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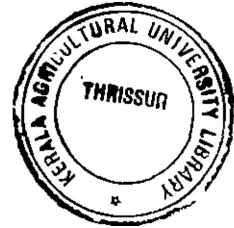
CHARACTERIZATION AND EVALUATION OF LANDRACES OF AMARANTHUS (*Amaranthus* spp.)



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Thesis submitted in partial fulfilment of the requirement
for the degree of

Master of Science in Horticulture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

2006

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DECLARATION

I hereby declare that this thesis entitled "**Characterization and evaluation of landraces of amaranthus (*Amaranthus* spp.)**" 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 of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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CERTIFICATE

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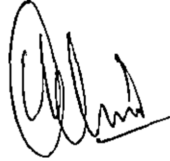

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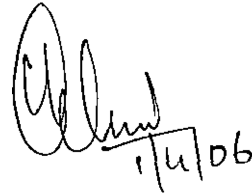

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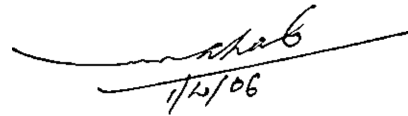

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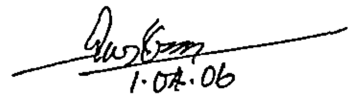

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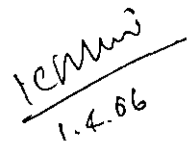

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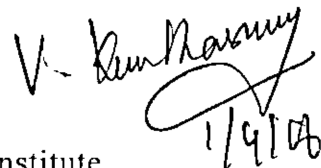

DEDICATED TO
MY FAMILY

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LIST OF ABBREVIATIONS

%	–	per cent
μl	–	microlitre
μg	–	microgram
μM	–	micromolar
A.O.A.C.	–	Association of Official Agricultural Chemists
ac	–	Acre
AFLP	–	Amplified Fragment Length Polymorphism
Ca	–	Calcium
cm	–	centimeter
DNA	–	Deoxy ribonucleic acid
dNTPs	–	deoxy nucleotide triphosphates
et al.	–	And others
Fe	–	Iron
F.I.B.	–	Farm Information Bureau
Fig.	–	*Figure*
g	–	gram
GCV	–	Genotypic Coefficient of Variation
h	–	hour
ha	–	Hectare
IIHR	–	Indian Institute of Horticultural Research
IISR	–	Inter Simple Sequence Repeat
ITS	–	Internal Transcribed Spacer
IPGRI	–	International Plant Genetic Resources Institute
I.U	–	International Unit
KAU	–	Kerala Agricultural University
KCl	–	Potassium chloride
kg	–	kilogram
mg	–	milligram
$MgCl_2$	–	Magnesium chloride
min	–	minutes
ml	–	millilitre
mM	–	Millimolar

ng	–	nanogram
No.	–	Number
^{o}C	–	Degree Celsius
PCR	–	Polymerase chain reaction
PCV	–	Phenotypic Coefficient of Variation
pM	–	picomolar
PVP	–	Poly vinyl pyrrollidone
RAPD	–	Random Amplified Polymorphic DNA
RFLP	–	Restriction Fragment Length Polymorphism
rpm	–	Revolutions per minute
s	–	seconds
SDS	–	Sodium dodecyl sulphate
SE	–	Standard error
t	–	tonnes
UPGMA	–	Unweighted Pair Group Method with Arithmetic Mean
var	–	variety

Introduction

1. INTRODUCTION

Amaranthus, belonging to family Amaranthaceae, is the only commercially grown leafy vegetable in Kerala. It is cultivated in an area of 1035 ha in Kerala (F.I.B, 2005). This crop is an attractive option for farmers because of its very short duration, high productivity, drought tolerance and relatively low incidence of pests and diseases.

Amaranthus is often referred to as poor man's spinach as it is a rich source of proteins, vitamins and minerals. The leaves contain proteins 4.0 g, fibre 1.0 g, vitamin A 9200 I.U., riboflavin 0.1 mg, thiamine 0.01 mg, vitamin C 99 mg, Fe 25.5 mg and Ca 397 mg per 100 g of edible portion (Choudhury, 1996). Though highly esteemed as a nutritious vegetable, the presence of antinutrients like oxalates and nitrates hinder its large-scale consumption (Marderosian *et al.*, 1980). Oxalates combine with dietary calcium forming kidney stones and nitrates interfere with the proper functioning of haemoglobin in the blood.

The family Amaranthaceae consists of about 60 species of which *Amaranthus tricolor* L., *A. dubius* Mart. ex Thell. and *A. tristis* L. are the major vegetable amaranthus species. The grain amaranthus species, *viz.*, *A. hypochondriacus* L., *A. caudatus* L. and *A. cruentus* L. are used as vegetable in the tender stage to some extent. However, the red leaved *A. tricolor*, a native of India remains the best choice of Keralites.

Tremendous variability for morphological characters like yield, leaf colour and size exists in most of the edible and wild species of amaranthus in Kerala. The collection, characterization and classification of this variability deserves utmost priority. But in amaranthus, it is difficult to characterize them taxonomically due to the similarity between species, small diagnostic parts, intermediate hybrids, wide geographical distribution and broad genetic diversity (Kulakow, 1990).

Information generated through DNA profiling using molecular techniques such as RAPD markers will give a more comprehensive picture on the diversity and relatedness of landraces in amaranthus. So in the present study, both morphological and molecular characterization have been conducted to analyse the genetic diversity within different cultivated amaranthus species namely *A. tricolor*, *A. dubius* and *A. hypochondriacus*.

Assessing the variability for desirable characters is a prerequisite for any crop improvement programme. So the characterization of local accessions of amaranthus would help us to determine the extent of genetic variability available in the germplasm of this crop which in turn would be helpful to identify the superior genotypes for yield, quality and resistance to biotic stress. The path analysis studies would facilitate effective selection for simultaneous improvement of one or more yield contributing characters .

Taking into consideration the above mentioned facts, the present study was carried out with the following objectives:

- To estimate the characters in terms of the extent of available variability, degree and pattern of association and genetic contribution in expression of each character.
- To identify superior landraces based on growth, yield and quality characters and pest and disease resistance.
- To characterize the landraces of amaranthus through morphological traits by genetic divergence analysis.
- To catalogue the available landraces of amaranthus morphologically.
- To characterize the landraces of amaranthus by RAPD analysis.

Review of Literature

2. REVIEW OF LITERATURE

Amaranthus is the most popular and widely grown leafy vegetable in Kerala. The present study involves characterization and evaluation of landraces of amaranthus using morphological characters and molecular markers. The available literature on morphological and genetic variability and characterization of amaranthus is reviewed in this chapter.

2.1 MORPHOLOGICAL CHARACTERIZATION

2.1.1 Biometric characters

Variability in biometric characters like plant height, stem girth, leaf length and width, petiole length, number of branches, days to 50 per cent bolting, leaf/stem ratio and yield were studied by several workers in amaranthus.

Kamalanathan *et al.* (1973) reported that the vegetable amaranthus variety CO 1, belonging to *Amaranthus dubius* yielded 19 t ha^{-1}.

Arakeerai (*A. tristis*) responded favourably to clipping and gave an increased yield of 11,736 kg greens per ha as compared to Sirukeerai (*A. blitum*) which gave 8680 kg ha^{-1} (Mohideen and Rajagopal, 1974).

Mathai (1978) reported that Co-1 can yield upto 15-16 x 103 kg ha^{-1}.

Mohideen (1978) observed increased leaf weight, stem weight, leaf length, leaf breadth, stem diameter and plant height in amaranthus 25 days after sowing and suggested that this was the optimum harvesting stage to get maximum yield.

In the varieties, CO 1 and CO 2, the green matter yield increased from 27th to 42nd day after sowing (Subbiah, 1979).

A study conducted by Vijayakumar (1980) on the performance of 19 types of amaranthus belonging to *A. dubius, A. tricolor, A. blitum,*

A. hypochondriacus, A. cruentus, A. tristis and *A. edulis* indicated green yield range from 0.920 kg m^{-2} to 4.700 kg m^{-2} on the 30th day of harvest.

Olufolaji and Tayo (1980) compared the growth and development of three cultivars of *A. cruentus*, 'Large Leaf', 'Light Red' and 'Local Green' under greenhouse conditions. There were small differences between cultivars for leaf area, number of branches, number of nodes and dry weight production of stem, roots, inflorescence and most especially leaves at the edible stage.

Mohideen and Muthukrishnan (1981) classified the amaranthus genotypes into high yielders, moderate yielders and low yielders. They reported that the mean yield of greens in most types was higher in summer when compared to rainy season.

In a study conducted among *A. tricolor* accessions, RRC 241 was reported to be top performer (Kauffman and Gilbert, 1981).

In a field study conducted by Campbell and Abbott (1982) involving three entries of *A. cruentus,* one of *A. dubius* and 16 of *A. tricolor*, *A. dubius* was found to be the highest yielder, whereas, *A. tricolor* exhibited the maximum leaf/stem ratio and therefore had the greatest market potential.

Devadas (1982) conducted a screening to locate non-bolting types of amaranthus suitable for year round planting in Kerala. The study revealed that days to flowering is a genetic character with much scope for improvement through simple selection.

The range of yield as reported by various workers were 4.0 to 16.5 t ha^{-1} (Campbell and Abbott, 1982) and 9.90 to 18.30 t ha^{-1} (Makus, 1984).

A high yielding clipping type of amaranthus, CO 3 was released by Tamil Nadu Agricultural University, Coimbatore, through selection and it recorded a yield of 10-12 t ha^{-1} (Mohideen *et al.*, 1985).

While comparing green yield among *A. tricolor*, *A. hybridus*, *A. cruentus* and *A. dubius* accessions, *A. hybridus* was found to be the highest yielder (Igbokwe *et al.*, 1988).

Seed colour was found to influence grain and vegetable yield in amaranthus as reported by Olufolaji and Dinakin (1988). The highest vegetable yield was noted in the black seeded cultivars while white seeded types recorded highest seed yield.

In an evaluation of 15 amaranth varieties, including four species, plant height ranged between 160 to 230 cm. Grain yield varied from 5129 kg per 27 m^2 in 848-K254C to 10484 kg per 27 m^2 in 848-1157 (Calderon *et al.*, 1991).

Devadas *et al.* (1993) reported that there were no significant differences between red and green amaranthus for plant height, stem girth and petiole length at 30 days after sowing, but the leaf length, leaf width and number of branches differed significantly and higher yield and frequency of harvests were seen in red types as compared to green.

A study on vegetable yield of amaranthus as influenced by species and harvesting frequency revealed that *A. hybridus*, *A. hypochondriacus* and *A. dubius* produced significantly higher yields than *A. flavus* and *A. hybridus* had the highest leaf/stem ratio (on dry weight basis). Harvesting at three weeks interval significantly increased vegetable yield, but higher leaf/stem ratio was obtained from fortnightly harvesting (Norman and Sichone, 1993).

Variability studies in 25 lines of vegetable amaranth gave mean values of 35.65 cm, 10.58 cm and 7.35 cm for plant height, leaf length and breadth respectively. Mean value for petiole length was 4.17 cm while that for stem girth was 3.02 cm (Varalakshmi and Reddy, 1994).

High yields of 50-55 t ha^{-1} and 35 t ha^{-1} were recorded for Pusa Kirti (*A. tricolor*) and Pusa Kiran (a natural cross between *A. tricolor* and *A. tristis*) (Sirohi and Sivakami, 1995).

Six amaranthus species were evaluated for productivity, taste and acceptability by Allemann *et al.* (1996). Maximum yield was obtained from *A. hypochondriacus* (43 t ha^{-1}). Yield obtained decreased steadily from first cutting to subsequent cuttings.

Nine accessions of amaranth, six from *A. tricolor*, one each from *A. hybridus, A. cruentus* and *A. dubius* were evaluated in the field for green and dry matter yields by Singh and Whitehead (1996). *A. tricolor* accession, RRC 241 showed highest leaf fresh and dry weights. *A. hybridus* and *A. cruentus* accessions had highest stem fresh and dry weights and green and dry matter yields.

Koppa *et al.* (1997) studied the growth and yield performance of seven cultivars of grain amaranthus and recorded that the grain yield varied from 0.42 to 0.83 t ha^{-1}. The cultivar IC 35463 was found to have the highest grain yield, tallest plants, maximum leaves per plant, highest dry matter content, longest inflorescence etc.

Pusa Lal Chaulai, a red pigmented vegetable amaranthus suited for both spring-summer and kharif seasons gave an average yield of 49 and 45 t ha^{-1} in spring and kharif seasons (Sirohi and Sivakami, 1997).

Mohanalekshmi *et al.* (1998) studied variability in relation to stages of growth in amaranthus. An overall analysis of growth pattern, yield of greens and component characters indicated the optimum harvest stage as 20 to 30 days after sowing. Optimum leaf/stem ratio was found to be 1.0 to 1.5.

Priya (1998) conducted studies in various genotypes of amaranthus and obtained highest yield for Amt 193 (304.5 g plant^{-1}). The genotypes

A 24 from *A. tricolor* showed highest leaf/stem ratio of 1.57. Days to 50 per cent bolting ranged from 36.38 in A 53 to 74.50 in A 41.

Hossain and Rahman (1999) assessed the stem production potential of 11 amaranth genotypes and results indicated that stem portion contributed more towards yield than leaf portion.

Effect of maturity on forage yield and quality of *A. cruentus* and *A. hypochondriacus* tissues was investigated by Stordahl *et al.* (1999). Dry matter yield was 1.3 t ac^{-1} at eight weeks after sowing, increased with subsequent harvests and peaked at 4.4 t ac^{-1} at 16 weeks after sowing. Degradation in forage quality was noticed as maturity increased from bud stage to flowering.

Growth analysis of four weedy amaranthus species, namely, *A. palmeri, A. rudis, A. retroflexus* and *A. albus* was undertaken by Horak and Laughin (2000). Results showed that rates of height increase were 0.21 and 0.18 for *A. palmeri,* 0.16 and 0.11 for *A. rudis*, 0.12 and 0.09 for *A. retroflexus* and 0.08 and 0.09 for *A. albus* in two consecutive years. *A. palmeri* had highest plant volume, dry weight and leaf area and *A. albus,* the lowest. Maximum relative growth rates (g g^{-1} day^{-1}) were 0.32 (*A. palmeri*), 0.31 (*A. rudis*), 0.3 (*A. retroflexus*) and 0.26 (*A. albus*).

Arka Arunima, a high yielding purple coloured multicut amaranth variety has been released by IIHR. Leaves are broad and purple and it yields about 27 t per ha in three cuttings (IIHR, 2000).

Pureline breeding experiments in Tamil Nadu resulted in the selection of a single coloured amaranthus (*A. tricolor*) plant from the germplasm type EC 536439. This had robust growth with broader leaves and fleshy stem weighing 300 g. It was released as CO 5 by Tamil Nadu Agricultural University (Kanthaswamy *et al.*, 2000).

Shukla and Singh (2000) showed that foliage yield per plant varies from 1.18 – 3.29 kg with an average of 2.25 kg. The range and mean of

individual cuttings for plant height, leaves/plant and foliage yield generally increased in ascending order of cuttings and was maximum in the fourth cutting, while range and mean were maximum in third cutting for leaf size (38.08 ± 3.99) and branches per plant (11.9 ± 0.78).

Effect of varieties and sowing dates on seed yield and quality in vegetable amaranthus (*Amaranthus* sp.) were studied by Srinivasaiah *et al.* (2000) using three *A. tricolor* cultivars, Arka Suguna, AG 114 and Local sown at 30 days interval from 15th July 1997 to 15th February 1998. Earliest flowering was got in August-sown crop and earliest maturity in July-sown crop. Highest seed yield with fairly good seed quality was noticed in November sown crop, but seed quality was still better in July and February sown crops. AG 114 recorded the highest value for all yield attributes irrespective of the sowing date.

Wu *et al.* (2000) recorded wide diversity for stem and leaf colour while evaluating the amaranthus genetic resources from China. They identified several genotypes that appeared to have favourable agronomic traits of immediate use in cultivar development.

Xiao *et al.* (2000) classified 31 vegetable amaranth varieties based on 17 biological characters, of which leaf shape and colour were most significant.

Shukla and Singh (2002a) compared the varietal performance and foliage yield among 10 cultivars of vegetable amaranth. AV-190 gave the highest yield (285.24 q ha^{-1}) following by AV-45 (270.66 q ha^{-1}). Maximum and minimum heights were recorded for AV-64 (24.35 ± 3.81 cm) and AV-151 (16.86 ± 2.36 cm). Maximum branches per plant were seen in AV-64 (11.80 ± 1.89) and minimum in AV-76 (8.07 ± 0.67).

An experiment was done to assess the genetic diversity of 66 amaranth genotypes by Shukla and Singh (2002b). Days to flowering

ranged from 44.33-75.33 days. Plant height varied between 31.67 cm and 125.33 cm. Number of branches varied from 4.33 to 19.67.

After studying the variability among *A. dubius* accessions for biometric characters, Sindhu (2002) reported that the yield varied from 464.80 g to 1555.94 g and that that the leaf/stem ratio ranged from 0.93 to 2.48. Days to 50 per cent bolting showed a variation from 47.80 days to 75.13 days.

Comparative growth of six amaranthus species was analysed by Sellers *et al.* (2003). Accessions of *A. retroflexus, A. rudis, A. spinosus, A. albus, A. hybridus* and *A. palmeri* were used for the study. Dry weight of *A. palmeri* was almost 65 per cent greater than those of other species two weeks after planting and its biomass accumulation was greater than those of the other species throughout the season. Final plant height ranged from 58 cm (*A. albus*) to 208 cm (*A. palmeri*).

Forty six accessions of vegetable amaranth maintained at IIHR were characterized and evaluated during kharif season by Varalakshmi (2004). Plant height, leaf width and petiole length were found to vary between 31 to 81.5 cm, 3 to 12 cm and 3 to 9 cm respectively. Days to flowering ranged from 29 to 69 days. This variation can be exploited for varietal improvement. Accessions AV-8, AV-33 and AV-39 had tall plants with more leaves while AV-64 showed greater height coupled with late flowering which is desirable. AV-7 and AV-45 were late bolters with large leaves.

2.1.2 Quality characters

2.1.2.1 Nutrient factors

Amaranth is one of the few vegetables in which several essential dietary components such as protein, vitamin C, vitamin A, Fe and Ca occur together (Mohideen and Subramanian, 1974; Martin and Telek, 1979).

Grubben (1976) reported that the ascorbic acid content in different species of amranthus exhibited significant variation from 325 to 1250 mg in 100 g of dry matter. Variety Co-2 was found to contain 3.5 g protein, 1.3 g crude fibre, 39.38 mg P, 310 mg Ca and 19 mg Fe in 100 g of edible matter (Rajagopal *et al.*, 1977).

Deutsch (1978) studied genetic variation in nutritional value in several *Amaranthus* species and suggested that genotype x environmental interactions were large for oxalate, Ca and protein content. Mean protein production was 5 kg per ha per day at first harvest.

Olufolaji and Tayo (1980) compared mineral content of three cultivars of *A. cruentus* namely 'Large Leaf', 'Light Red' and 'Local Green'. There were significant differences in mineral content of cultivars in the order, 'Local Green' > 'Large Leaf > 'Light Red' for concentration of N, Ca, Mg, Fe and Mn. 'Local Green' was the most nutritive among the three cultivars.

Protein content of the leaves ranged from 21 to 28 per cent in *A. caudatus* and 18.37 to 37.19 per cent in *A. tricolor* on the dry matter basis (Mathai *et al.*, 1980).

Appearance, flavour, texture and overall eating quality of 20 standard amaranthus entries (three *A. cruentus*, one *A. dubius* and 16 *A. tricolor*) and spinach were rated by consumer sensory panels(Abbott and Campbell, 1982). Of the *A. tricolor* entries, Chin was the best overall and Tampla was intermediate. Of all the entries, *A. dubius* was intermediate s, *A. dubius* was intermediate and *A. cruentus* was least acceptable.

Subbiah and Ramanathan (1982) conducted field experiments with two *A. blitum* cultivars CO 1 and CO 2 and noted that N application increased the crude protein, carotene and chlorophyll contents but simultaneously decreased the vitamin C content. K had no marked effect on carotene, vitamin C and chlorophyll levels but increased crude protein

content in case of late harvested crop. The crude protein was highest in amaranthus 27 days after sowing (Ramanathan and Subbiah, 1983).

Mohideen *et al.* (1985) evaluated the nutrient content of variety CO 3 and found that it contained 12.5 per cent protein on dry weight basis and 11.04 mg of carotene in 100 g of fresh matter.

Oxalates can comprise 0.2-11.4 per cent of the dry matter in vegetable amaranthus (Teutonico and Knorr, 1985). Vijayakumar and Shanmughavelu (1985) compared the nutritive value of seven types of amaranthus and reported that the vitamin C, carotene, protein and Ca contents ranged from 32.9 to 44.2 mg 100 g^{-1}, 9. to 10.9 mg 100g^{-1}, 12.5 to 14.5 per cent and 2.30 to 2.52 per cent respectively on dry weight basis.

Thirty germplasm lines belonging to three species *viz.*, *A. tricolor*, *A. dubius* and *A. cruentus* were evaluated for nutrient contents (George *et al.*, 1989). Acc 14 had the highest dry matter content (17.2 per cent), a red entry Acc 59 showed highest crude protein content (29.3 per cent) and Acc 28 contained maximum quantity of beta-carotene (36.1 mg 100 g^{-1} of dry matter). Red and green-red entries had high protein and beta-carotene contents.

Amaranth protein is a valuable contribution to the diet where protein intake is marginal (Shanmugavelu, 1989).

A study by Calderon *et al.* (1991) among fifteen amaranth varieties showed that the protein content varied from 12.74 per cent in A412 to 14.65 per cent in A622.

Prakash and Pal (1991) studied 61 accessions of amaranthus and reported variation for carotenoid content from 90 to 200 mg kg^{-1} in vegetable and from 60 to 200 mg kg^{-1} in grain types. Variation in leaf protein was 14 to 30 g kg^{-1} and 15 to 43 g kg^{-1} for vegetable and grain types respectively.

The carotenoid content in leaves picked at the flowering stage was determined in 31 accessions representing *A. tricolor, A. caudatus* and *A. cruentus*. Accessions K 49 and K 99 had highest carotenoid contents (4.95 and 4.23 mg g^{-1} respectively) while K 99 showed maximum amaranthin content of 34.2 mg/g (Kononkov *et al.*, 1995).

Carotenoid content of *A. viridis* was reported to be 15.4 mg 100 g^{-1} by Guill *et al.* (1997). Raja *et al.* (1997) analysed eight green vegetables including amaranthus for their nutritive value and reported the levels of crude protein to be 1.03 – 5.23 per cent on fresh weight basis.

Amaranth leaves have high content of protein (4.0 g), Ca (340.0 mg) and ascorbic acid (120 mg) per 100 g on fresh weight basis (Hemalatha *et al.*, 1999).

Hossain *et al.* (1999) analysed 11 genotypes of local amaranthus (*A. tricolor*) for the nutritional properties and reported that the genotype Bonefire had highest content of dry matter (10.07 per cent), protein (12.97 per cent), Ca (1.54) per cent, Mg (0.25 per cent) and Fe (576 ppm).

Nutrient evaluation of ten species of amaranth revealed that carotenoid varied from 9.0 to 20.0 mg 100 g^{-1} and protein from 1.4 to 3.0 per cent on fresh weight basis (Pal, 1999).

Effect of maturity on forage yield and quality of *A. cruentus* and *A. hypochondriacus* tissues indicated that crude protein was highest (23 per cent) at eight weeks after sowing when crop was vegetative and decreased with maturity to 13 per cent (Stordahl *et al.*, 1999).

Studies by Yadav and Sehgal (1999) showed that vitamin C content of amaranth leaves varied from 12.46-61.86 mg and that beta carotene varied from 1.14 – 14.52 mg per 100 g on dry weight basis. Gins *et al.* (2000) analysed chemical composition of *A. tricolor* leaves and recorded that they contained high amount of carotenoids (40 mg 100 g^{-1}) and considerable amount of ascorbic acid.

Kowsalya *et al.* (2001) reported the beta-carotene content of Araikeerai (*A. tristis*) as 19900 IU 100 g^{-1} and that of mullakeerai (*A. spinosus*) as 13941 IU 100 g^{-1}.

Leaves of *A. hybridus* contained 28.2-31.6 per cent protein, 25.2-37.3 mg per 100 g beta-carotene and 455-535 mg per 100 g vitamin C (Mziray *et al.*, 2001)

Sindhu (2002) noted that the minimum amd maximum protein contents were 9.03 per cent and 23.00 per cent respectively in *A. dubius* accessions whereas the vitamin A content ranged from 4331.50 I.U. to 8915.96 I.U.

In vegetable amaranths, carotenoids varied from 11.5-20.2 mg per 100 g, protein from 1.5 – 3.2 per cent and vitamin C from 105 to 180 mg per 100 g as reported by Tewari *et al.* (2002).

2.1.2.2 Antinutrient factors

Eventhough amaranthus is a highly nutritious vegetable, the presence of antinutrients like nitrates and oxalates is a main problem according to health experts. Excess of oxalates can combine with the dietary calcium forming calcium oxalate crystals which leads to kidney stone problems. Nitrates are also highly hazardous as they get converted into nitrites and join with the blood haemoglobin thereby blocking its function as an oxygen transporter.

Deutsch (1978) reported that genotype x environment interactions were high for oxalate content in several amaranthus species.

According to Martin and Telek (1979) the amount of amaranthus in the diet should be limited since it is high in oxalic acid content.

Mean nitrate levels were found to be 0.48 and 1.72 per cent in leaves and stems respectively (dry weight basis). Oxalate content of leaves and stems were 5.00 and 0.63 per cent respectively (Marderosian *et al.*, 1980).

Adverse nutritional effects are not seen with a consumption level of 100-200 g day^{-1} (Grubben and van Slotten, 1981). Analysis among eight amaranthus species for nitrate and oxalate content showed that *A. dubius* exhibited lowest content of both these antinutrients (Kauffman and Gilbert, 1981).

After analysing the oxalic acid and nitrate content of *A. retroflexus*, Hill and Rawati (1982) concluded that these factors would be important only if large amounts were eaten raw.

In a study using eight accessions of *A. tricolor*, Makus (1984) recorded that the contents of nitrates and soluble oxalates were 1.1 and 2.3 per cent respectively.

According to Teutonico and Knorr (1985), oxlates can comprise 0.2 to 11.4 per cent of the dry matter in vegetable amaranths.

A. dubius cv. Ibondwe proved to be superior among different species studied for carotene content (15.4 mg g^{-1} fresh weight) and low oxalate content (Sealy *et al.*, 1988).

George *et al.* (1989) compared the nutritive aspects of three species of vegetable amaranthus namely, *A. tricolor*, *A. dubius* and *A. cruentus* and noted that all green entries had low oxalate contents, while red and green-red entries had high oxalate contents ranging from 3.04 to 6.8 per cent. Vityakon and Standal (1989) also reported high levels of oxalates in *A. tricolor* (91 g kg^{-1}) on dry weight basis.

The oxalate content was found to range from 0.94 to 1.29 per cent and the least content of 0.94 per cent was seen in variety CO 3 (Devadas and Mallika, 1991). In the genus *Amaranthus*, section Blitopsis had higher content of antinutrients than section Amaranthus.

After conducting studies in 61 landraces involving vegetable and grain amaranthus, Prakash and Pal (1991) reported that the nitrate and oxalate contents varied from 1.8 to 9.2 g kg^{-1} and 3.0 to 19.2 g kg^{-1}

respectively on fresh weight basis. Oxalate content increased with advance in the growth period while nitrate content remained constant.

Red pigmented lines contained higher oxalate content when compared to green pigmented amaranthus lines (Devadas *et al.*, 1993; Priya and Celine, 2001).

After comparing 41 amaranthus lines, Thamburaj *et al.* (1994) reported that the oxalate content ranged from 0.820 to 0.921 per cent and that red types had higher oxalate content when compared to the green ones.

Guill *et al.* (1997) reported that in *A. viridis*, the nitrate content was 597 mg 100 g^{-1}. Bianco *et al.* (1998) reported extremely high levels of soluble oxalates in *A. retroflexus*.

According to Pal (1999) the variation in leaf nitrate was 0.18 – 0.8 per cent and that in oxalate was 0.51-1.92 per cent.

Mziray *et al.* (2001) analysed freshly harvested leaves of *A. hybridus* for antinutrients and reported a nitrate content of 501 to 560 mg per 100 g and oxalate content of 3383 to 4333 mg 100 g^{-1}.

Nitrate and oxalate contents were found to range from 0.29 to 0.89 per cent and 0.8 to 1.9 per cent respectively in vegetable amaranths (Tewari *et al.*, 2002).

Holubava (2002) evaluated the antinutrients in six amaranth genotypes and noted that the genotypes with highest amount of nitrate also contained the highest amount of oxalic acid.

The oxalate and nitrate levels were seen to vary from 0.62 - 3.85 per cent and 0.25 - 1.09 per cent respectively in *A. dubius* accessions as reported by Sindhu (2002).

2.1.3 Biotic stress

Diseases like leaf blight and pests like leaf webber are the major biotic stresses in amaranthus.

2.1.3.1 Leaf Blight

Leaf blight caused by *Rhizoctonia solani* Kuhn is a major problem that adversely affects amaranthus cultivation in Kerala. It greatly reduces the yield and lowers the market value of the crop.

Collar rot caused by Rhizoctonia solani was reported in amaranthus by Roy (1975).

A. tricolor is severely infected by *Rhizoctonia solani* during August – September in Kerala. Cream coloured spots are seen on the leaves which spread rapidly causing large scale damage and economic losses (Nayar *et al.*, 1996).

Gokulapalan *et al.* (1997) reported that the green amaranthus variety Co-1 exhibited excellent field tolerance to leaf blight and that the spread and severity of the disease can be lowered by raising of green and red amaranthus as a mixed crop.

After conducting a study in several amaranthus species, Priya (1998) concluded that *A. dubius* is the only species resistant to leaf blight.

Krishnakumary *et al.* (2001) screened 168 amaranthus accessions and noted that CO 1, a green variety, showed a disease incidence of less than 10 per cent.

Celine *et al.* (2002) evaluated the yield and leaf blight resistance of vegetable amaranth accessions and reported that *A. dubius* accessions were more superior in terms of yield and highly disease resistant. The *A. tricolor* accessions showed disease susceptibility of varying degrees.

Sindhu (2002) reported that *A. dubius* accessions are free from leaf blight under natural field conditions.

2.1.3.2 Leaf Webber

The infection of leaf webber will lower the yield and market value of the crop and it also makes the leaves unfit for culinary purposes.

The leaf webbers, *Psara basalis* Fab. and *Hymenia recurvalis* F. badly damage the leaves of vegetable amaranthus. Their caterpillars, web together the leaves of amaranthus and feed from within thereby completely skeletonising the leaves (Bhattacharjee and Menon, 1964; Nair, 1980). *Hymenia recurvalis* was reported as an important defoliator of *A. viridis* (Pande, 1973).

Leaf webber infestation on fourteen *Amaranthus hypochondriacus* cultivars was investigated in Orissa by Jena *et al.* (2001). Cultivars OGA-2, SKN-6, OGA-3 and Rasna-2 recorded the lowest leaf infestation rates (0.41, 0.48, 0.55 and 0.76 per cent respectively). Infestation was highest for SKN-7 (4.61 per cent).

2.2 GENETIC VARIABILITY, HERITABILITY AND CORRELATION

Mohideen and Subramanian (1974) suggested that leaf breadth, stem length and stem diameter are reliable characters for exercising selection based on a correlation study using the variety CO 1 belonging to *A. dubius*.

In a field experiment involving three weedy amaranths, three dominant species and naturally occurring crop-weed hybrids, very little intraspecific variation was seen (Hauptli and Jain, 1978).

Hauptli and Jain (1980) noted that there was large variation in flowering time, plant height, seed yield, harvest index and seed size in an amaranth population derived from a single plant. Late maturity, tallness and high yield correlated with harvest index. Red inflorescence and transluscent seed types had higher yield.

In his studies in amaranth, Vijayakumar (1980) found out that plant height had a positive and significant correlation with the green yield and

that leaf/stem ratio co-exhibited a significant negative association with yield of greens.

Mathai *et al.* (1980) estimated the leaf weight, stem weight, total green weight, protein and mineral contents of several varieties of *A. caudatus* and *A. tricolor* chosen from different countries. Protein content was positively correlated with plant weight in *A. caudatus* and Fe content was negatively correlated with leaf weight in *A. tricolor.*

Thirty F_1s and 30 F_2s involving six parents were grown and analysed for biometric and yield characters by Pandey (1981). The genotypic correlations were higher than corresponding phenotypic correlations. Grain yield was found to have high positive correlation with days to flowering, plant height, days to maturity etc and moderate positive correlation with all other characters except weight of grains/panicle and 1000 grain weight in which case, association was negative in parental populations.

Mohideen *et al.* (1982) studied the extent of variability in green yield and its components using 75 genotypes of amaranthus (*A. tricolor* L.) and reported that at the optimum harvest stage of 25th day, the genotypic coefficient of variation was high for stem weight, leaf/stem ratio, yield of greens and leaf weight. High heritability estimates along with high genetic advance for the above characters indicated that phenotypic selection for these traits will be more useful.

High heritability (broad sense) with high genetic advance was recorded for yield of greens, fresh weight of leaf, fresh weight of stem and number of leaves per plant. Plant height, leaf area and ascorbic acid content showed high heritability with moderate genetic advance. Considerably high heritability accompanied by low genetic advance were obtained for leaf/stem ratio, stem girth, dry weight of stem, dry weight of greens, percentage of crude protein and total chlorophyll content. Number

of branches per plant showed low heritability coupled with low genetic advance (Revanappa, 1985).

In a study involving 20 genotypes of *A. hypochondriacus,* wide variability for height, number of leaves per plant, leaf length and width, inflorescence length, number of spikelets per plant, days to maturity and 1000 seed yield per plant was recorded by Joshi (1986).

Kulakow and Jain (1987) assessed correlations between anthesis time and leaf length in *A. cruentus* and also studied single plant yield or yield components to evaluate correlated responses to selection. Selection for optimal flowering time is very likely to result in rapid yield improvement.

Correlation studies in amaranth highlighted leaf length to be the most important component of yield of greens followed by leaf width (Mohideen, 1988).

In amaranthus, the height and stem girth are positively correlated with yield (Hamid *et al.*, 1989).

In a study on heritability and yield correlations in 35 genotypes of *A. hypochondriacus*, panicle weight exhibited highest estimates of heritability and genetic advance (Das *et al.*, 1991).

Pan *et al.* (1991) in their studies on vegetable amaranthus (*A. tricolor*) reported that among the characters studied, days to flowering, number of clippings, duration of harvest, stem diameter and leaf width had high GCV values indicating the presence of greater extent of genetic variability. High heritability estimates, combined with high genetic advance as per cent of the mean were obtained for number of clippings, leaf width, duration of harvest, total green yield, stem diameter and leaf/stem ratio. They suggested that phenotypic selection for these traits would be most effective.

Highly significant positive genotypic as well as phenotypic association of total green yield with plant height, stem girth, number of nodes per plant, number of leaves, leaf length, fresh weight of leaves, days to 50 per cent bolting and total crude protein was recorded by Anuradha (1992) in her genetic studies on amaranthus.

Lohithaswa (1992) observed significant positive correlation of grain yield with plant height, inflorescence length, stem girth at collar, fresh weight of plant, number of rachis, fresh weight of inflorescence and dry weight of stem.

Pandey (1993) reported significant positive correlation between yield and yield contributing traits in amaranthus.

Varalakshmi and Reddy (1994) recorded high genotypic coefficient of variation for number of leaves, leaf weight, stem weight, leaf/stem ratio and green yield per plant. Yield exhibited significant and positive correlation with plant height, leaf length, leaf breadth, stem girth, leaf weight and stem weight.

Lohithaswa *et al.* (1996) observed high heritability coupled with moderate genetic advance for plant height and days to 50 per cent flowering in grain amaranthus.

Broad sense heritability was high for most characters studied but genetic advance was high for green yield per plant (61.07 %) and other yield parameters. Lowest genetic advance was observed for chlorophyll content (Revanappa and Madalgeri, 1997).

Evaluation of 40 genotypes of amaranthus for genetic variability led to the conclusion that PCV was higher than GCV for all characters (Revanappa and Madalgeri, 1998). PCV and GCV were maximum for leaf/stem ratio, number of leaves and fresh weight of leaves and minimum for stem girth.

Reddy and Varalakshmi (1998) studied heterosis, general and specific combining ability variances for nine characters in line x tester design in vegetable amaranth (*A. tricolor* L.). The results indicated the predominance of non-additive gene action for plant height, leaf number, leaf breadth, stem girth, stem weight and leaf weight. Additive gene action was preponderant for leaf/stem ratio.

Gowda *et al.* (1999) investigated correlation and regression in grain amaranthus and concluded that yield per ha was positively correlated with plant height, dry weight per plant, panicle length and weight and negatively correlated with leaf number, days to 50 per cent flowering and 1000 seed weight.

Coefficient of variability and genetic advance for foliage yield and its four main component traits were worked out by Shukla and Singh (2000). Broad sense heritability varied from 33.24 to 75.00 per cent. High heritability coupled with high genetic advance were seen for foliage yield (75.00 %), leaf size (74.98 %) and leaves per plant (73.43 %) indicating the prevalent role of additive gene effects.

Fifteen genotypes of amaranthus were evaluated for yield, yield attributes, quality characters, oxalate content and reaction to leaf blight and leaf webber. High values of PCV and GCV were obtained for most of the characters. High heritability together with high genetic gain was observed for leaf length, leaf width, leaf weight, fibre, oxalate and reaction to blight highlighting the chances for crop improvement through selection (Priya and Celine, 2001).

Phenotypic stability of eight genotypes of *A. hypochondriacus* was assessed during three consecutive years by Sharma *et al.* (2001) for five traits namely days to maturity, plant height, panicle length, harvest index and grain yield. Genotypes performed differently during different years and showed significant G x E interaction for all traits. Both linear and non-linear components showed dominant G x E interaction for harvest

index; only linear component for days to maturity; and non-linear for plant height, panicle length and grain yield.

Highest coefficient of variability was noted in number of inflorescence per plant (39.76) followed by leaf size (34.68 cm^2). Days to flowering contributed maximum towards genetic divergence followed by plant height, nodes per plant and leaf size (Shukla and Singh, 2002a).

Vaidya and Jain (2002) assessed 10 landrace populations of grain amaranths (*A. hypochondriacus* and *A. caudatus*) for genetic variation in quantitative and qualitative characters. Quantitative characters like leaf length, leaf width, petiole length, leaf number per plant, branches per plant and days to flowering showed significant genetic variation. Estimation of genetic variation between and within families showed that between family variances were significant for all metric characters.

Determination of genetic variability, heritability and genetic advance in several derivatives of *A. hypochondriacus* and *A. cruentus* revealed that genetic gain was highest for the number of inflorescence per plant, number of nodes per plant, leaf size and number of primary branches per plant. Co-heritability was high for all the trait combinations except for number of days to flowering with days to maturity and inflorescence length with grain yield (Shukla and Singh, 2003a).

In an experiment undertaken by Shukla and Singh (2003b) to analyse correlation and path analysis in grain amaranth genotypes, the genotypic correlation for yield and yield components was higher than the corresponding phenotypic correlation. At the phenotypic level, positive correlation was seen between grain yield per plant and plant height and leaf size. At genotypic level, grain yield per plant was positively associated with other traits. Leaf size was positively associated with all characters except inflorescence length.

Stability parameters for five quantitative traits (plant height, leaf number, leaf weight, stem weight and plant weight) were studied by Varalakshmi (2003) in 14 advanced breeding lines of vegetable amaranthus under three environments. One line each were found to be stable for plant height (AV 20), leaf weight (AV 29) and two for leaf number (AV 22 and AV 38). Significant positive correlation was seen between leaf number and leaf weight.

Fekova *et al.* (2003) suggested that thousand seed weight (TSW) decreased with plant height. No production trait was significantly influenced by plant height. There was a close relationship between genotype productivity, inflorescence length and TSW.

Twenty nine distinct strains of *A. tricolor* were evaluated to elucidate interrelationship among foliage yield and its seven contributing traits. Genotypic correlation values were generally higher than corresponding phenotypic correlation values in all cuttings for different traits. Foliage yield was positively correlated with plant height in all cuttings. Number of branches per plant was significantly correlated with number of leaves per plant and stem diameter in pooled as well as in all cuttings. Protein content had negative correlation with foliage yield and other traits. Plant height, leaf size and stem diameter were important characters for increasing yield (Shukla *et al.*, 2004).

2.3 PATH ANALYSIS AND D^2 ANALYSIS

Based on the results of a path coefficient analysis, Mohideen (1978) reported that weight of leaves, weight of stem, height, stem diameter and breadth of leaf contributed the highest direct and indirect positive effects on green yield.

According to Devadas *et al.* (1989), leaf width, plant height on bolting day, days to 50 per cent bolting and frequency of harvests are the

principal factors promoting the total vegetable yield. Das *et al.* (1991) have reported that 1000 grain weight contributed most to grain yield

Devadas *et al.* (1992) studied genetic divergence among 25 vegetable amaranthus accessions belonging to four botanical species and grouped them into seven clusters. Study of intracluster differences revealed that variability was maximum in *A. tricolor* when compared to other species.

Forty five indigenous and exotic genotypes of *A. tricolor* were evaluated for genetic divergence based on 10 quantitative traits. Analysis of variance revealed differences among the genotypes for all 10 characters and they were grouped into 10 clusters. Clustering pattern was associated with geographic distribution. Cluster 7 had high mean values for stem girth, leaf length and total yield. Cluster 3 had high mean values for leaf width and number of clippings. Duration of harvest and total yield accounted for most of the variation present (Pan *et al.*, 1992).

Following his studies on path coefficient analysis, Pandey (1993) suggested that harvest index had maximum direct effect on yield. Path coefficient analysis of a set of 20 amaranthus genotypes by Joshi and Rana (1995) showed that leaf length had maximum direct effect on grain yield followed by number of leaves, plant height and 1000 grain weight.

Sixty eight genotypes of grain amaranth (*A. hypochondriacus*) were evaluated for genetic divergence by Verma *et al.* (2002) and grouped into nine clusters. The clustering pattern indicated that genetic diversity is not related to geographical diversity. Genotypes in the different clusters showed substantial difference in the mean values of the various traits studied.

A path analysis study by Shukla and Singh (2003b) revealed that plant height was indirectly and positively associated to yield via number of days to flowering and leaf size. Leaf size, plant height, number of

inflorescence per plant, inflorescence length and days to maturity were major yield components.

2.4 MOLECULAR CHARACTERIZATION

Different types of markers such as morphological, biochemical and molecular markers are widely used to study the genetic variation in crop plants. Among these, molecular markers are used to characterize diversity at the molecular level, and therefore are environmentally independent. The use of these markers provide an effective selection technique for crop improvement and has an advantage over selection based on phenotype alone.

Molecular markers provide a chance to characterize genotypes and to measure genetic relationships more precisely than other markers (Soller and Beckmann, 1983). Various types of molecular markers are utilized to evaluate DNA polymorphism, the most important being polymerase chain reaction (PCR) based markers. Among the PCR markers, RAPD is the most widely used marker as it is highly cost effective, and requires the minimum time and labour.

2.4.1 Molecular characterization in amaranthus

Hauptli and Jain (1984) found that with the exception of the *A. caudatus* - *A. quitensis* pair, grain amaranthus are more closely related to each other than to their respective putative progenitors on the basis of isozyme data.

Transue *et al.* (1994) carried out RAPD analysis in 29 previously classified accessions of grain amaranth and 83 unclassified accessions. Known accessions were separated into three distinct groups corresponding to the morphological species identification and species designation was assigned to the unknown accessions. It was revealed that *A. caudatus* and *A. hypochondriacus* are genetically more similar than either species to *A. cruentus*.

On the basis of restriction- site variation in chloroplast and nuclear DNA, Lanoue *et al.* (1996) established that *A. caudatus* and *A. cruentus*

are more closely related to each other and to their respective putative and progenitors, *A. quitensis* and *A. hybridus* than either is to *A. hypochondriacus.* No close relationship was noted between *A. tricolor* and grain amaranthus.

Genetic diversity and relationships of 23 cultivated and wild *Amaranthus* species were examined using both isozyme and RAPD markers (Chan and Sun, 1997). High levels of inter-accessional genetic diversity were found within species, but genetic uniformity was observed within most accessions. Their studies revealed high levels of genetic similarity between *A. hypochondriacus* and *A. caudatus* and they suggested that the three species of grain amaranthus had a common ancestor namely *A. hybridus*. *A. dubius* was shown to be the next most closely related species to grain amaranthus after *A. hybridus*.

RAPD analysis has been carried out in amaranthus using 65 arbitrary sequence decamer primers which have been used to illustrate the inter and intra species relationships (Ranade *et al.*, 1997).

Dixit (1998) reported a simple, efficient and reliable method for isolation of total DNA from young leaves of amaranthus species. This procedure yielded high amount (600-800 μg DNA/g fresh leaf tissue) of good quality DNA free from contamination and suitable for digestion with restriction endonucleases, preparation of southern blots and PCR amplification. It was used for generating DNA fingerprint profiles and RAPD banding pattern in two species of amaranthus.

Sun *et al.* (1999) examined the genetic diversity and relationships among 24 cultivated and wild amaranthus accessions using the total low cot DNA and five individual repetitive sequences as probes which revealed different levels of polymorphism in the amaranthus genomes. Genetic diversity, measured as restriction fragment length polymorphism was high among interspecific accessions between the two species groups, *i.e.*, grain amaranthus and their wild progenitors, whereas at interspecific

level, grain amaranthus species are less divergent from each other than the wild types.

Studies were initiated by Wetzel *et al.* (1999) to develop a molecular marker identification system utilizing restriction enzyme analysis of amplified ribosomal DNA (rDNA) for the identification of weedy *Amaranthus* species. A set of PCR markers was developed to distinguish 10 such species.

Brenner *et al.* (2000) suggested that molecular techniques should be used together with morphological studies since RAPD and isozyme data do not always have the same dendrograms.

Polymorphic isozyme patterns were analysed in order to characterize eight commercial varieties and thirty five experimental strains obtained from an amaranth breeding programme (Renzo *et al.*, 2001). This provided a general overview of genetic diversity between the accessions and was found to be in coordination with the taxonomical classification and also illustrated interrelationships among the accessions.

Xu and Sun (2001) used internal transcribed spacer (ITS), amplified fragment length polymorphism (AFLP) and double-primer fluorescent inter simple sequence repeat (ISSR) to reexamine the taxonomic status and phylogenetic relationships of grain amaranthus and their wild relatives. Low ITS divergence in these taxa resulted in poorly resolved phylogeny. But, extensive polymorphisms exist at AFLP and ISSR loci both within and among species.

Mandal and Das (2002) conducted RAPD analysis to study the genetic diversity in three grain amaranthus species comprising a total of 17 accessions. Extent of polymorphism was highest in *A. cruentus* with 69.2 per cent followed by *A. caudatus* with 38.5 per cent and *A. hypochondriacus* having 15.4 per cent. Clustering pattern through dendrogram showed that *A. cruentus* stood apart while *A. hypochondriacus*

and *A. caudatus* overlapped, thereby suggesting higher level of genetic similarity between these two species.

Polymerase chain reaction (PCR) amplification of specific alleles(PASA) was adapted as a molecular marker based method for the rapid detection of point mutations in *A. retroflexus* and *A. rudis* leading to acetolactate synthase (ALS) inhibitor resistance. PASA is useful for identification of resistant weed biotypes (Wagner *et al.*, 2002).

In a study conducted by Jacobsen and Mujica (2003) to assess the genetic variability in Andean grain amaranths, it was revealed that a high degree of polymorphism exists both within and between populations of amaranth, even neighbouring ones, characterized by different alleles or isozymes. Manilay (2003) has assessed the genetic diversity in amaranthus using RAPD markers.

In order to determine which of the economically important weedy *Amaranthus* species are most genetically similar, Wassom and Tranel (2005) performed amplified fragment length polymorphism (AFLP)- based unweighted pair group method with arithmetic mean (UPGMA) analysis on eight such species represented by 98 accessions. Analysis grouped the specimens into four principal clusters.

2.4.2 Molecular characterization in related crops

Molecular characterization of genotypes using RAPD markers in related crops mainly salad crops, annuals like Capsicum, Phaseolus and Vigna are reviewed here.

Yang and Quiros (1993) screened 21 celery cultivars for polymorphic RAPD markers with 28 arbitrary decamer primers. Among a total of 309 bands observed, 29 (9.5 %) were polymorphic in the 23 cultivars screened. These markers were sufficient to distinguish each of the cultivars used.

Haley *et al.* (1994) evaluated the variation between and within the Andean and Middle American gene pools of common bean (*Phaseolus vulgaris*) using RAPD markers. A three-tired pattern of polymorphism was observed: between gene pools > between races (within same gene pool) > between genotypes (within same race).

Kesseli *et al.* (1994) constructed a genetic map from the F_2 population of a single intraspecific cross of *Lactuca sativa* comprising 319 loci using 152 RFLP and 130 RAPD markers. Both markers showed similar distributions throughout the genome and identified similar levels of polymorphism.

Nienhuis *et al.* (1995) studied 65 *Phaseolus lanatus* L. accessions from Caribbean and North, Central and South America. Based on 125 polymorphic random DNA bands, two major clusters which genetically correspond in seed size and geographic region to the Mesoamerican and Andean gene pools were identified.

Prince *et al.* (1995) examined interspecific variation among four *Capsicum annuum* cultivars using both RFLPs and RAPDs and reported the effectiveness of both the methods for DNA fingerprinting and discrimination of closely related *Capsicum annuum* genotypes.

Skroch and Nienhuis (1995) have done the qualitative and quantitative characterization of RAPD variation in *Phaseolus vulgaris*. Ten snap bean genotypes were screened for polymorphism with 400 RAPD primers. Polymorphic RAPDs were scored into three categories based on ethidium bromide staining intensity. They scored an average of 5.19 bands per primer for the 364 primers that gave scorable amplification products.

Liu (1996) evaluated genetic variation in 40 accessions of *Lablab purpureus* using RAPD markers. A high level of genetic variation in this species was detected but this was mainly restricted to the differences between cultivated and wild forms. In cultivated genotypes, genetic variation among Asian collections was significantly higher than that

among African collections. All the three most divergent cultivated genotypes were from Asia.

According to Rieseberg (1996) at the interspecific level, RAPD markers are considered less suitable for studying phylogenetic relationships because some of the co-migrating RAPD bands from different species may not be homologous.

Wang *et al.* (1996) surveyed 14 diverse *Capsicum* species by RAPD analysis and obtained high degree of polymorphism for four random decamer primers which produced 11 reproducible and effective amplification fragments useful for identification between species.

The genetic variability of 46 accessions of lima bean (*Phaseolus lanatus* L.) including wild forms and landraces were evaluated using RAPD markers which allowed the differentiation of two main groups (Fofana *et al.*, 1997). Wild forms and landraces were genetically differentiated and higher genetic diversity was seen among the latter than among the former.

Many Chilean landraces of common bean were found difficult to be classified into major gene pools by using morphological and ecological traits. With the help of RAPD technique, these Chilean landraces have been classified into two major gene pools-Chilean and Mesoamerican by John *et al.* (1997).

Random amplified polymorphic DNA analysis was widely used to evaluate genetic distance among accessions within and between different species of capsicum and of diverse geographic origin (Kang *et al.*, 1997; Wang *et al.*, 1997).

Genetic characterization of 51 pure lines from 13 landraces of three common bean (*Phaseolus vulgaris* L.) mixtures was undertaken by Briand *et al.* (1998) using RAPD analysis with 12 random decamers. The dendrogram generated by cluster analysis divided the individuals into two

main branches with less than 60 per cent similarity thereby suggesting that the 13 landraces might belong to two distinct gene pools of *P. vulgaris*.

Paran *et al.* (1998) examined genetic relationships among 34 pepper (*Capsicum annuum*) cultivars using RAPD and AFLP markers and compared their relative effectiveness. They noted that the percentage of polymorphic markers was lower for AFLP than for RAPD markers (13 and 22 % respectively). But AFLP primers amplified, on average, six times more products than RAPD markers.

Wang and Fan (1998) used misrosatellite DNA (ISSR) and RAPD markers to compare 90 accessions of *Capsicum annuum* from 16 different countries and observed that both ISSR and RAPD markers in addition to being simple and time efficient, allowed rapid identification of polymorphism within *C. annuum.*

Sultana *et al.* (2000) used RAPD markers to study phylogenetic relationships among 102 lablab bean accessions.101 RAPD fragments were generated using 11 single 12 mer primers of arbitrary nucleotide sequences. Two primers, CMN-A22 and CMN-A31 generated robust and easily interpretable markers between wild and cultivated accessions. A dendrogram constructed based on genetic similarity clearly separated wild type accessions from cultivated accessions.

Random amplified polymorphic DNA (RAPD) technique is the one which has been made use to its maximum in Phaseolus bean for variability studies (Galvan *et al.*, 2001)

Lefebvre *et al.* (2001) evaluated concordance of AFLP and RAPD markers for estimating genetic distances of 47 pepper inbred lines belonging to five varietal types. There was a general agreement between AFLP and RAPD markers and the molecular distances estimated by these methods were found to be in correlation with morphological distance based on agronomic traits.

Maciel *et al.* (2001) conducted a study to evaluate the variability among 15 cultivars and 18 landraces of common bean and an undefined species of *Phaseolus* by screening them with 15 primers in PCR reaction. A total of 304 amplification products were scored of which 88.8 per cent were polymorphic among *Phaseolus* genotypes.

Intraspecific relationships among 39 cultivars of *Lactuca sativa* var. *captata* were investigated by RAPD analysis using 50 primers out of which 12 gave amplification for all plants (Kioug and Seokwoo, 2003). 55 (78.6 %) of the 70 bands derived from the 12 primers showed polymorphism.

Lanteri *et al.* (2003) used RAPD and AFLP markers to assess genetic diversity within and between five populations of a landrace of *Capsicum annuum* grown in north-west Italy. Partitioning genetic variation revealed that 41.6 per cent occurred between and 58.4 per cent within populations. Analogous results were obtained when analysis was based only on RAPD or AFLP markers.

Ma *et al.* (2003) studied the genetic relationship among 46 chilli germplasm accessions by RAPD and genetic polymorphism was observed in 88.68 per cent of amplified bands from nine primers selected from a total of 160 primers. Accessions were classified into six groups by cluster analysis and results of RAPD were similar to those obtained using traditional methods.

A study was carried out by Nkongolol (2003) to determine the pattern and extent of RAPD marker variation within and among cowpea populations. Twenty of the 30 RAPD primers tested showed amplification of random loci and about 80 per cent of the scored loci were polymporhic. There was a general lack of agreement between clustering and morphological features.

A collection of 148 *Pisum* accessions including primitive and cultivated types were structured using 121 protein and PCR-based markers by Baranger *et al.* (2004). They noted that molecular marker based classification was largely consistent with available pedigree data and clearly resolved the different main varietal types.

DNA isolated from 14 cultivars of *Vigna radiata* was subjected to RAPD analysis by Betal *et al.* (2004). These cultivars revealed polymorphism with respect to RAPD markers and confirmed that two strongly aromatic cultivars, IC 1 and IC 4 were closely linked. RAPD results were in correlation with morphological characters.

Studies regarding the genetic variability in *Lactuca* species were carried out using isoenzymes and molecular markers (RFLP, RAPD, AFLP, SSR) by Dziechciarkova *et al.* (2004). The molecular markers contributed much to the elucidation of various aspects related to taxonomy, variability, biodiversity, genetics and breeding within *Lactuca* species.

Furini and Wunder (2004) characterized 94 Solanum accessions including egg plants and related species, both morphologically and molecularly by AFLP technique. AFLP data was found to correlate with the morphological classification.

Duran *et al.* (2005) compared the morphological characteristics, phenological traits and random amplified polymorphic DNA (RAPD) banding patterns of 54 Carribean bean landraces and cultivars with that of 11 Andean bean lines. RAPD polymorphism identified three groups, one corresponding to the genotypes with Mesoamarican morphologies, one to those with Andean morphologies, and another that had Andean phenotypes but proximity to Mesoamarican group, suggesting possible introgression between the genepools.

Serna *et al.* (2005) analyzed Mexican common bean (*Phaseolus vulgaris*) cultivars using amplified fragment length polymorphism (AFLP) fingerprinting to examine the genetic relationships within and among races. A Mexican *P. coccineus* cultivar was included for comparison. Broad genetic diversity was found within races, and diversity values between races were similar. The *P. coccineus* cultivar was quite distinct from all the *P. vulgaris* cultivars. A dendrogram based on AFLP analysis did not clearly match with that made on the basis of racial classification

Tosti and Negri (2005) conducted amplified fragment length polymorphism (AFLP) analysis in three neighbouring cowpea landraces to determine the distribution of genetic variation within and among them. Results showed that a relatively high level of diversity is still present within the landraces.

Materials and Methods

3. MATERIALS AND METHODS

The experiment entitled "Characterization and evaluation of landraces of amaranthus (*Amaranthus* spp.)" was conducted at the Department of Olericulture and Department of Plant Biotechnology, College of Agriculture, Vellayani, during the period 2003- 2005. The experimental site was located at 8° 5' N latitude and 77° 1' E longitude at an altitude of 29 m above mean sea level. Predominant soil type of the experimental site was red loam belonging to Vellayani series, texturally classified as sandy clay loam.

The experimental material consisted of 34 diverse accessions of amaranthus selected from the germplasm maintained in the Department of Olericulture, College of Agriculture, Vellayani based on morphological and geographical variations. Representative samples from the different districts of Kerala have been included. Among the selected 34 accessions, 25 belong to *Amaranthus tricolor*, six to *A. dubius* and and three to *A. hypochondriacus*. The details of the accessions and their source are given in Table 1.

Experiment was laid out in randomized block design with three replications during October (2004)-January (2005) (Plate 1). Seedlings were transplanted 25 days after sowing adopting a spacing of 30 x 20 cm. 20 plants were maintained in each plot. The crop received timely management practices as per package of practices recommendations of Kerala Agricultural University (KAU, 2002).

3.1 MORPHOLOGICAL CHARACTERIZATION.

Five plants were randomly selected from each plot and tagged for recording the morphological characters. Observations were recorded 30 days after transplanting and mean was worked out for further analysis.

Table 1. List of amaranthus accessions used for the study

Sl. No.	Accession number	Source	Special Features
1	Am 4	Thrissur	*Amaranthus tricolor*, red
2	Am 5	Thrissur	*A. tricolor*, red
3	Am 9	Manjeri, Malappuram	*A. tricolor*, red
4	Am 13	Ramapuram, Kottayam	*A. tricolor*, red
5	Am 14	Kakkamoola, Trivandrum	*A. tricolor*, red
6	Am 22	Bangalore	*A. tricolor*, green leaves, red stem
7	Am 25	Kollam	*A. tricolor*, red
8	Am 27	Alappuzha	*A. tricolor*, red
9	Am 28	NSC, Trivandrum	*A. tricolor*, Var. CO 2, green
10	Am 29	Kasaragode	*A. tricolor*, red
11	Am 31	Ernakulam	*A. tricolor*, red
12	Am 34	Ernakulam	*A. tricolor*, green leaves, pale red stem
13	Am 37	Wynad	*A. hypochondriacus*, purplish green leaves and stem
14	Am 40	CoA, Vellayani	*Amaranthus tricolor*, light green
15	Am 41	CoA, Vellayani	*A. tricolor*, light green
16	Am 42	CoA, Vellayani	*A. tricolor*, green leaves with purple centre, purple stem
17	Am 44	CoA, Vellayani	*A. tricolor*, green leaves with purple centre, purple stem
18	Am 45	CoA, Vellayani	*A. tricolor*, green leaves with purple veins and margins, purple stem
19	Am 47	CoA, Vellayani	*A. tricolor*, light green
20	Am 54	Idukki	*A. tricolor*, green leaves with purple veins and margins, purple stem
21	Am 55	Idukki	*A. tricolor*, green leaves, green stem
22	Am 58	Kalliyoor, Trivandrum	*A. tricolor*, red
23	Am 60	Kannur	*A. tricolor*, green leaves and stem
24	Am 63	Kannur	*A. tricolor*, green leaves and stem
25	Am 64	Palakkad	*A. hypochondriacus*, purplish green leaves and stem
26	Am 67	Palakkad	*A. hypochondriacus*, purplish green leaves and stem
27	Am 71	Vellayani, Trivandrum	*A. dubius*, green
28	Am 72	Pathanamthitta	*A. dubius*, green
29	Am 76	Venganoor, Trivandrum	*A. tricolor*, Red
30	Am 77	KAU, Vellanikkara	*A. tricolor*, Var. Arun
31	Am 78	KAU, Vellanikkara	*A. dubius*, Var. CO 1
32	Am 89	AD-14, CoA, Vellayani	*A. dubius*, green
33	Am 90	AD-23, CoA, Vellayani	*A. dubius*, green
34	Am 91	AD-30, CoA, Vellayani	*A.dubius*, green

Plate 1. General view of the field experiment

3.1.1 Growth Characters

3.1.1.1 Plant Height

Height of the plant was measured from the ground level to the top most leaf bud of all observational plants, average worked out and expressed in centimetres.

3.1.1.2 Stem Girth

The girth of main stem at the collar region was taken using a twine. Mean girth was worked out and expressed in centimetres.

3.1.1.3 Length of Leaf Lamina

The fifth leaf from top of the selected plants was used for recording the length. Length was measured and expressed in centimetres.

3.1.1.4 Leaf Width

The width of the same leaf used for recording the length was measured and expressed in centimetres.

3.1.1.5 Petiole Length

The petiole length of the same leaf used for recording the length was measured and expressed in centimetres.

3.1.1.6 Number of Branches

The total number of branches of each observational plant was counted and the average was worked out.

3.1.1.7 Days to 50 per cent Bolting

Days to 50 per cent bolting was recorded from the plants left unharvested.

3.1.2 Yield Characters

3.1.2.1. Yield per cutting

Three cuttings were taken from each plant. The first cutting was taken 30 days after transplanting and the next two cuttings were taken at

subsequent intervals of two weeks. The yield obtained per cutting was recorded and expressed in grams per plant.

3.1.2.2. Total yield

Yield per plant from the three cuttings was separately recorded and then added to get the total yield per plant and expressed in grams per plant.

3.1.2.3. Total leaf weight

The leaf weight per cutting was summed up and the total leaf weight was worked out.

3.1.2.4. Total stem weight

The stem weight per cutting was added up and the total stem weight was calculated.

3.1.2.5 Leaf / Stem Ratio

Leaf / stem ratio was obtained by dividing the weight of leaves by weight of stem. Leaf/stem ratio was worked out for the three cuttings.

3.1.3 Quality Characters

3.1.3.1 β-carotene

Carotene content of fresh leaves at first harvest (30 DAT) was estimated according to the method proposed by Srivastava and Kumar (1998).

Reagents

Acetone, anhydrous sodium sulphate, petroleum ether.

Procedure

Five gram of fresh sample was crushed in 10-15 ml acetone after adding a few crystals of anhydrous sodium sulphate, with the help of pestle and mortar. The supernatant was decanted into a beaker. The process was repeated twice and the combined supernatant was transferred

to a separatory funnel, 10-15 ml of petroleum ether was added and mixed thoroughly. The two layers were seperated on standing. The lower layer was discarded and the upper layer was collected in a 100 ml volumetric flask. The volume was made upto 100 ml with petroleum ether and the optical density was recorded at 452 nm using petroleum ether as blank.

$$\beta\text{-carotene } (\mu g\ 100\ g^{-1}) = \frac{\text{Optical density} \times 13.9 \times 10^{4} \times 100}{\text{Weight of sample} \times 560 \times 1000}$$

3.1.3.2 Ascorbic Acid

Ascorbic acid content of fresh leaves at first harvest (30 DAT) was estimated by 2,6-dichlorophenol indophenol dye method (Sadasivam and Manickam, 1996).

Reagents

(i) Oxalic acid (4 %)

(ii) Ascorbic acid standard

Stock solution was prepared by dissolving 100 mg of ascorbic acid in 100 ml of four per cent oxalic acid. 10 ml of this stock solution was diluted to 100 ml with four per cent oxalic acid to get working standard solution.

(iii) 2, 6-dichlorophenol indophenol dye

42 mg sodium bicarbonate was dissolved in a small volume of distilled water. 52 mg 2,6-dichlorophenol indophenol was added into this and made upto 200 ml with distilled water.

Procedure

5 ml of the working standard solution was pipetted out into a 100 ml conical flask and 10 ml of four per cent oxalic acid was added. It was titrated against the dye (V_1 ml). End point was the appearance of pink

colour which persisted for atleast five seconds. One gram of fresh leaf was extracted in an acid medium (4 % oxalic acid) and made upto a known volume (20 ml) and centrifuged. 5 ml of the supernatant was taken and titrated against the dye until pink colour appeared (V_2 ml). Ascorbic acid content was calculated using the formula.

$$\text{Amount of ascorbic acid (mg 100 g}^{-1}\text{ sample)} = \frac{0.5 \times V_2 \times \text{Vol. made up}}{V_1 \times 5 \text{ ml} \times \text{weight of sample}} \times 100$$

3.1.3.3 Oxalates

Estimated by the method suggested by A.O.A.C. (1984).

Reagents

(i) Tungsto phosphoric acid : 2.5 g sodium tungstate was dissolved in a mixture of 4 ml phosphoric acid and 50 ml water and made upto 100 ml with water.

(ii) Wash liquid : 12.5 ml acid was made upto 250 ml with water. After adding a pinch of calcium oxalate, it was shaken well and allowed to stand. The supernatant was decanted and filtered.

(iii) Acetate buffer (pH 4.5)

2.5 g of anhydrous calcium chloride was dissolved in 50 ml acetic acid (1: 1 diluted) followed by addition of a solution of 33 g of sodium acetate diluted to 5 ml.

(iv) Potassium permanganate : 0.01 N

(v). Sulphuric acid : 2 N

(vi). Hydrochloric acid : 0.25 N

Procedure

One gram of dried powder was extracted twice with 0.25 N hydrochloric acid in a water bath for one hour each. The extract was

centrifuged and the supernatant was collected in a conical flask. The extract was precipitated by adding 5 ml tungsto phosphoric acid, kept overnight and centrifuged the next day. It was neutralized with 1 : 1 dilute ammonia solution and precipitated by adding 5 ml acetate buffer containing calcium chloride (pH 4.5). The precipitate was centrifuged and washed twice with wash liquid (6 ml each). The precipitate was then dissolved in 10-15 ml 2 N sulphuric acid and transferred into a 100 ml conical flask. It was titrated against 0.01 N potassium permanganate solution at 60°C.

$$\text{Percentage oxalate} = \frac{0.063 \times V}{1\text{ g}}$$

3.1.3.4 Nitrate

Nitrate was estimated according to the procedure suggested by Middleton (1958).

Reagents

(i) Copper sulphate : 0.5 per cent solution

(ii) Silver sulphate : 0.35 per cent solution

(iii) Sodium phosphate : 138 g was dissolved in 500 ml water, followed by addition of strong NaOH solution to bring the pH to 6.5 and made upto 1 litre

(iv) Calcium hydroxide – magnesium carbonate mixture : One part of calcium hydroxide and two parts of magnesium carbonate was triturated in a mortar.

(v) Phenol-p-sulphonic acid : Mercuric chloride was added to 225 ml dilute sulphuric acid (1 : 5) at the rate of 1 g/5 ml of acid. This was kept overnight and the next day 25 g phenol and 10 ml ethyl alcohol was added to the acid. This mixture was heated on a water bath for two hours.

(vi) Ammonium hydroxide : 50 per cent (v/v)

(vii) Potassium nitrate : 0.0505 per cent solution

Procedure

0.1 g dried tissue was weighed in a beaker or flask. 9 ml silver sulphate was added and swirled quickly. One ml sodium phosphate was added immediately and it was allowed to stand for two hours. This solution was filtered. 2 ml of filtrate was measured into a 15 ml centrifuge tube, 2 ml copper sulphate solution was added and solution was mixed thoroughly followed by addition of water and made upto 6 ml. Approximately 0.5 g calcium hydroxide - magnesium carbonate mixture was added to this, mixed and allowed to stand for one hour and centrifuged at 3000 rpm for five minutes. 2 ml phenol-p-sulphonic acid was poured into a boiling tube, directly to the bottom. 2 ml supernatant was added drop by drop from above directly into the reagent, swirling carefully after the addition of each drop. This was cooled and 25 ml ammonium hydroxide was added with stirring. After proper cooling, the absorbance was read in a spectrophotometer at 475 nm with the instrument set at zero with water.

Standard : Aliquots of standard solution (potassium nitrate) from one to four ml were used and the above procedure was followed beginning with the addition of copper sulphate. The absorbance values of these solutions were taken and the standard graph was drawn.

One ml of 0.0505 per cent potassium nitrate solution contained 0.01 mg nitrogen. From the standard graph, the nitrogen content (mg/100 g) of sample was found out.

Nitrate content (mg/100 g sample) = Nitrogen content (mg/100 g) x 4.428

The nitrate content thus estimated was converted into percentage.

3.1.3.5 ***Protein***

Protein was estimated by Bradford method (Sadasivam and Manickam, 1996).

1. Dye concentrate: 100 mg of coomassie brilliant blue G 250 was dissolved in 50 ml of 95 per cent ethanol. 100 ml of concentrated orthophosphoric acid was added and final volume was made upto 200 ml with distilled water. It was stored under refrigerated conditions in amber bottles. 1 volume of concentrated dye solution was mixed with 4 volumes of distilled water for use. This was filtered with Whatman No.1 filter paper if any precipitate occurred.
2. Phosphate-buffer saline (PBS)
3. Protein solution (Stock standard): 50 mg of bovine serum albumin was accurately weighed and dissolved in distilled water and made upto 50 ml in a standard flask.
4. Working standard : 10 ml of the stock solution was diluted to 50 ml with distilled water in a standard flask. One ml of this solution contained 200 μg protein.

Procedure

500 mg of the fresh leaves was weighed and ground well with a pestle and mortar in 5-10 ml of the buffer. This was centrifuged and the supernatant was used for protein estimation.

0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard was pipetted out into a series of test tubes. 0.1 ml of the sample extract was pipetted out into 2 other test tubes. The volume was made upto 1 ml in all the test tubes. A tube with 1 ml of water is used as blank. 5 ml of diluted dye solution was added to each tube. This was mixed well and the colour was allowed to develop for five minutes, but not longer than 30 minutes. The absorbance was read at 595 nm. A standard curve was plotted using standard protein absorbance vs concentration. The protein in the sample was calculated using the standard curve.

3.1.4 Screening for Incidence of Pests and Diseases

3.1.4.1 Reaction to Leaf Blight

The accessions were monitored for the incidence and intensity of leaf blight and scoring was done on a 0 – 4 scale.

0 – No incidence

1 – Upto 25 per cent leaf area infected

2 – Upto 50 per cent leaf area infected

3 – Upto 75 per cent leaf area infected

4 – Upto 100 per cent leaf area infected

The scoring was done three times at biweekly intervals after transplanting and the average score was worked out.

3.1.4.2 Reaction to Leaf Webber

The leaf Webbers,*Hymenia recurvalis* and *Psara basalis*, attack amaranthus.Scoring was done by using the following score chart.

0 – No incidence

1 – Mild (25 per cent)

2 – Medium (50 per cent)

3 – Severe (75 per cent)

4 – Very severe (100 per cent)

The scoring was done thrice at fortnightly intervals after transplanting and the average score was calculated.

3.2 STATISTICAL ANALYSIS

The data collected were subjected to the following statistical analysis.

3.2.1 Analysis of Variance and Covariance

Analysis of variance and covariance was done according to Singh and Choudhary (1979) to test the significant difference among the accessions and to estimate variance components and other genetic parameters like correlation coefficients, heritability, genetic advance etc (Table 2).

	X	Y
Environmental variance (σ^2_e)	$= \sigma^2_{ex} = E_{xx}$	$\sigma^2_{ey} = E_{yy}$
Genotypic variance (σ^2_g)	$= \sigma^2_{gx} = \dfrac{G_{xx}-E_{xx}}{r}$	$\sigma^2_{gy} = \dfrac{G_{yy}-E_{yy}}{r}$
Phenotypic variance (σ^2_p)	$= \sigma^2_{px} = \sigma^2_{gx}+\sigma^2_{ex}$	$\sigma^2_{py} = \sigma^2_{gy} + \sigma^2_{ey}$

3.2.2 Coefficient of Variation

Phenotypic, genotypic and environmental coefficients of variation were estimated as :

$$GCV = \frac{\sigma_{gx}}{\bar{x}} \times 100$$

$$PCV = \frac{\sigma_{px}}{\bar{x}} \times 100$$

$$ECV = \frac{\sigma_{ex}}{\bar{x}} \times 100$$

where σ_{gx} = genotypic standard deviation

σ_{px} = phenotypic standard deviation

σ_{ex} = environmental standard deviation

$\bar{x}$ = mean of the character under study

Table 2 Analysis of variance / covariance

Source	df	Observed mean square XX	Expected mean square XX	Observed mean sum of products XY	Expected mean sum of products XY	Observed mean square YY	Expected mean square YY
Block	r-1	B_{xx}		B_{xy}		B_{yy}	
Genotype	v-1	G_{xx}	$\sigma^2_{ex} + r\sigma^2_{gx}$	G_{xy}	$\sigma^2_{exy} + r\sigma^2_{gxy}$	G_{yy}	$\sigma^2_{ex} + r\sigma^2_{ge}$
Error	(v-1) (r-1)	E_{xx}	σ^2_{ex}	E_{xy}	σ^2_{exy}	E_{yy}	σ^2_{ey}
Total	(rv-1)	T_{xx}		T_{xy}		T_{yy}	

3.2.3 Heritability (Broad sense)

$$H^2 = \frac{\sigma^2_{gx}}{\sigma^2_{px}} \times 100$$

Where H^2 is the heritability expressed in percentage (Jain, 1982). Heritability estimates were categorized as suggested by Johnson *et al.* (1955).

0 – 30 per cent → Low

31 – 60 per cent → Moderate

> 60 per cent → High

3.2.4 Genetic Advance as Percentage of Mean

$$GA = \frac{kH^2 \sigma_p}{\bar{x}} \times 100$$

Where k is the standardized selection differential. k = 2.06 at five per cent selection intensity (Miller *et al.*, 1958).

The range of genetic advance as per cent of mean was classified according to Johnson *et al.* (1955).

0 – 10 per cent → Low

11 – 20 per cent → Moderate

> 20 per cent → High

3.2.5 Correlation Analysis

$$\text{Genotypic correlation coefficient } (r_{gxy}) = \frac{\sigma_{gxy}}{\sigma_{gx} \times \sigma_{gy}}$$

$$\text{Phenotypic correlation coefficient } (r_{pxy}) = \frac{\sigma_{pxy}}{\sigma_{px} \times \sigma_{py}}$$

Environmental correlation coefficient (r_{exy}) = $\frac{\sigma_{exy}}{\sigma_{ex} \times \sigma_{ey}}$

3.2.6 . Path Analysis

The direct and indirect effects of yield contributing factors were estimated through path analysis technique (Wright, 1954; Dewey and Lu, 1959).

3.2.7 Mahalanobis's D^2 Analysis

Genetic divergence was studied based on 6 characters taken together using D^2 statistic. The landraces were clustered by Tocher's method as described by Rao (1952).

3.3 CATALOGUING OF THE GERMPLASM

3.3.1 Morphological Cataloguing

The accessions were described morphologically using modified descriptor developed from the standard descriptor for amaranthus by IPGRI (Appendix I).

The cataloguing was done on appropriate scales ranging from 0-9.

3.4 MOLECULAR CHARACTERIZATION

3.4.1 Materials

Out of the thirty four accessions of amaranthus used in the field experiment, 27 were subjectd to molecular characterization using RAPD markers. Seven accessions were left out because they were morphologically identical with those already included in the study. Out of these 27, three belong to *A. hypochondriacus*, five to *A. dubius* and the remaining 19 belong to *A. tricolor*.

3.4.2 Methods

3.4.2.1 Isolation of Genomic DNA

The method of isolation used was that of Murray and Thompson (1980) with modifications. Leaf samples were collected from young tender leaves of amaranthus seedlings. One gram of tender leaf material was first washed in running tap water and later in distilled water two times and finely chopped. These chopped leaves were dried using tissue paper and then pulverized in liquid nitrogen after adding a pinch of PVP in a pre-cooled mortar by rapid grinding and made into a fine powder. This dry powder was then transferred to a 2 ml centrifuge tube and extraction buffer (0.7 N NaCl, 1 % CTAB, 50 mM Tris HCl (pH 8.0), 10 mM EDTA) at the rate of 1 ml per 100 mg dry weight of powder was added so that clumps could easily be dispersed but the solution remained somewhat viscous. 5 μl of β-mercaptoethanol was also added to the centrifuge tube and then incubated in a water bath at 60°C for 60 minutes with occasional gentle shaking. Then the tube was taken out and 200 μl of phenol : chloroform : isoamyl alcohol (25 : 24 : 1) solution was added to it. The mixture was then subjected to centrifugation at 10000 rpm for 10 minutes. The clear supernatant was taken and remaining extraneous matter was discarded. After that 200 μl of chloroform : Isoamyl alcohol (24 : 1) solution was added to the centrifuge tube, the two phases were mixed gently and centrifuged at 10000 rpm for 10 minutes at 4.0°C. The upper aqueous phase was collected and the previous step was repeated until the interphase disappeared. The aqueous phase was collected after centrifugation (10000 rpm) and to that 100 μl of 3.0 M sodium acetate and double volume of chilled isopropyl alcohol were added. This mixture was kept in the refrigerator at 4°C overnight. It was then centrifuged at 10000 rpm for 10 minutes at 4°C to pellet the DNA. The supernatant was discarded and the pellet was washed in 500 μl of 70 per cent ethanol. Then it was centrifuged at 10000 rpm for five minutes at 4°C. The

supernatant was discarded and the washing was repeated. The final pellet obtained was air dried until the alcohol was fully evaporated and the pellet was dissolved in 100 µl of 0.1 x Tris EDTA buffer (1 mM Tris HCl, 0.1 mM EDTA, pH 8) and stored at -20°C.

All the materials used in the preparation and storage of reagents and final DNA suspension including reagent bottles, conical flasks, centrifuge tubes, spatula, pestle and mortar and tips of micro pipettes were washed with Labolin solution, rinsed with distilled water and autoclaved.

3.4.2.2 Quantification of DNA

DNA quantification was carried out with the help of UV-Vis spectrophotometer (Spectronic Genesys 5).

The buffer (0.1 x TE) was taken in a cuvette to calibrate the spectrophotometer at 260 and 280 nm wavelength. The optical density (OD) of the samples dissolved in the buffer is recorded at both 260 and 280 nm.

The quantity of DNA in the sample is estimated by using the following formula.

Amount of DNA (µg/ml) = A_{260} x 50 x dilution factor

Where, A – Absorbance at 260 nm

The quality of DNA can be judged from the ratio of the O.D. values recorded at 260 and 280 nm. A_{260} / A_{280} ratio between 1.6 and 1.8 indicates good quality of DNA, where A_{280} is the absorbance at 280 nm.

3.4.2.3 Agarose Gel Electrophoresis

Agarose gel electrophoresis was carried out in a horizontal gel electrophoresis unit supplied by Bangalore Genei. The required amount of agarose was weighed out (0.8 per cent for visualizing the genomic DNA and 1.2 per cent for visualizing the amplified products) and melted in 1 x TAE buffer (0.04 mM Tris acetate, 0.01 mM EDTA, pH 8) by boiling.

After cooling to about 50°C, ethidium bromide was added to a final concentration of 0.5 μg ml^{-1}. The mixture was then poured to a preset template with appropriate combs. After solidification, the combs and the sealing tapes were removed and the gel was placed in an electrophoresis tank filled with 1 x TAE buffer. The gel was completely covered on the surface by the buffer. The DNA sample was mixed with the required volume of gel loading buffer (6 x loading dye *viz.*, 40 per cent sucrose, 0.25 per cent bromophenol blue). Each well was loaded with 20 μl of sample. One of the wells was loaded with 5.0 μl of molecular weight marker along with the required volume of gel loading buffer. Electrophoresis was performed at 75 volts until the loading dye reached ¾th of the length of the gel. The gel was visualized using gel documentation system (BIO RAD, USA).

3.4.2.4 Random Amplified Polymorphic DNA (RAPD) Analysis

RAPD analysis was performed following the procedure recommended by Mandal and Das (2002) with required modifications.

Thirty eight arbitrarily designed primers (31 supplied by Operon Inc. USA and 7 from University of British Columbia) were used for DNA amplification. The Operon primers were taken from Kits A, E and U.

Polymerase chain reaction of genomic DNA (10 ng) was performed in 25 μl of reaction mixture containing 2.5 μl 10 x PCR buffer (10 mM Tris HCl pH 9.0, 1.5 mM $MgCl_2$, 50 mM KCl and 0.01 per cent gelatin), 10 pM primer, 200 μM each of dNTPs and 0.75 units of Taq DNA polymerase (Invitrogen, USA).Amplification was performed in a Programmable Thermal Controller (PTC-100, MJ Research Inc.) set for the following programme: An initial denaturation at 94°C for 4 min followed by 45 cycles of denaturation at 93°C for 30 seconds, annealing at 36°C for 1 min and extension at 72°C for 2 min and a final step of extension at 72°C for 5 min. Finally the products of amplification were

cooled to 4°C. A negative control containing sterile water instead of template was included in each reaction set.

Amplified products along with DNA molecular weight marker were size fractionated on a 1.2 per cent agarose gel prepared in 1 x TAE buffer and stained with ethidium bromide. DNA fragments were visualized and photographed using a gel documentation system (BIO RAD, USA). The RAPD bands were represented as '+' for presence and '-' for absence and recorded. The PCR was repeated twice in order to confirm the reproducibility. The amplified products of four primers alone which could produce amplification for most of the clones were used for further analysis.

Data Analysis

The reproducible bands were scored for their presence (+) or absence (-) for all the amaranthus accessions. A genetic similarity matrix was constructed using Jaccard's coefficient method (Jaccard, 1908).

$$Sj = a/(a+b+c)$$

Where,

a: number of bands present in both the landraces in a pair.

b: number of bands present in the first landrace, but not in the second one.

c: number of bands present in the second landrace, but not in the first.

Based on the similarity coefficient, the distance between the landraces was computed with the help of software package NTSYS (Version 2.02). Using the values of the distance between landraces, a dendrogram was constructed by UPGMA (Unweighted pair group method with arithmetic average). Association between the various landraces was found out from the dendrogram.

Results

4. RESULTS

Thirty four diverse accessions of vegetable amaranths were subjected to morphological characterization during 2003-2005 to evaluate the biometric characters, quality characters and incidence of pests and diseases. The data was subjected to statistical analysis to find the variability, heritability, correlation, path analysis and D^2 analysis. Molecular characterization of the accessions was also carried out using RAPD markers. The results obtained from the statistical analysis are presented below.

4.1 MORPHOLOGICAL CHARACTERIZATION.

4.1.1 Mean performance of the accessions

The analysis of variance revealed significant variation among the 34 accessions for all the characters studied. The mean values of the accessions for growth, yield and quality characters and incidence of pests and diseases are given below.

4.1.1.1 Growth

The mean performance of the accessions for growth characters like plant height, leaf length, leaf width, petiole length, number of branches, stem girth and days to 50 per cent bolting are given in Table 3. Plant height was maximum in Am 89 (74.46 cm) and minimum in Am 47 (17.01 cm).

Since leaf is the economic part in amaranthus, larger leaf size is preferable. The accession Am 67 had longest leaf lamina (25.16 cm) whereas Am 55 had the shortest (7.9 cm). Leaf width was maximum in Am 14 (11.86 cm) and minimum in Am 55 (5.02 cm). Longest petiole was seen in Am 37 (11.76 cm) and shortest in Am 22 (2.41 cm).

The accession Am 37 had the highest number of branches (12.29) and Am 47, the lowest (7.18). Maximum stem girth was noted in Am 67 (5.0 cm) and minimum in Am 14 (2.58 cm). Am 37 took maximum days to 50 per cent bolting (72.67) whereas Am 72 was the earliest (45.27).

Table 3. Mean performance of amaranthus accessions in terms of growth characters

Accessions	Plant height (cm)	Length of leaf lamina (cm)	Leaf width (cm)	Petiole length (cm)	Number of branches	Stem girth (cm)	Days to 50 per cent bolting
Am 4	47.53	10.99	6.40	3.91	8.49	4.14	50.01
Am 5	31.35	15.90	9.69	4.42	8.02	2.88	50.86
Am 9	26.83	14.08	10.70	5.18	9.29	3.12	59.42
Am 13	26.89	10.89	6.76	3.44	8.83	2.64	42.28
Am 14	23.74	14.59	11.86	5.98	9.01	2.58	60.73
Am 22	48.92	11.67	5.73	2.41	9.11	4.03	47.83
Am 25	21.89	15.76	10.12	4.99	8.90	3.67	60.04
Am 27	17.44	13.30	7.94	5.02	8.26	2.86	60.56
Am 28	49.39	14.12	7.61	4.71	8.90	3.78	47.92
Am 29	36.11	13.69	7.67	4.03	9.22	3.39	53.18
Am 31	48.22	15.47	9.19	3.66	9.78	3.79	62.38
Am 34	37.71	15.67	9.98	3.91	11.03	4.28	62.91
Am 37	71.62	22.41	9.82	11.76	12.29	4.89	72.67
Am 40	25.89	14.06	10.48	4.14	9.39	3.58	49.85
Am 41	22.19	13.86	8.43	3.47	9.89	4.17	49.59
Am 42	41.89	13.34	8.59	4.57	9.62	3.35	48.08
Am 44	36.67	13.84	8.02	5.02	9.81	4.11	59.79
Am 45	35.39	12.95	7.65	3.59	8.94	3.54	57.94
Am 47	17.01	13.52	9.99	4.34	7.18	3.33	65.05
Am 54	49.98	16.10	9.00	6.56	9.41	4.10	51.51
Am 55	32.98	7.90	5.02	3.08	9.49	3.64	60.25
Am 58	27.50	14.83	9.13	4.11	8.50	3.32	65.86
Am 60	42.29	17.10	8.86	5.47	9.36	3.72	50.36
Am 63	39.39	15.68	7.44	4.47	8.68	3.80	48.86
Am 64	43.50	13.31	7.45	3.95	9.62	4.23	56.88
Am 67	42.32	25.16	6.60	2.78	9.88	5.00	76.30
Am 71	6.92	15.89	10.97	8.35	9.91	4.14	57.35
Am 72	51.39	18.30	10.86	5.79	9.45	4.09	45.27
Am 76	34.83	15.19	9.89	4.42	9.51	3.26	49.26
Am 77	36.74	13.89	11.18	5.22	8.99	3.02	53.29
Am 78	30.80	15.62	8.68	7.28	8.01	3.63	60.82
Am 89	74.46	12.89	8.02	5.03	10.17	4.76	56.04
Am 90	51.28	14.38	8.63	5.18	11.39	4.39	58.02
Am 91	70.16	17.07	9.01	6.66	11.61	4.83	58.68
CD (0.05)	6.019	3.500	2.683	2.129	0.611	0.479	3.438

4.1.1.2 Yield

In the first cutting, highest total yield was obtained for Am 89 (166.11 g) and lowest for Am 27 (20.56 g). In the subsequent cuttings, Am 91 and Am 5 were the highest and lowest yielders respectively (Table. 4).

Total leaf weight was maximum for Am 91 (235.56 g) and minimum for Am 13 (30.0 g). In case of total stem weight, Am 71 (189.44 g) had the highest value and Am 13 (21.66 g) had the lowest. Highest total yield was recorded in Am 91 (387.22 g) and lowest in Am 13 (51.67 g). The highest yielding accessions among *A. tricolor*, *A. dubius* and *A. hypochondriacus* are shown in Plates 2, 3 and 4 respectively.

Leaf/stem ratio is an important factor influencing yield and was recorded for the individual cuttings as well as total yield. Highest leaf/stem ratio for the first cutting was observed in Am 89 (3.15) and in Am 13 for next two cuttings. Lowest ratios in first two cuttings were seen in Am 22 and in Am 71 for the third cutting. In case of total leaf/stem ratio, the highest value was noted in Am 77 (2.38) and lowest value in Am 22 (0.53).

4.1.1.3 Quality

Nutritional factors like β-carotene, vitamin C and protein and antinutritional aspects like oxalate and nitrate were estimated in the study (Table 5).

The β-carotene content was maximum in Am 5 (4655.54 μg/100 g) and minimum in Am 90 (1269.94 μg/100 g). Am 78 had the highest vitamin C content of 151.22 mg/100 g and Am 58 had the lowest (54.88 mg/100 g). Highest protein content was noticed in Am 91 (3.57 %) and lowest in Am 27 (0.67 %).

In general, the antinutrients were lower in the *A. dubius* accessions. The lowest oxalate content of 0.6 per cent was recorded in Am 90 and

Table 4. Mean performance of amaranthus accessions in terms of yield characters

Accessions	Leaf/ stem ratio (first cutting)	Leaf/ stem ratio (second cutting)	Leaf/ stem ratio (third cutting)	Total leaf/ stem ratio	Yield (first cutting), g $plant^{-1}$	Yield (second cutting), g $plant^{-1}$	Yield (third cutting), g $plant^{-1}$	Total yield (g $plant^{-1}$)	Total leaf weight (g $plant^{-1}$)	Total stem weight (g $plant^{-1}$)
Am 4	0.69	1.24	0.89	0.84	57.22	25.00	18.89	101.11	46.11	55.00
Am 5	0.67	1.76	0.75	0.82	42.78	15.00	12.78	70.55	31.67	38.89
Am 9	1.89	1.51	0.75	1.39	33.33	26.67	19.44	79.45	45.55	33.89
Am 13	1.00	2.23	2.07	1.55	18.89	18.89	13.89	51.67	30.00	21.66
Am 14	2.02	1.10	1.50	1.55	46.66	27.22	28.89	102.78	62.22	40.55
Am 22	0.56	0.58	0.69	0.53	65.00	56.11	51.11	172.22	65.00	127.22
Am 25	1.12	1.20	1.80	1.19	30.00	20.56	17.78	68.33	37.22	31.11
Am 27	1.64	1.49	0.83	1.29	20.56	27.78	19.99	68.33	38.33	29.99
Am 28	0.91	0.76	0.62	0.79	50.55	34.45	17.22	102.22	45.56	56.66
Am 29	0.80	1.00	1.25	0.93	39.44	17.78	21.67	78.89	37.78	41.11
Am 31	1.41	0.82	1.16	1.07	60.00	55.56	29.44	145.56	75.55	70.00
Am 34	1.02	0.94	0.88	0.95	84.44	47.22	31.66	163.33	79.45	83.89
Am 37	1.51	1.49	1.42	1.51	127.22	30.00	24.44	181.67	107.78	73.89
Am 40	1.71	1.09	1.41	1.41	68.33	40.56	28.89	137.78	80.55	57.22
Am 41	2.33	1.85	0.94	1.78	64.44	42.22	28.89	135.56	86.11	49.44
Am 42	1.47	1.34	1.49	1.41	56.67	37.22	37.78	131.67	76.11	55.55
Am 44	1.75	1.47	1.64	1.62	82.22	56.11	42.22	180.56	111.67	68.89
Am 45	1.02	1.25	1.35	1.14	38.34	26.11	21.11	85.56	45.56	40.00
Am 47	1.09	1.84	2.16	1.64	38.33	52.22	26.11	118.33	71.11	45.56
Am 54	1.13	0.83	0.65	0.97	50.55	47.78	36.11	134.45	61.11	73.33
Am 55	0.82	1.04	1.58	1.09	31.11	27.22	19.44	77.78	40.00	38.89
Am 58	1.25	1.05	0.89	1.03	30.56	24.45	19.44	74.44	36.67	37.78
Am 60	0.96	0.92	0.79	0.87	72.22	62.78	43.89	181.67	83.89	97.78
Am 63	0.79	1.62	0.82	0.97	160.56	74.44	35.56	270.56	132.22	138.33
Am 64	1.12	0.63	1.20	0.88	46.11	28.89	20.56	98.33	46.11	52.22
Am 67	1.03	0.77	0.86	0.87	37.22	36.67	26.11	100.00	46.67	53.33
Am 71	0.83	0.89	0.34	0.73	165.00	99.44	62.78	327.22	137.78	189.44
Am 72	2.22	0.68	0.40	0.92	105.55	128.33	66.11	300.00	143.33	156.67
Am 76	1.69	1.41	1.18	1.39	51.11	65.00	52.78	168.89	97.22	71.67
Am 77	2.95	2.17	1.83	2.38	87.22	66.11	30.56	183.89	128.89	55.00
Am 78	1.45	1.51	1.39	1.45	91.11	91.11	68.89	251.11	148.33	102.78
Am 89	3.15	0.85	1.43	1.62	166.11	128.89	78.33	373.33	230.33	142.99
Am 90	1.44	0.93	1.30	1.19	159.44	120.56	55.00	337.22	183.33	153.89
Am 91	2.44	1.20	1.45	1.56	137.22	166.66	81.67	387.22	235.56	151.66
CD (0.05)	0.431	0.718	0.695	0.398	14.614	11.216	11.665	22.333	15.322	16.779

Table 5. Mean performance of amaranthus accessions in terms of quality characters

Accessions	Protein (%)	β-carotene (μg/100 g)	Vitamin C (mg/100g)	Nitrate (%)	Oxalate (%)
Am 4	1.83	3739.75	82.32	0.84	1.04
Am 5	2.42	4655.54	73.18	1.04	1.61
Am 9	1.61	1414.38	94.52	1.01	1.81
Am 13	1.41	2391.19	100.61	0.85	1.34
Am 14	1.48	2717.47	94.52	0.75	1.72
Am 22	0.67	3718.01	92.68	0.71	0.65
Am 25	0.82	4299.75	67.08	0.86	1.21
Am 27	0.67	1783.16	94.51	0.99	1.15
Am 28	1.09	3437.72	107.32	1.62	1.15
Am 29	3.08	1967.10	91.46	0.77	1.53
Am 31	1.27	2417.18	73.17	0.80	1.54
Am 34	1.58	2184.10	119.51	0.66	1.38
Am 37	0.97	2998.51	68.29	0.04	0.71
Am 40	1.68	4137.43	91.46	0.68	0.78
Am 41	2.44	3577.93	97.56	0.68	0.71
Am 42	2.17	4025.32	72.97	0.68	1.05
Am 44	3.46	2072.52	151.22	0.61	1.54
Am 45	0.73	2431.26	100.00	0.55	0.75
Am 47	0.74	2499.96	87.02	0.79	0.96
Am 54	1.17	3467.06	87.81	0.76	0.88
Am 55	3.48	4120.71	112.19	0.85	0.92
Am 58	1.51	2504.75	54.88	0.71	1.18
Am 60	3.38	2662.63	109.76	0.61	1.18
Am 63	1.70	1847.57	102.44	0.47	1.96
Am 64	0.75	3633.84	146.34	0.05	0.78
Am 67	1.56	4241.30	89.03	0.05	0.73
Am 71	3.30	2803.25	79.27	0.57	0.86
Am 72	3.43	3353.82	113.69	0.61	1.32
Am 76	2.57	2765.02	67.43	1.58	2.08
Am 77	1.49	2541.93	67.08	0.75	1.59
Am 78	3.39	3470.99	151.22	0.28	1.03
Am 89	2.60	1778.05	98.89	0.50	0.79
Am 90	1.61	1269.94	93.09	0.38	0.60
Am 91	3.57	3967.82	109.76	0.50	0.63
CD(0.05)	0.451	309.813	22.606	0.145	0.184

Plate 2. Highest yielding *Amaranthus tricolor* accession

Plate 3. Highest yielding *Amaranthus dubius* accession

Plate 4. Highest yielding *Amaranthus hypochondriacus* accession

maximum in Am 76 (2.08 %) whereas the nitrate content was lowest in Am 37 (0.04 %) and highest in Am 28 (1.62 %).

4.1.1.4 Incidence of Pests and Diseases

Leaf blight caused by *Rhizoctonia solani* and leaf webber (*Psara basalis* and *Hymenia recurvalis*) were noted in the experimental plot. Scoring of the accessions are given in Table 6.

The highest leaf blight incidence was observed in Am 14 and Am 77 (2.00) while the lowest score of zero was observed in Am 89 and Am 91 both belonging to the species *A. dubius*. Leaf webber occurrence was most severe in Am 78 (2.07) and least in Am 42 (0.63).

4.1.2. Genetic variability, heritability and genetic advance

The population mean, range, genotypic coefficients of variation (GCV) and phenotypic coefficients of variation (PCV), heritability, genetic advance and genetic gain (as percentage of mean) for the 24 characters were studied and are presented in Table 7.

4.1.2.1. Growth characters

Plant height ranged from 17.01 to 74.46 cm with a mean of 39.89 cm. The GCV was 36.64 and PCV was 37.79. Genetic gain as percentage of mean was 75.18. Heritability was as high as 94 per cent while genetic advance was only 29.19.

Leaf length ranged from 7.9 – 25.16 cm and had a mean value of 14.35 cm. The GCV and PCV values were 18.72 and 23.66 respectively. Heritability was only 63 per cent and genetic gain was 31.49. Genetic advance was calculated to be 4.52.

Leaf width showed a range of 5.02 – 11.86 cm and the mean was 8.75 cm. GCV was found to be 14.99 and PCV was 24.03. Heritability was very low, only 39 per cent. Genetic gain was 19.31 and the genetic advance, 1.69.

Table 6. Mean performance of amaranthus accessions in terms of reaction towards leaf blight and leaf webber

Accessions	Leaf blight (Average score)	Leaf webber (Average score)
Am 4	1.40	1.40
Am 5	1.17	1.20
Am 9	1.47	2.00
Am 13	1.77	0.93
Am 14	2.00	1.37
Am 22	1.30	2.00
Am 25	1.10	0.87
Am 27	1.80	1.43
Am 28	0.93	0.90
Am 29	1.37	0.80
Am 31	1.10	0.77
Am 34	0.93	1.10
Am 37	0.57	1.13
Am 40	1.27	1.83
Am 41	1.30	1.50
Am 42	1.20	0.63
Am 44	1.67	1.80
Am 45	1.80	1.90
Am 47	0.93	1.43
Am 54	0.93	1.03
Am 55	0.50	1.97
Am 58	1.33	1.90
Am 60	1.33	1.67
Am 63	0.10	1.03
Am 64	0.53	0.90
Am 67	0.33	1.10
Am 71	0.07	1.13
Am 72	0.03	1.13
Am 76	1.57	1.30
Am 77	2.00	1.87
Am 78	0.07	2.07
Am 89	0.00	1.13
Am 90	0.03	0.80
Am 91	0.00	1.17
CD (0.05)	0.809	0.464

Table 7. Range, mean, PCV, GCV, heritability, genetic advance and genetic gain as percentage of mean in amaranthus accessions for important characters

Characters	Range	Mean ± SE	GCV	PCV	Heritability (%)	Genetic advance	Genetic gain (as per cent of mean)
Plant height (cm)	17.01-74.46	39.89±2.128	36.64	37.79	94.00	29.19	73.18
Length of leaf lamina (cm)	7.90-25.16	14.35±1.238	88.72	23.66	63.00	4.52	31.49
Leaf width (cm)	5.02-11.86	8.75±0.948	48.99	24.03	39.00	1.69	19.31
Petiole length (cm)	2.41-11.76	4.91±0.753	32.06	41.64	59.00	2.49	50.71
Number of branches	7.18-12.29	9.41±0.215	10.75	11.46	88.00	1.96	20.83
Stem girth (cm)	2.58-5.00	3.77±0.163	16.02	17.81	81.00	1.12	29.71
Days to 50 per cent bolting	45.27-72.67	56.17±1.215	13.35	13.86	93.00	14.87	26.47
Leaf/stem ratio (1st cutting)	0.56-3.15	1.41±0.152	44.30	48.09	85.00	1.18	83.69
Leaf/stem ratio (2nd cutting)	0.58-2.23	1.22±0.253	28.07	45.67	38.00	0.43	35.27
Leaf/stem ratio (3rd cutting)	0.34-2.07	1.17±0.245	32.59	48.87	44.00	0.52	44.44
Total leaf/stem ratio	0.53-2.38	1.22±0.140	29.07	35.30	68.00	0.59	48.36
Yield (g) (1st cutting)	18.89-166.11	71.04±5.166	61.14	62.42	96.00	87.64	123.37

Table 7. Continued

Characters	Range	Mean ± SE	GCV	PCV	Heritability (%)	Genetic advance	Genetic gain (as per cent of mean)
Yield (g) (2nd cutting)	15.00-166.66	53.68±3.965	68.85	70.03	97.00	74.85	139.44
Yield (g) (3rd cutting)	12.78-81.67	34.98±4.124	52.86	56.67	87.00	35.53	101.57
Total yield (g)	51.67-387.22	160.05±7.895	58.37	58.98	98.00	190.40	118.96
Total leaf weight (g)	30.00-235.56	86.02±5.417	62.76	63.69	97.00	109.57	127.38
Total stem weight (g)	21.66-189.44	74.59±5.931	58.98	60.57	95.00	88.26	118.33
Protein (per cent)	0.67-3.57	1.93±0.156	49.85	51.15	95.00	1.93	1.00
β-carotene (μg 100 g^{-1})	1269.94-4655.54	2967.56± 107.651	30.52	30.95	97.00	1840.09	62.01
Vitamin C (mg 100 g^{-1})	54.88-155.22	93.39± 7.854	22.77	25.58	79.00	39.82	42.64
Nitrate (per cent)	0.04-1.62	0.67 ± 5.047	48.37	49.46	96.00	0.68	101.49
Oxalate (per cent)	0.60-2.08	1.17 ± 6.395	35.04	35.91	95.00	0.81	70.43
Leaf blight	0.00-2.00	0.999 ±0.286	56.69	75.39	57.00	0.88	88.89
Leaf webber	0.63-2.07	1.33 ± 0.164	29.65	36.55	66.00	0.66	49.62

Petiole length ranged from 2.41 – 11.76 cm and showed a mean value of 4.91 cm. The GCV and PCV were 32.06 and 41.64 respectively. Heritability was 59 per cent and genetic gain was 50.71. Genetic advance was only 2.49.

Stem girth varied from 2.58 to 5.0 cm and the mean was 3.77. The GCV was 16.02 and PCV was 17.81. Heritability was 81 per cent, genetic gain was 29.71 and genetic advance was 1.12.

Mean number of branches per plant was 9.41 and the range was 7.18 – 12.29. GCV and PCV values were 10.75 and 11.46 respectively. Heritability noted was 88 per cent and genetic gain was 20.83. Genetic advance was very low, only 2.49.

Days to 50 per cent bolting ranged from 45.27 – 72.67 days with an overall mean of 56.17. GCV was 13.35 and PCV was 13.86. Heritability was found to be 93 per cent. Genetic gain and genetic advance were 26.47 and 14.87 respectively.

4.1.2.2 Yield Characters

In the first cutting, leaf/stem ratio ranged from 0.56 – 3.15 and the general mean was 1.41. The GCV and PCV were 44.30 and 48.09 respectively. Heritability was 85 per cent and genetic gain was 83.69. Genetic advance was noted to be 1.18.

The range was 0.58 to 2.23 with an overall mean of 1.22 in case of the second cutting ratio. GCV was 28.07 with a PCV of 45.67. Heritability was only 38 per cent, genetic gain was 35.25 and genetic advance was found to be only 0.43. For the third cutting, the ratio varied from 0.34 – 2.07 and mean value was 1.17. GCV and PCV values were 32.59 and 48.87 respectively. Heritability was only 44 per cent which is a low value. Genetic gain was 44.44 and the value of genetic advance was 0.52.

In case of total leaf/stem ratio, range was 0.53 – 2.38 and mean value was 1.22. GCV was 29.07 and PCV was 35.30. Heritability showed

a value of 68 per cent. Genetic gain was 48.36 and genetic advance was 0.59.

Yield at first cutting ranged from 20 – 166.11 g and overall mean was 71.04 g. GCV and PCV values were 61.14 and 62.42 respectively. Heritability was 96 per cent, a high value and genetic gain was 123.37. Genetic advance was as high as 87.64.

Yield at second cutting varied from 15 – 166.66 g and mean value was 53.65 g. GCV was 68.85 and PCV was 70.03. Heritability was very high, upto 97 per cent. Genetic gain was 139.44 with a genetic advance of 74.85.

Yield at third cutting ranged from 12.78 – 81.67 g with a mean value of 34.98 g. GCV was 52.86 and PCV was 56.67. Heritability was 87 per cent and genetic gain was 101.57. The value of genetic advance was 35.53 which is the lowest of the three cuttings.

As for total yield, the overall mean was 160.05 with a range of 51.67 – 387.22. GCV and PCV values were 58.37 and 58.98 respectively. Heritability was very high upto 98 per cent and genetic gain was 118.96. A high genetic advance of 190.40 was recorded for this character.

Total leaf weight ranged from 30 g to 235.56 g and overall mean was 86.02 g. GCV and PCV values were 62.76 and 63.69 respectively. Heritability was 97 per cent. High values of 127.38 and 109.57 were seen for genetic gain and genetic advance respectively.

The range and general mean for total stem weight were 21.66 to 189.44 g and 74.59 g respectively. GCV was 58.98 and PCV was 60.57. Heritability was as high as 95 per cent. Genetic gain was 118.33 and genetic advance was noted to be 88.26.

4.1.2.3 Quality Characters

Protein content ranged from 0.67 to 3.57 per cent with a mean value of 1.93 per cent. GCV and PCV values were 49.85 and 51.15 respectively.

Heritability was 95 per cent and genetic gain was 100 while the genetic advance was only 1.93.

The range and overall mean of β-carotene was 1269.94 to 4655.54 μg/100 g and 2967.56 μg/100 g respectively. GCV was 30.52 and PCV was 30.95. Heritability was high, upto 97 per cent and genetic gain was 62.01. A high genetic advance of 1840.09 was seen for this character.

Vitamin C value ranged from 54.88 to 151.22 mg/100 g with a general mean of 93.39 mg/100 g. GCV was 22.77 and PCV was 25.58. Heritability was only 79 per cent and genetic gain was 42.64. A low value of 39.82 was observed for genetic advance.

Nitrate content varied from 0.04 to 1.62 per cent and 0.67 per cent was the mean value. GCV was 48.37 and PCV was 49.46. Heritability was 96 per cent. Genetic gain and genetic advance were 101.49 and 0.68 respectively.

Oxalate content ranged from 0.6 to 2.08 per cent and 1.15 per cent was the general mean. PCV and GCV were 35.91 and 35.04 respectively. Heritability was 95 per cent and genetic gain was 70.43. Genetic advance was only 0.81.

4.1.2.4 Incidence of Pest and Disease

Leaf blight incidence ranged from 0.00 – 2.00 and 0.99 was the overall mean. GCV was 56.69 and PCV was 75.39. Heritability was 57 per cent which is very low. Genetic gain and genetic advance were 88.89 and 0.88 respectively.

Leaf webber incidence ranged between 0.63 – 2.07 and mean was 1.33. GCV was 29.65 and PCV was 36.55. Heritability was only 66 per cent and genetic gain was 49.62. The value of genetic advance was 0.66.

4.1.3 Correlation studies

The phenotypic, genotypic and error correlation among 19 morphological and yield characters were worked out and are presented in Tables 8, 9 and 10 respectively. Similarly the correlation among the five

Table 8. Phenotypic correlation coefficients in amaranthus accession for growth, yield and reaction to biotic stress

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	1																		
2	0.2934	1																	
3	-0.0463	0.4146*	1																
4	0.3936*	0.4483**	0.5026**	1															
5	0.6541**	0.3374	0.0942	0.3702*	1														
6	0.0286	0.4027*	0.0861	0.2311	0.2304	1													
7	0.6843**	0.4078*	-0.1389	0.2147	0.6462**	0.2888	1												
8	0.6130**	0.1947	0.1911	0.3800*	0.4773**	-0.0031	0.4870**	1											
9	0.6767**	0.2161	0.1002	0.3056	0.4056	-0.1257	0.5381**	0.7921**	1										
10	0.6753**	0.2244	0.1706	0.3757*	0.4712**	-0.0546	0.5384**	0.9616**	0.9253**	1									
11	0.1653	0.0688	0.3278	0.1951	0.2618	0.0145	0.0774	0.5864**	0.1515	0.4201*	1								
12	-0.3464*	-0.1478	0.0586	-0.0022	-0.2324	-0.0610	-0.3827*	-0.0208	-0.3242	-0.1596	0.1699	1							
13	-0.1995	-0.2364	-0.0142	-0.0489	-0.0502	0.1475	-0.2219	0.0631	-0.3219	-0.1105	0.1594	0.4753**	1						
14	-0.4893**	-0.2332	0.0297	-0.2328	-0.3022	-0.1608	-0.4948**	-0.5062**	-0.5718**	-0.5767**	-0.0104	0.1983	0.0822	1					
15	0.6896**	0.2410	0.1706	0.4553**	0.5243**	-0.0076	0.5495**	0.8729**	0.8753**	0.9236**	0.3142	-0.1196	-0.1469	-0.5507**	1				
16	0.5849**	0.1799	0.1544	0.2493	0.3791*	-0.0798	0.4646**	0.9279**	0.8588**	0.9501**	0.4630**	-0.152	-0.0555	-0.5484**	0.7744**	1			
17	0.5584**	0.1885	0.1363	0.2981	0.3434*	-0.0947	0.4473**	0.8593**	0.8121**	0.8858**	0.4168*	-0.1984	-0.0974	-0.4643**	0.6874**	0.8850**	1		
18	-0.1673	-0.0954	0.2297	0.1488	0.0517	0.0903	-0.2004	0.3214	-0.2209	0.0932	0.6989**	0.7028**	0.6445**	0.1457	0.0537	0.1256	0.0884	1	
19	-0.3007	-0.1754	0.0282	-0.0572	-0.2587	0.0701	-0.1865	-0.0602	-0.1229	-0.1051	0.1118	0.1122	-0.0363	0.2346	-0.1492	-0.0797	-0.0103	0.1572	1

*Significant at 5 per cent level **significant at 1 per cent level

1. Plant height (cm)
2. Length of leaf lamina (cm)
3. Leaf width (cm)
4. Petiole length (cm)
5. Number of branches
6. Days to 50 per cent bolting
7. Stem girth (cm)
8. Total leaf weight (g)
9. Total stem weight (g)
10. Total yield (g)
11. Leaf/stem ratio (1st cutting)
12. Leaf/stem ratio (2nd cutting)
13. Leaf/stem ratio (3rd cutting)
14. Leaf blight
15. Yield (g) (1st cutting)
16. Yield (g) (2nd cutting)
17. Yield (g) (3rd cutting)
18. Total leaf/stem ratio
19. Leaf webber

Table 9. Genotypic correlation coefficients in amaranthus accession for growth, yield and reaction to biotic stress

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	1																		
2	0.3269	1																	
3	-0.1164	0.2596	1																
4	0.5090**	0.4691**	0.4904**	1															
5	0.6754**	0.4079*	0.0611	0.4313**	1														
6	0.0343	0.5376**	0.1211	0.2908	0.2679	1													
7	0.7618**	0.5050	-0.3168	0.2585	0.7024**	0.3370	1												
8	0.6323**	0.1862	0.2537	0.4552**	0.5062**	-0.0043	0.5225**	1											
9	0.7063**	0.2279	0.115	0.3919*	0.4306**	-0.1322	0.5714**	0.8182**	1										
10	0.6922**	0.2218	0.216	0.4576**	0.4964**	-0.0571	0.5674**	0.9662**	0.9389**	1									
11	0.1741	0.0763	0.5479**	0.2552	0.3029	0.3019	0.0965	0.6193**	0.1799	0.4521**	1								
12	-0.5633**	-0.1896	0.2837	0.1418	-0.3624*	-0.1700	-0.6053**	-0.0844	-0.4370	-0.2409	0.2439	1							
13	-0.312	-0.3176	0.0256	-0.0384	-0.0481	0.1767	-0.2409	0.1653	-0.4035**	-0.1450	0.3259	0.5420**	1						
14	-0.6754**	-0.4439**	0.1644	-0.3829*	-0.4400**	-0.2060	-0.8389**	-0.6898**	-0.8241**	-0.7855**	-0.0212	0.6055**	0.3691*	1					
15	0.7156**	0.2516	0.2029	0.5399**	0.5529**	-0.0107	0.5925**	0.8871**	0.8893**	0.9338**	0.3358	-0.1534	-0.1567	-0.7683**	1				
16	0.6067**	0.1876	0.2181	0.3442*	0.4137*	-0.0814	0.5016**	0.9443**	0.8823**	0.9608**	0.5138**	-0.2537	-0.1177	-0.7414**	0.8042**	1			
17	0.5941**	0.1514	0.1814	0.3541*	0.3752*	-0.1030	0.4612**	0.9000**	0.8807**	0.9274**	0.4730**	-0.3384	-0.1349	-0.6708**	0.7539**	0.9525**	1		
18	0.2004	-0.0981	0.4837**	0.2398	0.0879	0.0857	-0.2114	0.3474*	-0.1924	0.1226	0.8024**	0.6777**	0.7361**	0.3606*	0.0927	0.1517	0.1015	1	
19	-0.3540*	-0.3194	-0.0835	-0.1132	-0.3343	0.1152	-0.2453	-0.0813	-0.1554	-0.1303	0.1209	0.2891	0.1043	0.3542*	-0.2041	-0.0939	0.0168	0.2572	1

*Significant at 5 per cent level **significant at 1 per cent level

1. Plant height (cm)
2. Length of leaf lamina (cm)
3. Leaf width (cm)
4. Petiole length (cm)
5. Number of branches
6. Days to 50 per cent bolting
7. Stem girth (cm)
8. Total leaf weight (g)
9. Total stem weight (g)
10. Total yield (g)
11. Leaf/stem ratio (1st cutting)
12. Leaf/stem ratio (2nd cutting)
13. Leaf/stem ratio (3rd cutting)
14. Leaf blight
15. Yield (g) (1st cutting)
16. Yield (g) (2nd cutting)
17. Yield (g) (3rd cutting)
18. Total leaf/stem ratio
19. Leaf webber

Table 10. Error correlation coefficients in amaranthus accession for growth, yield and reaction to biotic stress

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	1																		
2	0.2852	1																	
3	0.1263	0.5991**	1																
4	0.0869	0.4165*	0.5355**	1															
5	0.4700**	0.1641	0.2157	0.2654	1														
6	-0.0518	-0.0404	0.0631	0.0905	-0.1236	1													
7	0.1863	0.1814	0.1138	0.1279	0.3536*	-0.0253	1												
8	0.2131	0.4732**	0.2628	0.3177	0.1596	0.0225	0.3211	1											
9	0.2216	0.2913	0.1705	0.0813	0.1557	-0.0283	0.3787*	0.1837	1										
10	0.3161	0.5728**	0.3289	0.2933	0.2094	-0.0069	0.5282**	0.7947**	0.6270**	1									
11	0.1031	0.0552	0.0427	0.0568	0.0008	-0.0201	-0.0149	0.3667*	-0.112	0.1431	1								
12	-0.0551	-0.1153	-0.0814	-0.1378	-0.0857	0.1856	-0.1393	0.2244	-0.3493*	-0.1143	0.1035	1							
13	0.0123	-0.1511	-0.0427	-0.0614	-0.0778	0.169	-0.2376	0.1673	-0.3531*	-0.137	-0.1408	0.4277*	1						
14	0.0198	0.0766	-0.0922	-0.0263	0.0358	-0.0653	0.2523	0.0439	0.2118	0.0817	0.0168	-0.157	-0.2096	1					
15	0.1847	0.3734*	0.2958	0.3738*	0.2343	0.0462	0.3130	0.4885**	0.5897**	0.6384**	0.1440	-0.1709	-0.2965	0.1146	1				
16	0.1458	0.3046	0.1445	-0.0961	-0.0387	-0.056	0.2632	0.4218*	0.3386	0.5848**	-0.0322	0.0092	0.1592	-0.0017	0.0008	1			
17	0.2389	0.3483*	0.1092	0.1906	0.1208	-0.0221	0.3832*	0.5225**	0.1473	0.5724**	0.0741	-0.0153	-0.0503	0.0263	-0.019	0.1745	1		
18	-0.0524	-0.0909	-0.0424	-0.009	-0.0823	0.1455	-0.1768	0.4075*	-0.5166**	-0.0807	0.4083*	0.8039**	0.5682**	-0.2076	-0.1846	0.0267	0.0508	1	
19	-0.1558	0.0825	0.1541	0.036	-0.0216	-0.1256	-0.0295	0.0477	-0.0009	-0.0057	0.0942	-0.0692	-0.2127	0.0481	0.11	-0.0455	-0.1091	-0.0439	1

*Significant at 5 per cent level **significant at 1 per cent level

1. Plant height (cm)
2. Length of leaf lamina (cm)
3. Leaf width (cm)
4. Petiole length (cm)
5. Number of branches
6. Days to 50 per cent bolting
7. Stem girth (cm)
8. Total leaf weight (g)
9. Total stem weight (g)
10. Total yield (g)
11. Leaf/stem ratio (1st cutting)
12. Leaf/stem ratio (2nd cutting)
13. Leaf/stem ratio (3rd cutting)
14. Leaf blight
15. Yield (g) (1st cutting)
16. Yield (g) (2nd cutting)
17. Yield (g) (3rd cutting)
18. Total leaf/stem ratio
19. Leaf webber

nutrient characters was calculated and are shown in Tables 11, 12 and 13 respectively.

4.1.3.1 Phenotypic Correlation Coefficients

Plant height had significant positive correlation with yield at first cutting (.6896). It exhibited positive correlation with several other characters such as stem girth (0.6843), total stem weight (0.6767), total yield (0.6753), number of branches (0.6541) and total leaf weight (0.6130). Plant height had significant negative correlation with leaf blight incidence (-0.4893) followed by the leaf/stem ratio for the second cutting (-0.3464) and leaf webber incidence (-0.3007).

Leaf length showed significant positive correlation with petiole length (0.4483), leaf width (0.4146), stem girth (0.4078) and days to 50 per cent bolting (0.4027). It showed negative correlation with leaf blight scoring (-0.2332).

Leaf width showed significant positive correlation of 0.5026 with petiole length. It exhibited correlation values of 0.3278 and 0.2297 with leaf/stem ratio (first cutting) and total leaf/stem ratio respectively. It had negative correlation with stem girth (-0.1389).

Petiole length exhibited significant positive correlation with first cutting yield (0.4553). A positive correlation of 0.3800, 0.3757 and 0.3702 were seen for total leaf weight, total yield and number of branches respectively.

Number of branches showed significant positive correlations of 0.6462 with stem girth and 0.5243 with yield at first cutting. It had positive correlations of 0.4773 and 0.4712 with total leaf weight and total yield respectively.

Days to 50 per cent bolting showed positive correlation with stem girth (0.2888). It showed negative correlation of –0.1608 and –0.1257 with leaf blight scoring and total stem weight respectively.

Table 11. Phenotypic correlation coefficients for quality characters

	1	2	3	4	5
1	1				
2	-0.3256	1			
3	-0.0679	-0.0948	1		
4	0.0908	0.1105	0.3099	1	
5	-0.0256	0.5022**	-0.2621	-0.0396	1

Table 12. Genotypic correlation coefficients for quality characters

	1	2	3	4	5
1	1				
2	-0.3478*	1			
3	-0.0573	-0.1342	1		
4	0.0859	0.1038	0.3606	1	
5	-0.0277	0.5155**	-0.3377*	-0.0391	1

Table 13. Error correlation coefficients for quality characters

	1	2	3	4	5
1	1				
2	0.2493	1			
3	-0.2334	0.2180	1		
4	0.2204	0.2415	-0.0288	1	
5	0.0308	0.2215	0.3363	-0.0505	1

*Significant at 5 per cent level **significant at 1 per cent level

1. β-carotene (μg 100 g^{-1})
2. Oxalate (per cent)
3. Vitamin C (mg 100 g^{-1})
4. Protein (per cent)
5. Nitrate (per cent)

Stem girth had significant positive correlation values of 0.5495, 0.5384 and 0.5381 with yield at first cutting, total yield and total stem weight respectively, whereas it showed high negative correlation of –0.4948 and –0.3827 with leaf blight scoring and leaf/stem ratio for second cutting respectively.

Total leaf weight showed highly significant positive correlation with total yield (0.9616). Leaf weight had correlation values of 0.9279, 0.8729 and 0.8593 with yield at second, first and third cuttings respectively. Negative correlation value of –0.5062 was seen for leaf blight scoring.

A similar trend was noticed in case of total stem weight. It had significant positive correlation with total yield (0.9255) followed by yield at first (0.8753), second (0.8588) and third (0.8121) cuttings respectively. Negative correlation was maximum for leaf blight incidence (-0.5718).

Total yield had significant positive correlation of 0.9501 with second cutting yield followed by first (0.9236) and third cuttings (0.8858). Here also, negative correlation was high with leaf blight scoring (-0.5767).

Leaf/stem ratios for first, second and third cuttings exhibited significant positive correlation values of 0.6989, 0.7028 and 0.6445 respectively with total leaf/stem ratio. Total leaf/stem ratio had negative correlation with total stem weight (-0.2209) and stem girth (-.2004).

Leaf blight had positive correlation with leaf webber incidence (0.2346). It had significant negative correlation with yield at first cutting (-0.5507) and second cutting (-0.5484).

β-carotene content showed a positive correlation with protein content (0.0908) and negative correlation with oxalate (-0.3256). Oxalate content showed significant positive correlation of 0.5022 with nitrate.

Vitamin C had a positive correlation of 0.3099 with protein and negative correlation of –0.2621 with nitrate content.

4.1.3.2 Genotypic Correlation Coefficients

Plant height had significant positive correlation with stem girth (0.7618),, first cutting yield (0.7165), total stem weight (0.7036), total yield (0.6922) and number of branches (0.6754). Height showed significant negative correlation values with leaf blight scoring (-0.6754) and leaf/stem ratio for second cutting (-0.5633).

Leaf length showed significant positive correlation values with days to 50 per cent bolting (0.5376), stem girth (0.5050), petiole length (0.4691) and number of branches (0.4079). It had negative correlation of -0.4439 and –0.3194 with leaf blight incidence and leaf webber scoring respectively.

Leaf breadth was found to have significant positive correlation with leaf/stem ratio for first cutting (0.5479), petiole length (0.4904) and total leaf/stem ratio (0.4837). It had negative correlation value of –0.3168 with stem girth.

In case of both petiole length and number of branches high positive correlation was seen with yield at first cutting (0.5399 and 0.5562) and total yield (0.4576 and 0.4964). Negative correlation in both cases was highest with leaf blight incidence (-0.3829 and –0.4400) followed by leaf webber scoring (-0.1132 and -0.3343).

Days to 50 per cent bolting showed positive correlation with stem girth (0.3370) and leaf/stem ratio for third cutting (0.1767) and negative correlation with leaf blight scoring (-0.2060) and leaf/stem ratio for second cutting (-0.1700).

Stem girth had significant positive correlation with yield at first cutting (0.5925) followed by total stem weight (0.5714), total yield (0.5674) and total leaf weight (0.5225). It showed significant negative

correlation with leaf blight incidence (-0.8389) and leaf/stem ratio for second cutting (-0.6053).

Total leaf weight showed significant positive correlation with total yield (0.9662) followed by yield at second, third and first cuttings (0.9443, 0.9000, 0.8871) and stem weight (0.8182). It had significant negative correlation with leaf blight scoring (-0.6898).

Total stem weight exhibited very high correlation with total yield (0.9389). It showed positive correlation with yield at first cutting (0.8893), second cutting (0.8823) and third cutting (0.8807). A negative correlation of –0.8241 was seen between total stem weight and leaf blight incidence.

Total yield had significant positive correlation of 0.9338, 0.9608 and 0.9274 with yield at first, second and third cuttings respectively. Negative correlation between total yield and leaf blight scoring was significant (–0.7855).

Leaf/stem ratios for first, second and third cuttings were found to have significant positive correlations of 0.8024, 0.6777 and 0.7361 respectively with total leaf/stem ratio. Leaf/stem ratios for second and third cuttings had negative correlation with yield at first cutting (-0.1534, -0.1567), second cutting (-0.2537, -0.1177) and third cutting (-0.3384, -0.1349).

Leaf blight showed positive correlation with total leaf/stem ratio (0.3606) and leaf webber incidence (0.3542) and significant negative correlation with yield at first cutting (-0.7683), second cutting (-0.7414) and third cutting (-0.6708).

First cutting yield had significant positive correlation with second cutting yield (0.8042) and third cutting yield (0.7539). First cutting yield showed negative correlation with leaf webber attack (-0.2041).Yield at second cutting had significant positive correlation with that at third

cutting (0.9525) and negative correlation with leaf webber incidence (-0.0939).

β-carotene content showed positive correlation with protein content (0.0859) and significant negative correlation with oxalate content (-0.3478). Oxalate showed high positive correlation with nitrate (0.5155) and negative correlation with vitamin C (-0.1342). In case of vitamin C, positive correlation was seen with protein (0.3606) and negative correlation with nitrate (-0.3377).

4.1.3.3 Error Correlation Coefficients

Plant height showed significant positive correlation of 0.4700 with number of branches. Leaf length had high positive correlation with leaf breadth (0.5991) and total yield (0.5728). Leaf breadth exhibited significant positive correlation with petiole length (0.5355).

Petiole length showed positive correlation of 0.3738 with yield at first cutting. Number of branches showed positive correlation with stem girth (.3536). Stem girth had highest positive correlation with total yield (0.5282).

Total leaf weight showed significant positive correlation with total yield (0.7947), yield at first cutting (0.4885), second cutting (0.4218) and third cutting (0.5525).

Total stem weight exhibited significant positive correlations with total yield (0.6270) and yield at first cutting (0.5897). The negative correlation was also significant between total stem weight and total leaf/ stem ratio (-0.5166).

Total yield showed significant positive correlation with yield at first cutting (0.6384), second cutting (0.5848) and third cutting (0.5724).

Leaf/stem ratios for first, second and third cuttings had positive correlations with total leaf/stem ratio (0.4083, 0.8039 and 0.5682).

Error correlation values between the nutrients were insignificant.

4.1.4 Path coefficient analysis

Genotypic correlation between yield and its component characters were portioned into different components to find out the direct and indirect contribution of each character on yield. Plant height, leaf length, leaf width, number of branches, stem girth and total leaf weight were selected for path coefficient analysis (Table 14).

Direct effects and correlation of these yield components are presented in Fig. 1.

Plant height had a genotypic correlation of 0.6922 with yield. In this, the direct effect was only 0.1624. Major portion of the indirect effect was through total leaf weight (0.5404). Indirect effect of plant height on yield through leaf length (-0.0039), leaf width (-0.0073), number of branches (-0.813) and stem girth (0.0820) were very low.

Genotypic correlation of leaf length with yield was 0.2218. Its direct effect is only –0.119. But its indirect effect on yield through plant height, leaf width, number of branches, stem girth and total leaf weight were 0.0531, 0.163, -0.491, 0.0543 and 0.1591 respectively.

The direct effect of leaf width on yield was only 0.0626 but genotypic correlation with yield was 0.2160. This is mainly by the indirect effect of leaf width on yield through total leaf weight (0.2168). Indirect effects due to plant height (-0.0189) leaf length (-0.0031), number of branches (-0.0074) and stem girth (-0.341) were negative.

Genotypic correlation of number of branches with yield was 0.4964 in spite of the fact that it had a negative direct effect of –0.1204. Major portion of the correlation was contributed by its indirect effect through total leaf weight (0.4326) followed by plant height (0.1097), stem girth (0.0756) and leaf width (0.0038). Indirect effect through leaf length was negative (-0.0048).

Table 14. Direct and indirect effects of yield components on total yield of amaranthus accessions

Characters	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Number of branches	Stem girth (cm)	Total leaf weight (g)	Genotypic correlation with yield
Plant height (cm)	0.1624	-0.0039	-0.0073	-0.0813	0.0820	0.5404	0.6922
Leaf length (cm)	0.0531	-0.0119	0.0163	-0.0491	0.0543	0.1591	0.2218
Leaf width (cm)	-0.0189	-0.0031	0.0626	-0.0074	-0.0341	0.2168	0.2160
Number of branches	0.1097	-0.0048	0.0038	-0.1204	0.0756	0.4326	0.4964
Stem girth (cm)	0.1237	-0.0060	-0.0198	-0.0846	0.1076	0.4465	0.5674
Total leaf weight (g)	0.1027	-0.0022	0.0159	-0.0610	0.0562	0.8546	0.9662

Residue = 0.2230

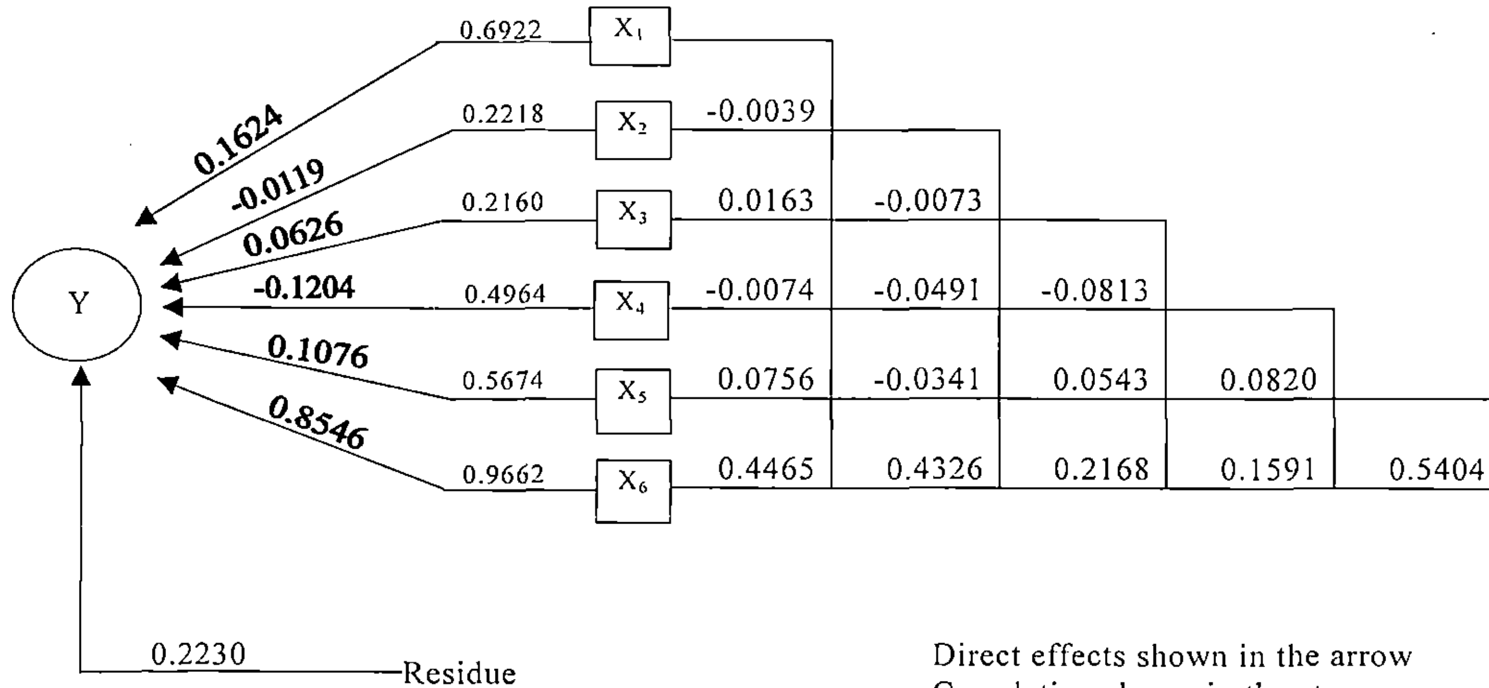


Fig. 1 Path diagram showing direct effects and correlation of yield components on total yield of amaranthus accessions

Stem girth had a genotypic correlation of 0.5674 with yield of which the direct effect was 0.1076. As in the previous case, the indirect effect was maximum through total leaf weight (0.4465) followed by plant height (0.1237). Indirect effects through leaf length (-0.0060), leaf width (-0.0198) and number of branches (-0.0846) were negative.

Total leaf weight had the highest genotypic correlation with yield (0.9662). Its direct effect on yield was as high as 0.8546. Indirect effects through plant height, leaf length, leaf width, number of branches and stem girth were 0.1027, -0.0022, 0.0159, -0.0610 and 0.0562 respectively.

The residue was 0.2230 indicating that the selected six characters contributed the remaining seventy eight per cent.

4.1.5 Genetic divergence analysis

Following Mahalanobis's statistic, the 34 genotypes of amaranthus were subjected to D^2 analysis based on seven characters, *viz.*, plant height, leaf length, leaf width, petiole length, number of branches, stem girth and days to 50 per cent bolting.

The 34 genotypes were grouped into ten clusters (Fig. 2). The clustering pattern is furnished in Table 15.

Cluster II was the largest with nine genotypes, closely followed by cluster III with eight genotypes. Cluster IV and cluster I had five and four genotypes respectively. Cluster V and cluster VI had two genotypes each. Clusters VII, VIII, IX and X contained only one genotype each.

The cluster means of the seven characters are presented in Table 16.

The highest cluster mean for plant height (72.13 cm) was shown by cluster VI, while the lowest was seen in cluster IV (26.14 cm).

Cluster V had the maximum cluster mean for leaf length (16.99 cm) and cluster VII had the minimum value (11.67 cm). Cluster mean for leaf

Table 15. Clustering pattern

Clusters	Accessions
I	Am 4, Am 28, Am 64, Am 67
II	Am 5, Am 9, Am 13, Am 25, Am 27, Am 29, Am 45, Am 55, Am 58
III	Am 31, Am 34, Am 37, Am 44, Am 54, Am 60, Am 76, Am 77
IV	Am 14, AM 40, Am 41,Am 42, Am 47
V	Am 63, Am 72
VI	Am 89, Am 91
VII	Am 22
VIII	Am 71
IX	Am 90
X	Am 78

Table 16. Cluster means for the various characters

Character	Clusters									
	I	II	III	IV	V	VI	VII	VIII	IX	X
Plant height	45.69	28.49	44.76	26.14	45.39	72.31	48.92	61.92	51.28	30.8
Leaf length	15.89	13.26	16.21	13.87	16.99	14.98	11.67	15.89	14.38	15.62
Leaf width	7.02	8.29	9.49	9.87	9.15	8.52	5.73	10.97	8.63	8.68
Petiole length	3.84	4.21	5.75	4.50	5.13	5.85	2.41	8.35	5.18	7.28
Number of branches	9.22	8.83	10.02	9.02	9.07	10.89	9.11	9.91	11.39	8.01
Stem girth	4.29	3.23	3.89	3.40	3.97	4.79	4.03	4.14	4.39	3.63
Days to 50 per cent bolting	57.78	56.71	57.77	54.66	47.07	57.36	47.83	57.35	58.02	60.82

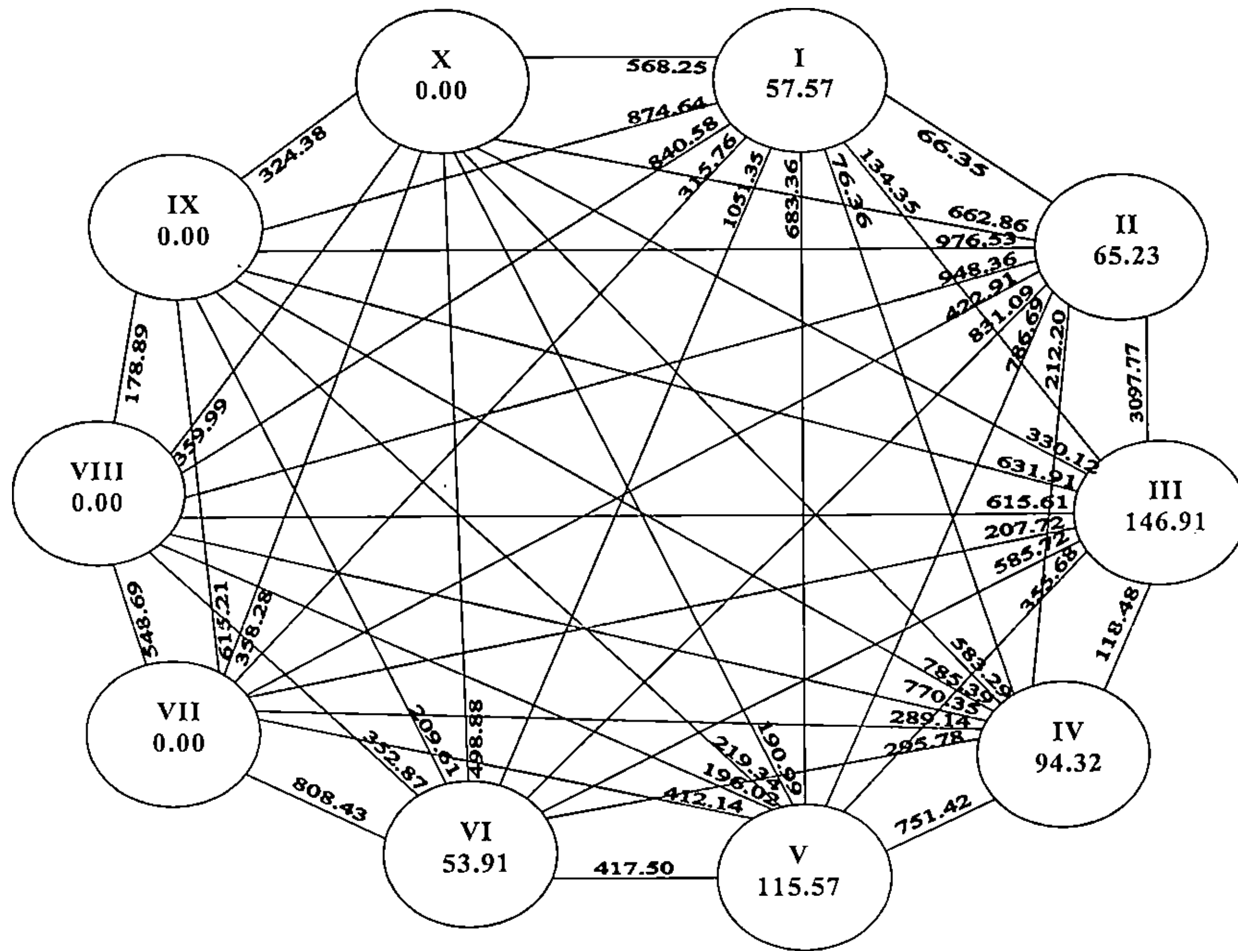


Fig. 2. Cluster diagram

width was highest in cluster VIII (10.97 cm) and lowest in cluster VII (5.73 cm).

Cluster VIII exhibited the maximum value for petiole length (8.35 cm) while cluster I had the minimum value (3.84 cm). Cluster mean for number of branches was highest in cluster IX (11.34) and lowest in cluster X (8.01).

Stem girth cluster mean showed highest value in cluster VI (4.79 cm) and lowest value in cluster II (3.23 cm). The maximum value for days to 50 per cent bolting was seen in cluster X (60.82) and minimum value in cluster V (47.07).

The average inter and intracluster distances are furnished in Table 17.

The average inter and intracluster distances were estimated based on the total D^2 values. The intracluster (D value) distances varied from 0 to 146.91 whereas the intercluster (D value) distance ranged from 66.35 to 3097.77. The intracluster distances were seen to be lower than intercluster distances. The maximum intracluster distance was observed in cluster III (146.91). Clusters VII, VIII, IX and X had only one genotype each and hence the intracluster distance was 0. The maximum intercluster distance was noticed between clusters II and III (3097.77) while the minimum distance was between clusters I and II (66.35).

4.1.6 Cataloguing of the germplasm

All the 34 accessions were described morphologically using the modified descriptor developed from the standard descriptor for amaranthus by IPGRI . The accessions were scored for 20 morphological characters on appropriate scales ranging from 0-9 (Table 18).

All the accessions had erect growth habit and had branches distributed all along the stem. In a few accessions, many branches were

Table 17. Average intra and inter cluster distances (D values)

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X
I	57.57	66.35	134.35	76.36	683.36	1051.35	315.76	840.58	874.64	568.25
II		65.23	3097.77	212.20	786.69	831.09	422.91	948.36	976.53	662.86
III			146.91	118.48	355.68	585.72	207.72	615.61	631.91	330.12
IV				94.32	751.42	295.78	289.14	770.35	785.39	583.29
V					115.57	417.50	412.14	196.02	219.34	190.99
VI						53.91	808.43	352.87	209.61	498.88
VII							0	548.69	615.21	358.28
VIII								0	178.89	359.99
IX									0	324.38
X										0

Table 18 Morphological cataloguing of amaranthus accessions

Sl. No.	Descriptor	Am4	Am5	Am9	Am13	Am14	Am22	Am25	Am27	Am28	Am29	Am31	Am34	Am37	Am40	Am41	Am42	Am44
1	Growth habit	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	Plant height	5	2	1	1	1	5	1	1	5	2	5	2	7	1	1	2	2
3	Branching index	4	4	4	3	4	3	3	4	4	4	2	3	4	4	3	3	3
4	Stem pubescence	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	Stem pigmentation	4	5	5	4	5	4	5	4	2	4	5	4	2	2	2	4	4
6	Spines in leaf axil	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	Leaf length	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
8	Lead width	3	3	3	3	6	3	3	3	3	3	3	3	3	3	3	3	3
9	Leaf pubescence	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	Leaf pigmentation	1	1	1	1	1	9	1	1	9	1	1	9	7	8	8	3	3
11	Leaf shape	4	3	4	3	3	3	4	4	3	3	4	3	2	7	7	3	3
12	Leaf margin	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	Prominence of leaf veins	2	2	2	3	3	1	3	2	2	3	2	3	1	1	3	3	3
14	Petiole pigmentation	4	4	4	4	4	3	4	4	3	4	4	3	1	1	1	3	3
15	Terminal inflorescence shape	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1
16	Terminal inflorescence attitude	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	Axillary inflorescence	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
18	Inflorescence colour	4	3	4	4	4	2	4	3	4	4	3	2	1	2	2	3	3
19	Days to 50 per cent bolting	2	2	2	1	3	2	2	3	2	2	3	3	3	2	2	2	2
20	Seed colour	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Table 18 Continued

Sl. No.	Descriptor	Am45	Am47	Am54	Am55	Am58	Am60	Am63	Am64	Am67	Am71	Am72	Am76	Am77	Am78	Am89	Am90	Am91
1	Growth habit	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	Plant height	2	1	5	2	1	2	2	2	2	7	5	2	2	2	7	5	7
3	Branching index	4	2	4	4	3	3	3	4	4	4	4	4	3	4	4	4	4
4	Stem pubescence	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	Stem pigmentation	4	1	4	1	5	1	2	3	3	1	1	5	5	1	1	1	1
6	Spines in leaf axil	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	Leaf length	5	5	5	3	5	5	5	5	5	5	5	5	5	5	5	5	5
8	Lead width	3	3	3	3	3	3	3	3	3	5	5	3	6	3	3	3	3
9	Leaf pubescence	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	Leaf pigmentation	6	8	6	9	1	9	9	7	7	9	9	1	1	9	9	9	9
11	Leaf shape	3	4	3	3	4	4	3	2	2	5	5	3	3	5	5	5	5
12	Leaf margin	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	Prominence of leaf veins	2	2	2	2	3	2	2	1	1	2	2	2	3	4	4	4	4
14	Petiole pigmentation	3	1	3	2	4	2	1	3	3	1	1	4	4	1	1	1	1
15	Terminal inflorescence shape	1	1	1	1	1	1	1	2	2	3	2	1	1	3	2	3	3
16	Terminal inflorescence attitude	1	1	1	1	1	1	1	2	1	1	1	1	1	2	1	2	1
17	Axillary inflorescence	1	1	1	1	1	1	1	0	0	0	0	1	1	0	0	0	0
18	Inflorescence colour	3	2	3	2	3	2	3	4	4	2	2	4	4	2	2	2	2
19	Days to 50 per cent bolting	2	3	2	2	3	2	2	2	3	2	2	2	2	3	2	2	2
20	Seed colour	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

seen clustered at the base of the stem. And in two particular accessions, Am 31 and Am 47, only a few branches were seen around the basal region.

The plant height exhibited a wide range of variation. A total of 14 accessions were found to have height in the range of 30-45 cm. The height was less than 30 cm in nine accessions and in the range of 46-60 cm in five accessions. Four accessions, namely Am 37, Am 71, Am 89 and Am 91 had a height of more than 60 cm.

Stem pubescence was totally absent in all the 34 accessions. Stem pigmentation showed a wide variation from pale green to deep purple (Plate 5). Among the *A. tricolor* accessions, eight had deep purple stem and 11 had purple stem. The *A. tricolor* accessions, Am 14, Am 41 and Am 63 and *A. hypochondriacus* accession, Am 37 had pale green stem. Am 64 and Am 67 had purplish green stem. All the *A. dubius* accessions (Am 71, Am 72, Am 78, Am 89, Am 90 and Am 91) and three other accessions belonging to *A. tricolor*, namely Am 47, Am 55 and Am 60 had green stem. All the accessions were free of spines in the leaf axil.

In all the accessions except Am 55, leaf length was 11 cm or above. For leaf width, 30 of the accessions showed values in the range of 5-10 cm. In the remaining four, Am 14, Am 71, Am 72 and Am 77, higher values in the range of 11-16 cm were recorded. Variation in leaf length is depicted in Plate 6.

Leaf pubescence was absent in all the accessions. Leaf pigmentation ranged from purple to dark green. In most of the *A. tricolor* accessions, the entire lamina was purple or red. In the accessions, Am 40, Am 41 and Am 47 light green leaves were seen whereas green leaves with purple margins and veins were present in case of Am 45 and Am 54. In the two accessions, Am 42 and Am 44, the leaves were green with purple centre. The *A. tricolor* accessions, Am 22, Am 34, and Am 55, Am 60 and Am 63 and all the *A. dubius* accessions (Am 71, Am 72, Am 78, Am 89, Am 90 and Am 91) had dark green leaves. The three accessions Am 37, Am

Plate 5. Variation in stem pigmentation

Plate 6. Variation in leaf length

64 and Am 67 belonging to *A. hypochondriacus* had purplish green leaves. Variation in leaf colour is shown in Plate 7.

The leaf shape was ovate or broad ovate in the *A. tricolor* collections with two exceptions, Am 40 and Am 41, in which the leaves were rhombic in shape. In the three *A. hypochondriacus* accessions, Am 37, Am 64 and Am 67, the leaves were elliptic and in the *A. dubius* accessions (Am 71, Am 72, Am 78, Am 89, Am 90 and Am 91), the leaves were seen to be triangle ovate.

Leaf margin was entire in all the 34 landraces. Leaf veins were either slightly prominent or very prominent in the *A. tricolor* accessions. In the *A. dubius* accessions (Am 71, Am 72, Am 78, Am 89, Am 90 and Am 91), slightly prominent leaf veins were noticed. Leaf veins were smooth in case of Am 37, Am 64 and Am 67 (*A. hypochondriacus*).

13 of the 25 *A. tricolor* accessions had deep purple petiole and six of them showed purple petiole. Among the remaining six, four (Am 40, Am 41, Am 47 and Am 63) had green petiole pigmentation and the other two (AM 55 and Am 60) had dark green petioles. Among the *A. hypochondriacus* accessions, Am 37 had green petiole while purple petioles were seen in case of Am 64 and Am 67. Am 71, Am 72, Am 78, Am 89, Am 90 and Am 91 (*A. dubius*) had green petiole.

Inflorescence characteristics showed much variation among the different species (Plate 8). Terminal inflorescence was erect in all the 34 accessions. Axillary inflorescence was present in *A. tricolor* accessions and absent in *A. dubius* and *A. hypochondriacus* accessions. In the case of *A. tricolor*, terminal inflorescence was a spike. In Am 37, Am 64 and Am 67 (*A. hypochondriacus*) inflorescence was a panicle with short branches. Among the *A. dubius* types, Am 72 and Am 89 had short branched panicles while the rest (Am 71, Am 78, Am 90 and Am 91) showed long branched panicles. Inflorescence colour was green in all the six *A. dubius* accessions. In Am 37 (*A. hypochondriacus*) the colour was yellow whereas in Am 64 and Am 67, it was red. 10 among the 25 *A. tricolor* types were

Plate 7. Variation in leaf pigmentation

Plate 8. Variation in inflorescence characters

red and nine were pink in colour. The remaining six had green inflorescences.

In 24 accessions, days to 50 per cent bolting was observed to be in the range of 46-60 days while in 9 accessions, a higher range of 61-75 days was recorded and in a single accession, Am 13, the time taken for bolting was very low, only 30-45 days. Seed colour was black in all the 34 accessions.

4.2 MOLECULAR CHARACTERIZATION.

In this study, 27 accessions of amaranthus from diverse geographical locations in Kerala were analysed using RAPD markers to assess the extent of genetic diversity. Seven accessions were not included because they were found to be identical to those already included in the study. Out of these 27 accessions, 19 belonged to *A. tricolor*, five to *A. dubius* and the last three to *A. hypochondriacus*.

4.2.1 DNA Isolation

DNA isolation was done in 27 morphologically different accessions following the procedure of Murray and Thompson (1980) with slight modifications.

Addition of 1 per cent PVP and beta- mercaptoethanol to the extraction buffer along with the other reagents reduced the browning of the pellet. The DNA yield of accessions ranged from 0.12120 μg/ml to 0.93930 μg/ml. The purity of DNA (A_{260}/A_{280}) ranged from 1.4 to 2.0 (Table 19).

4.2.2 Gel Electrophoresis

Gel electrophoresis was done to assess the quality of DNA isolated by using 0.8 per cent agarose. For RAPD analysis, 1.2 per cent agarose was used. The genomic DNA was observed as a single crisp band showing

Table 19. Quality and Quantity of DNA isolated from amaranthus accessions

Sl. No	Accession number	A_{260} (nm)	A_{280} (nm)	A_{260}/A_{280}	DNA yield (µg/ml)
1	Am 4	0.023	0.014	1.64	120
2	Am 9	0.016	0.008	2.00	480
3	Am 13	0.012	0.006	2.00	360
4	Am 14	0.015	0.009	1.66	450
5	Am 22	0.005	0.003	1.66	150
6	Am 25	0.014	0.010	1.40	420
7	Am 27	0.008	0.005	1.60	240
8	Am 28	0.015	0.009	1.66	450
9	Am 29	0.031	0.021	1.47	930
10	Am 31	0.007	0.005	1.40	210
11	Am 34	0.017	0.009	1.88	510
12	Am 37	0.010	0.005	2.00	300
13	Am 40	0.009	0.006	1.50	270
14	Am 42	0.014	0.007	2.00	420
15	Am 45	0.015	0.010	1.50	450
16	Am 47	0.008	0.005	1.60	240
17	Am 54	0.012	0.008	1.50	360
18	Am 55	0.008	0.005	1.60	240
19	Am 63	0.009	0.006	1.50	270
20	Am 64	0.007	0.004	1.75	210
21	Am 67	0.004	0.002	2.00	120
22	Am 71	0.007	0.005	1.40	210
23	Am 72	0.008	0.004	2.00	240
24	Am 77	0.022	0.015	1.47	660
25	Am 78	0.013	0.008	1.63	390
26	Am 89	0.008	0.005	1.60	240
27	Am 91	0.012	0.008	1.50	360

unsheared DNA. The PCR products were well separated in the 1.2 per cent agarose.

4.2.3 Polymerase Chain Reaction

Polymerase chain reaction was done for the molecular analysis of 27 accessions of amaranthus. 38 primers (31 from Operon series and 7 UBC primers) were screened for their efficiency using the DNA isolated from Am 22 as the representative sample. Out of the 38 primers screened, 25 primers namely, OPA-1, OPA-3, OPA-6, OPA-7, OPA-8, OPE-1, OPE-2, OPE-5, OPE-6, OPE-7, OPE-10, OPE-11, OPE-12, OPE-13, OPE-14, OPE-15, OPE-16, OPE-17, OPE-19, OPU-14, UBC-16, UBC-17, UBC-18, UBC-23 and UBC-29 yielded amplification products with the genomic DNA. The total number of bands, number of faint bands and number of intense bands produced by all the primers used for screening are presented in Table 20.

The 25 primers produced 77 bands, of which 40 per cent (31 bands) were polymorphic and the rest were monomorphic (46 bands). The highest numbers of RAPDs (7 nos) were produced by the primers UBC-17, UBC-23 and OPE-14. Of these primers, UBC-23 produced the highest number of intense bands (5 nos). The primer UBC-17 produced four intense bands and the number of faint bands were 3. UBC-18 produced four intense bands and one faint band. OPE-14 produced three intense bands and four faint bands.

The four primers namely UBC-17, UBC-18, UBC-23 and OPE-14 were used for amplifying the DNA from the 27 accessions. These primers yielded 39 scorable bands with an average of 9.75 bands per primer. Primer UBC-17 produced a maximum number of 12 bands while eight bands were got in case of UBC-18. The nucleotide sequence and number of informative markers given by each primer are presented in Table 21.

Table 20. Primer associated banding patterns with the DNA of Am 22 using 38 primers

Sl. No	Primer	No. of intense bands	No. of faint bands	Total No. of Bands
1	OPA-01	0	1	1
2	OPA-02	0	0	0
3	OPA-03	0	1	1
4	OPA-04	0	0	0
5	OPA-05	0	0	0
6	OPA-06	1	1	2
7	OPA-07	1	0	1
8	OPA-08	0	2	2
9	OPA-09	0	0	0
10	OPA-10	0	0	0
11	OPE-01	1	2	3
12	OPE-02	1	1	2
13	OPE-03	0	0	0
14	OPE-04	0	0	0
15	OPE-05	2	0	2
16	OPE-06	2	2	4
17	OPE-07	0	2	2
18	OPE-08	0	0	0
19	OPE-09	0	0	0
20	OPE-10	1	3	4
21	OPE-11	2	0	2
22	OPE-12	2	1	3
23	OPE-13	0	3	3
24	OPE-14	3	4	7
25	OPE-15	1	1	2
26	OPE-16	1	0	1
27	OPE-17	0	2	2
28	OPE-18	0	0	0
29	OPE-19	2	1	3
30	OPE-20	0	0	0
31	OPU-14	3	1	4
32	UBC-13	0	0	0
33	UBC-16	0	1	1
34	UBC-17	4	3	7
35	UBC-18	4	1	5
36	UBC-23	5	2	7
37	UBC-29	2	2	4
38	UBC-30	0	0	0

Table 21. Nucleotide sequence of primers and total number of informative RAPD markers amplified with them

Primer	Sequence	Number of informative RAPD markers
UBC-17	CCTGGGCCTC	7
UBC-18	GGGCCGTTTA	5
UBC-23	CCCGCCTCCC	7
OPE-14	TGCGGCTGAG	7

Primer UBC-17 could amplify the DNA samples from all the accessions. A total of 12 scorable bands were obtained. Three bands were monomorphic for the three *A. hypochondriacus* accessions and five *A. dubius* accessions among which the second band was absent only in Am 78. One band was monomorphic for all the *A. tricolor* accessions except Am 22, Am 29 and Am 63. The maximum number of bands were recorded for the *A. hypochondriacus* accessions. Am 67 showed nine bands followed by Am 37 with eight bands and Am 64 with seven bands. Among the *A. dubius* accessions Am 71, Am 72 and Am 91 had six bands each while Am 89 and Am 78 showed five and three bands respectively. In case of the *A. tricolor* accessions, the highest number of bands were noted for Am 42 and Am 47. Much variation was seen in the band distribution among the tricolor accessions compared to the other two species (Plate 9).

Primer UBC-18 produced eight scorable bands. All the five *A. dubius* accessions produced two monomorphic bands among which Am 71, Am 72 and Am 78 produced faint bands and Am 89 and Am 91 produced intense bands. Three bands were seen to be monomorphic for the three *A. hypochondriacus* accessions. *A. tricolor* accessions Am 4, Am 9, Am 27, Am 34 and Am 55 produced four bands each whereas Am 13, Am 25, Am 28, Am 42, Am 45, Am 47, Am 54 and Am 63 had three bands each. A minimum number of two bands each were seen each in Am 14, Am 22, Am 29, Am 31, Am 40 and Am 77 (Plate 10).

Primer UBC-23 amplified ten scorable bands and they were all polymorphic. Maximum of six bands were recorded for Am 89 and Am 28 and five bands in Am 37, Am 67, Am 9, Am 14 and Am 55. Minimum of two bands were noted in accessions Am 13, Am 22, Am 29, Am 34, Am 45, Am 54, Am 63 and Am 77 (Plate 11).

Nine scorable bands were obtained in case of primer OPE-14 (Plate 12). Two bands were monomorphic for the *A. dubius* and *A. hypochondriacus* accessions. Two bands are monomorphic for the

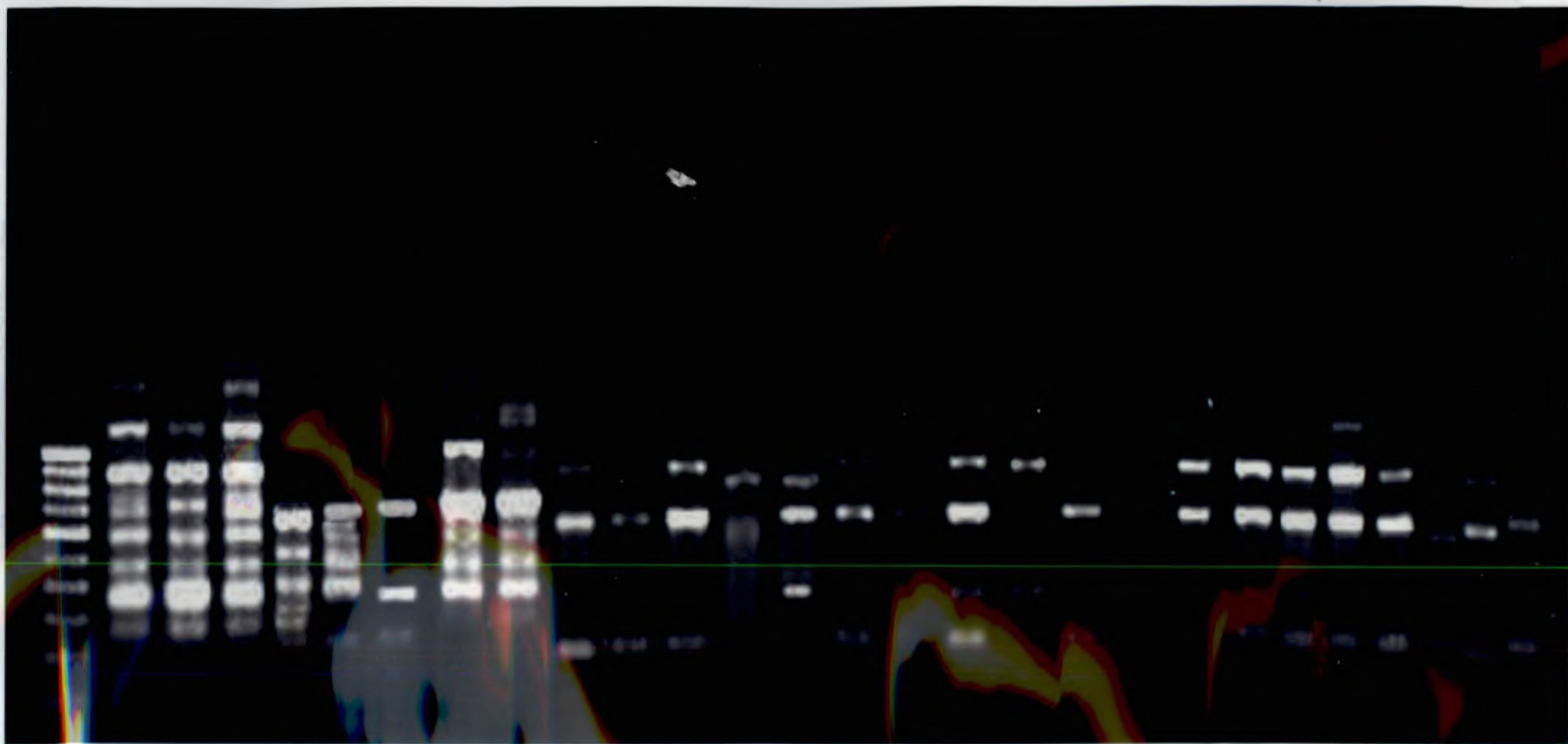
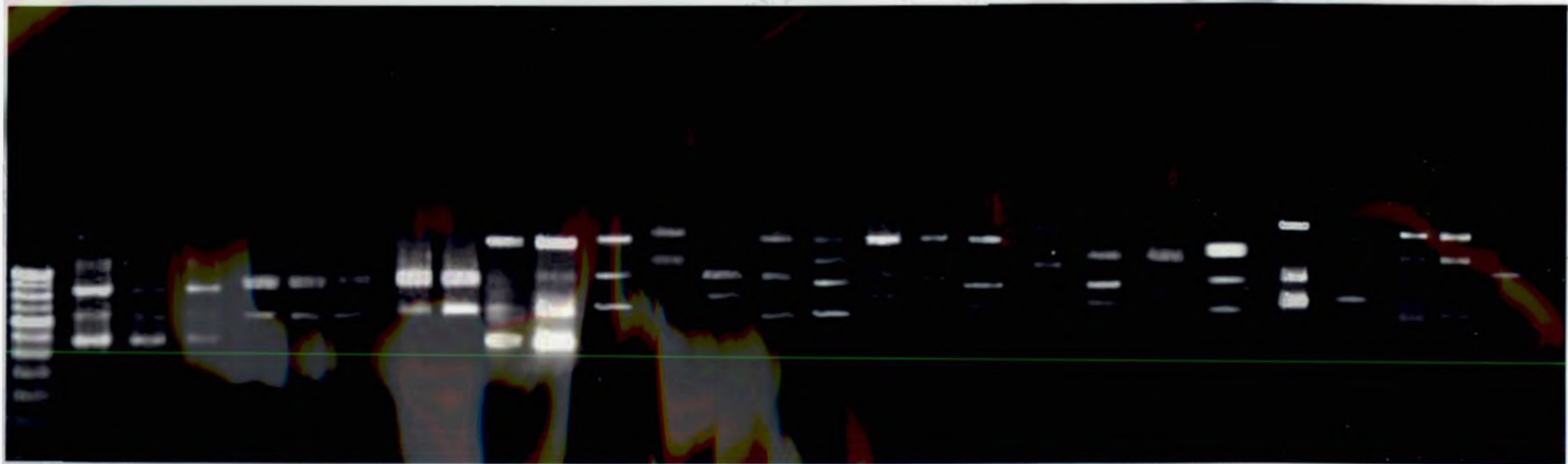


Plate 9

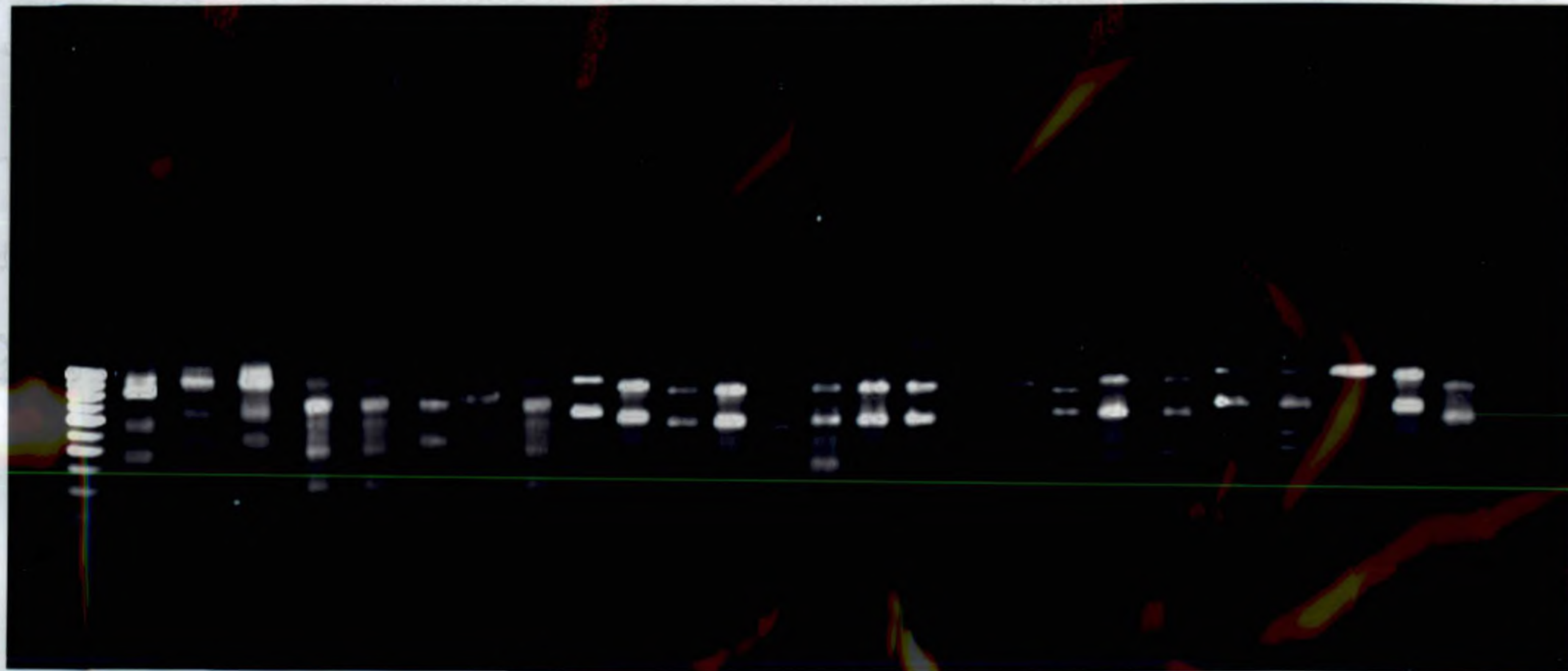
Amplification profile of the DNA of 27 amaranthus accessions using the primer UBC-17



37 64 67 71 72 78 89 91 4 9 13 14 22 25 27 28 29 31 34 40 42 45 47 54 55 63 77

Plate 10

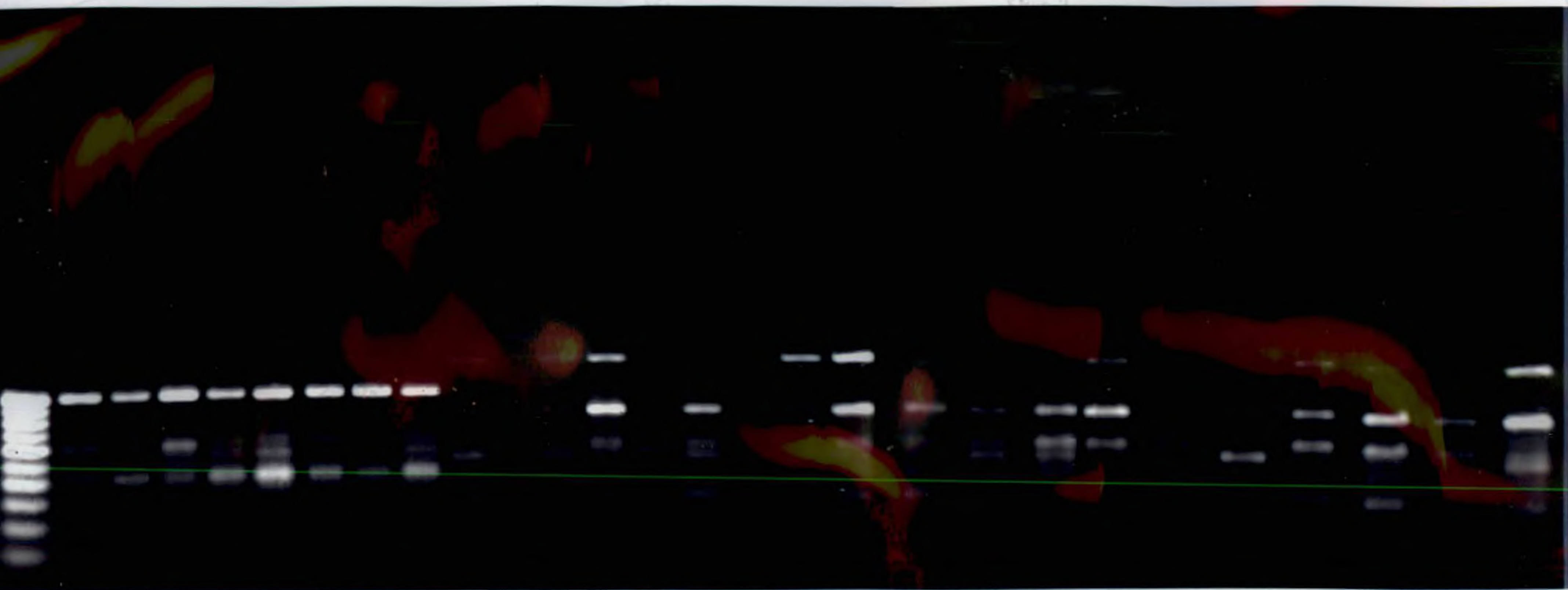
Amplification profile of the DNA of 27 amaranthus accessions using the primer UBC-18



37 64 67 71 72 78 89 91 4 9 13 14 22 25 27 28 29 31 34 40 42 45 47 54 55 63 77

Plate 11

Amplification profile of the DNA of 27 amaranthus accessions using the primer UBC-23



37 64 67 71 72 78 89 91 4 9 13 14 22 25 27 28 29 31 34 40 42 45 47 54 55 63 77

Plate 12

Amplification profile of the DNA of 27 amaranthus accessions using the primer OPE-14

A. tricolor accessions. Among these, the upper one is absent in Am 9, Am 27, Am 47 and Am 63. The lower one is absent in Am 4, Am 22, Am 27 and Am 47. Accessions Am 25 and Am 55 exhibited maximum amplification with six bands each while five bands each were seen in Am 14, Am 29, Am 34 and Am 40. Only faint bands were produced by Am 4, Am 13, Am 22, Am 31, Am 34, Am 45 and Am 63. Amplification was totally absent in case of Am 9 and Am 27 while Am 47 produced only a single band. The PCR was repeated twice in order to check the reproducibility.

Amplification profiles of the DNA of the amaranthus accessions using primers UBC-17, UBC-18, UBC-23 and OPE-14 are presented in Fig. 3, 4, 5 and 6 respectively. The data obtained from all these four primers were subjected to statistical analysis.

4.2.4 Data Analysis

RAPD marker data were subjected to cluster analysis using NTSYS programme to estimate the similarity indices and genetic relatedness among the accessions. The reproducible bands were scored for their presence (+) or absence (-) for all the accessions studied. A genetic similarity matrix was constructed using the Jaccard's similarity coefficient method (Table 22). The pairwise coefficient values varied between 0.07 and 0.81. The least similarity coefficient values were that of accessions Am 14 with Am 91 (0.07), Am 54 with Am 91 (0.08), Am 22 with Am 55 (0.08) and Am 9 with Am 54 (0.09). The highest similarity index values were obtained for accessions Am 4 with Am 13 (0.81) and Am 64 with Am 67 (0.80).

The dendrogram generated by UPGMA cluster analysis based on the similarity coefficient values is shown in Fig. 7. Cluster analysis revealed that at 0.27 similarity coefficient, the 27 accessions can be grouped into two main clusters. The first cluster includes the *A. hypochondriacus* and *A. dubius* accessions in one subcluster and an

37	64	67	71	72	78	89	91	4	9	13	14	22	25	27	28	29	31	34	40	42	45	47	54	55	63	77
-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	+	+	-	-	-	+	+	-	-	-	-	-	+	+	+	+	-	-	-	+	-	+	-	+	-	-
+	+	+	-	+	-	-	-	+	-	+	+	+	-	-	-	+	-	-	+	+	+	+	+	-	+	+
+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+
+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
+	+	+	-	+	-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	+	+	-	-	-	+	+	+	+	-	-	+
-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	+	+	+	-	-	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	-	+
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-

\+ denotes presence of bands

\- denotes absence of bands

Fig. 3. Representation of amplification profile of DNA using primer UBC-17

37	64	67	71	72	78	89	91	4	9	13	14	22	25	27	28	29	31	34	40	42	45	47	54	55	63	77
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-
-	-	-	-	-	-	-	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+
+	-	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	+	+	+	+	+	+	+	+	-
+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	-	+	-	+
-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-	+	-	+	-	-	+	-	-	-
+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	-	-	-	-	-	+	-	-	+	+	-
+	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

\+ denotes presence of bands

\- denotes absence of bands

Fig. 4. Representation of amplification profile of DNA using primer UBC-18

37	64	67	71	72	78	89	91	4	9	13	14	22	25	27	28	29	31	34	40	42	45	47	54	55	63	77
+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-
-	-	-	-	-	+	+	-	-	+	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	-	+	-
-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	+	-	+
-	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-
-	+	+	-	-	+	+	-	+	+	-	+	-	+	+	+	-	+	-	+	-	-	+	-	+	-	-
+	-	-	+	+	+	+	+	+	+	-	+	-	+	-	+	-	+	-	+	+	-	+	-	+	-	-
-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ denotes presence of bands
- denotes absence of bands

Fig. 5. Representation of amplification profile of DNA using primer UBC-23

37	64	67	71	72	78	89	91	4	9	13	14	22	25	27	28	29	31	34	40	42	45	47	54	55	63	77
-	-	-	-	-	-	-	-	+	-	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	-	+
-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-
+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
-	-	-	-	-	-	-	-	+	-	+	+	-	+	-	+	+	+	+	+	+	+	-	+	+	+	+
+	+	+	+	+	+	+	-	-	-	-	+	-	+	-	-	+	+	+	+	+	+	-	+	+	+	+
-	-	-	-	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	+	-	-	+	-	-	-	-
+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	+	+	+	+	+	-	+	+	+	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

+ denotes presence of bands

- denotes absence of bands

Fig. 6. Representation of amplification profile of DNA using primer OPE-14

Table 22. Similarity matrix of 27 amaranthus accessions based on Jaccard's similarity index

	37	64	67	71	72	78	89	91	4	9	13	14	22	25	27	28	29	31	34	40	42	45	47	54	55	63	77
37	1.00																										
64	0.63	1.00																									
67	0.73	0.80	1.00																								
71	0.41	0.45	0.38	1.00																							
72	0.45	0.50	0.42	0.72	1.00																						
78	0.33	0.42	0.36	0.64	0.61	1.00																					
89	0.52	0.65	0.54	0.55	0.60	0.70	1.00																				
91	0.41	0.45	0.38	0.57	0.72	0.47	0.63	1.00																			
4	0.30	0.39	0.33	0.30	0.40	0.33	0.29	0.30	1.00																		
9	0.25	0.33	0.28	0.30	0.28	0.41	0.35	0.23	0.62	1.00																	
13	0.22	0.29	0.25	0.26	0.36	0.28	0.25	0.26	0.81	0.47	1.00																
14	0.17	0.19	0.20	0.16	0.20	0.28	0.20	0.07	0.38	0.31	0.40	1.00															
22	0.22	0.31	0.26	0.15	0.26	0.16	0.20	0.21	0.27	0.18	0.37	0.22	1.00														
25	0.25	0.32	0.27	0.29	0.33	0.38	0.33	0.29	0.55	0.42	0.50	0.50	0.26	1.00													
27	0.25	0.33	0.33	0.23	0.22	0.41	0.35	0.23	0.36	0.57	0.31	0.31	0.26	0.50	1.00												
28	0.25	0.28	0.24	0.25	0.24	0.40	0.34	0.25	0.50	0.44	0.61	0.38	0.35	0.55	0.52	1.00											
29	0.24	0.31	0.26	0.17	0.21	0.19	0.21	0.17	0.42	0.21	0.44	0.44	0.33	0.55	0.27	0.42	1.00										
31	0.19	0.26	0.22	0.28	0.27	0.38	0.27	0.17	0.50	0.43	0.52	0.52	0.33	0.75	0.43	0.58	0.50	1.00									
34	0.14	0.11	0.13	0.17	0.21	0.19	0.12	0.12	0.28	0.15	0.36	0.44	0.25	0.47	0.27	0.35	0.33	0.50	1.00								
40	0.26	0.29	0.29	0.26	0.36	0.35	0.25	0.20	0.52	0.31	0.55	0.64	0.37	0.66	0.38	0.45	0.44	0.73	0.52	1.0							
42	0.36	0.33	0.33	0.30	0.34	0.33	0.29	0.25	0.42	0.23	0.52	0.52	0.43	0.55	0.36	0.57	0.58	0.58	0.50	0.70	1.0						
45	0.33	0.30	0.30	0.33	0.38	0.36	0.31	0.21	0.33	0.14	0.28	0.35	0.23	0.38	0.26	0.27	0.38	0.38	0.38	0.50	0.55	1.00					
47	0.33	0.30	0.30	0.27	0.38	0.36	0.38	0.33	0.33	0.20	0.35	0.22	0.16	0.31	0.33	0.33	0.19	0.25	0.19	0.42	0.40	0.44	1.00				
54	0.24	0.20	0.22	0.17	0.21	0.19	0.16	0.08	0.28	0.09	0.36	0.36	0.25	0.33	0.21	0.35	0.41	0.41	0.60	0.44	0.58	0.66	0.31	1.00			
55	0.37	0.29	0.30	0.32	0.30	0.34	0.41	0.26	0.32	0.26	0.28	0.45	0.08	0.47	0.26	0.32	0.30	0.36	0.30	0.39	0.37	0.47	0.34	0.30	1.00		
63	0.25	0.27	0.28	0.23	0.28	0.26	0.17	0.13	0.30	0.22	0.25	0.56	0.26	0.42	0.37	0.23	0.35	0.43	0.53	0.56	0.52	0.50	0.20	0.43	0.31	1.00	
77	0.24	0.26	0.22	0.28	0.33	0.31	0.27	0.17	0.35	0.15	0.36	0.23	0.25	0.33	0.21	0.35	0.41	0.41	0.33	0.36	0.42	0.66	0.38	0.60	0.30	0.27	1.0

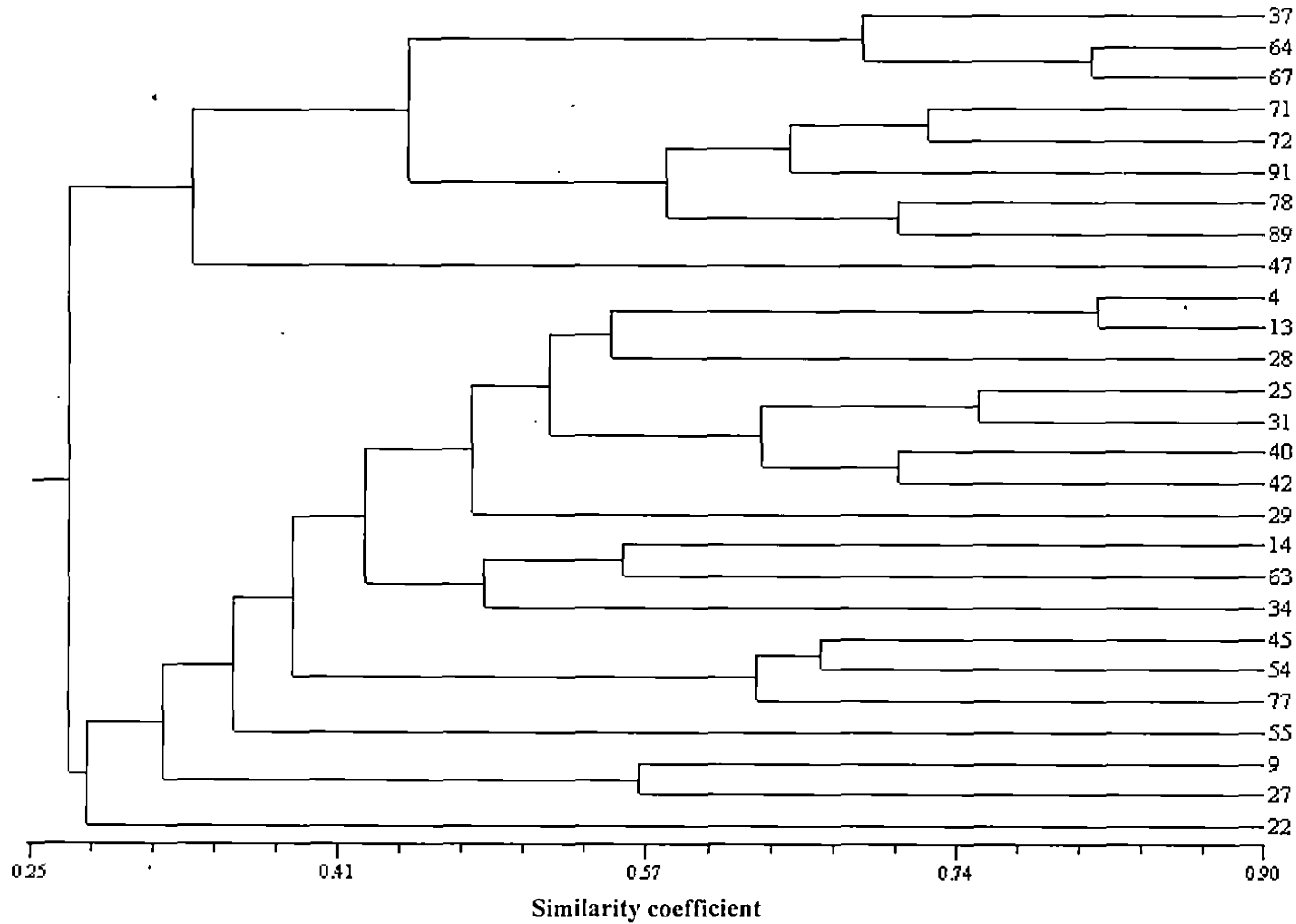


Fig. 7 Dendrogram obtained from RAPD analysis based on similarity coefficient values

individual accession, Am 47 (*A. tricolor*), in the next subcluster. Thus a total of nine members are seen in this cluster. The second cluster comprises of 18 members, all belonging to *A. tricolor*, showing a wide range of genetic variation.

At approximately 0.35 similarity coefficient, the first main cluster gets divided into two subclusters. One subcluster is split into two subgroups of which one contains the three *A. hypochondriacus* accessions namely Am 37 and Am 64 and Am 67 as a pair. The remaining subgroup includes the five *A. dubius* accessions Am 71, Am 72, Am 78, Am 89 and Am 91. Among these, Am 71 and Am 72 form a pair with 72 per cent similarity while 70 per cent similarity can be noted between Am 78 and Am 89. The second subcluster contains a single *A. tricolor* accession, Am 47. Eventhough Am 47 occurs in this cluster, it remains separated from the other members in this group.

The second main cluster contains 18 *A. tricolor* accessions. At 42 per cent similarity, it is further subdivided into six subclusters. The largest subcluster contains eight accessions namely Am 4, Am 13, Am 25, Am 28, Am 29, Am 31, Am 40 and Am 42. Am 4 and Am 13, Am 25 and Am 31 and Am 40 and Am 42 form pairs with 81 per cent, 75 per cent and 70 per cent similarity respectively. Among the remaining five subclusters, two were single accession clusters representing Am 22 and Am 55 and the third one included Am 9 and Am 27 in a pair with 0.57 similarity coefficient. The last two subclusters have three accessions each. Am 14, Am 63 and Am 34 were seen in one of which the first two form a pair. The remaining three accessions Am 45, Am 54 and Am 77 formed the last cluster. Am 45 and Am 54 showed a pairwise similarity coefficient of 0.66.

Discussion

5. DISCUSSION

Amaranthus is a green leafy vegetable extensively grown and consumed in Kerala and other parts of south India. It is a rare example of a vegetable where all the dietary components such as protein, vitamin A, vitamin C, Fe and Ca are combined in one (Mohideen and Subramanian, 1974). So it is an ideal crop for combating the problems of under nutrition and malnutrition.

Collection and characterization of the existing variability is the first step *a priori* to any breeding programme. Morphological characterization based on growth, yield and quality characters and response to biotic stress is the simplest way to assess the diversity in the germplasm of this crop. But this method is not very effective due to the interrelationships between the different species, existence of intermediate hybrids and effect of environmental variations on the morphological characters. The use of molecular markers will help us to get a clear picture of the variation and relatedness of landraces of amaranthus and will provide the means for their authentic classification based on genetic diversity.

In the present study, 34 diverse accessions of amaranthus including those collected from different districts of Kerala were subjected to both morphological and molecular characterization. The accessions were grouped into clusters based on both methods. Attempts were made to compare the two methods of characterization. The results obtained are discussed here under.

5.1 MORPHOLOGICAL CHARACTERIZATION

5.1.1 Mean performance of the accessions

In a leafy vegetable like amaranthus, leaf length, leaf width, plant height, stem girth, days to 50 per cent bolting, petiole length and number of branches are traits as important as yield and leaf : stem ratio. Much variation was noted in the morphological characters which is in accordance with the findings of Calderon *et al.* (1991), Shukla and Singh (2002a), Shukla and Singh (2002b) and Varalakshmi (2004).

In any crop improvement programme, yield is the most important character which decides the superiority of the accessions. In the present study, total yield was highest in Am 91 (387.22 g) followed by Am 89 (373.33 g) and Am 90 (337.22 g) belonging to *A. dubius* and lowest in the A. tricolor accession, Am 13 (51.67 g). The results are in agreement with the findings of Campbell and Abbott (1982) who reported the superior performance of *A. dubius* compared to the other amaranthus species. The yield decreased steadily in the ascending order of cuttings (Allemann *et al.*, 1996).

The extremely low yields obtained in most of the *A. tricolor* accessions can be attributed to the high rainfall during the crop duration (Appendix II and Fig. 8) especially in October and November which in turn led to severe incidence of leaf blight. Similar yield pattern of low yield in amaranthus during rainy season has been reported by Mohideen and Muthukrishnan (1981).

According to Mohanalekshmi *et al.* (1998) the optimum leaf/stem ratio in amaranthus is 1.0 to 1.5 and is to be aimed at in selection. Among the 34 accessions, the leaf/stem ratio was found to range from 2.38 in Am 77 (*A. tricolor*, var. Arun) to 0.53 in Am 22. This indicates the wide variation for this character both within and among different species of amaranthus as reported by Campbell and Abbott (1982) and Priya (1998).

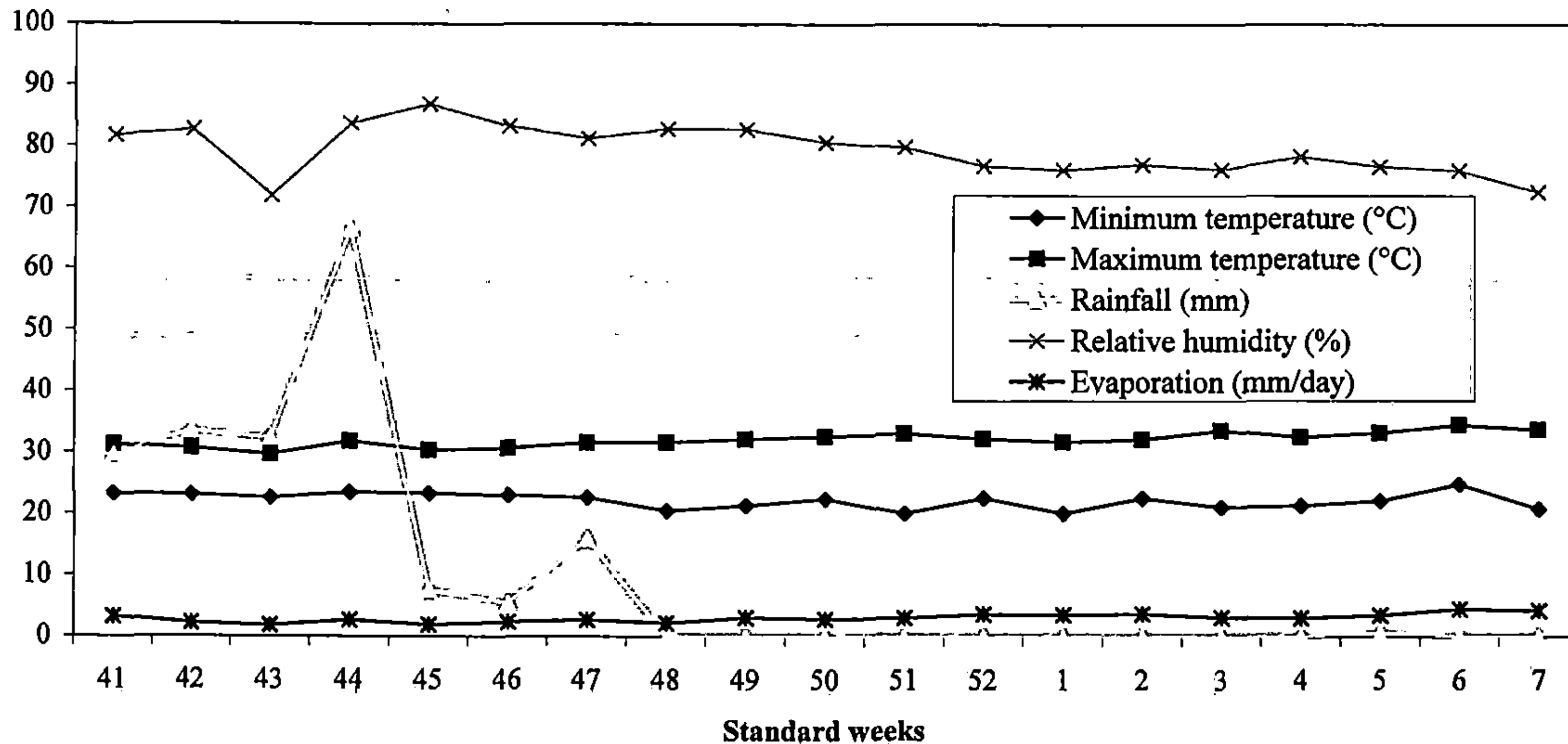


Fig. 8. Weather parameters during the cropping period (October 2004 to February 2005)

The ratio decreased slightly with subsequent harvests. This was due to the fact that the incidence of leaf blight and leaf webber caused stunting of growth in the plants after the first harvest and retarded the development of the lateral branches.

As amaranth is a leafy vegetable, bolting or premature flowering is an undesirable character since it retards the vegetative growth thereby lowering the yield. Genetic as well as environmental factors such as short day conditions contribute to this condition. In the present study, days to 50 per cent bolting was maximum for Am 37 (72.67 days) and minimum for Am 72 (45.27 days). These values indicate a wide variation among the accessions for this character which is in accordance with the results obtained by Devadas (1982), Priya (1998), Shukla and Singh (2002b) and Varalakshmi (2004). So there is much scope for breeding of late bolting types of amaranthus.

The important nutrients in amaranthus are vitamin A, vitamin C and proteins and antinutrients include nitrate and oxalate. An ideal variety should contain more quantity of nutrients and lower quantity of antinutrients.

Variability in the nutritive composition of different varieties and species of amaranthus has been reported by several workers (Vijayakumar and Shanmughavelu, 1985; Prakash and Pal, 1991; Hossain *et al.*, 1999; Pal, 1999). The β-carotene content was found to range from 1269.94 μg/100 g in Am 90 to 4655.54 μg/100 g in Am 5. Similar values have been obtained by several workers (Mohideen *et al.* 1985; Vijayakumar and Shanmughavelu, 1985; Pal 1999). The maximum vitamin C content of 151.22 mg/100 g was seen in Am 78 and minimum of 54.88 mg/100 g in Am 58 which is accordance with the findings of Hemalatha *et al.* (1999) and Tewari *et al.* (2002).

Protein content was highest in Am 91 (3.57 %) and lowest in Am 27 (0.67 %) on fresh weight basis. Chemical analysis of amaranthus by

other researchers have also revealed similar results (Prakash and Pal, 1991; Raja *et al.*, 1997; Pal, 1999; Tewari *et al.*, 2002).

Since excess consumption of nitrate and oxalate can adversely affect health, the quantity of amaranthus in the diet should be limited (Martin and Telek, 1979). Much variability is noted in the content of these two factors which can be utilized in selection programmes to develop varieties with lower content of antinutrients. The highest and lowest contents of oxalate were recorded in Am 76 (2.08 %) and Am 90 (0.6 %) respectively while those for nitrate were seen in Am 28 (1.62 %) and Am 37 (0.04 %) respectively. These values are in agreement with the reports of Teutonico and Knorr (1985), Pal (1999), Mziray (2001) and Tewari *et al.* (2002). The fact that the antinutrients were lower in the *A. dubius* accessions has been reported earlier by several workers (Kauffman and Gilbert, 1981; George *et al.*, 1989; Thamburaj *et al.*, 1994; Priya and Celine, 2001).

In the current experiment, the accessions were screened for leaf blight and leaf webber incidence.

Leaf blight caused by *R. solani* lowers the yield and quality of amaranthus thereby causing huge economic losses to farmers all over Kerala. The results revealed that the *A. tricolor* accessions irrespective of geographic location were severely infested by leaf blight whereas the *A. dubius* and *A. hypochondriacus* accessions were rather resistant.

Maximum disease incidence was recorded in the *A. tricolor* accessions Am 14 and Am 77 (2.00) while the *A. dubius* accessions Am 89 and Am 91 showed a zero score. This agrees with the observations made by Gokulapalan *et al.* (1997), Krishnakumary *et al.* (2001), Celine *et al.* (2002) and Sindhu (2002). The continuous and heavy rainfall during the initial phase of the crop (Appendix II) was highly conducive for the spread of the leaf blight disease resulting in very low yields especially in the susceptible *A. tricolor* accessions.

Leaf webbers, *viz.*, *Psara basalis* and *Hymenia recurvalis* occur widely in amaranthus causing severe damage. All accessions were infested by leaf webber and the intensity ranged from 0.63 in Am 42 to 2.07 in Am 78. No geographic or species level differences could be noted for leaf webber incidence.

A perusal of the results on performance of the accessions revealed that the *A. dubius* accessions Am 91, Am 90 and Am 89 were the superior ones due to their high yield, disease resistance and low antinutrient factors.

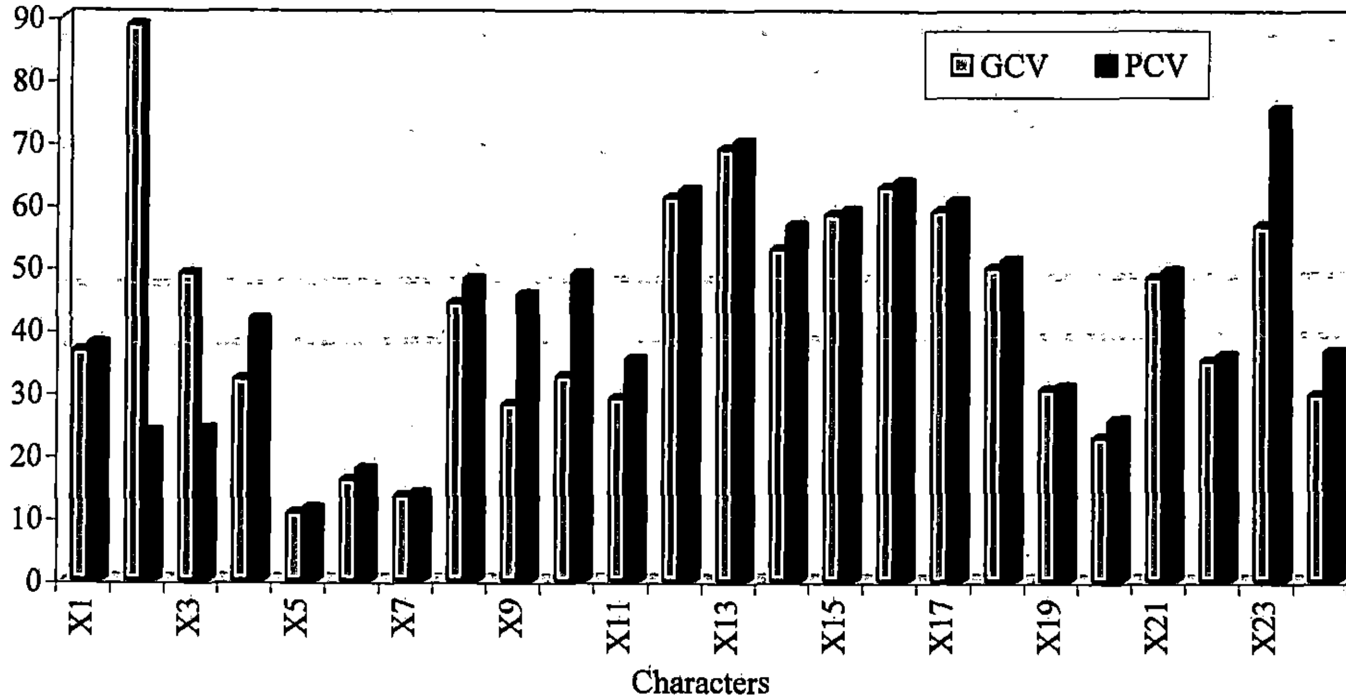
5.1.2 Genetic variability, heritability and genetic advance

A detailed analysis of the genetic variability of different characters is an absolute necessity for the development of improved varieties of amaranthus. The variation may be genetic or due to environmental factors.

In the present study, variability was noticed for growth, yield and quality characters and response to biotic stress. High variability for growth, yield and yield attributes in amaranthus has been reported by earlier workers (Mohideen *et al.*, 1982; Devadas *et al.*, 1992; Pan *et al.*, 1992; Shukla and Singh, 2002a; Vaidya and Jain, 2002; Verma *et al.*, 2002)

Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) are better indices for comparison of characters with different units of measurement, than estimates of quantitative variation like range and variation around mean (Fig. 9).

PCV varied from 11.46 to 75.39 and GCV varied from 10.75 to 68.85. Among the biometric characters, plant height, petiole length, leaf/stem ratio, total yield, total leaf weight and total stem yield exhibited high GCV and PCV values. Among the five quality parameters analyzed, protein, nitrate and oxalate had higher values. Leaf blight incidence had higher coefficients of variation when compared to leaf webber incidence.



X1 Plant height (cm)
X2 Length of leaf lamina (cm)
X3 Leaf width (cm)
X4 Petiole length (cm)
X5 Number of branches
X6 Stem girth (cm)
X7 Days to 50 per cent bolting
X8 Leaf/stem ratio (1st cutting)
X9 Leaf/stem ratio (2nd cutting)
X10 Leaf/stem ratio (3rd cutting)
X11 Total leaf/stem ratio
X12 Yield (g) (1st cutting)
X13 Yield (g) (2nd cutting)
X14 Yield (g) (3rd cutting)
X15 Total yield (g)
X16 Total leaf weight (g)
X17 Total stem weight (g)
X18 Protein (per cent)
X19 β-carotene (μg 100 g^{-1})
X20 Vitamin C (mg 100 g^{-1})
X21 Nitrate (per cent)
X22 Oxalate (per cent)
X23 Leaf blight
X24 Leaf webber

Fig. 9. Genotypic and phenotypic coefficients of variation for different characters

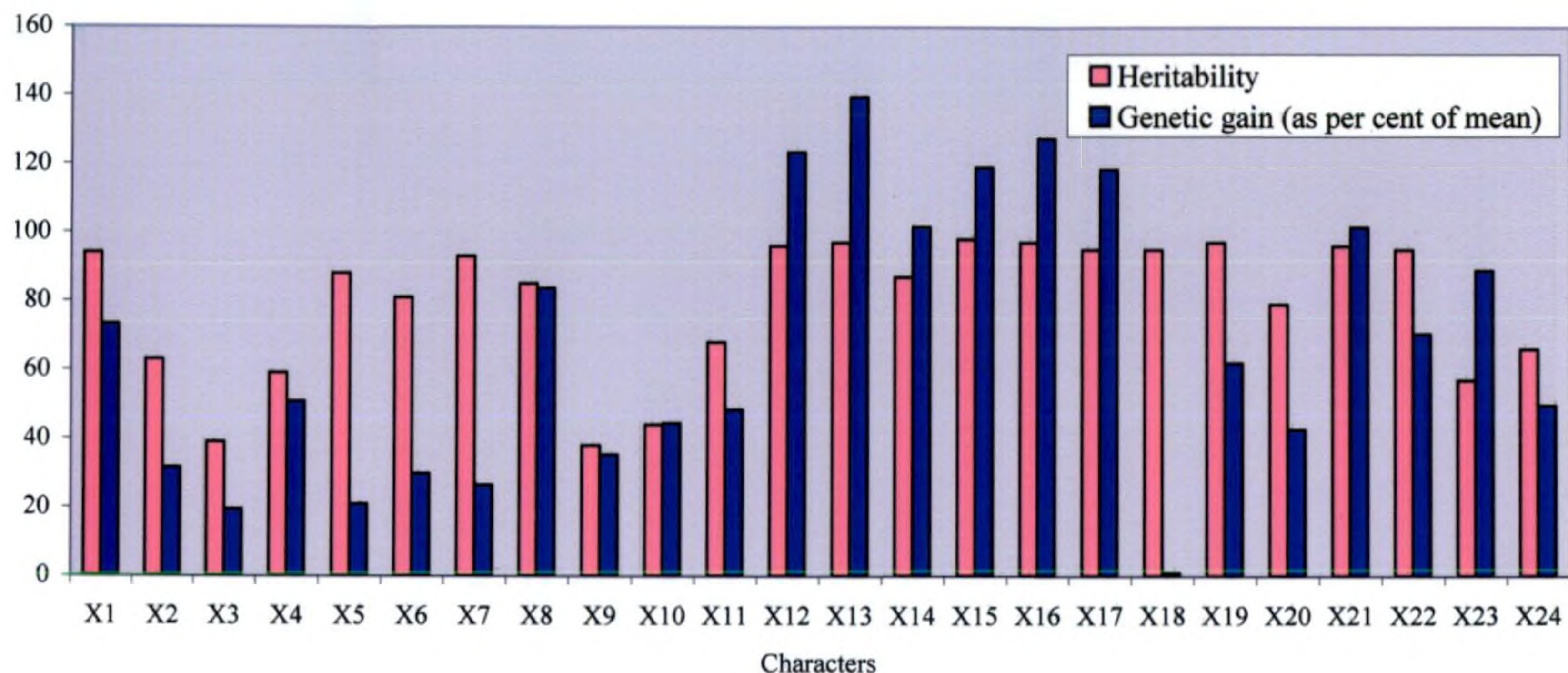
These results are in agreement with the findings of Pan *et al.* (1991), Revanappa and Madalgeri (1998) and Priya and Celine (2001).

Higher GCV and PCV values indicate the great extent of variability for these characters thereby offering good scope for improvement through selection. The PCV was seen to be higher than GCV for all the characters as reported by Revanappa and Madalgeri (1998). But still, the magnitude of genetic variation nearly approached the phenotypic variation, in all the characters, denoting that selection on phenotypic basis will hold good scope for genotypic upgradation.

Both heritability and genetic advance are important selection parameters. Heritability estimates together with genetic advance are more effective in predicting the gain under selection than heritability estimates alone. In the present study, characters such as yield in the individual cuttings, total yield, total leaf weight, total stem weight and β-carotene content exhibited high heritability along with high genetic advance. Similar results were obtained by earlier workers (Pan *et al.*, 1991; Revanappa and Madalgeri, 1997; Shukla and Singh, 2000). This denotes that phenotypic selection for these traits will be more useful.

Plant height, days to 50 per cent bolting and vitamin C content showed high heritability together with moderate genetic advance. These results are in accordance with the conclusions of other workers like Revanappa (1985) and Lohithaswa *et al.* (1996). Stem girth, leaf/stem ratio and protein content showed high heritability coupled with low genetic advance as reported by Revanappa (1985).

Genetic gain as percentage of mean showed wide variation among the different characters studied. Plant height, yield and yield attributes, protein, nitrate and oxalate contents and reaction to leaf blight were found to have high heritability along with high genetic gain thereby highlighting the chances for crop improvement through selection. Priya and Celine (2001) have reported similar results (Fig. 10).



X1	Plant height (cm)	X7	Days to 50 per cent bolting	X13	Yield (g) (2nd cutting)	X19	β-carotene (μg 100 g^{-1})
X2	Length of leaf lamina (cm)	X8	Leaf/stem ratio (1st cutting)	X14	Yield (g) (3rd cutting)	X20	Vitamin C (mg 100 g^{-1})
X3	Leaf width (cm)	X9	Leaf/stem ratio (2nd cutting)	X15	Total yield (g)	X21	Nitrate (per cent)
X4	Petiole length (cm)	X10	Leaf/stem ratio (3rd cutting)	X16	Total leaf weight (g)	X22	Oxalate (per cent)
X5	Number of branches	X11	Total leaf/stem ratio	X17	Total stem weight (g)	X23	Leaf blight
X6	Stem girth (cm)	X12	Yield (g) (1st cutting)	X18	Protein (per cent)	X24	Leaf webber

Fig. 10. Heritability and Genetic gain as percentage of mean in amranthus accessions for different characters

5.1.3 Correlation studies

Correlation coefficient is a statistical measure used to find out the degree and direction of relationship between two or more variables. This helps in understanding the change caused in one character by doing selection based on another character.

In the present study, characters such as plant height, leaf length, leaf breadth, petiole length, stem girth, leaf weight and stem weight exhibited positive correlation with yield. Similar correlations were reported by Anuradha (1992) and Varalakshmi and Reddy (1994). From this, we can conclude that selection based on these characters will help in the development of improved varieties with higher yield. Total leaf/stem ratio had a low positive correlation with yield contradictory to the findings of Vijayakumar (1980).

On comparing the phenotypic and genotypic correlation values, it was observed that the genotypic correlation was higher than the corresponding phenotypic values, which is in accordance with the findings of Shukla and Singh (2003b). Leaf weight was positively correlated with plant height and branches per plant was significantly correlated with stem diameter as reported by Shukla *et al.* (2004). Yield and yield attributes showed negative correlation with leaf spot and leaf webber incidence which explains the reduction in yield due to the occurrence of these two problems.

Protein content was seen to have positive correlation with β-carotene and vitamin C contents which means that selection programmes for one of these nutrients will result in the development of varieties richer in the other two nutrients. The antinutrients, nitrate and oxalate exhibited positive correlation with each other (Holubava, 2002).

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5.1.4 Path coefficient analysis

Path coefficient analysis is simply a standardised regression coefficient which splits the correlation coefficient into the measures of direct and indirect contribution of independent variables on dependent variable. The path analysis reveals whether the association of these characters with yield is due to their direct effect or is a consequence of their indirect effects via other component characters.

In the present study, plant height, leaf length, leaf width, number of branches, stem girth and total leaf weight were the characters included for path analysis.

The genotypic correlation of plant height on total yield was high (0.6922). The major portion of this was contributed by its indirect effect on total leaf weight and the direct effect was very low. So indirect selection through leaf weight will be effective.

Leaf length had a low genotypic correlation with yield while that of the number of branches was moderate. But the direct effects of both these components were negative. Their contribution towards yield was mostly due to their indirect effect through total leaf weight. Their indirect effects through other characters were negligible.

Leaf width showed a low genotypic correlation with yield and its direct effect was only 0.0626. Its indirect effect through leaf weight compensated for its low direct effect.

Though the genotypic correlation of stem girth on yield was high (0.5674), the direct effect was only 0.1076 and the major portion was contributed by its indirect effect through leaf weight. Stem girth showed a low indirect effect through plant height also indicating that indirect selection through height will be effective. The data obtained from these five characters clearly indicate that indirect selection through leaf weight is the most effective method in amaranthus to get higher yield.

Total leaf weight had the maximum genotypic correlation with yield (0.9662) and high direct effect (0.8546). So its direct selection is also very effective.

5.1.5 Genetic divergence analysis

One of the present techniques of measuring genetic divergence is by Mahalanobis's D^2 statistic. This technique measures the force of differentiation at the intracluster and intercluster levels and thus provides a reasonable basis for selection of genetically divergent parents in breeding programmes.

The 34 accessions of amaranthus were subjected to D^2 analysis based on seven characters. They were grouped into ten clusters on the basis of relative magnitude of D^2 values. The greater the distance between two clusters, greater is the divergence between the accessions belonging to the two clusters and vice versa.

The existence of ten clusters within 34 accessions indicates wide genetic diversity between the accessions selected. Cluster VI exhibited the maximum cluster mean for plant height and in case of mean leaf length, cluster V was superior. Cluster VIII had highest value for leaf width and petiole length. In case of days to 50 per cent bolting, cluster X had highest value. This indicates the superiority of these clusters with respect to those particular characters.

The highest intercluster distance was seen between cluster II and III meaning that these two clusters show the maximum genetic divergence which can be utilized in hybridization programmes to get heterotic advantage. Cluster I and II with least intercluster distance are genetically most similar. The intracluster distances were seen to be lower than intercluster distances thereby suggesting homogeneity among the genotypes within a cluster and heterogeneity between clusters.

The clustering pattern is not in coordination with the geographical distribution. This can be attributed to several reasons. The same genetic material might have spread to different locations by the free exchange of seeds. The slight morphological variations may be due to environmental influences.

5.1.6 Cataloguing of the germplasm

Cataloguing of accessions based on a standard descriptor is useful in international exchange of information in a precise and scientific way. Moreover it helps in locating morphological characters linked with economic traits, which can be utilized for indirect selection.

The 34 accessions involved in the experiment were noted to have erect growth habit and in the majority of them, branches were distributed all along the stem. In very few accessions, the branches were concentrated in the basal region.

Plant height showed a wide variation from less than 30 cm to more than 60 cm. Majority of the landraces had height in the intermediate range, *i.e.*, 30-45 cm. Stem and leaf pubescence were completely absent. Spines were not seen in any of the accessions.

Higher leaf length of 11 cm or above was recorded in all accessions except Am 55. In majority of the accessions, leaf width was in the intermediate range of 5-10 cm. The accessions of *A. hypochondriacus* had uniformly elliptic leaves and those of *A. dubius* had triangle ovate leaves whereas the *A. tricolor* accessions showed varying leaf shapes.

Similar pattern was noticed in case of leaf pigmentation also. The three *A. hypochondriacus* accessions had purplish green leaves while the *A. dubius* accessions showed dark green leaves. In case of *A. tricolor* accessions, the leaf colour was noted to range from light green to purple. Hamid *et al.* (1989) have reported diversity in leaf colour among *Amaranthus* spp, *i.e.*, light green to green and reddish to red. Stem

pigmentation varied from green to deep purple. Variations in leaf and stem pigmentation in amaranthus have been observed by Wu *et al.* (2000).

Inflorescence was spike in all the *A. tricolor* accessions and panicle in the *A. hypochondriacus* and *A. dubius* accessions. The terminal inflorescence of *A. hypochondriacus* and *A. dubius* is another character which distinguishes them from the *A. tricolor* accessions with axillary inflorescence. Inflorescence colour ranged from yellow to purple.

In a single accession, Am 13, 50 per cent bolting was achieved very early, *i.e.*, within 30-45 days. But in the rest of the accessions, it took upto 46-60 days. Studies by Varalakshmi (2004) have shown that days to 50 per cent bolting showed much deviation among the accessions. Seed colour was black in all the accessions.

5.2 MOLECULAR CHARACTERIZATION

Polymerase Chain Reaction (PCR) based molecular markers have developed into powerful tools which are widely used to analyse genetic relationships and genetic diversity in crop plants. These markers are highly efficient with respect to their accuracy and ability to depict maximum genetic variability. RAPD technique is one of the most popularly used PCR markers. The information obtained from RAPD analysis opens up the avenues for proper identification and selection of the genotypes that can be used for varietal identification and future crop improvement programmes. RAPD analysis has been successfully employed to analyse genetic diversity in amaranthus by earlier workers (Chan and Sun, 1997; Mandal and Das, 2002; Manilay, 2003).

In the current study, an attempt was made to determine the extent of genetic diversity in 27 accessions of amaranthus, belonging to three species collected from different geographical regions of Kerala, using RAPD markers. Arbitrary primers were used to amplify random DNA sequences in the genome.

Isolation of genomic DNA of amaranthus was done using modified Murray and Thompson (1980) method. The yield and purity of the DNA extracted from the 27 accessions was found to be sufficient for PCR reaction. Dixit (1998) devised a method of DNA isolation in amaranthus that yielded high amount (600-800 µg /g fresh leaf tissue) of good quality DNA ideal for PCR amplification.

To identify the promising primers for RAPD analysis, 38 random primers were screened using the DNA of accession Am 22. The procedure standardized by Mandal and Das (2002) in grain amaranthus was used for PCR amplification after incorporating slight modifications. 25 primers, out of the 38 primers screened, yielded amplification products indicating the presence of sequence complementary to these primers in the DNA of Am 22.

A total of 77 bands were generated by the primers of which 40 per cent (31 bands) were polymormorphic and the remaining were monomorphic (46 bands). For further amplification of DNA from the 27 amaranthus accessions, four promising primers were identified based on performance in DNA amplification, production of highest number of polymorphic bands as well as intense bands and reproducibility. They were UBC-17, UBC-18, UBC-23 and OPE-14.

These four primers on RAPD analysis yielded 39 scorable bands (9.75 bands per primer). Among these four, UBC-17 produced the highest number of scorable bands (12 nos) while the minimum number of bands (8) were amplified by UBC-18. All the UBC primers produced amplification for all the accessions while OPE-14 failed to give amplification for Am 9 and Am 27. UBC-17 and OPE-14 produced bands which were monomorphic for the *A. hypochondriacus* and *A. dubius* accessions together. Separate bands monomorphic for the *A. tricolor* accessions were also generated by them. In case of UBC-18, two bands were monomorphic for the dubius accessions and another three were

monomorphic for the three hypochondriacus accessions. No such pattern was noticed in UBC-23 where all the bands were fully polymorphic.

The estimation of Jaccard's similarity coefficients and construction of dendrogram using UPGMA revealed the presence and extent of genetic similarities among the accessions of amaranthus. The pairwise similarity coefficient values ranged from 0.07 to 0.81. Cluster analysis revealed that at 0.27 similarity coefficient, the 27 accessions could be grouped into two main clusters.

The first cluster comprises of nine accessions and is divided into two subclusters. Among these, one subcluster is again split into two groups of which one includes the three *A. hypochondriacus* accessions and the other represents the five *A. dubius accessions.* The second subcluster contains a single *A. tricolor* accession, Am 47. The remaining 18 accessions all belong to *A. tricolor* and they are seen together in the second cluster. The fact that Am 47 has occurred in the first cluster along with the other two species indicates the probability of existence of genetic variation in this accession when compared to the other *A. tricolor* members. So further studies on this particular accession is necessary.

The pattern of distribution of the accessions clearly proves the marked genetic variation between the three different amaranthus species. Transue *et al.* (1994) carried out RAPD analysis for the seperation of grain amaranth accessions belonging to different species. The grouping of the *A. dubius* accessions in the first cluster suggests that they are more closely related to the *A. hypochondriacus* members when compared to the tricolor accessions. Chan and Sun (1997) have also reported the similarity between *A. dubius* and *A. hypochondriacus* in their studies on different amaranthus species using isozyme and RAPD markers.

The second cluster exhibited a wide range of similarity coefficient from 0.28 to 0.81 thereby establishing the wider genetic variability within *A. tricolor* accessions as reported by Devadas *et al.* (1991). This

observation can be further substantiated by the fact that morphological cataloguing revealed very little variation among the *A. dubius* and *A. hypochondriacus* accessions whereas the *A. tricolor* accessions exhibited extensive morphological diversity among themselves.

The clustering pattern is not related to the geographical distribution or morphological characterization by D^2 analysis. But upto a certain extent, it can be coordinated with a significant morphological trait, *viz.*, pigmentation of plant parts. At 42 per cent similarity, the second cluster can be split into six subclusters. In the largest subcluster, eight accessions are there of which six are seen as three pairs. The first pair (Am 4 and Am 13) have purple pigmentation of leaves, stem, petiole and inflorescence and same is the situation in case of the second pair (Am 25 and Am 31).

The second and third subclusters include three accessions each. Am 45 and Am 54 occur as a pair in the third cluster and both of them have green leaves with purple veins and margins along with purple stem. Among the remaining three subclusters, two are single accession clusters representing Am 22 and Am 55. The last one includes Am 9 and Am 27, both purple in colour.

Brenner *et al.* (2000) has suggested that morphological studies and molecular techniques should be used together. In the current experiment, morphological characterization by D^2 analysis failed to group the genotypes according to geographical location and clear demarcation between the different species was also not attained. Compared to that, molecular characterization by RAPD was able to differentiate the different species and group the accessions in accordance with plant pigmentation upto a limited extent. This clearly verifies the fact that molecular techniques are far more superior and precise than morphological techniques.

Thus the present study revealed that RAPD technology was successful and efficient in discriminating the various amaranthus accessions at species

level. The clusters obtained from RAPD analysis depict the wide genetic variation that exists both within and between different species of amaranthus. Polymorphism obtained in the present study will be very useful for DNA fingerprinting and higher molecular studies of these accessions. In order to obtain a more reliable marker that can be used in the identification of a significant trait useful for breeding purposes, greater number of primers are to be included in the RAPD analysis to get more accurate results.

Summary

6. SUMMARY

The present investigation on "Characterization and evaluation of landraces of amaranth (*Amaranthus* spp.)" was carried out at the Department of Olericulture and the Department of Plant Biotechnology, College of Agriculture, Vellayani during 2003-2005.

The study envisaged genetic cataloguing of the available germplasm in vegetable amaranths, assessment of variation in growth, yield, quality characters and resistance to biotic stress, genetic variability, genetic divergence, association among the characters including direct and indirect effects of various characters on yield and molecular characterization using RAPD analysis.

The experimental material consisted of 34 diverse accessions of amaranthus selected from the germplasm maintained in the Department of Olericulture, College of Agriculture, Vellayani based on morphological and geographical variations. Representative samples from the different districts of Kerala have been included. Among the selected 34 accessions, 25 belong to *Amaranthus tricolor*, six to *A. dubius* and and three to *A. hypochondriacus*.

The analysis of variance revealed significant variation among the accessions for all the characters studied. Am 91 had the maximum yield of 387.22 g and Am 13 had the minimum yield of 51.67 g. The highest and lowest leaf weights were recorded for Am 91 (235.56 g) and Am 13 (30.0 g) respectively. In case of total stem weight, Am 71 (189.44 g) was the most superior and Am 13 (21.66 g) was the most interior. Leaf/stem ratio was seen to vary from 0.53 in Am 22 to 2.38 in Am 77.

The quality parameters included in the study were β-carotene, vitamin C, protein, oxalate and nitrate. Am 5 had the maximum β-carotene content of 4655.54 μg per 100 g while Am 90 had the minimum content of

1269.94 per 100 g. Vitamin C was highest in Am 58 (151.22 mg per 100 g) and lowest in Am 58 (54.88 mg per 100 g). Protein content varied from 0.67 per cent in Am 27 to 3.57 per cent in Am 91.Oxalate was minimum in Am 90 (0.6 per cent) and maximum in Am 76 (2.08 per cent). Nitrate content varied from 0.04 per cent in Am 37 to 1.62 per cent in Am 28.

Much variation was noticed in the incidence of pests and diseases. Leaf blight incidence ranged from 0 in Am 89 and Am 91 to 2.0 in Am 14 and Am 77. Severity of leaf webber attack was maximum in Am 78 (2.07) and minimum in Am 42 (0.63).

Considering the mean performance with respect to yield, quality and resistance to leaf blight, the accessions Am 91, Am 90 and Am 89 were identified as superior.

Genetic variability was high for growth, yield and quality characters and response to biotic stress. In this study, the PCV ranged from 11.46 to 75.39 and GCV ranged from 10.75 to 68.85. Higher GCV and PCV values for yield and yield attributes indicate good scope for improvement through selection for these characters. High heritability along with genetic gain as per cent of mean was recorded for yield and quality characters. Genetic advance was higher in the case of yield and yield attributes.

The morphological characters such as plant height, leaf length, leaf width, petiole length, number of branches and stem girth had positive correlation with yield. Leaf/stem ratio had positive correlation with yield but the values were low and non-significant.Leaf blight and leaf webber attack were found to be negatively correlated with yield.

Path analysis was based on six characters *viz.*, plant height, leaf length, leaf width, number of branches, stem girth and total leaf weight. Total leaf weight had the maximum direct effect on yield suggesting that selection based on this character would help in the development of

varieties with higher yield. Among the other five characters plant height, leaf width and stem girth had low direct effects while leaf length and number of branches had negative direct effects on yield. Their influence on yield was mainly due to their indirect effect through total leaf weight.

By adopting Mahalanobis's D^2 statistic, the 34 accessions were divided into ten clusters. Cluster II had the maximum number of accessions (9) followed by cluster III (8). Cluster IV and cluster I had five and four accessions respectively. Both cluster V and cluster VI had two accessions each. Clusters VII, VIII, IX and X had only one accession each. With regard to the cluster means the seven characters involved showed highest values in different clusters. The intercluster distance was maximum for clusters II and III (3097.77) followed by clusters II and IV (976.53). Intracluster distance was maximum in cluster III.

The landraces were morphologically catalogued using the modified descriptor developed from the standard descriptor for amaranthus by IPGRI. The results revealed distinct variations among the landraces with respect to vegetative and floral characters. All the accessions showed erect growth habit, absence of spines in the leaf axil and black seed colour. Stem and leaf pigmentations were found to range from green to purple. Much variation was noted in the leaf shape and petiole pigmentation. The inflorescence characters were noted to differ with the species.

RAPD (Random amplified polymorphic DNA) analysis was used to characterize genetic variability and relationships at the molecular level among 27 accessions selected from the 34, based on morphological variation and geographical distribution. DNA was isolated from the tender leaves of young seedlings. DNA yield ranged from 120 μg /ml to 930 μg/ml. Purity of DNA (A_{260}/A_{280}) varied from 1.4 to 2.0. Out of the 38 arbitrary decamer primers screened, 25 yielded amplification products.

A total of 77 RAPDs were generated by the 25 primers. Out of this, 40 per cent (31 bands) were polymorphic and the rest were monomorphic (46 bands). Four promising primers, *viz.*, UBC-17, UBC-18, UBC-23 and OPE-14 were identified for RAPD analysis of all the accessions based on their performance in DNA amplification, reproducibility and production of highest number of polymorphic bands. The selected primers yielded 39 scorable bands (9.75 bands per primer). Primer UBC-23 could distinguish maximum polymorphism among the accessions studied, while UBC-17 produced maximum number of scorable bands.

Estimation of Jaccard's similarity coefficients and construction of dendrogram by UPGMA revealed the presence and extent of genetic similarities among the 27 accessions of amaranthus studied. The overall similarity coefficients ranged from 0.07 to 0.81. Cluster analysis showed that at 0.27 similarity coefficient, the 27 accessions could be grouped into two main clusters.

The first cluster containing nine members, is further subdivided into two subclusters. The larger subcluster has two subgroups of which, one includes the three *A. hypochondriacus* accessions and the other comprises of the five *A. dubius* members. The second subcluster includes a single *A. tricolor* accession, Am 47. The remaining 18 accessions, all belonging to *A. tricolor*, grouped together in the second subcluster which exhibits a wide range of similarity coefficient from 0.28 to 0.81.

The clustering pattern indicates the distinct genetic divergence between the different species of amaranthus. But the grouping is not in accordance with the geographical distribution or morphological characterization by D^2 analysis. The clustering within the *A. tricolor* accessions is seen to be in agrèement with the pigmentation of plant parts upto a limited extent.

From the results, it is evident that the *A. tricolor* accessions show a wider range of variability compared to the other two species. This is highly advantageous since *A. tricolor* is the most preferred species by Keralites. So the available genetic variability should be exploited to the maximum possible extent in future breeding programmes. The results clearly indicate that in a crop like amaranth having high genetic diversity, RAPD analysis can be used in the clear differentiation of various genotypes. Using more number of primers can help to generate a more reliable marker which in turn can be used in the identification of significant traits.

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*Original not seen

CHARACTERIZATION AND EVALUATION OF LANDRACES OF AMARANTHUS (*Amaranthus* spp.)

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Abstract of the
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ABSTRACT

The research project entitled "Characterization and evaluation of landraces of amaranth (*Amaranthus* spp.)" was carried out at the Department of Olericulture and the Department of Plant Biotechnology, College of Agriculture, Vellayani during 2004-2005. The objective of the study was to morphologically catalogue the landraces using the modified descriptor developed from the standard descriptor for amaranthus by IPGRI, to estimate the genetic parameters for different traits in the germplasm for identifying superior lines based on growth, yield, quality and pest and disease resistance and to characterize the landraces using molecular techniques (RAPD analysis).

Thirty four accessions for amaranthus were collected from various sources and grown in the field in RBD with three replications. Analysis of variance showed significant differences among the accessions for all the characters evaluated. The yield obtained ranged from 51.67 g (Am13) to 387.22 g (Am 91) and leaf/stem ratio varied from 0.53 (Am 22) to 2.38 (Am 77).

The range of values for quality characters were 1269.94 to 4655.54 μg 100 g^{-1} for β-carotene, 54.88 to 151.22 mg 100 g^{-1} for vitamin C, 0.67 to 3.57 per cent for protein, 0.62 to 2.08 per cent for oxalate and 0.04 to 1.62 per cent for nitrate.

Incidence of biotic stress ranged from 0 (Am 89 and Am 91) to 2.0 (Am 14 and Am 77) for leaf blight and 0.63 (Am 42) to 2.07 (Am 78) for leaf webber.

High PCV and GCV values were obtained for yield characters. Heritability ranged from 38 to 97 per cent. High heritability with high genetic advance was seen for yield and quality characters. All the morphological characters except days to 50 per cent bolting had positive correlation with yield. Leaf blight and leaf webber incidence exhibited

negative correlation with yield. Total leaf weight had the maximum direct effect on yield in path analysis.

The 34 accessions were split into ten clusters by using D^2 statistic. A maximum number of nine accessions were noted in cluster II. Four clusters (Clusters VII, VIII, IX and X) were found to have only one accession each. A maximum intercluster distance of 3097.77 was seen between clusters II and III and intracluster distance was maximum in cluster III. Morphological cataloguing of the landraces based on 20 characters using modified descriptor revealed distinct variations among the accessions for most of the characters.

Out of the 34 accessions involved in the study, 27 were selected for RAPD analysis. Screening was done using 38 primers out of which 25 produced amplification. A total of 77 bands were produced out of which 40 per cent (31 bands) were polymorphic and the rest were monomorphic (46 bands). Four primers *viz.*, UBC-17, UBC-18, UBC-23 and OPE-14 gave high level of polymorphism and were chosen for PCR amplification. Maximum polymorphism was seen in case of UBC-23 and maximum number of scorable bands were obtained for UBC-17. The primers gave 39 scorable bands (9.75 bands per primer).

The overall similarity coefficients ranged from 0.07 to 0.81. The 27 accessions got divided into two main clusters. The first cluster had nine members and included the three *A. hypochondriacus* and five *A. dubius* accessions. A single *A. tricolor* accession, Am 47 was also seen in this cluster. The second cluster was comprised of the remaining 18 *A. tricolor* accessions. This distribution revealed the distinct genetic variation between the three different species of amaranthus. It was also evident that the genetic divergence was much higher in *A. tricolor* compared to the other two species. This higher variation should be exploited to the maximum possible extent in future crop improvement programmes. Using more number of primers for RAPD analysis will make it possible to get more accurate results and this will help in the identification of reliable markers.

Appendices

APPENDIX-I

Descriptor for amaranthus

1. Growth habit
    1. Erect
    2. Prostrate

2. Plant height (measured in cm)
    - 1 < 30
    - 2 30-45
    - 5 46-60
    - 7 > 60

3. Branching index
    - 1 No branches
    - 2 Few branches all near the base of the stem
    - 3 Many branches all near the base of the stem
    - 4 Branches all among the stem

4. Stem pubescence
    - 0 None
    - 3 Low
    - 5 Conspicuous

5. Stem pigmentation
    - 1 Green
    - 2 Pale green
    - 3 Purplish green
    - 4 Pink/purple
    - 5 Deep purple

6. Spines in leaf axis
    - 0 Absent
    - 1 Present

7. Leaf length (measured in cm on 5th leaf)
    - 1 <5
    - 3 5-10
    - 5 11 and above

APPENDIX-I Continued

8. Leaf width (measured in cm on 5th leaf)

    1 < 5
    3 5-10
    6 11-16

9. Leaf pubescence

    0 None
    3 Low
    5 Conspicuous

10. Leaf pigmentation

    1 Entire lamina purple or red
    2 Basal area pigmented
    3 Green with deep purple centre
    4 Two stripes (V shaped)
    5 One stripe (V shaped)
    6 Green with margin and veins pigmented
    7 Purplish green
    8 Normal green
    9 Dark green

11. Leaf shape

    1 Lanceolate
    2 Elliptic
    3 Ovate
    4 Broad ovate
    5 Triangle ovate
    6 Rhombic ovate
    7 Rhombic

12. Leaf margin

    1 Entire
    2 Crenate
    3 Undulate

13. Prominence of leaf veins

    1 Smooth
    2 Slightly prominent
    3 Very prominent

APPENDIX-I Continued

14. Petiole pigmentation

    1 Green
    2 Dark green
    3 Purple
    4 Deep purple

15. Terminal inflorescence shape

    1 Spike (dense)
    2 Panicle with short branches
    3 Panicle with long branches
    4 Club shaped at tips

16. Terminal inflorescence attitude

    1 Erect
    2 Drooping

17. Axillary inflorescence

    0 Absent
    1 Present

18. Inflorescence colour

    1 Yellow
    2 Green
    3 Pink
    4 Red

19. Days to 50 per cent bolting

    1 30-45
    2 46-60
    3 61-75

20. Seed colour

    1 Pale yellow
    2 Pink
    3 Red
    4 Brown
    5 Black

APPENDIX – II

Weather data for the cropping period
(October 2004 to February 2005)

Standard week	Temperature (°C)		Rainfall (mm)	Relative humidity (%)	Evaporation (mm/day)
	Minimum	Maximum			
41	23.25	31.28	29.8	81.74	3.27
42	23.18	30.75	33.6	82.71	2.25
43	22.63	29.7	32.4	71.92	1.82
44	23.52	31.87	66.4	83.71	2.68
45	23.38	30.43	7.63	86.92	1.92
46	23.05	30.72	5.53	83.33	2.41
47	22.70	31.65	16.0	81.34	2.76
48	20.56	31.71	0	82.83	2.23
49	21.42	32.25	0	82.83	3.18
50	22.53	32.71	0	80.71	2.93
51	20.21	33.25	0	79.91	3.15
52	22.72	32.36	0	76.87	3.76
1	20.21	31.85	0	76.25	3.67
2	22.72	32.24	0	77.14	3.81
3	21.21	33.75	0	76.35	3.28
4	21.41	32.63	0	78.41	3.09
5	22.25	33.32	0.67	76.78	3.54
6	24.95	34.63	0	76.14	4.60
7	20.91	33.85	0	72.64	4.31