 planting (1.29) (Table 34). When different accessions were considered averaged over the different dates of planting, the highest mean value for leaf stem ratio was observed in A-210 (2.45) and the lowest in A-204 (1.04) and A-227 (0.98). Maximum leaf stem ratio observed in March crop ranged from 1.13 (A- 227) to 2.9 (A-210)

4.3.3.9 Total vegetable yield

Among different dates of planting, the maximum overall mean yield was observed in March crop (973 g) and minimum in June (318 g) and July (320 g) crops (Table 35). The highest mean yield was recorded in A-227 (837g) and the lowest in A-210 (363 g).

The March crop recorded maximum overall mean yield. The genotype A-3 recorded the highest yield (1355 g) and A-210 recorded the lowest yield (620 g). In June, where the overall mean yield was minimum which varied from 116 g (A-210) to 550 g (A-227).

4.3.4. Correlation studies

Correlation between yield attributes and weather parameters were

Table 34. Seasonal influence on leaf stem ratio in selected amaranth accessions

Accessions	Leaf stem ratio												
	May	June	July	August	September	October	November	December	January	February	March	April	Mean
A.227	0.83 e	0.73 ef	0.80 de	1.07 e	1.03 ef	0.83 d	1.13 cd	0.87 c	1.00 e	1.13 d	1.13 f	1.20 E	0.98 F
A.204	0.93 de	0.57 f	0.67 e	0.93 e	0.80 f	0.93 d	1.00 d	1.23 bc	1.30 cde	1.30 cd	1.57 ef	1.27 de	1.04 f
A.3	1.30 cde	0.93 def	1.00 cde	1.20 de	1.37 de	1.03 cd	1.27 cd	1.27 bc	1.33 cde	1.50 cd	1.77 de	1.60 bcde	1.30 de
A.194	2.00 ab	1.53 bc	1.27 cd	1.70 bcd	1.87 bc	1.43 bc	1.57 bc	1.47 ab	1.80 bc	2.00 b	2.13 bcd	2.03 b	1.73 c
A.225	2.20 ab	1.63 b	1.37 bc	2.07 b	2.10 ab	1.93 b	1.93 ab	1.70 ab	2.10 ab	2.13 ab	2.50 ab	2.10 b	1.98 b
A.210	2.40 ab	2.30 a	2.27 a	2.83 a	2.43 a	2.60 a	2.20 a	1.90 a	2.37 a	2.53 a	2.90 a	2.70 a	2.45 a
A.6	1.77 bc	1.50 bc	1.80 b	1.80 bc	2.10 ab	1.80 b	1.80 ab	1.70 ab	1.67 bcd	2.03 b	2.37 bc	2.10 b	1.87 b
A.191	1.73 bc	1.33 bcd	1.40 bc	1.70 bcd	1.73 bcd	1.80 b	1.90 ab	1.23 bc	1.30 de	1.70 bc	1.90 cde	1.83 bc	1.63 c
A.189	1.30 cde	1.10 cde	1.10 cde	1.43 cde	1.43 cde	1.60 b	1.13 cd	1.27 bc	1.20 cde	1.77 bc	1.87 de	1.73 bcd	1.41 de
A.182	1.37 cd	1.30 bcd	1.20 cd	1.23 de	1.27 def	0.90 d	0.87 d	1.17 bc	1.00 e	1.43 cd	1.73 de	1.50 cde	1.25 e
Mean	1.58 d	1.29 f	1.29 f	1.60 cd	1.61 cd	1.49 de	1.48 de	1.38 ef	1.51 de	1.75 bc	1.99 a	1.81 b	

Table 35 Seasonal influence on yield in selected amaranth accessions

Accessions	Yield (gm/plant)												
	May	June	July	August	September	October	November	December	January	February	March	April	Mean
A.227	750.17 a	550.23 a	615.53 a	710.57 a	925.30 a	670.57 a	810.17 a	750.40 a	850.77 b	975.57 a	1290.40 b	1150.40 b	837.51 a
A.204	620.37 c	471.87 c	415.60 c	610.40 c	515.20 d	488.77 e	587.23 c	680.53 d	730.30 f	860.20 d	1116.37 c	985.20 c	673.51 c
A.3	678.73 b	510.30 b	560.20 b	676.93 b	750.43 b	580.20 c	790.37 b	695.30 c	820.40 c	950.47 b	1355.50 a	1175.37 a	795.36 b
A.194	225.60 h	185.50 h	142.10 i	230.20 i	505.27 d	415.37 f	372.53 g	515.33 g	650.57 h	710.47 f	815.43 h	790.97 f	463.28 g
A.225	368.67 f	238.87 f	262.67 f	370.27 f	472.13 e	508.60 d	472.17 e	645.43 e	760.73 e	810.33 e	918.79 f	860.30 e	557.41 e
A.210	351.80 g	116.87 j	144.37 i	208.60 j	292.03 h	323.67 h	407.07 f	346.20 i	446.93 j	510.57 h	620.88 j	590.90 h	363.32 i
A.6	668.80 b	410.47 d	372.17 d	413.77 d	503.60 d	618.83 b	580.20 c	708.60 b	866.83 a	912.00 c	1030.42 d	979.03 c	672.06 c
A.191	558.70 d	215.43 g	217.27 g	340.33 g	422.30 f	380.27 g	513.67 d	560.43 f	682.10 g	709.53 f	871.90 g	795.57 f	522.29 f
A.189	610.30 c	318.60 e	295.23 e	385.20 e	583.67 c	478.67 e	592.00 c	677.13 d	792.00 d	850.63 d	960.63 e	908.93 d	621.09 d
A.182	477.03 e	169.77 i	177.00 h	275.27 h	355.47 g	278.67 i	317.13 h	375.53 h	473.77 i	590.50 g	750.79 i	677.13 g	409.84 h
Mean	531.02 g	318.79 j	320.23 j	422.15 i	532.54 g	474.36 h	544.25 f	595.49 e	707.44 d	788.03 c	973.11 a	891.40 b	

worked out and presented in table 36.

4.3.4.1 Plant height

The plant height was negatively correlated with maximum temperature during vegetative phase and reproductive phase (correlation coefficients –0.866 and –0.730 respectively) (Table 36). Maximum temperature from sixth day onwards influenced plant height. Minimum temperature neither affected positively or negatively with plant height in the early vegetative stage but at later stage it was negatively correlated with minimum temperature (Correlation coefficient –0.801). Minimum temperature during 21-30 days influenced plant height at flowering. A positive correlation was noticed between relative humidity and rainfall. Correlation coefficient was the lowest (0.597) between plant height at vegetative phase and rainfall.

4.3.4.2. Stem girth

The interaction between weather elements and stem girth showed a significant positive association with maximum and minimum temperature (correlation coefficients more than 0.7) while relative humidity and rainfall had significant negative correlation with correlation coefficients of –0.631 to –0.732 and –0.583 to –0.603 respectively.

4.3.4.3. Leaf characters

Leaf length, width and leaf stem ratio was positively associated with maximum and minimum temperatures (correlation coefficients more than 0.7) whereas negative association was observed between

Table 36. Correlation between yield attributes and weather parameters

Charecters	Maximum temperature	Minimum temperature	Mornig RH	Evening RH	Rainfall
Plant height (30th day)	-0.866 **(6-30)	NS	0.608 *(11-30)	0.769 **(6-30)	0.597 *(26-30)
Plant height at flowering	-0.730 **(6-45)	-0.801 **(21-30)	0.688 *(11-30)	0.651 *(6-45)	0.657 *(11-30)
Stem girth	0.854 **(6-30)	0.721 **(26-30)	-0.631 *(21 - 25)	-0.732 **(6-30)	-0.583 *(6-10) -0.603 *(26-30)
Leaf length	0.869 **(6-30)	0.713 **(26-30)	-0.584 *(21-30)	-0.844 **(6-30)	-0.699 *(1-30)
Leaf width	0.883 **(6-30)	0.740 **(21-30)	-0.640 *(21-30)	-0.886 **(1-30)	-0.729 **(1-30)
Leaf stem ratio	0.867 **(6-45)	0.739 **(41-60)	-0.622* *(21-30)	-0.637 *(11-45)	-0.658 *(41-45)
Branch number at flowering	0.831 **(16-45)	0.858 **(41-45)	-0.690 **(16-35)	-0.887 **(11-45)	-0.737 **(1-45)
Yield	0.881 **(6-60)	0.844 **(21-60)	-0.637 *(11-30)	-0.710 **(6-60)	-0.667 *(1-45)

Figures in parantheses show the period in days identified to have influence on different characters
*** Significant at 5 % level**
**** Significant at 1 % level**

leaf characters and relative humidity and rainfall. Leaf length, leaf width and leaf stem ratio were influenced by maximum temperature from sixth day onwards and morning relative humidity during 21-30 days. Rainfall from first day itself influenced leaf length and leaf width whereas leaf stem ratio was influenced by rain during 41-45 days.

4.3.4.4. Number of branches at flowering

Unlike the plant height, the number of branches at flowering responded positively to maximum and minimum temperature (correlation coefficients 0.831 and 0.858 respectively). There was negative association with relative humidity and rainfall (correlation coefficient -0.690 to -0.887 and -0.737) may be due to the interrelationship between relative humidity, rainfall and maximum temperature.

4.3.4.5. Yield

Vegetable yield was positively associated with maximum and minimum temperatures (correlation coefficients 0.881 and 0.844) and negatively with relative humidity and rainfall. Maximum temperature from sixth day onwards had significant influence on yield whereas minimum temperature from 21st day onwards influenced yield. Results showed that rainfall from the first day itself influenced yield negatively.

4.4. Anatomy of leaves

Leaf sections of the ten accessions showed the following structure (Plate VII). The upper cuticle in the two accessions A-227 and A-204 (green types) was comparatively thicker and the upper

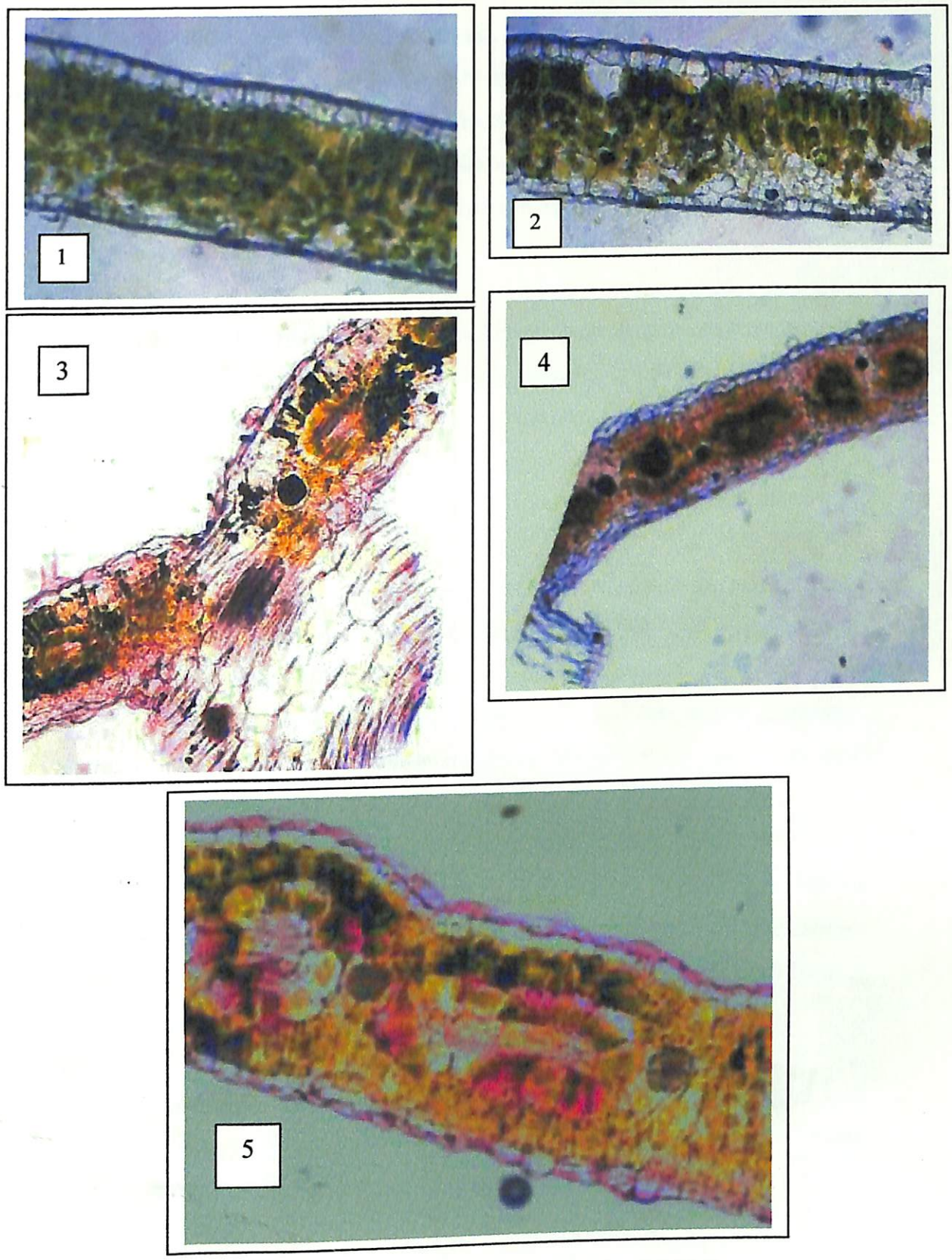


Plate VII. Leaf anatomy of different amaranth accessions :

1. Immune (A-227) ; 2- Resistant (A-3) ; 3- Moderately Susceptible (A-210) ; 4- Moderately resistant (A-194) ; 5- Highly susceptible (A-6).

epidermal cells were closely packed. Palisade layers of two to three were present and the palisade parenchyma contained a high degree of chlorophyll. The spongy layers of parenchyma was traversed at regular intervals by muscilage canals which were comparatively lesser in number. The lower epidermis was made of comparatively smaller epidermal cells.

The genotype A-3 (green type) was essentially like that of A-227 and A-204. In this type, the muscilage canals were distributed just below the upper epidermis in the palisade parenchyma and their size and frequency were higher. This accessions had higher content of chlorophyll in the palisade layers.

In the reddish green genotype, A-194 the upper and lower epidermal cells were more or less of the same size with thin cuticle. The palisade contained chlorophyll at intermittent group of cells and in between palisade cells contained anthocyanin pigment. Muscilage canals were comparatively very small in size and frequency was also very less.

In the two accessions A-210 and A-225 (green types), the upper and lower epidermal cells were smaller in size with trichomes. Muscilage canals were present in the middle of the leaf blade and their frequency was less.

In A-6 (red type) , epidermal cells were larger in size and not compact in arrangement. Muscilage canals were larger and their frequency was high in this genotype.

In the green genotype A-191. the content of chlorophyll was high in palisade layer. Muscilage canals were smaller in size with less frequency.

In the red accessions, viz. A-189 and A-182, the epidermal cells were smaller in size and the palisade and spongy parenchyma cells were loosely arranged. Muscilage canals were located towards the middle of the lamina and the frequency was less.

4.5. Muscilage content in amaranth accessions

Muscilage content in amaranth accessions as estimated by the procedure of Smitha Nandini (1998) revealed no significant difference between them (Table 37).

Table 37. Muscilage content in amaranth accessions

Accessions	Muscilage content (%)
A-227	1.39[a]
A-204	1.41[a]
A-3	1.37[a]
A-194	1.33[a]
A-225	1.35[a]
A-210	1.26[a]
A-6	1.36[a]
A-191	1.28[a]
A-189	1.37[a]
A-182	1.32[a]

4.6 Biochemical bases of leaf spot resistance

This study was carried out to assess the biochemical status of the ten selected amaranth accessions. The stem and leaf samples were analysed during summer and rainy season and the results are given in Table 38-45.

4.6.1. Total phenol

Contents of total phenol varied with plant parts (Table 38 and Fig. 8). Higher phenol content was observed in leaves as compared to stems. Among the accessions, A-227 and A-204 expressed the highest phenol content in both stem (3.538 mg/g and 3.242mg/g) and leaves (4.936 mg/g and 4.98mg/g) during summer and rainy seasons whereas A-182 had the lowest concentration (0.5773mg/g in stem and 0.948 mg/g in leaves). Resistant accessions like A-3 and A-194 had significantly higher content of total phenol compared to susceptible varieties.

Pooled analysis data for two seasons showed higher phenol content in rainy seasons in all the accessions than in summer. Total phenol content in leaves of A-227 increased from 4.193mg/g to 5.678mg/g from summer to rainy season (35.42 % increase) and that of A-182 increased from 0.802 to 1.093mg/g (36.28%). In stems, the increase was 24.82 per cent in A-227 and 61.7 per cent in A-182. Generally accessions which are immune and resistant to leaf spot diseases recorded higher contents of total phenol than susceptible accessions.

4.6.2. O.D. phenol

In general, immune and resistant lines had significantly higher amounts of OD phenol as compared to susceptible accessions in both seasons as well as all the parts tested (Table 39 and Fig. 9). In immune accessions (A-227 and A-204), the OD phenol content in stems was 0.9102 mg/g and 0.8567 mg/g whereas in leaves it was 1.245 and 1.237 mg/g respectively. The resistant genotype, A-3, also recorded significantly higher amount of OD phenol both in

Table 38. Total phenol content (mg/g) in amaranth accessions (stem and leaf)

Sl. No.	Accessions	Disease reaction	Total phenol (stem)			Total phenol (leaf)		
			Summer	Rainy	Pooled mean	Summer	Rainy	Pooled mean
1	A-227	Immune	3.147^{a}	3.928^{a}	3.538^{a}	4.193^{a}	5.678^{a}	4.936^{a}
2	A-204	Immune	2.906^{a}	3.578^{a}	3.242^{a}	4.384^{a}	5.579^{a}	4.981^{a}
3	A-3	Resistant	1.919^{b}	2.381^{b}	2.150^{b}	2.450^{b}	.4.637^{ab}	3.544^{b}
4	A-194	Moderately resistant	1.518^{bc}	1.772^{bc}	1.645^{bc}	1.874^{bc}	3.662^{bc}	2.768^{bc}
5	A-225	Moderately susceptible	1.144^{bcd}	1.573^{bcd}	1.359^{cd}	1.610^{bc}	2.645^{cd}	2.128^{cd}
6	A-210	Moderately susceptible	1.170^{bcd}	1.506^{cd}	1.338^{cd}	1.682^{bc}	2.607^{cd}	2.144^{cd}
7	A-6	Highly susceptible	0.7323^{cd}	0.8247^{d}	0.7785^{de}	0.9293^{bc}	1.655^{d}	1.292^{de}
8	A-191	Highly susceptible	0.6418^{cd}	0.8453^{d}	0.7432^{e}	0.9993^{bc}	1.642^{d}	1.321^{de}
9	A-189	Highly susceptible	0.8033^{cd}	0.8000^{d}	0.8017^{de}	1.010^{bc}	1.564^{d}	1.287^{de}
10	A-182	Highly susceptible	0.4397^{d}	0.7110^{d}	0.5773^{e}	0.8020^{c}	1.093^{d}	0.948^{e}
Pooled mean			1.442	1.792		1.993	3.076	

Table 39. OD phenol content(mg/g) in amaranth accessions (stem and leaf)

Sl. No.	Accessions	Disease reaction	OD phenol (stem)			OD phenol (leaf)		
			Summer	Rainy	Pooled mean	Summer	Rainy	Pooled mean
1	A-227	Immune	1.114^{a}	0.7067^{a}	0.9102^{a}	1.526^{a}	0.9630^{a}	1.245^{a}
2	A-204	Immune	1.094^{a}	0.6193^{a}	0.8567^{ab}	1.447^{a}	1.027^{a}	1.237^{a}
3	A-3	Resistant	0.9633^{a}	0.4753^{ab}	0.7193^{b}	1.163^{a}	0.7440^{ab}	0.9537^{b}
4	A-194	Moderately resistant	0.3083^{b}	0.2933^{bc}	0.3008^{cd}	0.5770^{b}	0.4953^{bc}	0.5362^{c}
5	A-225	Moderately susceptible	0.2107^{b}	0.2073^{c}	0.2090^{cdc}	0.2703^{b}	0.2563^{c}	0.2633^{cd}
6	A-210	Moderately susceptible	0.3340^{b}	0.3242^{bc}	0.3292^{c}	0.4403^{b}	0.4327^{bc}	0.4365^{cd}
7	A-6	Highly susceptible	0.1957^{b}	0.2143^{c}	0.2050^{cde}	0.2843^{b}	0.2940^{c}	0.2892^{cd}
8	A-191	Highly susceptible	0.2010^{b}	0.1947^{c}	0.1978^{cde}	0.2983^{b}	0.2837^{c}	0.2870^{cd}
9	A-189	Highly susceptible	0.0680^{b}	0.0707^{c}	0.0693^{e}	0.1778^{b}	0.1767^{c}	0.1768^{d}
10	A-182	Highly susceptible	0.1163^{b}	0.1240^{c}	0.1202^{de}	0.2133^{b}	0.2080^{c}	0.2107^{d}
Pooled mean			0.461	0.323		0.639	0.488	

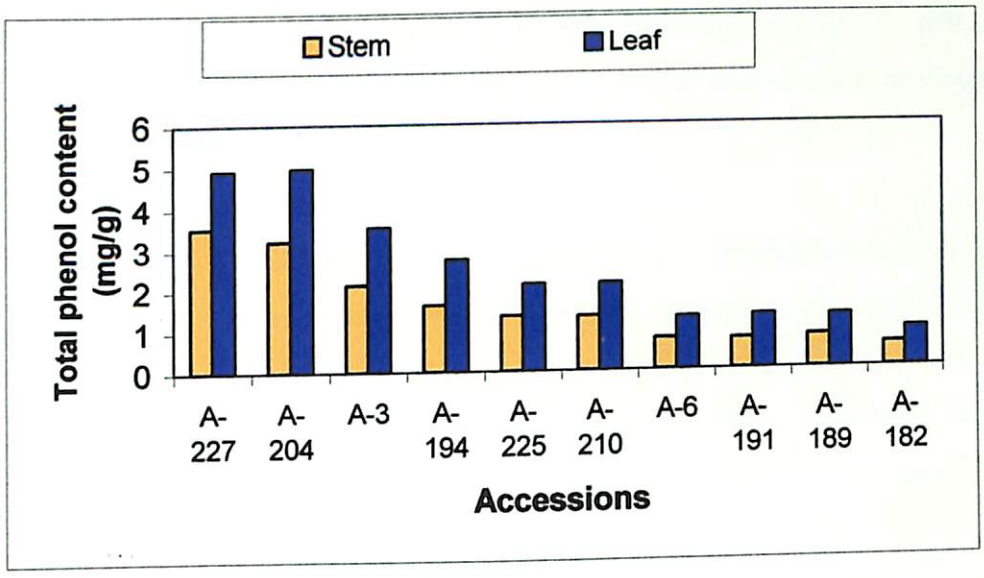


Fig. 8. Total phenol content in amaranth accessions (Leaf and Stem)

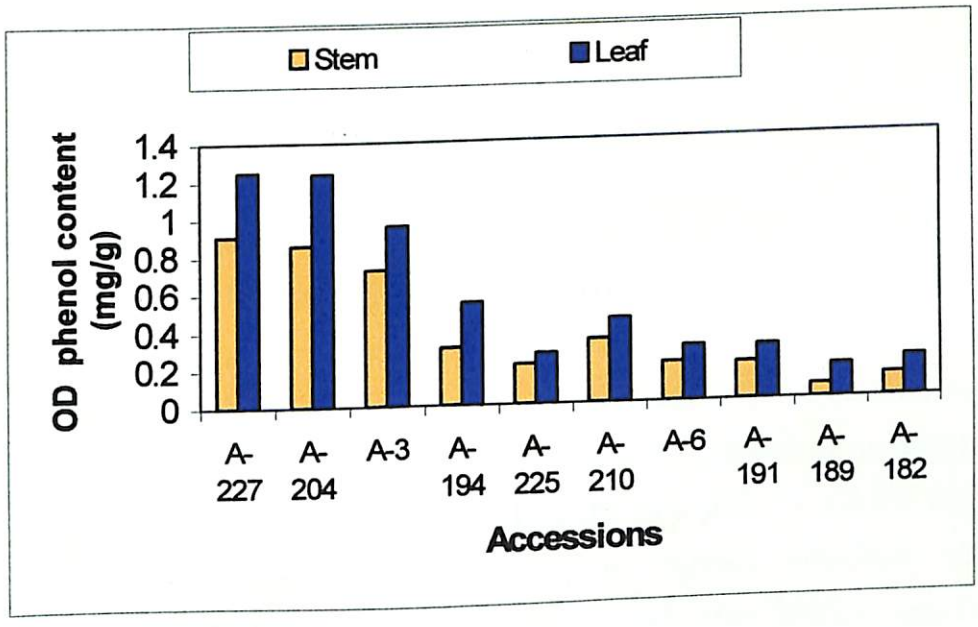


Fig. 9. OD phenol content in amaranth accessions (stem and leaf)

stems (0.7193 mg/g) and in leaves (0.9537 mg/g). A-189 recorded the lowest content of `OD phenol which was 0.693 mg/g in stems and 0.1768 mg/g in leaves.

Significant difference was observed for OD phenol content in stem and leaf during summer and rainy seasons as revealed by the result of the pooled analysis. In rainy season, the OD phenol content in the stem of A-227 decreased from 1.114 to 0.7067 mg/g (36.56% decrease) and in leaves it decreased from 1.526 to 0.963mg/g (36.89 % decrease.

In immune and resistant accessions, OD phenol content decreased significantly in the rainy season, whereas in moderately resistant, moderately susceptible and highly susceptible accessions OD phenol content did not vary significantly after infection.

4.6.3. Ascorbic acid

Ascorbic acid content of the accessions differed significantly in stems and leaves (Table 40). Immune and resistant accessions had higher ascorbic acid content in stems and leaves in both seasons than in susceptible accessions. Generally the ascorbic acid content was low in stem than in leaves. The immune genotype, A-204 recorded the highest ascorbic acid content of 72.94 mg/100g in stems and 104.5 mg/g 100g in leaves whereas the highly susceptible genotype A-182 recorded the lowest ascorbic acid content of 15.55 mg/100g and 37.18 mg/100g in stem and leaf respectively.

Table 40. Ascorbic acid content in amaranth accessions (stem and leaf) (mg/100g)

Sl. No.	Accessions	Disease reaction	Ascorbic acid (stem)			Ascorbic acid (leaf)		
			Summer	Rainy	Pooled mean	Summer	Rainy	Pooled mean
1	A-227	Immune	66.24^{ab}	64.24^{a}	65.24	96.23^{a}	94.48^{ab}	95.36^{ab}
2	A-204	Immune	74.39^{a}	71.50^{a}	72.94	102.4^{a}	106.5^{a}	104.5^{a}
3	A-3	Resistant	63.27^{ab}	62.36^{a}	62.82	92.30^{ab}	91.03^{ab}	91.66^{ab}
4	A-194	Moderately resistant	51.76^{bc}	48.03^{b}	49.89	86.58^{abc}	81.65^{b}	84.11^{b}
5	A-225	Moderately susceptible	28.50^{d}	32.59^{c}	30.55	64.14^{bcd}	58.13^{c}	61.14^{c}
6	A-210	Moderately susceptible	31.68^{cd}	30.51^{cd}	31.10	59.04^{cd}	48.35^{cd}	53.70^{cd}
7	A-6	Highly susceptible	26.38^{d}	24.51^{cde}	25.45	61.14^{bcd}	46.09^{cd}	53.62^{cd}
8	A-191	Highly susceptible	19.40^{d}	19.37^{de}	19.39	44.14^{d}	29.38^{d}	36.76^{d}
9	A-189	Highly susceptible	21.63^{d}	19.63^{de}	20.63	59.35^{cd}	46.30^{cd}	52.83^{cd}
10	A-182	Highly susceptible	16.38^{d}	14.73^{e}	15.55	41.94^{d}	32.42^{d}	37.18^{d}
	Pooled mean		39.96	38.75		70.73	63.44	

Pooled analysis of two season's data of ascorbic acid content in stems revealed absence of interaction, whereas ascorbic acid content of leaves indicated lower values in rainy season in all the accessions than in summer. In A-227, ascorbic acid content of leaves decreased from 96.23 mg/100g in summer to 94.48 mg/100g in rainy season (1.8%) and in highly susceptible genotype A-182 the content decreased from 41.94 mg/100g in summer to 32.42 mg/100g in rainy season (22.70%). The percentage decrease was more in highly susceptible accessions than in resistant types.

4.6.4 Chlorophyll 'a' and ' b'

There was significant difference in Chlorophyll 'a' and ' b' among accessions and among seasons (Table 41 and 42 and Figs. 10 and 11). Generally varieties, viz. A-227, A-204, A-3, A-225, A-210, A-191 recorded higher content of chlorophyll 'a' and ' b' than the varieties like A-194, A-6, A-189 and A-182. Leaf samples of all the accessions recorded more chlorophyll content than stem. The variety A-3 showed maximum chlorophyll 'a' content of 1.204 mg/g and 1.906 mg/g in stems and leaves respectively. The variety A-182 recorded lowest values of 0.265 mg/g and 0.590 mg/g in stems and leaves respectively. Chlorophyll ' b' content was also the highest in A-3 in summer (0.365 mg/g in stem and 0.568 mg/g in leaf) and the least in A-182 (0.163 mg/g in stem and 0.291 mg/g in leaf). Among green types, A-225 and A-210 recorded lower content of chlorophyll 'a' and ' b'.

Pooled analysis data showed a decrease in chlorophyll content in rainy season than in summer. Chlorophyll 'a' content of the variety A-3 decreased from 1.216 to 1.192 mg/g (1.97%) in_stem and in leaves from 1.921 to 1.892 mg/g (1.5%). Chlorophyll 'b" content of the same variety decreased by about 4.11 per cent in stem and

Table 41. Chlorophyll 'a' (mg/g) in amaranth accessions (stem and leaf)

Sl. No.	Accessions	Disease reaction	Chlorophyll 'a' (stem)			Chlorophyll 'a' (leaf)		
			Summer	Rainy	Pooled mean	Summer	Rainy	Pooled mean
1	A-227	Immune	1.09^{a}	1.120^{a}	1.105^{abc}	1.69^{ab}	1.520^{a}	1.605^{ab}
2	A-204	Immune	1.17^{a}	1.080^{a}	1.125^{ab}	1.871^{a}	1.810^{a}	1.840^{a}
3	A-3	Resistant	1.216^{a}	1.192^{a}	1.204^{a}	1.921^{a}	1.892^{a}	1.906^{a}
4	A-194	Moderately resistant	0.432^{bc}	0.392^{bc}	0.4120^{e}	0.984^{bc}	0.6340^{b}	0.8090^{cde}
5	A-225	Moderately susceptible	0.924^{a}	0.862^{ab}	0.8930^{bed}	1.526^{abc}	0.9380^{b}	1.232^{bc}
6	A-210	Moderately susceptible	0.884^{ab}	0.781^{ab}	0.8325^{cd}	1.416^{abc}	0.9260^{b}	1.171^{cd}
7	A-6	Highly susceptible	0.412^{bc}	0.421^{bc}	0.4165^{e}	0.898^{c}	0.6230^{b}	0.7603^{de}
8	A-191	Highly susceptible	0.893^{ab}	0.713^{abc}	0.8030^{d}	1.396^{abc}	0.8360^{b}	1.116^{cd}
9	A-189	Highly susceptible	0.321^{c}	0.298^{c}	0.3095^{e}	0.823^{c}	0.4280^{b}	0.6255^{e}
10	A-182	Highly susceptible	0.284^{c}	0.246^{c}	0.2650^{e}	0.791^{c}	0.3890^{b}	0.5900^{e}
Pooled mean			0.763	0.710		1.332	1.000	

Table 42. Chlorophyll 'b' (mg/g) in amaranth accessions (stem and leaf)

Sl. No.	Accessions	Disease reaction	Chlorophyll 'b' (stem)			Chlorophyll 'b' (leaf)		
			Summer	Rainy	Pooled mean	Summer	Rainy	Pooled mean
1	A-227	Immune	0.326^{ab}	0.3180^{ab}	0.3220^{a}	0.560^{a}	0.528^{ab}	0.5440^{a}
2	A-204	Immune	0..281^{abc}	0.2860^{abc}	0.2835^{abc}	0.431^{ab}	0.448^{abc}	0.4395^{ab}
3	A-3	Resistant	0.365^{a}	0.3497^{a}	0.3573^{a}	0.568^{a}	0.546^{a}	0.5570^{a}
4	a-194	Moderately resistant	0.212^{bc}	0.1950^{bc}	0.2035^{cd}	0.308^{b}	0.258^{cd}	0.2830^{cd}
5	A-225	Moderately susceptible	0.216^{bc}	0.2110^{abc}	0.2135^{bcd}	0.438^{ab}	0.326^{abcd}	0.3820^{bc}
6	A-210	Moderately susceptible	0.208^{bc}	0.1980^{bc}	0.2030^{cd}	0.426^{ab}	0.382^{abcd}	0.4040^{bc}
7	A-6	Highly susceptible	0.196^{bc}	0.1860^{bc}	0.1910^{cd}	0.309^{b}	0.261^{cd}	0.2850^{cd}
8	A-191	Highly susceptible	0.318^{ab}	0.2920^{abc}	0.3050^{ab}	0.595^{a}	0.312^{bcd}	0.4535^{ab}
9	A-189	Highly susceptible	0.187^{bc}	0.1860^{bc}	0.1865^{cd}	0.319^{b}	0.251^{cd}	0.2850^{cd}
10	A-182	Highly susceptible	0.163^{c}	0.1480^{c}	0.1555^{d}	0.291^{b}	0.198^{d}	0.2445^{d}
Pooled mean			0.247	0.237		0.425	0.351	

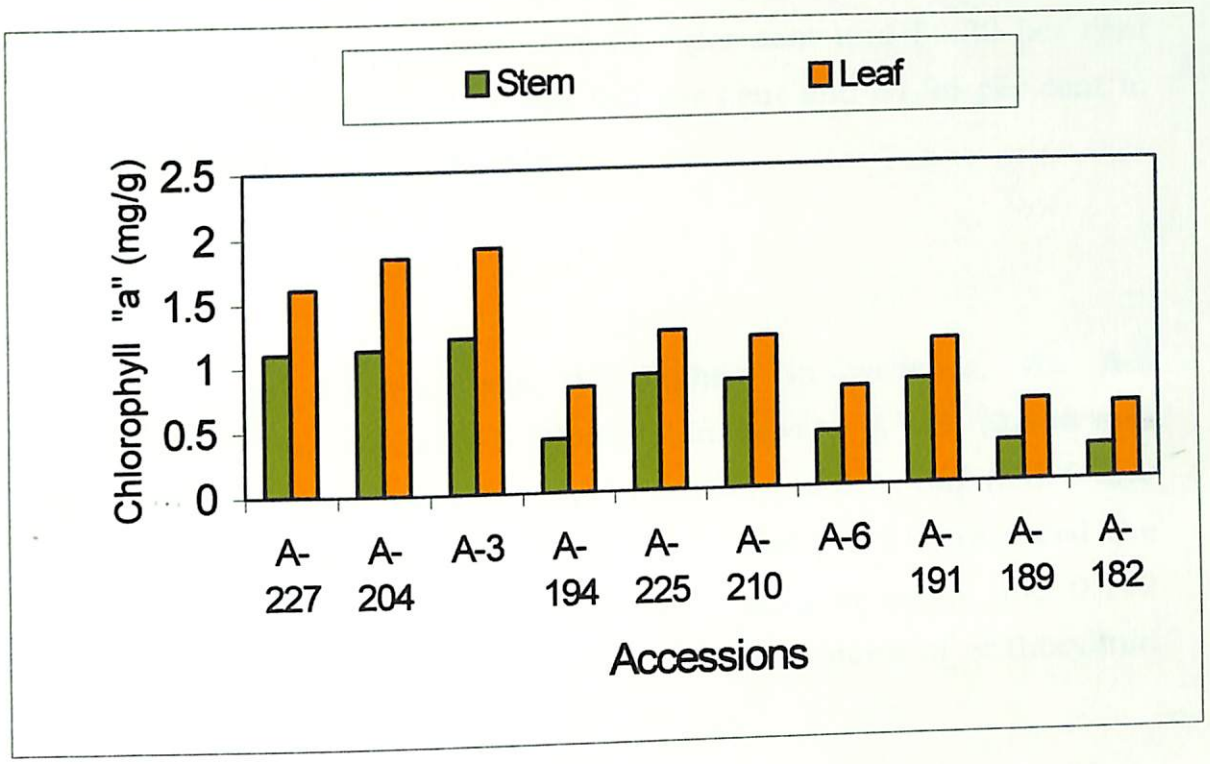


Fig. 10. Chlorophyll 'a" content in amaranth accessions (Stem and leaf)

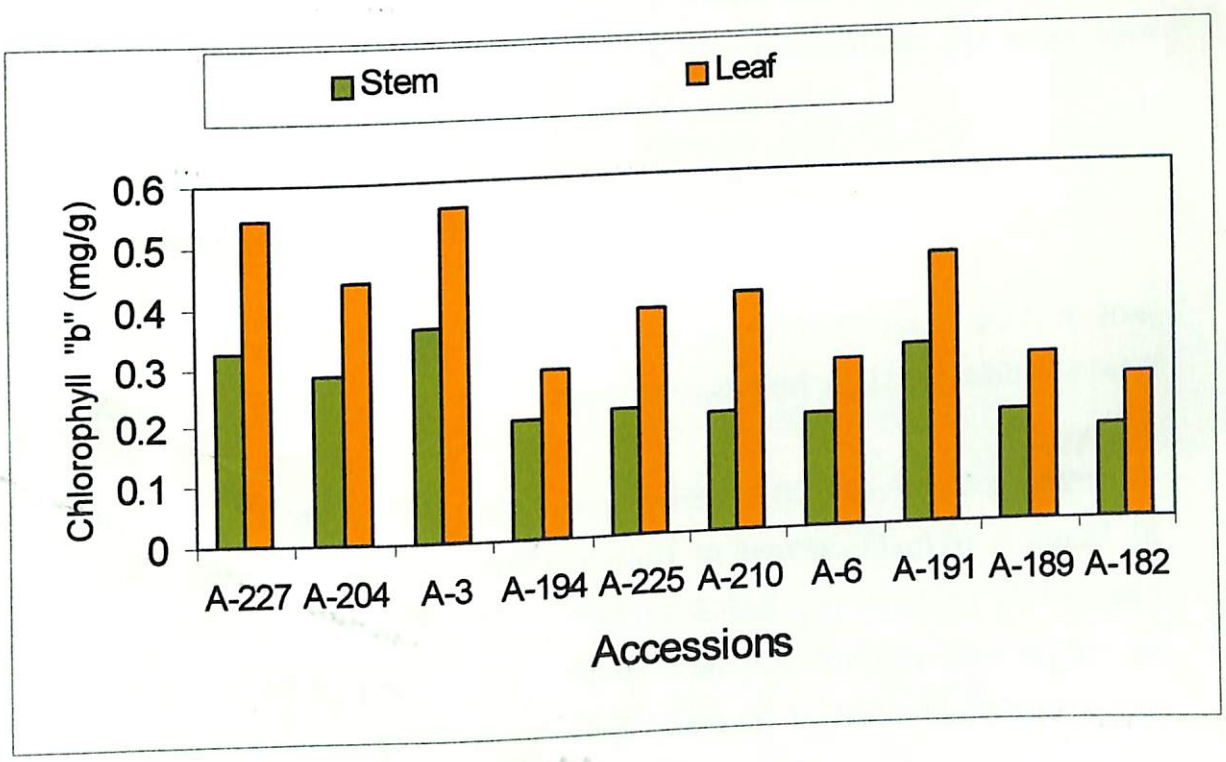


Fig. 11. Chlorophyll "b" content in amaranth accessions (Stem and leaf)

3.87 per cent in leaves. In the variety A-182, the percentage decrease of chlorophyll 'a' was 13.3 per cent and 50.82 per cent and that of chlorophyll 'b' was 9.2 per cent and 31.96 per cent in stems and leaves respectively.

4.6.5 Anthocyanins

Anthocyanin content was the highest in varieties, viz. A-6 (0.34mg/g in stem and 0.402mg/g in leaves), A-189 (0.356 and 0.474mg/g in stem and leaves respectively) and A- 182 (0.309 and 0.351 mg/g in stem and leaves respectively) A-210 recorded the lowest content of anthocyanin, 0.115 mg/g in stems and 0.142 mg/g in leaves. In general leaves contained more of anthocyanin than in stems (Table 43).

Anthocyanin content was high in summer season in all the accessions. In A-189, the percentage decrease in rainy season was 18.11 and 17.5 whereas in A-210, the percentage decrease was 4.23 and 5.48 in stems and leaves respectively.

4.6.6 Oxalates and Nitrates

Accumulation of oxalates was higher in varieties viz. A-227, A-204, A-194, A-189, A-182, and lower in A-3 and A-210 (Table 44 and 45)..

In summer oxalate content ranged from 1.628 to 3.823 per cent in stems and 1.208 to 3.910 per cent in leaves. Oxalate content in rainy season ranged from 1.098 to 3.318 per cent in stems and 0.980 to 3.131 per cent in leaves. Oxalate content was higher in summer season in all the accessions and in both the plant parts studied than in rainy season.

Table 43. Anthocyanin content (mg/g) in amaranth accessions (stem and leaf)

Sl. No.	Accessions	Disease reaction	Anthocyanin (stem)			Anthocyanin (leaf)		
			Summer	Rainy	Pooled mean	Summer	Rainy	Pooled mean
1	A-227	Immune	0.142^{c}	0.1360^{b}	0.1390^{bcd}	0.1720^{c}	0.1680^{c}	0.1700^{def}
2	A-204	Immune	0.192^{c}	0.185^{b}	0.1885^{bc}	0.2210^{c}	0.2280^{c}	0.2245^{cde}
3	A-3	Resistant	0.209^{c}	0.188^{b}	0.1985^{b}	0.2420^{bc}	0.2380^{bc}	0.2400^{cd}
4	A-194	Moderately resistant	0.228^{bc}	0.193^{b}	0.2105^{b}	0.3310^{b}	0.2160^{c}	0.2735^{c}
5	A-225	Moderately susceptible	0.149^{c}	0.135^{b}	0.1420^{bcd}	0.1860^{c}	0.1690^{c}	0.1775^{def}
6	A-210	Moderately susceptible	0.118^{c}	0.113^{b}	0.1155^{d}	0.1460^{c}	0.1380^{c}	0.1420^{f}
7	A-6	Highly susceptible	0.362^{a}	0.319^{a}	0.3405^{a}	0.4690^{a}	0.3360^{ab}	0.4025^{b}
8	A-191	Highly susceptible	0.121^{c}	0.119^{b}	0.1200^{cd}	0.1550^{c}	0.1460^{c}	0.1505^{ef}
9	A-189	Highly susceptible	0.392^{a}	0.321^{a}	0.3565^{a}	0.5200^{a}	0.4290^{a}	0.4745^{a}
10	A-182	Highly susceptible	0.328^{ab}	0.291^{a}	0.3095^{a}	0.4750^{a}	0.2280^{c}	0.3515^{b}
Pooled mean			0.224	0.20		0.292	0.230	

Table 44. Oxalate content (%) in amaranth accessions (stem and leaf)

Sl. No.	Accessions	Disease reaction	Oxalate (stem)			Oxalate (leaf)		
			Summer	Rainy	Pooled mean	Summer	Rainy	Pooled mean
1	A-227	Immune	3.121^{ab}	2.813^{ab}	2.967^{a}	3.300^{ab}	2.810^{ab}	3.055^{ab}
2	A-204	Immune	3.316^{ab}	2.864^{ab}	3.098^{a}	3.123^{ab}	2.921^{a}	3.022^{ab}
3	A-3	Resistant	1.823^{bc}	1.612^{cd}	1.717^{c}	1.916^{bc}	1.413^{bc}	1.664^{de}
4	A-194	Moderately resistant	3.410^{a}	2.583^{abc}	2.997^{a}	3.202^{ab}	2.612^{ab}	2.907^{abc}
5	A-225	Moderately susceptible	2.421^{abc}	1.768^{bcd}	2.095^{bc}	2.104^{abc}	1.812^{abc}	1.958^{cde}
6	A-210	Moderately susceptible	1.628^{c}	1.098^{d}	1.363^{c}	1.208^{c}	0.980^{c}	1.094^{e}
7	A-6	Highly susceptible	3.823^{a}	3.318^{a}	3.570^{a}	3.910^{a}	3.131^{a}	3.520^{a}
8	A-191	Highly susceptible	2.913^{abc}	2.623^{abc}	2.768^{ab}	2.818^{ab}	2.121^{abc}	2.470^{bcd}
9	A-189	Highly susceptible	3.418^{a}	2.714^{abc}	3.066^{a}	3.612^{ab}	2.812^{ab}	3.212^{ab}
10	A-182	Highly susceptible	3.312^{ab}	2.723^{abc}	3.017^{a}	3.131^{ab}	2.630^{ab}	2.881^{abc}
Pooled mean			2.919	2.412		2.832	2.324	

Nitrate accumulation in stem was the highest in A-204 (2.36%) and in leaves, A-227 recorded the highest value of 2.43 per cent. Genotype A-210 recorded the lowest nitrate content of 1.102 per cent in stems and 0.945 per cent in leaves (Table 45).

Nitrate content was lower in rainy season in all the accessions and in the plant parts studied. Nitrate content in A-204 decreased from 2.747 to 2.312 per cent and from 2.236 to 2.142 per cent in stems and leaves respectively. Nitrate content was the lowest in A-210 and its value decreased from 0.6473 to 0.5243 per cent in stems and 0.789 to 0.368 per cent in leaves.

4.6.7 Correlation studies between biochemical contents of leaf and disease

Correlation studies between various biochemical contents of leaf, viz. total phenol, OD phenol, ascorbic acid, anthocyanin, chlorophyll 'a', chlorophyll 'b', oxalate and nitrate contents and leaf spot disease were conducted in rainy season (July) at 30 days after planting for all the eight accessions except two immune accessions A- 227 and A-204 (Table 46). High negative correlation was observed between total phenol, OD phenol, ascorbic acid, chlorophyll 'a' and chlorophyll 'b' and disease incidence, disease severity and CODEX. Correlation coefficients were maximum with total phenol and disease incidence and disease severity (-0.866 and –0.853 respectively) and minimum with chlorophyll 'b' and disease incidence, disease severity and CODEX (-0.610, -0.611 and –0.551 respectively). Anthocyanin pigment, oxalate and nitrate content had no significant correlation with disease incidence, disease severity and CODEX

Table 45. Nitrate content (%) in amaranth accessions (stem and leaf)

Sl. No.	Accessions	Disease reaction	Nitrate (stem)			Nitrate (leaf)		
			Summer	Rainy	Pooled mean	Summer	Rainy	Pooled mean
1	A-227	Immune	2.212^{a}	2.011^{ab}	2.112^{abc}	2.631^{a}	2.234^{a}	2.432^{a}
2	A-204	Immune	2.747^{a}	2.312^{a}	2.363^{a}	2.236^{abc}	2.142^{a}	2.189^{ab}
3	A-3	Resistant	1.621^{abc}	1.314^{bc}	1.567^{cd}	1.161^{bcd}	1.181^{abc}	1.371^{bc}
4	A-194	Moderately resistant	1.731^{abc}	1.512^{ab}	1.622^{bcd}	1.812^{abcd}	1.313^{abc}	1.563^{bc}
5	A-225	Moderately susceptible	0.8120^{bc}	0.5163^{c}	1.498^{cd}	0.9120^{cd}	0.2897^{c}	1.418^{bc}
6	A-210	Moderately susceptible	0.6473^{c}	0.5243^{c}	1.102^{d}	0.7897^{d}	0.3680^{bc}	0.9455^{c}
7	A-6	Highly susceptible	1.916^{ab}	1.123^{bc}	1.520^{cd}	1.861^{abcd}	1.348^{abc}	1.605^{abc}
8	A-191	Highly susceptible	1.812^{abc}	1,614^{ab}	1.713^{abcd}	2.132^{abcd}	1.913^{a}	2.023^{ab}
9	A-189	Highly susceptible	2.310^{a}	1.921^{ab}	2.114^{abc}	2.210^{abc}	1.728^{ab}	1.969^{ab}
10	A-182	Highly susceptible	2.621^{a}	1.918^{ab}	2.270^{ab}	2.456^{ab}	1.819^{a}	2.138^{ab}
Pooled mean			1.843	1.477		1.820	1.434	

Table 46. Correlation coefficients between biochemical contents of leaf and disease incidence, disease severity and CODEX

	Biochemical contents in leaf							
	Total phenol	OD phenol	Ascorbic acid	Anthocyanin	Chlorophyll 'a'	Chlorophyll 'b'	Oxalate	Nitrate
Disease incidence	-0.866 **	-0.817 **	-0.832 **	0.347	-0.713 **	0.610 **	0.043	0.008
Disease severity	-0.853 **	-0.769 **	-0.799 **	0.406	-0.704 **	- ** 0.611	0.134	0.100
CODEX	-0.761 **	-0.670 **	-0.708 **	0.436	-0.634 **	- ** 0.551	0.181	0.217

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

.4.7 Isozyme analysis

Ten selected accessions of amaranth which were grown round the year at monthly intervals for field screening against leaf spot disease were analysed for variation in enzyme pattern for polyphenol oxidase and peroxidase. Isozyme analysis was done twice – one during summer when there was no disease incidence (March 98) and second during rainy season when there was heavy incidence of leaf spot (July 97).

Leaf samples of the accessions at seasonal harvest stage were taken for this study. The banding pattern had variation with respect to the accessions.

4.7.1 Polyphenol oxidase (PPO)

PPO zymograms of ten accessions grown during rainy season are presented in figures 12, plate VIII and table 47. Six bands were resolved in rainy season crop. Immune accessions expressed more number of bands than the susceptible accessions. The protein band PPO-5 (Rm - .750) was present in all the ten accessions and was thick in the case of immune (A-227 and A-204), moderately susceptible (A-225, A-210) and susceptible varieties (A-6, A-191, A-189 and A-182). However, in varieties like A-3 and A-194 this band was very feeble.

The protein bands like PPO-1 and PPO-3 with Rm values 0 .120 and 0 .210 were found mainly in immune accessions. PPO-1 band was very thick and PPO-3 was very feeble in immune accessions. PPO-4 ((Rm- 0.490) was present as feeble band only in resistant and moderately resistant accessions. PPO-2 (Rm- 0.170) was common in resistant, moderately resistant and moderately

susceptible accessions. PPO-6 (Rm- 0.850) was present in immune, moderately susceptible and highly susceptible accessions.

PPO zymograms of the same ten accessions during summer season are presented in figure 13, plate VIII and Table 48. No disease incidence was noticed during this period of analysis. Only 5 bands were resolved in summer season crops. The protein band PPO-3 (Rm- 0.210) which was present in the two immune accessions in rainy season crop had disappeared in summer crop. All the ten accessions expressed the protein band PPO-5 and the same band was thick in eight accessions except A-3 and A-194. PPO-1 (Rm-0.120) was present mainly in immune accessions (A-217 and A-204) whereas PPO-2 was present in four accessions coming in the categories of resistant (A-3), moderately resistant (A-194) and moderately susceptible (A-225) accessions. PPO-6 (rm- 0.850) was present in six accessions, viz. A-227, A-204, A-225, A-210, A-6 and A-189.

4.7.2. Peroxidase

In leaf samples of accessions, a total of five different peroxidase bands were obtained both at rainy and summer seasons. Leaf samples taken from the rainy season crop had four bands with Rm values of 0.114, 0.143, 0.671 and 0.771. The band PRX-3 (Rm-0.671) was found in all accessions and was darker in four accessions (Fig. 14 ; Plate IX ; Table 49). PRX-1 (Rm- 0.114) was found in immune, moderately resistant, moderately susceptible and highly susceptible accessions. PRX-2 (Rm-0.143) was found mainly in immune and resistant accessions. PRX5 (Rm- 0.771) was expressed in almost all categories of accessions.

Fig. 12. Zymogram of polyphenoloxidase (PPO) amranth leaves in rainy season

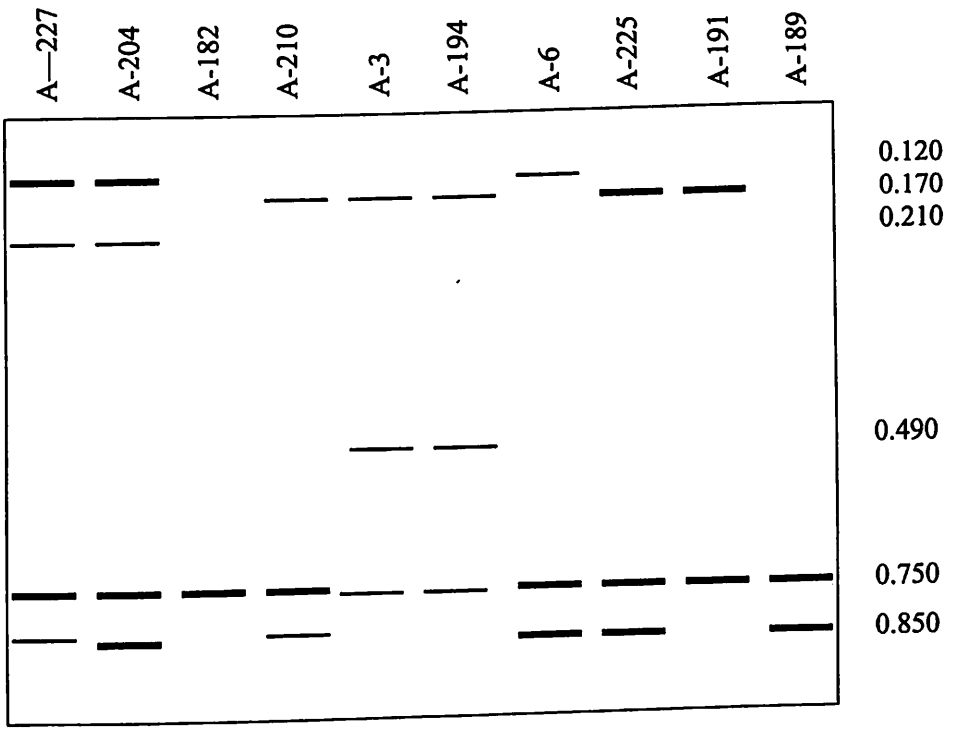
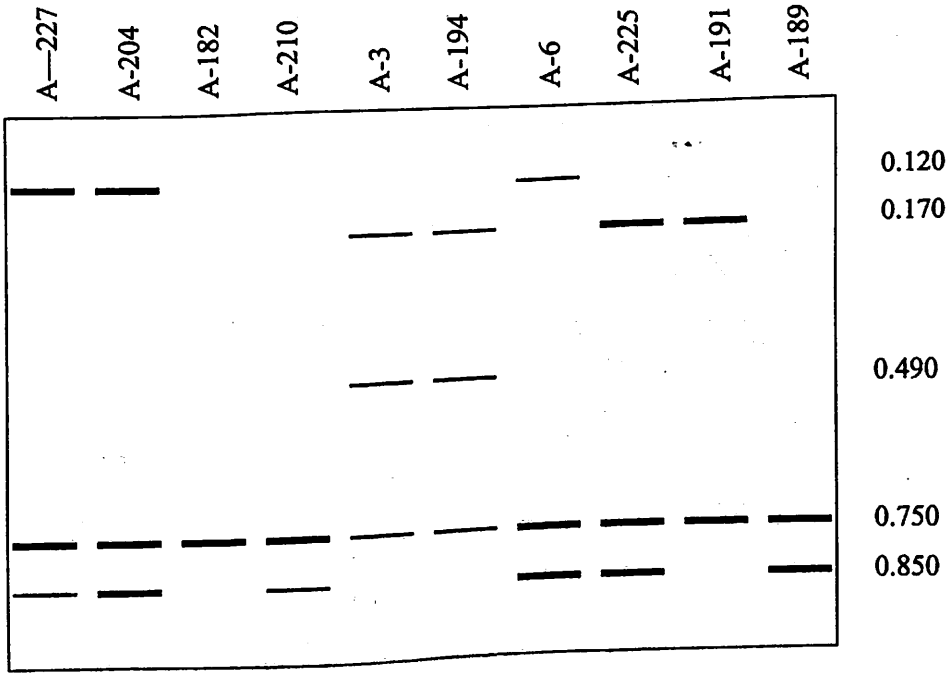


Fig. 13. Zymogram of polyphenoloxidase (PPO) in amaranth leaves in summer



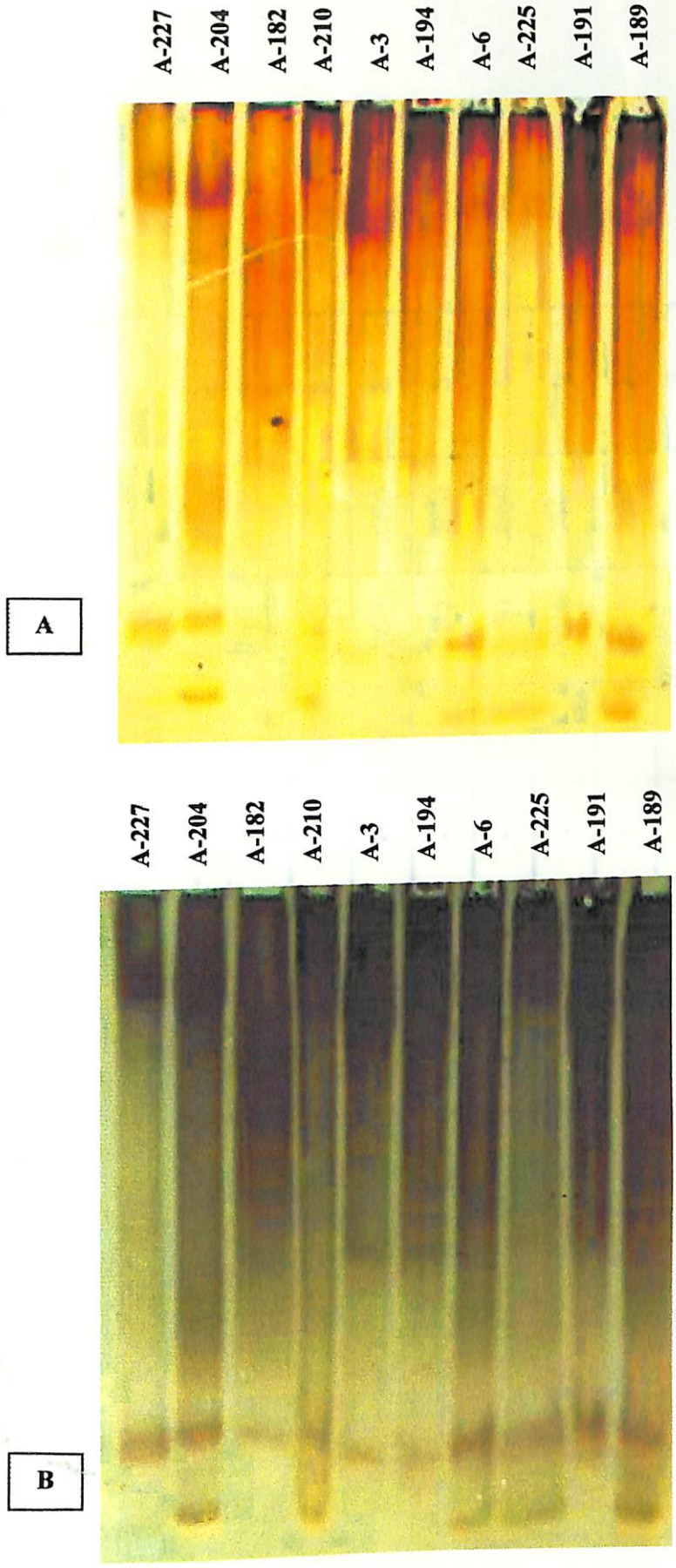


Plate VIII. Isoenzyme banding pattern in amaranth leaves in different seasons – Polyphenoloxidase (PPO) ; A- Summer ; B – Rainy season

Table 47 Rm value of different bands of polyphenol oxidase in amaranth leaves in rainy season

Accessions	Disease reaction	Rm						Total No. of bands
		Banding from origin						
		PPO1	PPO2	PPO3	PPO4	PPO5	PPO6	
A-227	Immune	.120	-	.210	-	.750	.850	4
A-204	Immune	.120	-	.210	-	.750	.850	4
A-3	Resistant	-	.170	-	.490	.750	-	3
A-194	Moderately resistant		.170	-	.490	.750	-	3
A-225	Moderately susceptible	-	.170	-	-	.750	.850	3
A-210	Moderately susceptible	-	.170	-	-	.750	.850	3
A-6	Highly susceptible	.120	-	-	-	.750	.850	3
A-191	Highly susceptible	-	.170	-	-	.750	-	2
A-189	Highly susceptible	-	-	-	-	.750	.850	2
A-182	Highly susceptibl	-	-	-	-	.750	-	1

Table 48 Rm value of different bands of polyphenol oxidase in amaranth leaves in summer

Accessions	Disease reaction	Rm						Total No. of bands
		Banding from origin						
		PPO1	PPO2	PPO3	PPO4	PPO5	PPO6	
A-227	Immune	.120	-	-	-	.750	.850	3
A-204	Immune	.120	-	-	-	.750	.850	3
A-3	Resistant	-	.170	-	.490	.750	-	3
A-194	Moderately resistant		.170	-	.490	.750	-	3
A-225	Moderately susceptible	-	.170	-	-	.750	.850	3
A-210	Moderately susceptible	-	-	-	-	.750	.850	2
A-6	Highly susceptible	.120	-	-	-	.750	.850	3
A-191	Highly susceptible	-	.170	-	-	.750	-	2
A-189	Highly susceptible	-	-	-	-	.750	.850	2
A-182	Highly susceptibl	-	-	-	-	.750	-	1

Fig.14. Zymogram of peroxidase (PRX) in amaranth leaves in rainy season

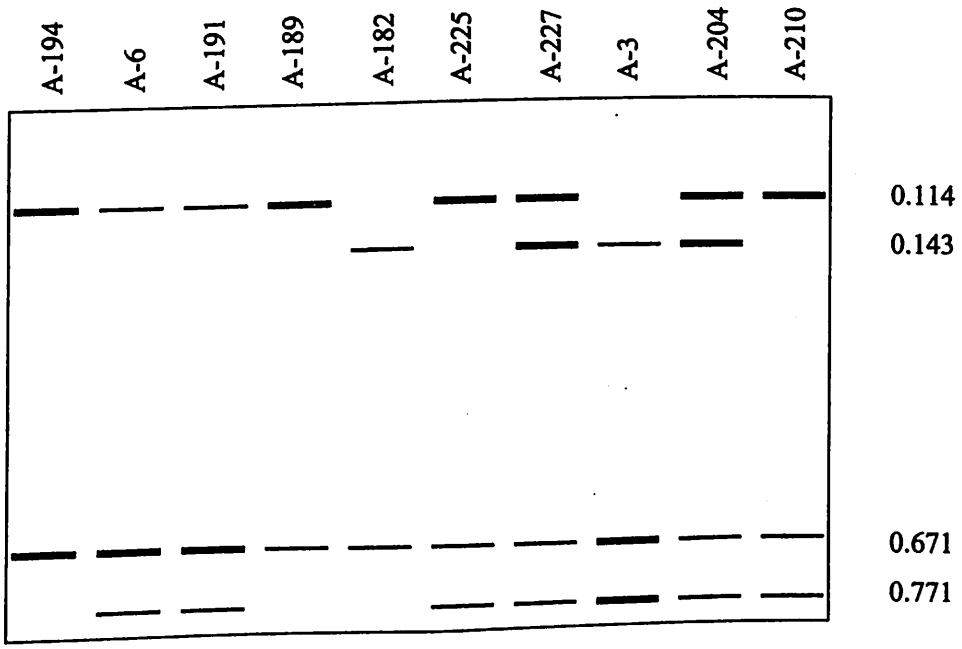
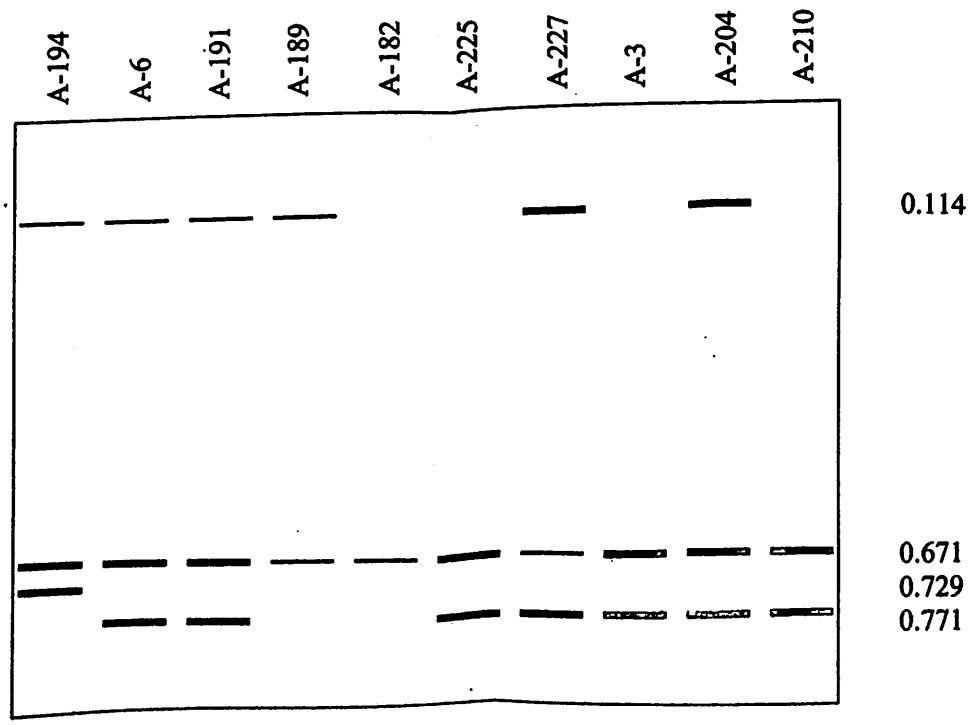
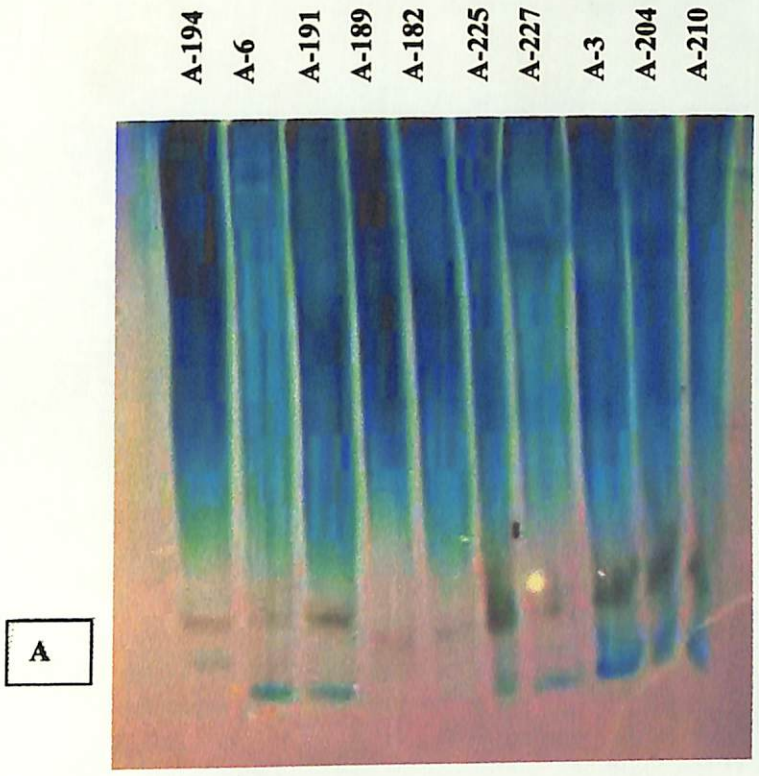


Fig.15. Zymogram of peroxidase (PRX) in amaranth leaves in summer season





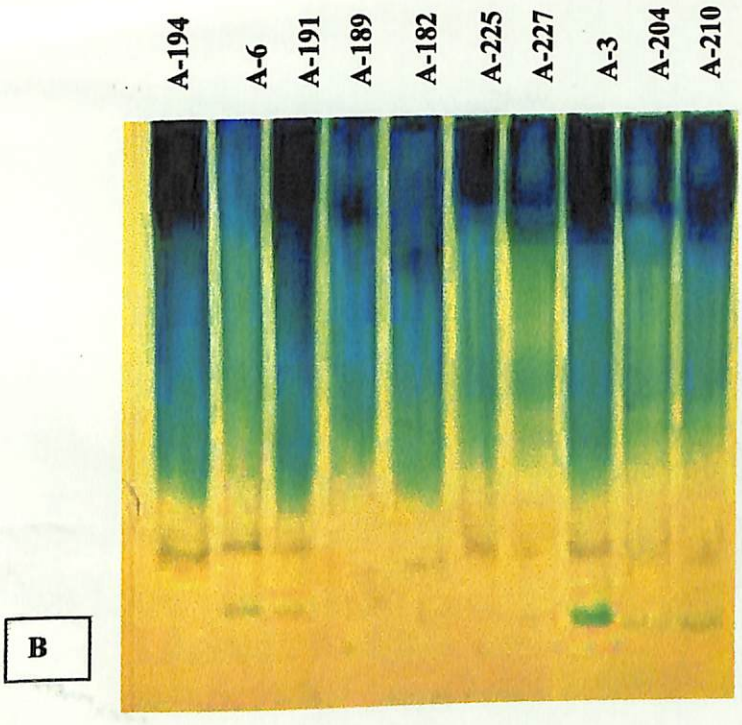


Plate IX. Isozyme banding pattern in amaranth leaves – Peroxidase (PRX) – A- Summer ; B- Rainy season

Table 49. Rm value of different bands of peroxidase (PRX) in amaranth leaves in rainy season

Accession	Disease reaction	Rm					Total No. of bands
		Banding from origin					
		PRX1	PRX2	PRX3	PRX4	PRX5	
A-227	Immune	.114	.143	.671	-	.771	4
A-204	Immune	.114	.143	.671		.771	4
A-3	Resistant	-	.143	.671	-	.771	3
A-194	Moderately resistant	.114		.671	-	-	2
A-225	Moderately susceptible	.114	-	.671	-	.771	3
A-210	Moderately susceptible	.114	-	.671		.771	3
A-6	Highly susceptible	.114	-	.671	-	.771	3
A-191	Highly susceptible	.114	-	.671	-	.771	3
A-189	Highly susceptible	.114	-	.671	-	-	2
A-182	Highly susceptible	-	.143	.671	-	-	2

Table 50. Rm value of different bands of peroxidase (PRX) in amaranth leaves in summer

Accessions	Disease reaction	Rm					Total No. of bands
		Banding from origin					
		PRX1	PRX2	PRX3	PRX4	PRX5	
A-227	Immune	.114	-	.671	-	.771	3
A-204	Immune	.114	-	.671		.771	3
A-3	Resistant	-		.671	-	.771	2
A-194	Moderately resistant	.114		.671	.729	-	3
A-225	Moderately susceptible	-	-	.671	-	.771	2
A-210	Moderately susceptible	-	-	.671	-	.771	2
A-6	Highly susceptible	.114	-	.671	-	.771	3
A-191	Highly susceptible	.114	-	.671	-	.771	3
A-189	Highly susceptible	.114	-	.671	-	-	2
A-182	Highly susceptible	-	-	.671	-	-	1

Leaf samples taken in summer season had expressed four bands with Rm values of 0.114, 0.671, 0.729 and 0.771. PRX-3 (Rm-0.671) was found in all accessions and was darker in seven accessions (Figure 15; Plate IX ; Table 50). PRX-1 (Rm-0.114) was expressed in six accessions only and absent in moderately susceptible accessions. PRX-2 (Rm- 0.143) was also absent in all the accessions grown during summer season whereas one additional band PRX-4 (Rm-0.729) appeared in one genotype (A-194), PRX-5 (Rm-0.771) was present in all categories of accessions.

The number of peroxidase bands were found to increase from highly susceptible accessions to immune accessions in both the seasons. In each genotype more number of bands were observed during rainy season as compared to summer season (Table 51).

Table 51. Comparison of polyphenol oxidase and peroxidase bands in amaranth accessions in two seasons

Enzyme	Number of bands in different amaranth accessions					
	Season	Immune	Resistant	Moderately resistant	Moderately susceptible	Highly susceptible
Polyphenol oxidase	Rainy	4	3	3	3	1-3
	Summer	3	3	3	2-3	1-3
Peroxidase	Rainy	4	3	2	3	2-3
	Summer	3	2	3	2	1-3

Discussion

5. DISCUSSION

Amaranth is an important vegetable cum grain crop grown for its high nutrient plant part. Among the various diseases affecting the crop, leaf spot disease was first observed in India during 1966. But it has become a serious problem in recent years in amaranth growing tracts of Kerala, causing great damage to the crop and thereby reducing yield and quality to a considerable extent.

The conventional plant protection measures like chemical means of control of the disease is inefficient and undesirable due to the residual hazards on health and environment. So the most effective and the cheapest alternative to combat the disease is the use of resistant varieties. Hence an investigation was carried out to study various aspects of leaf spot diseases of amaranth focussing on the source of resistance, etiology, pathogenicity, symptomatology of the disease and influence of climatic factors on disease development. The results of the studies on these aspects are discussed below.

5.1. Evaluation of amaranth accessions for resistance to leaf spot disease and genetic cataloguing of the germplasm

5.1.1. Evaluation of amaranth accessions against leaf spot disease

Genetic reaction of the host to the pathogen is an innate plant factors determining the success of infection. A genetically susceptible plant is infected readily and rapidly under sufficient inoculum potential while a resistant plant poses obstacle in the way of penetration and establishment of pathogen. Moreover, use of resistant varieties for controlling plant disease is a cheap, simplest and effective method. Screening of a large number of accessions of

a crop with considerable genetic diversity is a method for locating resistant types against disease which could further be utilized for the development of resistant varieties with desirable characters. It was with this objective, a collection of 168 accessions of amaranth were screened for host resistance against leaf spot disease under field condition during October 1996. The result showed that out of the 168 accessions, 14 accessions were completely free from disease and 15 accessions were resistant to the disease in which the disease severity and incidence ranged only from 2.3 to 9.7 per cent and 5.6 – 21 per cent respectively (Table 6). Ninteen accessions showed moderately resistant reaction while 34 were of moderately susceptible and 86 were found to be highly susceptible to the disease (Table 5). A-3 (Co-1), the popular green variety Kerala showed a disease incidence of 8.1 per cent only and disease severity of 6.8 per cent while the popular red varieties like A-6 (Kannara local) and A-189 (Arun) showed disease incidence of 95.9 per cent and 96.15 per cent and a disease severity of 69.9 per cent and 76.03 per cent respectively. Eighty percentage of the red accessions belonged to highly susceptible group as against 33.7 per cent of the green types. This result is in line with the findings of Gokulapalan and Reghunath (1995) that the red varieties are highly susceptible to the leaf spot disease than the green varieties.

Certain accessions which were immune at the early stage of plant growth were found susceptible to disease at a later stage. A variety may show resistant type of reaction when the conditions for infection are not conducive such as the inoculum load is not sufficient, the races of the pathogen present are not pathogenic or due to the nutrient status of the soil in which the crops are cultivated (Yarwood, 1978 and Khan, 1989). A variety could be called resistant only if it shows the resistant characters consistently under different sets of environmental conditions and

under uniform inoculum pressure. Thus the present study showed that 14 accessions were immune and 15 were resistant to leaf spot disease. In resistant accessions, infection appeared only at later stages of growth.

With regard to yield, the highest values were obtained from the green accessions, viz. A-3, A-172, A-185, A-227 and A-284 ranging from 1240 g to 1500 g per plant. Two among these (A-227 and A-284) were immune to the disease and the other three, A-3, A-172 and A-185 belonged to the resistant class where disease incidence was noticed towards the fag end of the crop (45 days after transplanting) (Table 6). This shows that late incidence of leaf spot does not apparently affect the yield and such accessions exhibit tolerance to the disease.

The varieties which were found immune and resistant under natural conditions showed the same type of reaction even on artificial inoculation as revealed by the immuned nature of A-227 and A-204 and resistant nature of A-3 and A-194.

5.1.2. Genetic cataloguing in amaranth and genetic divergence

The genetic cataloguing of the 168 accessions of the amaranth showed wide range of variation for leaf, stem and inflorescence characters (Table13). In growth habit, 97 per cent of accessions belonged to erect types. Though leaf colour ranged from green, light green, greenish red, red, reddish green to purple, 54 per cent accessions were green leaved and 20 per cent were red. Variations in leaf shape and size were also observed. The stem pigmentation showed variation and 52 per cent had green stem and 36 per cent had red stem. The inflorescence shape mostly belonged to spike (86 %) or panicle.

Selection of parents for hybridisation is based mainly on genetic diversity. The more divergent the parents are, more will be the magnitude of heterosis. Genetically divergent parents are essential to generate new variability and desirable recombinants.

Major reasons for creation of genetic diversity in plants are mutations, recombinations, disruptive selection and polyploidization, whether they are accomplished through natural agencies or through controlled means (Rai, 1979). Usually in many of the conventional heterosis breeding programmes, geographical diversity at times and phenotypic diversity in majority of cases are taken as criteria to choose genetically divergent populations to isolate inbred lines. Phenotypic divergence in a population is also considered as an index and criteria for genetic diversity (Rai, 1979).

In the present study, the 168 amaranth accessions of exotic and indigenous origin were grouped in to fourteen clusters, indicating considerable genetic diversity prevailing among them. The distribution of accessions into various clusters showed no uniformity. Maximum number of accessions (24) were in clusters VIII and X and a minimum number of two come under the cluster IV (Table 12).

Cluster IV which included accessions A-284 and A-285 recorded maximum values for yield (1364 g) and days to bolting (66) . The highest yield obtained may be due to the delayed bolting resulting in more number of harvests of greens. Cluster XII recorded maximum values for plant height (at 30th day 65.66 cm and at flowering 117.14 cm), stem girth (12.31 cm), leaf length (21 cm) and terminal inflorescence length (25.48 cm) and its yield value recorded was 1143 g. The higher yield in this cluster may be due to

the increased plant height, stem girth, leaf length and also due to more number of days taken to bolt (57 days). Cluster VI recorded minimum value for yield (70 g) which is apparently due to the shorter stature of the plant (mean plant height was 13.9 cm).

Clsuters having the largest genetic distance show maximum divergence. Hybridization between accessions of cluster VI and XII, VI and XI is likely to give high heterosis for yield attributes due to high divergence between these clusters

5.2. Isolation and identification of pathogens associated with leaf spot disease

Isolation of pathogen from infected plant showed the association of two fungi in culture media and its pathogenicity was proved by artificial inoculation under laboratory condition. Based on the morphological characters, the fungi were identified as *Rhizoctonia solani* Kuhn and *Colletotrichum capsici* (Syd.) Butler and Bisby. In red and purple accessions the pathogen associated with the disease was found to be *R. solani* only, whereas in the green, greenish red and reddish green accessions the association of both fungi were noticed, the major being *C. capsici* (Table 15 and Fig .1).

C. dematium was associated with the occurrence of leaf spot in amaranth according to Kumar and Rao (1976) and *C. gloeosporioides* was implicated as the cause of leaf spot in amaranth as per Suharban *et al.* (1994).

Kamalanayar *et al.* (1996) reported another causal organism *R. solani* producing leaf spot in *A- tricolor.* But in the present investigation in addition to *R. solani* , C. *capsici* was identified as the causal organisms of leaf spot based on the morphological description of Mordue (1971). Thus the involvement of *C. capsici*

and *R. solani* were unambiguously recorded in the development of leaf spot disease in amaranth at this location. The two organisms act either alone or in combination to produce leaf spot symptoms.

In the present study the morphological characters of *R. solani* resemble with that described by the workers such as Duggar (1915), Townsend and Willetts (1954) and Menon (1996). Similarly the characters of the fungus *C. capsici* observed are in agreement with the descriptions of Mordue (1971).

Studies on the symptomatology are essential for the early detection of the disease. In the present investigation, symptoms observed due to *R. solani* infection were the same as described by Kamalanayar *et al.* (1996). However, slight variation was observed in symptoms produced by *C. capsici* from that of *C. gloeosporioides* reported by Suharban *et al.* (1994). In the case of *C. capsici*, the initial symptoms which included appearance of chlorotic spot with an yellow halo and later the centre of lesion falls off showing shot hole symptom. Symptoms observed on artificial inoculation were similar to those produced under natural condition.

5.3. Crop weather relationship in amaranth

5.3. 1. Seasonal influence on leaf spot disease

Disease intensity in any crop is dependent upon the virulence of the pathogen, susceptibility of the host and prevailing environmental conditions. Absence of disease in January and February 1998 months may be due to the prevalence of high temperature (33–39 °C), low RH (63-68 %) and absence of rain which are the conditions unfavourable for the growth and development of the fungi.

Some accessions free from disease during the early growth phase were found infected in the later part of growth period which could be attributed to the favourable environmental conditions resulted due to the receipt of summer showers during the last week of March and April (11mm and 61mm respectively) as has been proved that rain would invariably predispose the disease occurrence. In the case of accessions in which infection occurred at later stage, the economic loss due to the disease was considerably low as by this time two to three harvest could be completed. But, in the case of the accessions in which infection occurred within 30 days of planting the impact of the disease was too devastating.

The disease incidence and disease severity percentage of different accessions in different planting months were maximum in the crops raised in July (Fig. 2-7). This may be due to the prevalence of the low temperature (28.8°C), high RH (89.6 %) and high rainfall (891.2mm) which are the optimal weather conditions favourable for disease development as reported by Colhoun (1973) and Siddaramaiah *et al.* (1978). The higher incidence of leaf spot during rainy season has been related to its dependence on rain splash for the dispersal of spores as revealed by Sukumar and Ramalingam (1989). Disease severity was however minimum during March and April planting and this may be due to the occurrence of higher temperatures, minimum RH and rainless conditions. Thus in the present study it was revealed that the environmental factors have a pivotal role on leaf spot disease development and severity as reported by Colhoun (1973). Seasonal influence on the incidence of spotted wilt virus in tomato (Prasadrao *et al.* 1980), *Alternaria* leaf spot in brinjal (Mohitsingh and Shukla, 1986) and anthracnose in cowpea Praveenkumar (1999) have already been reported.

In December crop, the occurrence of disease in the early stage may due to the carried over effect of higher RH (78%) and the rains existed during the previous few months. However, towards the end of December, absence of rain could have reduced the fungal inoculum to a lower level sufficient to make the situation unfavourable for disease development.

The disease incidence and severity in different planting months showed an increase with the advancing age in all accessions as evident from table 20.. These results are in close agreement with the findings of Praveen kumar (1999) in cowpea anthracnose diease and Kumar and Irulappan (1990) in tomato spotted wilt virus.

Correlation studies revealed that leaf spot disease in amaranth was negatively correlated with maximum and minimum temperatures whereas relative humidity and total rainfall were positively correlated with disease. Positive association of rain with disease development and adverse effect of temperature on disease have been reported by Siddaramaiah *et al.* (1978) and Dingar and Mohitsingh (1986) in brinjal.

A variety could be called as immune/resistant only if it shows the resistant characters consistently under different sets of environemental conditions and under uniform inoculum pressure. The genotypes examined in this study showed differential response to the changing environmental conditions. Certain accessions belong to different disease classes in different months and this may be due to the difference in weather parameters prevalent in different months. In July and August, since weather conditions are highly favourable for the fungus growth, the genotype A-191 can be classified under "highly susceptible" class. But in March and April, due to the unfavourable weather conditions, only mild infection

was noticed in this accessions shifted its position to "Resistant" class. This shows that a susceptible variety can be successfully grown by change of season.

5.3.2 Seasonal influence on yield contributing characters

The ten accessions included in this study exhibited significant variability for plant height, branches per plant, leaf length, leaf width, days to bolt, leaf stem ratio and total vegetable yield (Table 27-35). The crops raised in August and September have taller plants inspite of the longer vegetative phase in summer (March – April) due to the optimum availability of required environmental conditions. The branching capacity was lower in rainy season (June and July) compared to summer (March and April). Leaves were larger in all accessions in summer probably due to higher photosynthetic activity as light is not a limiting factor and also freedom from leaf spot disease which will help in better translocation of nutrients. In March planted crop in addition to delayed flowering, stem girth, branches per plant, length and width of leaf and leaf stem ratio were the highest and these factors contributed to the highest yield in this month. The lowest yield in June can be attributed to less number of branches per plant, the low value for leaf length and width and high incidence and severity of disease. Association of yield with stem girth, leaf length and leaf width has been reported earlier by Mohideen and Muthukrishnan (1979) and Mohanalakshmi (1995) in amaranth. Better performance of amaranth types in summer has already been reported by Devadas (1982) and George (1986).

November planted crop took the lowest number of days for bolting (48 days) and March planted had the maximum number of days (65) for bolting favouring March planting for vegetable yield and less incidence of disease. Early bolting has been one of the

limitations in the cultivation of vegetable type since it results in poor vegetable yield due to the fact that yield stops as a result of flowering. Bolting is affected by temperature (Zabka, 1957), soil factors (Grubben, 1976), height of cutting (Kauffmann and Gilbert, 1981), density of population (Enyi, 1965) and age of seedling (Sulekha, 1980). The reason for an early flowering in November may be due to their short day flowering nature.

The leaf stem ratio has its importance as an index of leafiness in selection programme of amaranth. Eventhough accessions with more leaves are preferred by consumers, a strong negative relation exists between yield and leaf stem ratio in the present study. Similar observation was reported by George (1986) and Mohanalakshmi (1995) in amaranth. Hence in selection programme, optimum leaf stem ratio of around 1.5 which has to be aimed at so that optimum yield will be obtained with acceptable palatability.

As literature suggested, it is the environmental factors which decide the plant characters and final yield for a given crop (Ramachandra and Thimmaraju, 1983 and Dang *et al.* 1995). It is presumed that among several environmental factors, weather is the resource, which drives the plant husbandry if the crop management practices are strictly adhered to. Under the above situation , all attempts have been made to develop a relationship between various weather elements and crop yield through correlation techniques. Vegetable yield was associated positively with maximum and minimum temperatures and negatively with RH and rainfall. This result is in agreement with Ramachandra and Thimmaraju (1983) who obtained higher yield in summer due to positive association of maximum temperature with stem girth, leaf length, leaf width, branch number and yield.

Information on the extent of genotype and environment interactions is required in any breeding programme aimed at developing phenotypically stable varieties. Sreerangaswamy *et al.* (1980) and Devadas (1982) reported the existence of a strong genotype and environment interaction in the diverse genetic population of amaranth. In the present investigation, genotype and environment interactions were highly significant for most of the characters studied. This indicates that the above characters are unstable and fluctuate considerably with a change in the environment. This signifies the need for judicious selection of optimum season to realise the maximum productivity and disease free condition.

5.4 Biochemical bases of resistance to leaf spot

Different defence mechanisms are attributed in resistant varieties for its survival and hence it is important to study the biochemical factors contributing to resistance. Based on the above fact the study was carried out in the selected ten amaranth accessions for total phenols, OD phenol, ascorbic acid, chlorophyll 'a' and 'b', anthocyanin and isozymes. These selected accessions had different reaction to the pathogens. The study was conducted in two seasons, first in summer when there was no disease incidence and second in rainy season when there was heavy incidence of disease.

5.4.1. Total phenols

The total phenol content of all the leaf spot resistant accessions were higher than susceptible accessions in stems and leaves in the two seasons studied. High content of phenols in resistant plants suggests its role of phenols in imparting resistance to diseases. Walker (1923) has already reported protective role of phenolics against disease incidence. Menon and Schachinger (1957)

illustrated the role of phenolics in combating diseases in tomato. Increased level of phenolics in resistant accessions as compared to susceptible ones has been reported by Sadankumar (1995) in tomato and Paul (1998) in solanaceous vegetables. Ndubizu (1976), Godfrey and Clements (1978) and Gupta et al. (1995) reported that phenolic contents inhibited germination of conidia of many pathogens.

Among the two seasons studied, all the accessions recorded high phenol content in rainy season when there was a serious incidence of leaf spot disease. This indicated that infection by the pathogen increased the total phenol content significantly in both resistant and susceptible accessions of amaranthus (Table 38). The finding of Chowdhury (1995) on the increased biosynthesis of phenolics in response to infection by *Puccinia arachidis* in groundnut is in agreement with the present results. Sridhar and Ou (1974), Sharma et al. (1983) and Luthra et al. (1988a) also reported an increase in total phenol content after infection.

5.4.2. O.D. Phenol

The stems and leaves of immune and resistant accessions viz. A-227, A-204 and A-3 had higher OD phenol content compared to susceptible accessions in both the seasons. The findings of Rajan (1985), Geetha (1989), Sadankumar (1995) , Markose (1996) and Paul (1998) are in line with the present observations.

The OD phenol content in plant samples of immune and resistant accessions decreased after infection by the pathogen in rainy season whereas in susceptible accessions OD phenol content did not vary significantly between summer and rainy seasons. This suggests that lower OD phenol level in plant samples during rainy

season can be correlated with disease resistance in amaranth when the total phenol was high. The reports of Paul (1998) in tomato, brinjal and chilli for OD phenol content and that of Bose (1999) in tomato are in line with the present results. Comparatively low OD phenol content in resistant varieties may be contributed by the immediate oxidation of OD phenol to more toxic compounds like quinones by the oxidising enzymes like polyphenol oxidase and peroxidase (Mahadevan, 1970). The reaction of the genotype as resistant or susceptible would be having a bearing on the relative content of the total phenols and OD phenol in plants. The difference in level is also affected by the enzyme activity in plants.

5.4.3. Ascorbic acid

The role of L– ascorbic acid in various metabolic pathways and its biosynthesis in plants have been discussed by Isherwood and Mapson (1962). Immune and resistant varieties had higher ascorbic acid content than susceptible varieties (Table 40) and this result is in agreement with the findings of Chanal and Grover (1972).

It was observed that infection by the pathogen (rainy season) reduced the ascorbic acid content in the leaves and the reduction was higher in highly susceptible varieties . Gangawane and Datar (1978) also made similar observation in tomato leaves infected by *Alternaria solani.* Ascorbic acid functions as one of the biological oxidation reduction substance. It is oxidised to dehydro-L-ascorbic acid by the enzyme ascorbic acid oxidase or by other oxidative enzymes. It is therefore, probable that the decline in the ascorbic acid is due to the production of ascorbic acid degenerating enzymes either by the fungus itself or by the host-parasite interaction as postulated by Gosh et al. (1965).

5.4.4. Chlorophyll 'a' and 'b'

Generally green varieties recorded higher content of Chlorophyll 'a' and 'b' than the red varieties. Dark green variety A-3 recorded maximum Chlorophyll 'a' and 'b' content and the least in the red variety A-182.

In rainy season, the infection by the pathogen decreased the Chlorophyll 'a' and 'b' content and the percentage decrease was more in highly susceptible accessions. It was also observed that percentage reduction was more in leaves than in stem. This is because the pathogens attack only leaves causing leaf spot disease resulting in drying up of affected portion of leaf in patches. This result is in agreement with the report of Sharma and Chowfla (1991) that the infection by amaranthus mosaic virus in *A-caudatus* decreased the Chlorophyll 'a' and 'b' content.

5.4.5. Anthocyanin

Anthocyanin content was higher in red varieties than green varieties. Leaves contained more of anthocyanin than stem. The variety A-210 (moderately susceptible) which has light green leaves recorded lowest content of anthocyanin in both the season.

Anthocyanin content decreased in rainy season upon infection by the pathogen and the percentage decrease was more in highly susceptible varieties. Dhan-Prakash et al. (1995) also reported a reduction in the content of pigments in amaranthus upon infection by cucumber mosaic virus.

5.4.6. Oxalates and Nitrates

All the red varieties recorded higher amount of oxalates in both the seasons and in all plant parts studied. Similarly all the grain types (A-227 and A-204) accumulated higher amounts of oxalates, similar to the red varieties in both seasons. This result is in agreement with the observation of Figueroa (1989), George et al. (1989), George (1986) and Thamburaj *et al.* (1994).

Oxalate content was high in summer season in all the accessions and in all the plant parts studied than in rainy season. Same result was reported by George (1986) and Sukumar and Rajan (1998).

Nitrate accumulation was higher in grain types like A-227 and A-204 which is comparable with that of A-189 and A-182. Nitrate content was the lowest in A-210 which has light green leaves which are closely arranged. This observation is in agreement with that of Liu *et al.* (1988) who reported that amaranth accessions with low nitrate content were characterized by light leaf colour, short leaf stalk, wide leaf blade and small plant spread.

Similar to oxalate, nitrate content was also higher in summer season for all accessions in both stem and leaf samples. This result is in agreement with that of George (1986). There was no significant difference in the oxalate and nitrate content between immune and susceptible types which indicates that they have no influence on disease incidence and severity.

Correlation studies between biochemical contents of leaf and disease recorded a high negative correlation between phenols, ascorbic acid, chlorophyll 'a' and 'b' with disease incidence, severity and CODEX. Anthocyanin pigment, oxalate and nitrate contents had no significant correlation with the disease.

5.5 Anatomical studies

Leaf anatomy of the ten accessions revealed presence of thick cuticle in immune accessions A-227 and A-204. Thick cuticle coupled with high content of phenol may give resistance to the penetration of the fungus in these accessions. Salisbury and Ross (1974) reported that the cuticle provides protection against some plant pathogens and against minor mechanical damage and presence of lignin in cell walls offer resistance to pathogen since the walls may not be easily degradable by the pathogen enzyme.

Though the size and frequency of muscilage canals were higher in the resistant genotype A-3, they were lower in immune accessions A-227 and A,204. This indicate that muscilage canals may not have any role in disease resistance.

Loose arrangement of epidermal and mesophyll cells as observed in A-6, A-189 and A-182 resulted in more chances of becoming susceptible to leaf spot disease.

Muscilage content in the leaf tissue of a variety is known to affect its palatability (Dhua, 1986). In the leaf analysis carried out, no significant differences in the muscilage content was observed between the accessions even though in A-3 the muscilage canals were observed to be larger and with higher frequency.

5.6 Isozyme analysis

Isozyme analysis by electrophoresis provides a well defined and effective method to detect genetic differences among individuals. Isozyme profiles might be used as a tool to compare healthy and pathological stages of plants for extracting valuable information in

pathogenesis. Isozyme studies complement the conventional biochemical and genetic analysis. The utility of electrophoretic method of isozyme study in the biochemical mechanism of disease resistance was reported by Kato *et al.* (1978) and Kato and Jodo (1989) in *C. melo.*

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

In the present study, isozyme pattern of polyphenol oxidase and peroxidase were studied in ten amaranth genotype during two seasons – one in the rainy season (July 1997) when there is heavy incidence of leaf spot disease and in another in summer (March 1998) when there is no leaf spot disease incidence.

5.6.1 Polyphenol oxidase (PPO)

In rainy season six bands were resolved. Immune accessions, viz. A-227 and A-204 expressed four number of bands in rainy season whereas the highly susceptible genotype A-182 expressed only one band. When the number of bands increased from one to four, the disease reaction also differed from high susceptibility to immunity. The protein band PPO-3 (Rm- 0.210) is specific to two immune accessions (A-227 and A-204) which can be linked to immunity and is absent in all the other eight accessions. Similarly, PPO-4 (Rm-0.490) was present only in two accessions (A-3 and A-194) which found resistant to the disease. Susceptible types had only less number of bands, as recorded in the genotype A-182 (one band)

which got affected by the disease to the extent of 81.4 per cent disease severity (Table 20).

Five bands were resolved in summer season and no disease incidence was noticed during this period in all the ten accessions. Number of bands expressed by the immune accessions was lower in summer season when compared to rainy season. Immune accessions A-227 and A-204 expressed only three bands in summer and they lacked PPO-3. This additional band of PPO-3 enzyme with Rm value 0.210 is expressed only in rainy season. The complementary effect of these four proteins contributed to their immunity in rainy season. In general not much difference in banding pattern was noticed in summer season when compared to rainy season, except the absence of PPO-3 (Rm- 0.210) in A-227 and A-204 and PPO-2 (Rm- 0.170) in A-210.

In leaf samples PPO-5 (Rm- 0.750) can be considered as the base band which was recorded for all the ten accessions. Immune genotype had a total of 3-4 bands, resistant and moderately resistant types had 3 bands, whereas moderately susceptible types had a total of 2-3 bands, while the highly susceptible accessions had only 1-3 bands (Table 51). This was in confirmation with the reports of Ganguly and Dasgupta (1988) in tomato roots for nematode resistance. The observation of Fan *et al.* (1996) in leaves of pear cultivars for resistance to *Venturia nashicola* and Bose (1999) in leaves of tomato cultivars for resistance to bacterial wilt point out that resistant cultivars have more number of PPO bands.

The nature and properties of proteins available in rainy season and summer season can also be related with total phenols and O.D. phenol content in plant parts. The zymogram of rainy season showed that the presence of protein band PPO-3 and PPO-4 may be

responsible for the high total phenol and O.D. phenol content of immune resistant and moderately resistant accessions like A-227, A-204, A-3 and A-194 in rainy season. This can be used as a marker for screening specifically the resistant accessions. This result was in line with Gupta *et al.* (1995) in *Brassica* for *Alternaria* leaf blight resistance

5.6.2 Peroxidase

In leaf samples a total of five peroxidase bands were observed. Leaf spot incidence was observed in rainy season and during this period more number of peroxidase bands were observed in all accessions when compared to summer season. PRX –2 (Rm - 0.143) was observed only in rainy season in immune and resistant accessions like A-227, A-204 and A-3 and it was absent in the same genotype in summer. Hence this can be considered as marker for resistance in these accessions. Peroxidase may act as the biological catalyst for the production of high phenol content rather than oxidation/degradation of phenol to more toxic quinone or other organic molecules as effected by polyphenol oxidase. The phenolics in the selected accessions showed a positive trend in support of the above statement. High phenolics were recorded in the sample in which the peroxidase showed more number of protein bands. This result is in agreement with results of Deyu *et al.* (1995) in barley ; Lebeda and Dolezal (1995) in millets ; Barcelo *et al.* (1996) and Morales *et al.* (1997) in grapes. Generally resistant varieties had more number of bands than in susceptible varieties (Table 51). This result is in confirmation with reports of Fei *et al.* (1997) in soybean and Liu *et al.* (1988) in millet.

The resistance mechanisms of leaf spot disease as revealed by different accessions, their reaction to environmental factors show

that resistance to leaf spot disease is imparted by genetic as well as biochemical factors of the host which get often modified by environmental influences. The resistance in certain lines, viz. A-227 and A- 204 remained unaltered with change of season or levels of inoculum load being they are immune . But the resistant lines (A-3 and A-194) behaved differently with season. The line A-194 was free from disease during January to April whereas mild infection (DS <10%) was noticed in six months except July and August (DS 11-19%). This shows that tolerant lines can be successfully cultivated in a wide range of climatic conditions provided they are ideally chosen. The susceptible lines, viz. A-6, A-191, A-189 and A-182 became totally free from disease when the maximum temperature, RH and rain reached 39.5° C, 63 per cent and zero respectively showing the utility of such lines under a very narrow temporal conditions.

Anatomical character like presence of thick cuticle and biochemical factors such as high content of phenols and ascorbic acid are strongly associated with resistance in amaranth.

The protein band PPO 3 with Rm value 0.210 contributed immunity in A-227 and A-204 and presence of thick PRX 2 band (Rm 0.143) can also be construed as biochemical marker for immunity.

Summary

6. SUMMARY

The investigation on " Genotypic and seasonal influence on leafspot disease in amaranth" was undertaken in the Department of Olericulture, College of Horticulture, Vellanikkara, Thrissur during the period from 1996 to 1998. The findings of above investigations are summarised hereunder.

One hundred and sixty eight amaranth accessions belonging to vegetable and grain types were screened under natural conditions against the leafspot disease. Out of these, 14 accessions were immune, 15 resistant, 19 moderately resistant, 34 moderately susceptible and 86 highly susceptible to the disease. Red types were found more susceptible to the disease than green types.

Out of the fourteen immune accessions, eleven belonged to *Amaranthus hypochondriacus* , one to *A. viridis* and two to *A. spinosus.*

Disease infection was noticed immediately after transplanting(within 15 days) in 110 genoptypes which resulted in higher percentage of disease severity.

The highest yield in the range of 1250-1500 g/plant was obtained from the green amaranth accessions, viz. A3, A.172, A.185. A.227 and A.284.

The morphological studies of the 168 amaranth accessions revealed that 97 per cent were erect in growth habit, 54 per cent had green leaves, 20 per cent red, 2 per cent light green and 7 per cent each under greenish red, reddish green and purple leaves. Most of the accessions had green stem (53%) followed by red (36%) and the rest belonged to reddish green and pink stem.

Based on Eucledian distance cluster analysis, the total 168 accessions were grouped into 14 clusters and cluster IV included accessions A. 284 and A. 285 which recorded the highest yield. Genetic divergence was maximum between cluster VI and cluster XII (8.74) followed by cluster VI and cluster XI (8.56).

Investigations on the symptomatology revealed that two types of leafspot symptoms are present and are caused by two types of pathogens, *Rhizoctonia solani* Kuhn. and *Colletotrichum capsici* (Syd.) Butler and Bisby. *R. solani* was found associated with dirty white grey spot symptoms and *C. capsici* was found causing brown spots surrounded by an yellow halo which at later stage produce shot hole symptoms.

Classification of amaranth accessions based on infection by pathogens revealed that all the 35 red accessions were infected by *R. solani* only whereas out of 92 green types, 66 accessions were infected by *C. capsici* and 14 by both pathogens.

Ten selected accessions belonging to various disease infection classes were evaluated for disease response to leafspot for one

year at monthly plantings from May 1997 to April 1998. All the accessions were free from disease during January and February 98 and prevalence of high temperature, low RH and absence of rain was associated with this. Maximum disease severity was recorded in July crop when favourable weather conditions for disease development like low temperature, high RH and high rainfall were prevailed. Disease severity was the minimum in March and April crop which was associated with the occurrence of unfavourable weather conditions.

Correlation studies conducted revealed that leafspot in amaranth was negatively correlated with maximum and minimum temperatures whereas relative humidity and total rainfall were positively correlated with the disease.

Accessions showed differential response to the changing environmental conditions. Accessions resistant to the disease in certain months, were found to be moderately resistant, moderately susceptible or even highly susceptible in certain other months of the year.

Seasonal influence on yield revealed maximum values in March crop due to the maximum stem girth, number of branches, leaf length, leaf width and delay in flowering. Yield was associated positively with maximum and minimum temperatures and negatively with RH and rainfall.

Investigations on biochemical bases of resistance revealed that the total phenol, OD phenol and ascorbic acid content in all the resistant accessions were higher than the susceptible accessions. Their content was observed higher in leaves than

in stems. Among seasons, higher total phenol content was noticed in rainy season where as the content of OD phenol, ascorbic acid and pigments decreased upon infection by pathogens. Oxalates and nitrates had no influence on leafspot disease.

Correlation studies between various biochemical contents of leaf and leafspot disease revealed a high negative association between total phenol, OD phenol, ascorbic acid, chlorophyll 'a' and 'b' and disease incidence, severity and CODEX. Anthocyanin pigment, oxalate and nitrate content had no significant correlation with disease.

Electrophoretic studies of isozymes revealed that more number of bands were expressed for both polyphenol oxidase and peroxidase enzymes in immune and resistant accessions than the susceptible ones. The number of bands observed were more in rainy season (in diseased condition) than in summer with respect to all the accessions. In immune accessions (A. 227 and A. 204), the protein PPO-3 with Rm value of 0.210 was expressed only in rainy season. The complementary effect of this protein along with other three proteins contributed immunity in them in rainy season. For peroxidase enzyme also, PPX-2 (Rm 0.143) expressed only in rainy season along with other three proteins (PRX1, PRX3 and PRX5) which could be responsible for immunity in A.227 and A.204.

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GENOTYPIC AND SEASONAL INFLUENCE ON LEAFSPOT DISEASE IN AMARANTH

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ABSTRACT OF THE THESIS

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ABSTRACT

The investigation on " Genotypic and seasonal influence on leafspot disease in amaranth" was undertaken in the Department of Olericulture, College of Horticulture, Vellanikkara, Thrissur during 1996 – 1998. The objectives of the study were to identify amaranth accessions resistant to leaf spot disease, to isolate and identify the pathogens associated with the disease, to study the seasonal influence on leaf spot and yield and to find the possibility for biochemical cataloguing of amaranth accessions.

Evaluation of 168 amaranth accessions for leaf spot disease resulted in identification of 14 immune, 15 resistant, 19 moderately resistant, 34 moderately susceptible and 86 highly susceptible accessions. Red types were found highly susceptible as compared to green types. Disease infection occurred within 15 days of planting resulted in maximum percentage of disease severity.

Two types of leaf spot symptoms were inflicted and causal organisms identified were *Rhizoctonia solani* Kuhn. and *Colletotrichum capsici* (Syd.) Butler and Bisby. The two organisms either alone or in combination caused the incidence and development of leaf spot disease. Red types were infected by *Rhizoctonia solani* alone where as the green types were infected by both pathogens.

Seasonal influence on disease recorded maximum disease severity in July crop and minimum in April crop. Low temperature, high relative humidity and high rainfall were the favourable weather conditions for disease development. Leaf spot in amaranth was negatively correlated with maximum and minimum temperatures where as relative humidity and rainfall were positively correlated with the disease.

Highest yield was realised in March planted crop and lowest in June crop. Yield and yield attributes except plant height were positively correlated with maximum and minimum temperatures where as relative humidity and rainfall were positively correlated with them.

High content of total phenols, OD phenol and ascorbic acid were recorded in immune and resistant accessions than susceptible. Total phenol content was higher in rainy season. OD phenol, ascorbic acid and pigments decreased upon infection in rainy season. Oxalates and nitrates had no influence on leaf spot disease development. High negative correlation was found between total phenol, OD phenol, ascorbic acid, chlorophyll 'a' and 'b' and disease.

Generally immune and resistant accessions had more number of PPO and PRX bands than susceptible types. The additional band expressed in diseased condition in certain accessions can be taken as the biological marker for leaf spot resistance.