GENOME ANALYSIS IN THE GENUS *Amaranthus*

By

V K MALLIKA

THESIS

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DECLARATION

I hereby declare that this thesis entitled "Genome analysis in the Genus *Amaranthus*" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or any other similar title, of any other University or Society.

Vellanikkara,
7.12.1987

Mallika
V.K. MALLIKA

CERTIFICATE

Certified that this thesis entitled "Genome analysis in the Genus *Amaranthus*" is a record of research work done independently by Smt. V.K. MALLIKA, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Vellanikkara,
7.12.1987

Dr. K.V. PETER,
Chairman, Advisory Board,
Professor and Head,
Department of Olericulture,
College of Horticulture,
Vellanikkara, Trichur.

CERTIFICATE

We, the undersigned, members of the Advisory Committee of Smt. V.K. MALLIKA, a candidate for the degree of Doctor of Philosophy in Horticulture with major in Horticulture, agree that the thesis entitled "Genome analysis in the Genus *Amaranthus*" may be submitted by Smt. V.K. MALLIKA, in partial fulfilment of the requirement for the degree.

CHAIRMAN — Dr. K.V. Peter

MEMBERS — Dr. C. Sreedharan

Dr. P.K. Gopalakrishnan

Dr. K.M. Narayanan Namboodiri

Dr. T.V. Viswanathan

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Malika

V.K. MALIKA

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Introduction

INTRODUCTION

Amaranths include one of the ancient groups of crop plants having great potential for combating under and malnutrition in the world. Amaranths are divided into grain and vegetable types based on the parts used for consumption. While *A. tricolor* and *A. dubius* are the important species grown as leafy vegetables, *A. hypochondriacus*, *A. caudatus* and *A. cruentus* are the major grain species in the tropical and subtropical regions of the world. With a protein content of about 16%, amaranth seed compares very well with that of wheat (12-14%), rice (7-10%) and maize (9-10%). Again, amaranth protein has nearly twice the lysine content of wheat protein, three times of maize and in fact as much as in milk and would be a nutritional complement to conventional cereals. Similar to grasses, amaranth plants are also belayed with C_4 photosynthetic pathway, thus enabling them to produce more carbohydrates and to withstand adverse conditions like drought than C_3 plants. Because of the nutritional and physiological superiority of amaranths, recently National Academy of Sciences, USA selected amaranth for global research and development in order to make the crop universally prominent (National Research Council, USA, 1984)

Cytogenetical studies serve as an essential prelude to scientific crop improvement. Such studies have been successful in many crop plants to understand the phylogeny, evolution and domestication of various species. Investigations on the interspecific hybrids of *Amaranthus* to elucidate systematically the species relationship were made by some workers (Grant, 1959; Sauer, 1967; Pal and Khoshoo, 1973 a & b). However many gaps

and dissenting hypotheses, exist on the phylogeny, evolution and domestication of amaranths. For example, grain amaranths are believed indigenous to the New World, where the crop had been growing for about 6750 years (Agogino 1957). But at present Asia is the largest producer of grain amaranths. How did the three species move to India and establish in the Himalayas and Nilgiris would be an interesting topic to support the New World origin of grain amaranths. Also, the cultivation of vegetable species is predominantly confined to Asia and Africa. Cytogenetical studies on the interspecific hybrids between vegetable and grain species would add to our present understanding on the origin and distribution of vegetable species.

Amaranth taxonomy is so confused and the available taxonomic keys for identification of the species are too cumbersome for application (Sauer, 1967; Feine, 1980). Incidently these keys are based on minute floral details like shape and proportion of pistillate flower parts. Hence a simplified provisional key for eight *Amaranthus* species including major vegetable and grain species has been developed in the present study. Later, this key and cytomorphological studies were used to classify the amaranth germplasm of the College of Horticulture, into different species.

The genus *Amaranthus* contains 40 species and the taxonomists divided the genus into two sections Blitopsis and Amaranthus. The members of the section Blitopsis are characterised by prominent axillary inflorescences and trimerous flowers, where as members of the section Amaranthus have terminal panicles and pentamerous flowers. It is not clear whether the species belonging to these two sections are genomically related or not. Hybridization between species belonging to these two sections is seldom reported. In the present investigation hybrids are

developed within each section and also between the two sections and the interrelationship between sections and species are ascertained through cytomorphological analysis of parents and hybrids.

Many of the cytogeneticists in the past failed to fully establish the wild progenitors of cultivated amaranths (Pal and Khoshoo, 1973 a & b). *A. spinosus* is a pantropical cosmopolitian semiwild species, used sparingly as a vegetable. However it belongs to the section Amaranthus to which all grain species are grouped. *A. spinosus* shares its genome with *A. dubius*, an allopolyploid leafy vegetable (Grant, 1959). In the present study, hybrids between *A. spinosus* and other vegetable and grain species are analysed cytologically and morphologically, with a view to educe the role of this semiwild species towards the evolution of others. In this event the relationship between the two basic chromosome numbers in the genus (x = 16 and x = 17) is also delineated.

Most of the amaranth species are sensitive to day length (NRC, USA 1984). Bolting is one of the serious handicaps in the culture of vegetable amaranth. The information on the photoperiodic requirement of different species and varieties of *Amaranthus* is useful in various ways. First of all this would facilitate the selection of species or varieties for particular climatic regions and periods. Secondly the planting time of vegetable or grain amaranths can be correctly adjusted, so that there will be maximum production of edible greens or grains. Further, seedsmen can easily use the information on photoperiodic requirement for quick and early production of seeds by growing the plants at the proper time of flowering. Thirdly the knowledge on the photoperiodic requirement of species and varieties will be highly essential for any breeder to synchronize the flowering time for the success of any hybridization programme.

In the present investigation, the influence of varying photoperiodis on vegetative growth and flowering of different *Amaranthus* species is also investigated.

Amaranth contains an array of antinutritional factors such as oxalate, nitrate, polyphenols etc. in the leaves. Studies on adverse nutritional effects due to the higher consumption of amaranth leaves showed that an intake of more than 100 g/day causes the formation of calcium oxalate crystals in the human urinary systems (NRC, U S A 1984). Hence under the present level of consumption (18.8 g/person/day), nitrate and oxalate in the leaves do not pose any nutritional problem. The eight different species were evaluated for their oxalate and nitrate contents in a preliminary study. *In toto* this dissertation embodies the results of investigations on the following objectives.

1. Genome analysis
   a. Detailed studies on morphology and cytology of the eight species.
   b. Modification of the existing key for identification of the species.
   c. Interspecific hybridization and analysis of morphology and cytology of the hybrids.
2. Classification of the existing germplasm into different species based on detailed cytomorphological studies.
3. Analysis of reasons for low seed set in a widely accepted cultiver A_6 ('Kannara local') of *A. tricolor*.
4. Classification of the eight species based on response to photoperiodism.
5. Estimation of antinutrient factors (nitrate and oxalate) in the eight species.

Review of Literature

REVIEW OF LITERATURE

A. Origin and Distribution

Amaranths were widely dispersed through the worlds temperate and tropical regions even before man converted some of them into cosmopolitan weeds and domesticates. Sauer (1967) reported that about 60 amaranth species were native to the Americas and about 15 others to Europe, Asia, Africa and Australia. Most were pioneer annuals of naturally open habitats in mountain and desert canyons, river banks, lake shores, tidal marshes and ocean beaches.

The vegetable amaranths (section Blitopsis) were widely distributed in the tropical and subtropical regions with some in the temperate zones. The taxa used mainly as pot herbs were confined to tropical Asia and Africa which probably represented regions of their origin (De Candolle, 1886). Archaeological studies in South and Central America revealed that grain amaranths were associated with man since prehistoric times and were among the most ancient crops cultivated as early as 4800 B.C. (Pal and Khoshoo, 1974).

Many investigators believed that grain amaranths were cultivated in Southern Asia from time immemorial and probably originated there (De Candolle, 1886, Hooker, 1885, Merril, 1959; Vavilov, 1950). De Candolle (1886) and Vavilov (1950) considered Indo-Burma region as the centre of origin of grain amaranths, like *A. cruentus*. But there is no mention of these grains in the ancient sanskrit literature and there is no sanskrit name for this crop and so the Indo-Burman origin of grain amaranths appears quite untenable (Singh and Thomas, 1978). Further, Pal and Khoshoo (1973) showed that the four domesticates collected from Old world and New world were perfectly conspecific and no differences existed either morphologically and/or genetically.

Although grain amaranth is widely distributed in Asia and the most widespread in India, it is not clear as to when it was introduced into this country. Merril (1954) suggested that amaranth was introduced from Brazil into India (Malabar coast) by early Portuguese traders after 1500 AD from where it reached northern parts of India. It was grown along the whole length of Himalayas and appeared as a staple food crop in the Nilgiri hills of South India. They were since noted over an increasingly wider area of India as well as across the interior of China to Manchuria. Sauer (1976) reported that the crop later declined to a vanishing relic in its homeland and far more amaranth grain is now produced in Asia (especially in India) than in America. He also expressed the view that all the three cultivated grain amaranth species - *A. hypochondriacus*, *A. caudatus* and *A. cruentus* - were introduced to the Old world via Eruope and they were grown in European gardens as ornamentals and curiosities for at least 250 years. At the time of Spanish Conquest, grain amaranths were important in rituals of the Aztecs and other Mexican people. In Spanish America, after the sixteenth century, grain amaranth cultivation was regarded as a symbol of paganism and repressed, thus the crop nearly disappeared from history.

According to Sauer (1967), each of the three grain species originated from its own progenitor, viz., *A. cruentus* (n = 17) from *A. hybridus* (n = 16), *A. hypochondriacus* (n = 16) from *A. powellii* (n = 17) and *A. caudatus* (n = 16) from *A. quitensis* (n = 16). Alternatively he also hypothesised that *A. hybridus* (n = 16) gave rise to *A. cruentus* (n = 17) in the Central Americal region, *A. cruentus* moved northwards later and picked up *A. powellii* (n = 17) as its weed and by repeated interspecific hybridization gave rise to the Mexican grain amaranth, *A. hypochondriacus*

(n = 16). Similarly *A. cruentus* moved southwards, picked up *A. quitensis* (n = 16) and by continued crossing gave rise to *A. caudatus*. Pal and Khoshoo (1973a) opined that the exact mode of origin of grain amaranths was difficult to unravel since critical data based on experimental hybrids were not available to decide on the nature of genetic differentiation between the progenitor species on the one hand and between them and grain type on the other in all the pertinant combinations. Hence they found it difficult to comment on the two hypotheses put forward by Sauer (1967) about the descendance of grain amaranths.

The details of different species including synonyms and wild relatives are summarised in Table 1.

B. Morphology and Taxonomy

Amaranthus belong to the genus *Amaranthus* of the family *Amaranthaceae* (Amaranthus - sometimes spelled as Amarantus - Greek - Unfading in allusion to the unwithering bracts). The family comprises of 65 genera and 850 species, of mostly weedy plants but a few are grown for ornament and also for food as potherb, most abundant in warm countries. The genus *Amaranthus* are mostly erect or decumbent annual herbs with alternate, exstipulate leaves. Flowers are small, monoecious in axillary clusters or dense terminal thyrsoid panicles. The basic units of inflorescence are small dichasial cymes usually called glomerules, each ordinarily consisting of an initial staminate flower and an indefinite number of pistillate flowers. The glomerules are crowded on a leafless axil to form complex inflorescences, technically thyrses which are generally called spikes. Flowers are small, regular, tri/pentamerous, bracteate, perianth parts 2-5, imbricate, stamens 2-5, free, anthers 2 celled, dorsifixed. Ovary superior

Table 1. Botanical name, synonyms, origin, chromosome number, importance and wild relatives of *Amaranthus* species.

Sl No	Name of the species	Synonyms*	Place of Origin	Chromosome Number	Author	Economic Importance	Wild relatives
Vegetable type							
1	*Amaranthus tricolor* L.	*A gangeticus* L *A mangostanus* L *A tristis* L *A polygamous* L *A melancholicus* L	India or South China	34 34 32	Grant (1959) Takagi (1933) Takagi (1933)	Most popular vegetable type	-
2	*A lividus* L	*A blitum* L	Southern or Central Europe	34	Grant (1959)	Cultivated and weed	
3	*A dubius* Mart ex.Thell	-	Central America	64	Grant (1959)	Cultivated and weed	-
4	*A viridis* L	*A gracilis* Desf		34	Krishnaswami and Raman (1949)	Weed, not cultivated	-
5	*A graecizans* L	*A blitoides* S. Wats *A angustifolius* Lam		32	Helser and Whitaker (1948)	"	
6	*A albus* L	-	Western North America	32	Helser and Whitaker (1948)	"	
7	*A spinosus* L			34	Polya (1949)	"	
8	*A retroflexus* L			34	Grant (1959)		
Grain type							
9	*A hypochondriacus* L	*A flavus* L *A leucocarpus* S.Wats *A frumentaceus* Buch.Hamilt	North Western and Central Mexico	32	Murray (1940a)	Cultivated for grain and as ornamental	*A. powellii* S. Wats *A. bouchonii* Thell
10	*A cruentus* L	*A paniculatus* L.	Southern Mexico and Central America	32 34	Takagi (1933) Grant (1959)	Cultivated for grain vegetable and as ornamental	*A. hybridus* L *A. chlorostachys* Willd *A. patulus* Bertol
11	*A caudatus* L	*A mantegazzianus* Passr. *A edulis* Speg	Argentina (South America)	32	Takagi (1933)	Cultivated for grain and as ornamental	*A. quitensis* H.B.K.

* Wildspread nature of many species and variability resulted in considerable synonimity (Grant, 1959)

1-celled, with one campylotropous ovule, stigmas 2-4 and pubescent. Fruit is a one seed utricle enclosed by persistent perianth parts; circumscissile or indehiscent. Seeds are black, brown or white, compressed, smooth and shiny with floury endosperm (Gamble, 1967; Bailey, 1973; Singh and Thomas, 1978 and Muthukrishnan and Irulappan, 1986).

Many taxonomists attempted classification of genus *Amaranthus* (Thellung, 1914; Standley 1917; Schinz, 1934; Kowal, 1954; Sauer, 1950, 1955, 1957, 1967; and Aellen, 1961). Thellung (1914) and Schinz (1934) recognised two sections . Amaranthotypus Dumort and Blitopsis Dumort, Kowal (1954), based on seed morphology and anatomy of 21 species of *Amaranthus* proposed a few changes such as shifting of *A. spinosus* from the section Amaranthotypus to the section Blitopsis and also the creation of a new section, Puncticulate. Only two species were included in the section Puncticulatae of which one was *A. gracilis (= A. viridis)*. Bailey (1966) classified the genus essentially in the same way, wherein he described Blitopsis as a section, with all species having flower clusters in axils, while species under section Amaranthus types had only terminal flower clusters. Section Amaranthotypus included the important grain types while section Blitopsis consisted of the green types.

Sauer (1967) renamed section Amaranthotypus Dumort as section Amaranthus Sauer. The section Amaranthus included most of the domesticated ornamentals, all the grain and dye amaranths and common weeds. In order to support the renaming, he quoted the rules of nomenclature that the section bear the same name as the genus, as it includes the type species. *A. caudatus*, L. The section Amaranthus was distinguished from the bulk of the genus by plants monoecious cymes continuing above the uppermost leaves to form large compound terminal inflorescences, tepals and stamens 5, and utricle circumscissile. The monoceious habit,

the dehiscent utricle allowing easy threashing and winnowing, and large and compound inflorescences producing enormous quantities of seed make members of this section successful grain crops.

Sauer (1967) expressed the view that the genus *Amaranthus* is not a taxonomically difficult group as described by Grant (1959c). He pointed out that clear cut characters exist to distinguish each species within the genus. The marker key characters which distinguish qenetically isolated species are particularly shape and proportion of pistillate flower parts. Primary classification of the genus into species is based on bract size in relation to tepal size. Bailey (1973) formulated a key for identification of the species based on size and shape of flower clusters. The final key character is the utricle size compared to tepal size. Feine (1980) proposed a key which was only a modification of Sauer's classification (1967). The initial classification was based on number of tepals and stamens while the final emphasis was on bract structure, style branches and the tepal shape.

C. Floral arrangement

Murray (1940) described the arrangement and development of individual flowers within a flower cluster. In the monoceions species of grain amaranths used in the study, he observed that the inflorescence axis was usually branched and the shape of the inflorescence was determined by the length and number of branches and their angle with the axis. On the inflorescence axis, flower clusters developed in an alternate fashion. The first flower was terminal on its branch and at two branches located at base developed the second and third flowers. Each of these flowers in turn was terminal and at its base developed the next two flowers. The development was usually symmetrical up to the third or fourth series of flowers. At that time, the setting of the first seed usually inhibited growth and upset the symmetry. Unpollinated flower clusters had an exceptionally large number of flowers.

In an attempt to study the genetics of sex determination in *Amaranthaceae,* Murray (1940) observed that all the species used in his study were strictly monoecious. The different species used exhibited two types of arrangement of staminate and pistillate flowers. In the first type of arrangement, the first flower of each cluster was staminate and all the succeeding ones were pistillate. The only one staminate flower in each flower cluster of the inflorescence abscissed soon after shedding the pollen. All the monoecious species of *Amaranthus* except *A. spinosus* belonged to this group. In the second type, all the flowers of each cluster were of the same sex but the cluster of pistillate flowers developed only in the axils of branches and at the base of the terminal inflorescence. The clusters of staminate flowers were borne terminally on the main axis and lateral branches. *A. spinosus* belonged to this group.

Pal and Khoshoo (1974) observed that in the grain amaranths each glomerule contained one male flower and about 250 female flowers. Evidently the higher number of female flowers in the section Amaranthus was claimed to be advantageous for its exploitation as grain. Madhusoodhanan and Nazeer (1983) observed that in *A. tricolor,* glomerules contained up to 30 per cent male flowers. In other species of the section Blitopsis viz., *A. albus, A. graecizans* and *A. lividus* the percentage of male flower were lower (5-15%) except in *A. viridis* which showed up to 28% male flowers. Muthukrishnan and Irulappan (1986) recorded the percentage of male flowers/glomerule as 0.5 to 1% in grain types and 10-25% in the green types.

D. Pollination

Murray (1940) reported that the monoecious species of grain amaranths are mainly self pollinated although the stigmas of pistillate flowers remain receptive several days prior to the opening of staminate flowers (Protogyny). According to Sauer (1967) all the dioecious species (about 10)

were confined to a small area in North America, though sporadic occurrence of few dioecious species were reported in other regions. Walton (1968) found 15.7 to 34.9% cross pollination in *A. caudatus*. According to Khoshoo and Pal (1972) Amaranths are characteristically, wind pollinated but the grain species with colourful inflorescences are occasionally visited by bees. Pal and Khoshoo (1973a) expressed the view that the two sections of *Amaranthus* differ in their mode of pollination. In section Amaranthus there is only a single male flower/glomerule and there is a huge showy inflorescence leading to more cross pollination. In the section Blitopsis there are many male flowers/glomerule, small unshowy terminal inflorescence (when present) and greater development of axillary glomerules leading to self pollination. The difference between the two sections in their breeding systems was also correlated with cytogenetical variation.

According to Sauer (1976) grain species and their close relatives are monoecious and self fertile. Arrangement and sequence of anthesis of the unisexual flowers favour a combination of self and cross pollination. Each of the many cymes of the inflorescence is initiated by a single staminate flower followed by an indefinite number of pistillate flowers, often over a hundred. Stigmas of the earliest pistillate flowers are receptive before the staminate flower opens; most of the later pistillate flowers develop after the staminate flower has abscised. However, cymes of different ages are present on each indeterminate inflorescence and pollen transfer among them makes selfing more common than crossing.

Singh and Thomas (1978) suggested that the genus *Amaranthus* possessed three types of mating systems viz., obligate outcrossing in the dioecious species, relatively greater outcrossing in monoecious species of section Amaranthus while relatively greater self pollination in section Blitopsis.

Kauffman (1979) observed that grain amaranths are primarily self pollinated, though the mechanisms to assure outcrossing are present. He produced true to type lines from unbagged single plant selections made from a 'Mexican Bulk lot'. He viewed that available pollen do not come from the male flower in the same glomerule but from staminate flowers from other glomerules.

E. Crossing technique

Murray (1940) opined that the small closely arranged flowers of the monoecious species made emasculation extremely difficult. He suggested that the most satisfactory method of making crosses in the monoecious species was to pollinate heavily as soon as the stigmas were receptive and to remove the staminate flowers by hand. Even so, 5-25% self pollination usually occurred. The hybrids were easily distinguished from the monoecious parent and in several crosses dominant genes were used to distinguish hybrids in the seedling stage.

Madhusoodanan (1976) produced interspecific hybrid between *A. graecizans* and *A. viridis* after the removal of young unopened male flowers from each glomerule of the female parent followed by heavy dusting of pollen from the male parent. Even then more than half the progeny comprised of the selfed plants. The selfing resulted from subsequent opening of the male flowers in the emasculated glomerules which could not be removed without damaging the whole glomerule.

Kauffman (1981) suggested that it is very important to emasculate before anthesis in order to avoid selfing. Male and female flowers can be distinguished before anthesis and it is easy to identify the staminate flowers by the use of head-set magnifying glasses and to remove them by fine forceps. Since stigmas are receptive before the anthers dehisce and since the sequence of maturation of flowers is from bottom to the top, Kauffman (1981) recommended trimming of the lower florets with a safety razor prior to emasculation. The apical portion of the inflorescence

should also be removed to prevent pollination from later opening male flowers. This process left only 10-20 mm of florets for pollination. Pollen is collected by shaking the stamens of donor plant into a half a size 000 gelatin capsule. Maximum pollen production occur between 8 AM to 11 AM. The gelatin capsule is then placed oven the emasculated flower head and used as a protective cover to prevent later pollen contamination. It was also found that seed set is the highest when pollinations are repeated on three consecutive days following emasculation. The indeterminate nature of the inflorescence makes it unlikely that all stigmas become receptive on the same day.

F. Chromosome number

Grant (1959c) complied the information on chromosome numbers in 30 species of *Amaranthus* of which 8 were reported for the first time. Of these 4 had 2 n = 32 while others had 2n = 34. With the exception of one tetraploid species *A. dubius* (2n = 64) all other species has a diploid number of either 32 or 34 chromosomes.

According to Khoshoo and Pal (1972) the genus is dibasic with x = 16 and 17. While both basic chromosome numbers are almost equally distributed in section Amaranthus, a preponderance of x = 17 in section Blitopsis and therefore in the genus as a whole was observed. They also reported that out of the total 30 *Amaranthus* spp, 20 belonged to section Amaranthus and 10 to section Blitopsis. Half the species under the section Amaranthus had x = n = 16, one had n = 34 and nine had x = n = 17. Out of the 10 species in Blitopsis 2 1/2 species had x = n = 16 and 7 1/2 species had x = n = 17 (one species *A. graecizans* was dibasic and hence considered under both basic numbers and hence the half value).

Pal and Khoshoo (1974) suggested that *A. cruentus* got derived from *A. powellii* based on the analogy of chromosome number, 2n = 34. But Sauer (1976) reported that the comparative morphology of the species did not support this. Moreover somatic counts of 32 and 34 were noted between closely related species and within certain species of section Blitopsis without much taxonomic meaning. In a study of grain amaranthus and their wild relatives viz., *A. hypochondriacus, A. cruentus, A. caudatus, A. powellii, A. hybridus* and *A. quitensis*. Sauer (1976) observed that all the six species are diploids with 2n = 2x= 32 consistently, except that counts of both 32 and 34 were reported in *A. cruentus* and *A. powellii* respectively.

Madhusoodanan and Pal (1981) studied the cytology of five species of the genus *Amaranthus* (section Blitopsis) viz., *A. tricolor, A. lividus A. graecizans, A. viridis* and *A. albus*. They observed that all the species were diploid with x = 17 or x = 16, the former being more common. One bivalent was always associated with the nucleolus and meiosis was normal in all the five species studied. They observed that the modal number of ring bivalents/cell in different forms varied between 3 and 9 in *A. tricolor,* 5 and 8 in *A. graecizans*, 8 and 9 in *A. viridis,* 5 and 9 in *A. lividus* and was between 5 and 6 in *A. albus*. Usually one or two and sometimes three chiasmata were formed randomly in each bivalent. Chiasma frequency in wild species was higher in the cultivars. In most of the species, one or two bivalents underwent early disjunction ('Precentric bivalents'). Anaphase I was normal in all cases with equal distribution of chromosomes to the poles. Bivalents with more than two chiasmata sometimes had disjunction difficulties and were reponsible for late disjunction bridges.

Madhusoodanan and Nazeer (1983) studied the comparative morphology of the somatic karyotypes of vegetable maranths, viz., *A. tricolor*, *A. lividus*, *A. graecizans*, *A. viridis* and *A. albus*. Root tip cells in *A. albus* had 32 chromosomes in 16 pairs while the other species had 34 chromosomes in 17 pairs.

G. Basic number

The genus *Amaranthus* is dibasic with x = 16 and x = 17. Grant (1959a) reported that perhaps 16 is the original chromosome number, as the addition rather than a loss of chromosome is tolerated. Tucker and Sauer (1958) also postulated a phylogenetic increase in basic number on morphological grounds in two members of the section Amaranthotypus, *A. cruentus* (2 n = 34) and *A. hybridus* (2n = 32). But these hypotheses were not supported by any scientific evidence. Madhusoodanan and Pal (1976) noticed that addition of one chromosome to even a 17 set was not causing any disturbance to its genetic set, while reporting a primary trisomic in *A. tricolor*. Pal *et al.* (1982), based on the study of a dibasic interspecific semifertile hybrid between *A. hypochondriacus* (n = 17) suggested the cytogenetic relationship between the two basic chromosome numbers. Meiosis in the dibasic interspecific hybrid (2n = 33) was characterised by the presence of 15 bivalents and 1 trivalent at metaphase I in nearly 98 per cent of the PMCs. The presence of a trivalent in the dibasic F_1 hybrid indicated an aneuploid relationship between the two basic numbers. In the F_2, plants were observed with 2n = 34, 2n = 33 and 2n = 32. The presence of quadrivalent in F_2 plants with 2n = 34 was an additional evidence of the origin of x = 17 through primary

trisomy. The natural occurrence of a primary trisomic as observed in *A. tricolor* by Madhusoodanan and Pal (1976) further supported this.

H. Polyploidy

Murray (1940) observed that one application of 0.25 per cent acqueous solution of colchicine to the growing point of *A. caudatus* gave polyploids. The tetraploids showed increase in size of the various plant organs. The tetraploids flowered a week later than diploids. Pal and Khoshoo (1968) studied the cytogenetics of *A. edulis* an autotetraploid. In comparison to the diploid lines, the raw autotetraploids were shorter, sturdier and nonlodging with 83% seed fertility and had an increase in seed weight up to 2-5 times suggesting agroeconomic potential. In contrast to the diploids, the tetraploids had predominantly male flowers. Misra *et al.* (1971) produced polyploids in *A. hypochondriacus, A. edulis* and *A. caudatus* by treating with colchicine. After a thorough study of diploid and tetraploid grain amaranths, they showed that polyploidy apart from increasing grain size and weight without much loss in fertility, had generally maintained the nutritive value found at the diploid level.

Pal (1972) observed that *A. dubius* is the only polyploid species of the genus, commonly used as a pot herb. Meiotic studies in the tetraploid *A. dubius* by many workers have not revealed any multivalent formation. (Grant, 1959b; Clifford 1959; Pal and Khoshoo, 1965; Pal, 1972). Sauer (1976) reported that no spontaneous polyploids were known among the grain amaranths but colchicine induced tetraploids and amphiploids had been bred in a few of them.

Madhusoodanan and Pal (1984) observed autotriploids in *A. tricolor* as a result of cross between autotetraploids and diploids. The triploids exhibited gigantism in morphological features than both 2n and 4n parents. The foliage had a thicker texture,a trait undesirable for a leafy vegetable. Stomatal size was intermediate to the parents. Cytological investigations revealed higher trivalent frequency, about 65 per cent of chromosomes being involved in trivalent formation. Subsequent course of meiosis was abnormal resulting in considerable reduction in the pollen fertility. However, triploids were suggested to be commercially unexploitable because the plant was not amenable to vegetative multiplication and also because of the practical difficulty in the production of triploids.

I. Intergeneric and interspecific hybridization

Hybridization within the genus *Amaranthus* or between *Amaranthus* and other genera were attempted by a few workers, though not with the objective of breeding new promising varieties of grain or vegetable amaranths. The more important earlier work on intergeneric and interspecific hybridization in *Amaranthaceae* was by Murray (1940). He attempted crosses between *Amaranthus* and *Acnida, Gomphrena, Celosia* or *Achyranthes*. However genuine hybrids were obtained only with the genus *Acnida*. In the monoecious species of *Amaranthus* including grain amaranths Murray (1940) recognised two types of arrangement of staminate and pistillate florets in the glomerule of the inflorescence. In all species except *A. spinosus* the first flower of each glomerule is staminate and the rest pistillate while in *A. spinosus* each glomerule bears flowers of the same sex either pistillate on staminate. Hybridization between these

two types with differing sex arrangement was reported to be comparatively difficult. The first type of sex arrangement was suggested to be epistatic to the second type (spinosus type) when *A. caudatus* was crossed with *A. spinosus*. The hybrid also inherited two dominant leaf colour genes carried by the male parent. The hybrid had normal flowers but the staminate and pistillate flowers had no precise positional arrangement with reference to each other.

Evidence for the close homology of the chromosomes in crosses between the related grain species was provided by Covas (1950), who observed complete bivalent pairing (16 II's) in a natural interspecific hybrid between *A. edulis* (2n = 32) and *A. hybridus* (2n = 32). Pollen Fertility was less than 50% in the hybrid.

Tucker and Sauer (1958) reported a number of hybrids among closely related species viz., *A. caudatus*, *A. cruentus*, *A. hybridus*, *A. powellii* and *A. retroflexus* from California. These belonged to section Amaranthotypus and seven out of the ten collections studied were resultants of triple hybridization. The cytological study of one of these hybrids, namely, *A. cruentus*, *A. hybridus*, *A. powellii*, *A. retroflexus* showed that this hybrid had a somatic chromosome number 2n = 32 during mitosis and 16 bivalents at metaphase I during meiosis with little meiotic irregulatrities in contrast to 2n : 34 in parental species. According to Singh and Thomas (1978) the above hybrid resulted by the loss of a pair of chromosomes through non-homology of the chromosomes in the initial cross.

Grant (1959c) did not observe any reduction in pollen fertility in the hybrids examined by him. One hybrid plant between *A. cruentus* (2n = 34)

and *A. hybridus* (2n = 32), having 2n = 34 was most likely a segregate of the initial hybrid as no chromosomal irregularities were observed in meiosis. Khanna *et al.* (1960) noticed five natural interspecific hybrids, three from the 3-stamen group and two from the 5-stamen group(*A. cruentus x A. hypochondriacus* and *A. dubius x a. spinosus*). The hybrids were mostly sterile, which showed that the parent had independent phylogenetic relationship. Seth (1963) also reported several interspecific hybrids in the 5-stamen group, viz., *A. hypochondriacus x A. dubius*, *A. cruentus x A. hypochondriacus* and *A. dubius x A. spinosus*.

Grant (1959b) observed the presence of 17 bivalents and 15 univalents in the hybrid between *A. dubius x A. spinosus* and indicated that 17 chromosomes of *A. dubius* were homologous with the chromosome complement of *A. spinosus* (n = 17). On this basis he suggested that *A. spinosus* is one of the putative parents of *A. dubius*. Pal and Khoshoo (1965, 1966) reported the same dibasic triploid hybrid and observed that the hybrid arose spontaneously wherever *A. dubius* (2n = 64) and *A. spinosus* (2n = 34) were grown in sufficient proximity, whereas the parents had normal meiosis with 32 and 17 bivalents, respectively, the hybrid individuals possessed 17 II and 15 I at metaphase I in 50% of PMCs. Fertility of the hybrid was about 4%. Srivastava *et al.* (1977) studied the pollen morphology in the above mentioned parents and hybrid. Occurrence of two pollen size groups was reported in the hybrids in the hybrids viz. macropollen (>14 µm) and micropollen (6-14 µm), the latter being characterised by a lesser number of chromosomes (1-4).

Sauer (1967) proposed free and frequent intercrossing between both cultivers and weedy species based on his observation of the extensive

variation both within and between the species of grain amaranths and their weedy relatives.

Pal (1972) did not support the suggestion of Grant (1959b) that *A. spinosus* is one of the putative parents of *A. dubius*. This conclusion was based on the cytogenetical study of *A. dubio-spinosus* amphidiploid (2n = 49). In contrast to the expectation of a high number of quadrivalents, the 6x amphiploid produced a low multivalent frequency with high fertility accompanied by vigorous growth. From a comparison of the meiotic behaviour in the 3x hybrid, 6x amphiploid and 2x *A. dubius* - polyhaploid, he suggested that 17 bivalents in the F_1 resulted from homeologous pairing, and homologous preferential pairing restored in the amphiploid resulting in a low multivalent frequency. Furthermore, based on the difference in flower arrangement between the species and also the absence of dominant characters like spinosity in *A. dubius*, Pal (1972) suggested that *A. spinosus* (n=17) was not one of the parents of *A. dubius* (n = 32) and the occurrence of the other parent with n = 15 was very much doubted by him. From an analysis of ten experimental interspecific hybrids in *Amaranthus* Pal and Khoshoo (1972) has shown the existence of hybrid inviability, weakness and sterility that ranged from probable endosperm malfunction in the maternal plant itself, to seedling mortality, stunted and deformed plants with tumorous stems and even roots, virus like syndrome in leaves, to deformed and malformed flowers and finally to pollen and ovule sterility. Furthermore, they indicated that in the absence of involvement of pathogens, all these developmental defects had a genetic basis, indicating genic disharmony between the parental genomes. Based on these results they concluded that there are no barriers to crossability in the genus and that hybridization is the major factor in initiating variation and promoting speciation appeared to be an exaggeration.

Pal and Khoshoo (1973 a and b) utilised the available information on the natural crossability among different species in a systematic programme of hybridization to test the cross compatibility of *Amaranthus* spp. experimentally. The results of their hybridization experiments are abstracted in Table 2.

Based on crossability, chromosome number and geographical distribution, the seven species were later classified into three groups. The two cultivated species *A. caudatus* and *A. edulis* and the weedy *A. quitensis* formed a group with n = 16 chromosomes, indigenous to South American Andes. Later the Central and North American species were divided into second and third groups based on chromosome number. The cultivated *A. hypochondriacus* and the wild *A. hybridus* with n = 16 formed the second group, and the cultivated *A. cruentus* and the wild *A. powellii* with n = 17 formed the third group. Within a group, wild and cultivated species crossed and gave fertile offspring, but between groups crossing failed or gave rise to inviable or sterile hybrid plants. Based on crossing studies, they related *A. hypochondriacus* to *A. hybridus* and not to *A. powellii* as done by Sauer (1967). They observed that *A. caudatus* and *A. edulis* are very close genetically as their F_1 hybrids exhibited heterosis. It was also concluded that the three cultivar groups *A. hypochondriacus*, *A. cruentus* and *A. caudatus* represented the end point of three distinct evolutionary lines genetically, so as to preclude gene exchange among them.

Pal and Khoshoo (1973b) also studied the cytogenetic relationship in the vegetable amaranths belonging to the section Blitopsis. Based on the cytogenetic analysis of three hybrids *A. graecizans* x *A. tricolor*, *A. lividus* x *A. tricolor* and *A. gracilis* x *A. tricolor*, they suggested that differentiation between parent species was chiefly as a result of interchanges and paracentric inversions. The interchange complexes would have

Table 2. Results of experimental crosses among *Amaranthus* spp. (Pal and Khoshoo, 1974)

	A. hybridus	*A. hypochondriacus*	*A. powellii*	*A. cruentus*	*A. quitensis*	*A. caudatus*	*A. edulis*
A. hybridus							
A. hypochondriacus	F						
A. powellii	St	St					
A. cruentus		x					
A. quitensis	Not available						
A. caudatus	d	ab					
A. edulis	d	ab					

x = failed

d = seedlings died

ab = seedling abnormal

st = F_1 normal but sterile

F = F_1 partially fertile

involved four *(A. gracilis x A. tricolor)* to fourteen *(A. lividus x A. tricolor)* chromosomes indicating that the parents differed from each other in 1-6 interchanges. The interchanged segments were most likely small and sterility in the hybrid was entirely chromosomal, because of the preferential pairing and restoration of fertility in the *A. lividus - tricolor* amphidiploid. According to Pal and Khoshoo (1973b) this situation was in strong contrast to the one in section Amaranthus where in species differentiation involved gene differences and cryptic structural hybridity that resulted in bivalent pairing and varying degrees of sterility in the interspecific hybrids.

J. Seed yield in amaranths

Rajagopal *et al.* (1972) reported that seed yield/plant in two promising lines Co-2 *(A. gangeticus)* and Co-1 *(A. dubius)* as 48.4g and 10.4g, respectively. In reporting the seed production techniques in amaranth, Grubben (1976) suggested cutting the principal stock at a height of a few centimeters under the terminal bud one month after harvesting the edible grees to stimulate branching out. He did not recommend this method for those varieties with a large apical inflorescence. Grubben (1977) also reported that the utriculi of *A. cruentus* did not dehisce at maturity causing scattering of seeds. All the seeds matured within a short period and a yield of 1500 kg/ha was recovered. In *A. dubius* the yield was only 600 kg/ha as there was premature shattering of the ripened seeds. Singh and Thomas (1978) reported that no proper seed yield data are available In grain amaranths. Individual grain amaranth plants yielded on an average 30g of seeds.

Hauptli and Jain (1977), in an evaluation of genetic variation in Amaranth collections, reported that seed yield was negatively correlated with stem growth in two weedy types while it was positively correlated

with yield in domesticated types. In *A. lividus*, Prasad *et al.* (1979) observed positive association between yield and leaf size. The yield got increased with increase in leaf length and leaf width. But as the leaf number increased, the yield, leaf length and leaf width decreased. Hence they recommended that more emphasis should be given during selection to leaf size than to leaf number which was negatively correlated with yield.

The vegetable amaranths were characterised by prominent axillary inflorescences and trimerous flowers (Madhusoodanan and Pal, 1981). All the species were monoecious. The most widely cultivated species was *A. tricolor*. The glomerules contained up to 30% male flowers. In other species like *A. lividus*, *A. graecizans* and *A. viridis*, the percentage of male flowers was very low (5-15%) compared to *A. tricolor*. Based on variability studies in *A. tricolor* Mohideen *et al.* (1982) reported that high heritability estimates are associated with high genetic advance for stem weight, leaf/stem ratio, yield of greens, leaf weight and leaf number indicating additive gene effects governing these characters and hence phenotypic selection will be more useful.

Devadas (1982) observed that the line A_6 was the highest yielder (796.09 g/45 m^2) of edible greens among the 25 vegetable types evaluated. The line A_{13} was also found high yielding (740.46 g/45 m^2) and stable for bolting. The line A_6 was not recommended for seed production programmes as it is not stable for days to bolting. This line flowered only under short day conditions available during October-November months. Both A_6 and A_{13} are *A. tricolor* species of the section Blitopsis.

K. Photoperiodic requirement

Allard (1932) and Allard and Garner (1940) reported an indeterminate photoperiodic response in *Amaranthaceae* as a whole. In many studies on photoperiodism in flowering plants, meagre attention was only given to the members of *Amaranthaceae*. Fuller (1949) concluded that *A. caudatus* is a short day (SD) plant. It required a minimum, of two SD cycles for floral induction and 4-5 days for the appearance of the macroscopic inflorescence primordia irrespective of the post inductive photoperiodic conditions. This species failed to flower under continuous illumination and under the natural long days of spring.

Panigrahi (1951) studied the photoperiodic response of *A. gangeticus* var. *oleraceus* Roxb. and found that under a six hour photoperiod, flower buds were formed within 32 days after sowing compared to 39 days under normal illumination. Plants receiving 12, 18 and 24h respectively of illumination remained vegetative. Under 18h illumination, they had the best vegetative growth. The studies indicated a short day response in *A. gangetus* var. *oleraceus*.

Downs (1956) described *A. caudatus* as a short day plant. The inflorescence development was more rapid in winter than in summer indicating an annual rhythm of readiness to flower in this grain species (Chaudri, 1956). Zabka (1957) reported that *A. caudatus* required short days for inflorescence development. The intensity, quality and duration of light exposure affected the flowering behaviour of this species. He also stated that temperature was also an important factor to decide the time of inflorescence initiation. Zabka (1961) concluded that *A. caudatus* became sensitive to day length, 30 days from germination after which two short days were sufficient to initiate inflorescence primordia. The same species

also started flowering in long days of about 18h, 60 days after germination. This indicated that *A. caudatus* is not an obligate short day plant.

Seth (1963) reported that *A. leucocarpus* is a day neutral plant like *A. cruentus*. He confirmed the short day response in *A. caudatus* as reported by Zabka (1957). Samson (1972) working at Wageningen revealed that a Surinam cultivar showed no difference in flowering response to day lengths of 10.5h and 13.5h while a reddish leaved Ethiopean cultivar showed considerable delay in flowering in day lengths above 12.5h. This indicated the short day response in the Ethiopean cultivar. Detailed studies by Grubben (1976) revealed that *A. cruentus* and *A. dubius* were day neutral types and cultivars of cereal amaranths, *A. caudatus* and *A. hypochondriacus* were quantitatively short day plants. These cereal amaranths flowered only by the end of September when the days became sufficiently short. Grubben (1976) also suggested that photoperiodic reaction alone, may not be the only factor responsible for flowering, because certain varieties were practically indifferent to photoperiodicity and early flowering occurred irregularly and more over in all the seasons. Mathai (1978) reported that amaranths are in general short day plants and when planted towards short days they bolted. On the other hand, Harwood (1980) reported varying degrees of photoperiod sensitivity in amaranths. Amaranths are generally adapted to a wide range of day lengths and is not nearly as sensitive as soybean.

Sawhney *et al.* (1980) determined the critical photoperiods of *A. caudatus* f. *albiflorus*, *A. caudatus* f. *caudatus* and *A. tricolor* var. *tristis* as being 16. 15.5 and 15h respectively, and the minimum number of eight hour photoperiodic inductive cycle required were 6, 3, and 5 respectively, for the macroscopic appearance of the inflorescence

primordia. They clearly demonstrated that all the three tested plant types were qualitative short day plants since all the plants in each case produced inflorescence primordia when exposed to photoperiods shorter than the critical photoperiods and none bore flowers when exposed to longer photoperiods. They also observed that photoperiods did not appear to affect vegetable growth.

Grubben (1980) studied the influence of light intensity, temperature and day length on growth and flowering in the popular vegetable amaranth, *A. cruentus* at South Benin (Africa). He concluded that all tested amaranth types behaved as more or less pronounced short day plants but significant differences existed among cultivars.

Kauffman (1981) reported that many 'late' varieties required short days for floral initiation. This was especially true for many *A. hypochondriacus* types, originated at lower latitudes. He found that the green house crops of *A. hypochondriacus* planted during fall began to initiate flowers when the plant had only four leaves approximately. On the other hand, *A. cruentus* did not initiate flower until the plant had eight to twelve true leaves. Kauffman (1981) concluded that *A. cruentus* should be planted three weeks earlier than *A. hypochondriacus* in the fall for synchronization of flowering. He observed that September and October plantings were the most difficult to handle as the plants began to bolt very early. He recommended that mid winter plantings of *A. cruentus* and *A. hypochondriacus* should be approximately one week apart and spring and summer green house plantings were not recommended for these species.

National Research Council (1984) reported on the photoperiod sensitivity of amaranths. The report indicated that the strains of *A. hypochondriacus* from South Mexico did not set flower during summer in Pennsylavania (USA) but they matured in the green house during short day conditions of winter. The reverse happened with *A. cruentus* from Nigeria. It remained vegetative for a long period in its equatorial home. However it went to seed very early when introduced into the long day conditions in Pennsylvania.

L. Antinutritional factors in amaranths

The presence of antinutritional factors like oxalate and nitrate in amaranth leaves is considered as the major limiting factor in its large scale use as a vegetable. Srivastava and Krishnan (1959) reported that oxalate
the soluble/content of *A. gangeticus* was 4.4% and 7.44% in the leaves and stems respectively on dry weight basis. Grubben (1976) observed considerable differences in the oxalic acid content among 25 varieties of *Amaranthus*. Studies on the consumption of leafy vegetables revealed a daily intake of 5g/caput in Latin America, 11g in Central and South West Asia, and 21g in Africa (Grubben, 1977). This intake is definitely non-consequential as compared to 100g required for causing nutritional defects due to the higher nitrate and oxalate levels.

Deutsch (1977) indicated that healthy adults need not be concerned about the presence of these compounds as the leafy greens make up only a fraction of the daily food intake. One would need a daily intake of more than 100g of fresh green to raise nitrate and oxalate levels. He also indicated that oxalates become more of a problem when plants are grown under stress.

Marderosian *et al.* (1980) studied the presence of both these anti-nutritive factors. Mean nitrate levels were 0.48%, in leaves and 1.72%, in stems on dry weight basis. Oxalate levels were 4.5% and 0.63% in stems. The mean percentage of nitrates over two growing seasons were 0.51%, 0.19%, 0.39%, 0.54%, 0.29% and 0.65% and that for oxalates were 5.37%, 5,95%, 3.52%, 6.95%, 2,45% and 4.33% respectively in the leaves of *A. gangeticus, A. blitum, A. dubius, A. cruentus, A. caudatus* and *A. hypochondriacus*. They also reported that nitrates and oxalates in amaranths are similar to those found in spinach and chard. Devadas (1982) observed free oxalate content ranging from 0.94% to 1.29% and nitrate content from 0.58 to 1.00% on dry weight basis in the 25 vegetable amaranth types. In a study of eight accessions of *A. tricolor*, Makus (1984) reported that collectively the amaranth leaf blades contained 1% nitrate nitrogen and 2.3% soluble oxalates on dry weight basis.

The foregoing review reveals that the available information on the centre of origin, distribution, genome relationship, taxonomy, photoperiodic requirement etc. of the *Amaranthus* species are not complete. The keys to identification of different species are quite complex and cumbersome for use in the field conditions. The vegetable amaranth species like *A. tricolor* and *A. dubius* are predominantly cultivated in Asian countries, where as grain amaranths are cosmopolitan in distribution in the new world. If the American origin of *Amaranthus* is accepted, one is compelled to trace out the introduction, distribution and domestication of vegetable types in Asia. The cytogenetical studies undertaken and envisaged in the present thesis would be useful for such analysis. Moreover, it will be useful for establishing relationship between sections and species. Available information on oxalate and nitrate contents in *Amaranthus* spp spells doubt on the part of consumers, as far as these anti-nutritional factors are concerned. Photosensitivity of *Amaranthus* species

is an established fact though the available information is not complete in all the species.

Materials and Methods

MATERIALS AND METHODS

A. Genome analysis

The experimental material included eight species of *Amaranthus*. These consisted of three vegetable species viz. *A. tricolor*, *A. lividus* and *A. dubius*, three grain species viz. *A. hypochondriacus*, *A. cruentus* and *A. caudatus* and two related wild species viz. *A. viridis* and *A. spinosus*. Seeds of the six cultivated species used in the study were obtained from National Botanical Research Institute, Lucknow and those of the wild species were collected locally. *A. tricolor*, *A. lividus* and *A. viridis* belong to Section Blitopsis and the rest five species to Section Amaranthus.

1. Morphological studies

The eight species were studied for their morphology. The vegetative characters observed included plant height (cm), nature, colour and girth of stem, number of branches, length (cm) and colour of petiole, number, size (length and breadth in cm), shape, apex, base, margin and colour of leaf, as well as duration of plant life. The inflorescence characters studied included position, orientation, colour, feel and length (cm) of panicle and number and length (cm) of lateral branches as well as density of flowers on inflorescence axis. In order to study the minute floral details florets were dissected out under a dissection microscope and floral characters invisible to the naked eye were scored. The features studied using the dissection microscope included number of glomerules/cm of terminal panicle, number of florets/glomerule, percentage of male flowers/panicle, nature, length (mm) and tip of bract, size of male flower (length and breadth in mm), stamen characters like length (mm) of filament and

anther at anthesis, tepal length (mm), number of style branches, nature of style, length (mm) of utricle, nature of the utricle, seed colour, seed size (mm) and 1000 seed weight(g).

Microscopic observation of the glomerule structure revealed that the development and arrangement of flowers within a cluster was not as a typical dichasial cymose pattern as reported by Murray (1940). Necessary modifications were hence made to this development pattern.

2. Modification to the existing key

Available keys on the genus *Amaranthus* (Sauer, 1967,Feine, 1980) are based on minute microscopic floral details like bract structure, tepal shape etc. which are difficult to adopt in the field conditions. Hence necessary simplifications and modifications were made using gross plant morphology and nature of inflorescence (terminal - axillary, slender-huge etc.) as primary characters in the existing keys of Sauer (1967) and Feine (1980). In order to differentiate the species from the common groups further secondary characters like tepal and stamen number (3-5), leaf characters (tip, shape etc.) branching of inflorescence, nature of utricle etc. were employed. Finally a provisional simplified key was developed for the identification of eight species used in the present investigation.

3. Meiotic analysis

Meiotic studies were made from pollen mother cells. Young inflorescences were fixed in Carnoyis II fluid (1 acetic acid: 3 chloroform: 6 absolute alcohol) mixed with a few drops of saturated ferric acetate solution. After 24 hrs of fixation the anthers were squashed in 1% acetocarmine (Pal and Khoshoo, 1973a). The inflorescence after 24 hrs

of fixation can also be stored in 70% alcohol for 1 or 2 weeks in refrigerator and can be analysed. Prolonged storage produced chromosome clumping. For prearing slides, the anthers were dissected out using a dissection microscope and squashed in a drop of 1% acetocarmine with slight warming and sealed after removal of excess stain. The preparations were then observed for meiotic stages.

Observations at the metaphase I stage included the following.

(a) Number of ring bivalents

(b) Number rod bivalents

(c) Mean chiasmata/PMC

(d) Mean chiasmata/bivalent

Chiasma frequency was calculated from 25 PMCs in each species. Data on number of chiasmata/bivalent were then subjected to 'F' test (Panse and Sukatme, 1978). PMCs were also observed for the presence of abnormalities during anaphase I, telophase I, anaphase II and telophase II stages of meiosis.

Photomicrographs of melotic chromosomes were taken from temporary preparations using an Olympus PM-6 camera.

4. Pollen studies

Pollen studies were carried out in all the eight species of *Amaranthus*. Fertility was assessed on the basis of stainability of pollen grains in acetocarmine-glycerine mixture. Pollen grains were extracted from fully matured anthers just before anthesis using the tip of a sharp needle and stained in a drop of acetocarmine glycerine mixture on a clean side and kept aside for one hour. All the pollen grains that were stained were counted as fertile. Pollen fertility was calculated after observing at

least 500 grains from three plants in each species. Pollen diameter was measured using an ocular micrometer after calibration. For comparative studies 100 pollen grains were measured in each species. Later they were classified on the basis of diameter into micrograins (6-12 μm), medium grains (12-24 μm) and macrograins (> 24 μm). Micrograins were observed only in hybrids while medium and macrograins were found in all the species in varying percentages. Photomicrographs of pollen grains of different species were taken using an Olympus PM-6 Camera.

5. Interspecific hybridisation in *Amaranthus*

Eight species of *Amaranthus* viz., *A. tricolor*, *A. lividus*, *A. viridus*, *A. spinosus*, *A. dubius*, *A. hypochondriacus*, *A. cruentus* and *A. caudatus* were included in a systematic hybridization programme. Pot grown plants were used for the study and artificial hybridization was undertaken in all possible combinations including reciprocals. The hybridization programme was started during 1984, but the unsuccessful crosses were repeated during 1985 and 1986. Lack of synchronization in flowering times in different species was the major draw back for the recovery of enough hybrids in the early stages.

Emasculation and pollination

Amaranth is a monoecious plant bearing both male and female flowers on the same inflorescence and hence it was very important that emasculation was done before anthesis to avoid selfing of the female parent in the hybridization programme. Emasculation in *A. spinosus* was restricted to the topping of the upper part of the panicle carrying male flowers above the female flowers. After emasculation the flower heads were bagged with butter paper bags. In all other species emasculation process was very tedious due to the intermixed nature of the male and

female florets within the same glomerule. The head set magnifying glass was not used during emasculation as the mature male flowers were quite visible to the naked eye and immature ones were invisible and unidentifiable with the magnifying lenses. Emasculation was incomplete in all the species except *A. spinosus* because all the male flowers could not be removed without damaging the whole glomerule. Even then emasculation was done to the maximum extent possible by removing terminal part of the flower heads which mostly contained male flowers as well as removing all the swollen male flowers before anthesis. Even after careful emasculation, protection and pollination selfing was more common than crossing due to the subsequent opening of the male flowers within the same glomerule. The hybrid seedlings were then located from selfed seedlings by the use of marker characters.

In order to pollinate the flower heads, pollen was collected from the male parents at the time of anthesis (8.30 - 9.30 A.M.) in small petridishes. Gentle tapping of the inflorescence was sufficient to collect pollen in all the species of the section Amaranthus, while in section Blitopsis pollen collection was rather difficult due to the smaller nature of the inflorescences, positional arrangement of male flowers at random along the whole length of the plant especially in leaf axils, as well as the meagre amount of pollen produced in each flower. Hence just before anthesis anthers were collected and used for pollination in these species. A piece of wet cotton was placed in each petridish to prevent pollen from being dried off. Pollen from the desired male parent was then dusted on to the emasculated flower heads using a camel hair brush. Pollination was repeated for three consecutive days since all the female flowers were not receptive on the same day. Length of panicle axis included in hybridization varied from 2 to 5cm, depending upon the

availability of pollen. The fully matured seeds were harvested after 3 - 4 weeks.

In the repeated attempts to obtain the unsuccessful crosses indiscriminate hybridization was done without any emasculation. The female plant was sorrounded by male plants and maximum chance was provided for cross pollination in all possible ways. At the time of anthesis, pollen was dusted heavily on to the female plant by shaking the flower heads of the male plants. This method of hybridization was also found to be successful to a lesser extent.

a) Morphology of interspecific hybrids

The seeds collected from the female plants were sown in pots and their germination and further growth were closely observed. Selfed seeds could easily be sorted out at the seedling statge by marker characters. Many of the hybrid seedlings exhibited seedling mortality and they failed to grow beyond 2-3 leaf stage especially due to the dissolution of the apical meristem. However a few hybrids grew and flowered even though they exhibited sterility and other abnormalities. The following morphological characters of the interspecific hybrids were recorded.

The vegetative characters studied included plant height (cm), number and length (cm) of branches, internodal length (cm), nature, colour and spines on the stem, number of leaves, size of the leaf, (length x breadth in cm), shape, colour, apex, base and margin of the leaf. Inflorescence characters included orientation, branching, type, colour and feel of the panicle. The male flowers were studied in detail with respect to their position on the panicle, proportion of male flowers, nature of opening and colour of the anthers. The varied pattern of development, of axillary female clusters in different hybrids were also studied. Duration of plant

life was also observed and those hybrids which did not perish even after 8 months were classified as perennial. Seed set was examined periodically.

b) Cytogenetical studies

Melotic studies of species hybrids were carried out from pollen mother cell squashes after fixation of young inflorescences in Carnoy's II fluid. The technique used for meiotic analysis of the hybrid is similar to the one employed for the analysis of species and it has been already described. Meiosis in one interspecific hybrid viz. *A. cruentus x A. caudatus* could not be studied as it produced only female flower. Meiotic stages were studied both at first and second divisions and the following observations were made after detailed observation of 10-25 PMCs.

(i) Range and mean of chromosome associations at metaphase I like multivalents, bivalents and univalents. Multivalents observed included associations involving 6, 5, 4 and 3 chromosomes.

(ii) Percentage of cells showing equal segregation and unequal segregation at anaphase I and telophase II.

(iii) Percentage of cells showing abnormalities like bridges and laggards at anaphase I.

(iv) Number and percentage of PMCs with abnormal number of nuclei at telophase II (above or below 4 nuclei)

(v) Mean number of chiasmata/PMC.

The mean number of chiasmata/PMC in the different hybrids were statistically analysed using the one way analysis of variance (Panse and Sukhatme, 1978). The interspecific hybrids were grouped into different clusters using chromosome associations at metaphase I as variables by the Mahalanobis (1936) D^2 statistics.

c) Pollen studies

Pollen grains were stained in acetocarmineglycerine (1:1) mixture and the stained pollen grains were counted as fertile. Staining in hybrid pollen grain was not so deep as in the parent species. Fertility percentage was worked out after observing 500 grains from each hybrid. Pollen grains of size less than 12 um were classified as micropollen, 12-14 um as medium pollen and above 24 um as macropollen.

Photomicrographs of the different chromosome associations at metaphase I, abnormalities at anaphase I, and telophase II as well as pollen grains of all hybrids were taken from temporary preparations using Olympus PM-6 camera.

B. Classification of the existing germ plasm into different species

The forty amaranth accessions available in the existing germplasm at the Department of Olericulture, College of Horticulture, Vellanikkara were scored for their morphological, cytological and pollen characters to ascertain their correct specific status.

1. Morphological studies

The keys used for identification were those of Sauer (1967) and Feine (1980) and also the modified key developed in the present investigation. Representative plant from each accession was used for observation. The different characters observed included height at flowering (cm), stem colour, branching habit, length (cm) and colour of the petiole, shape, size (length x breadth in cm), colour and apex of the leaf, type of inflorescence and its orientation, colour, density of flowers, number and length (cm) of lateral branches, floral characters like number of stamens and tepals, style length in relation to tepals and bract length, nature

of dehiscence of the utricle and seed colour. The photoperiod requirement was also noted based on the time of flowering.

2. Meiotic studies

Meiotic studies were carried out in the forty accessions to ascertain the chromosome number. Young inflorescences were fixed in Carnoy's solution to which a few drops of ferric acetate were added. After keeping the material in the fixative for at least 24h, the buds were squashed in 1% acetocarmine.

The number of bivalents were counted at diakinesis/metaphase I stages in 25 PMCs of a typical plant in each accession of the germ plasm. The number of ring bivalents and rod bivalents were noted separately. Mean number of chiasmata/PMC was also determined based on observation of the 25 PMCs.

3. Pollen morphology

Pollen fertility and diameter (µm) were determined using one typical plant within each accession. Pollen grains were classified as medium (12-24 µm) and macrograins (> 24 µm) and their percentages were worked out. 500 pollen grains were observed in each accession.

C. Investigations on low seed set in two *A. tricolor* accessions.

Two accessions A_6 and A_{13} flowered only during October-November and were shy seed bearers. They were sown separately in pots filled with potting mixture. One month old seedlings were transplanted singly in pots, with ten pots for each accession. Morphological observations were made from seed germination to harvest. The axillary glomerules

were examined for the different type of florets. Meiotic studies were made from pollen mother cells. Details of the techniques have already been described. Pollen fertility (%) was calculated from the number of stained grains to the total number of grains in acetocarmine-glycerine mixture (1:1). The pollen size in each accession was not uniform and they were classified as macro (>24μm) and medium pollen (12-24 μm) based on diameter.

D. Photoperiodic requirement of Amaranth species

This experiment was conducted to classify the eight species viz. *A. tricolor, A. lividus, A. viridis, A. spinosus, A. dubius, A. hypochondriacus, A. cruentus* and *A. caudatus* based on their response to photoperiodism. The seeds of eight species were sown in pots filled with rich thoroughly mixed loam soil during September 1985. After the cotyledonary leaves had unfolded the seedlings were subjected to continuous illumination, which consisted of sunlight from 6.00 to 18.00h supplemented with artificial light from 18.00 to 6.00h.

The experiment was laid out in CRD with five replications. The treatments consisted of a factorial combination of the eight species and five photoperiods, viz. natural day (T_1), 14h (T_2), 15h (T_3), 16h (T_4) and 17h (T_5) photoperiods. Three weeks old seedling from the nursery pots were transplanted to pots filled with equiproportion of sand, garden soil and cattle manure. The separation of plants for different treatments were done at the time of transplanting. Twenty five uniform 3-4 cm tall seedlings in each species with 3-4 unfolded leaves were divided into 5 equal lots. These were subjected to different photoperiodic treatments ranging from 14 to 17h, for 60 photoperiodic cycles. The long days were obtained by extending the natural day light period with two white fluorescent tubes and four low intensity incandescent lamps of 60 W mounted at a height of 100-150 cm above the plants. This arrangement provided

about 5000 lux of artificial light at the plant levels. (Fig. 1)

Weekly observations were made on plant height and number of expanded leaves borne on the main stem. The number of days to flower were also noted in each plant under different treatments. Weather data pertaining to the season are given in Table 3.

E. Nitrate and oxalate content in the different species

Nitrate and oxalate in the eight species of *Amaranthus* were determined. The species were grown in pots with three replications under uniform agronomic management. The leaf and stem samples were harvested in all the species after 50 days of planting. Samples were rinsed in tap water and dried to constant weight in an oven at 60-70 C. The dried plant materials were ground to pass through 0.5 mm mesh sieve. Oxalate content of the dried plant sample was assayed using Ferron reagent as suggested by Marderosian *et al.* (1980) and the nitrate content by Nesslers reagent method as suggested by Snell and Snell (1977).

FIG 1 LIGHT ARRANGEMENT FOR PHOTOPERIODIC STUDIES

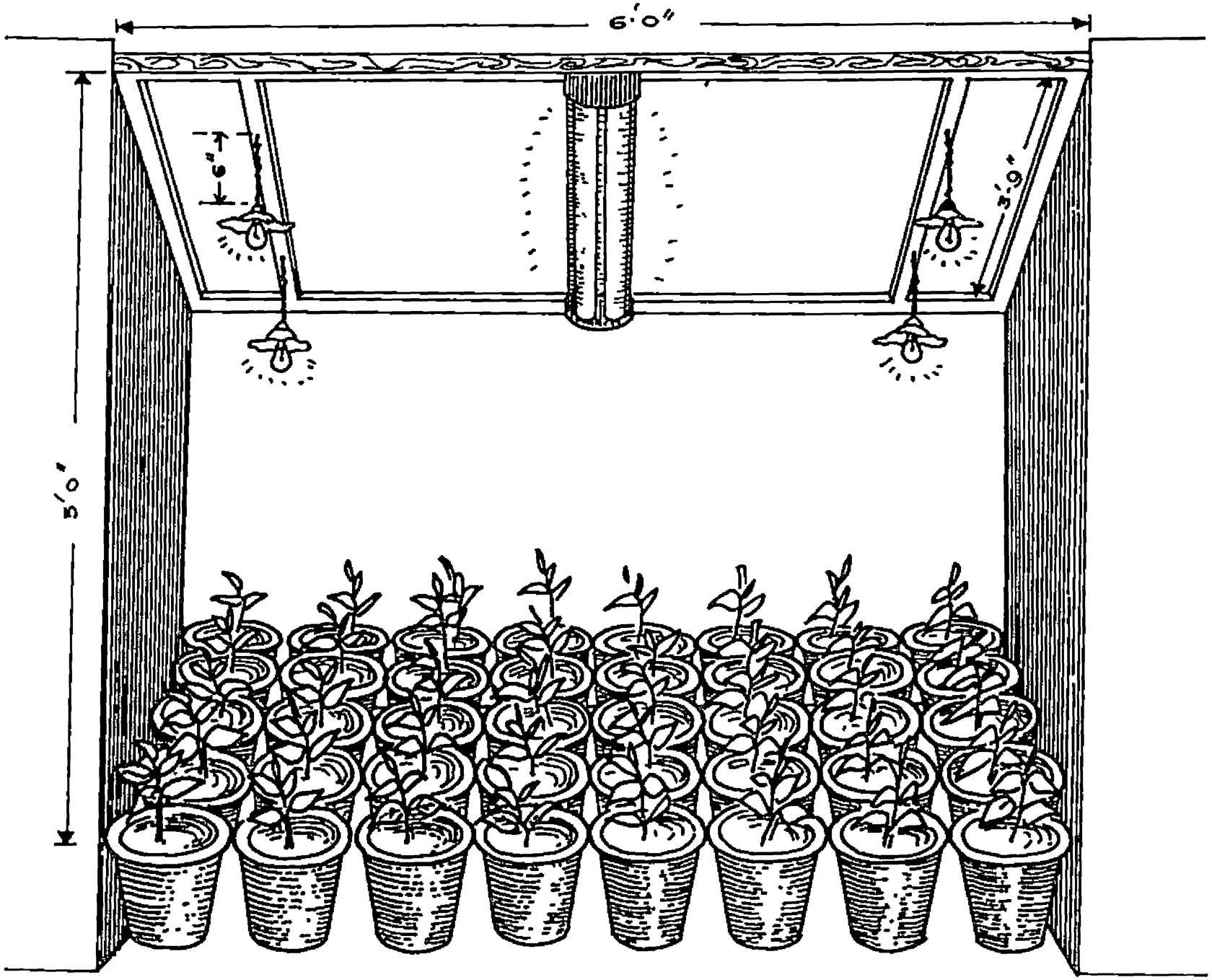


Table 3. Meterological data averaged over weekly intervals during August 1985 to February 1986

Standard week	Meteorological parameters							
	Maximum temperature C	Minimum temperature C	Total rainfall (mm)	No. of rainy days	Relative humidity (%)		Hours of Bright sunshine	
					M	E	Total	Mean
1	2	3	4	5	6	7	8	9
32nd week	28.6	22.8	109.2	6	93	82	20.4	2.9
33	28.6	23.3	105.3	7	85	79	15.1	2.2
34	29.6	23.5	6.7	1	93	74	20.4	2.9
35	29.5	23.6	24.8	3	94	75	32.0	4.6
36	30.1	23.1	6.0	1	93	72	29.6	4.2
37	29.1	22.2	42.2	4	92	71	36.0	5.2
38	31.4	23.1	4.4	1	92	60	54.7	7.8
39	31.1	23.3	12.1	1	89	72	37.0	5.3
40	30.6	22.8	133.2	7	92	75	29.0	4.1
41	30.5	22.4	34.1	3	92	67	41.9	6.0
42	30.9	22.0	98.9	3	83	64	57.2	8.2
43	32.1	22.4	110.9	3	87	62	47.7	6.8

(contd.)

Table 3 (Contd.)

1	2	3	4	5	6	7	8	9
44	32.0	23.5	-	-	80	59	53.9	7.7
45	30.5	22.9	11.7	1	87	72	17.5	2.5
46	32.7	22.3	-	-	86	62	43.6	6.2
47	32.6	22.4	2.7	1	85	54	59.3	8.5
48	31.5	20.5	-	-	71	48	70.0	10.0
49	31.9	23.8	2.2	-	76	53	52.8	7.5
50	32.2	22.3	56.6	2	69	47	51.0	7.3
51	32.5	23.9	-	-	77	43	69.2	9.9
52	32.2	22.8	-	-	72	39	83.2	10.4
1/86	31.8	21.3	-	-	64	37	63.7	9.1
2	31.9	23.7	-	-	67	55	25.8	4.3
3	32.3	23.1	1.2	-	81	53	46.2	6.6
4	33.2	23.1	-	-	64	38	73.0	10.4
5	33.9	21.5	-	-	79	38	69.9	10.0
6	34.6	22.2	-	-	74	38	67.1	9.6
7	32.9	21.8	0.7	-	83	54	49.7	7.1
8	34.7	22.3	1.2	-	72	37	65.2	9.3
9	35.4	23.4	-	-	73	31	62.4	8.9

Results

RESULTS

Interspecific relationship among *Amaranthus* species were studied based on morphological and cytogenetical characters and valuable information on genome relationship were obtained. Photoperiodic requirement and content of antinutritional factors in different species were also determined. The results are presented under the following heads.

A. Morphological description of *Amaranthus* species

B. Development and arrangement of flower cluster

C. Modification of the existing key

D. Meiotic studies in *Amaranthus* species

E. Pollen morphology

F. Interspecific hybridization in *Amaranthus*

G. Classification of the existing germplasm into different species

H. Investigations on seed yield in *A. tricolor* accessions

I. Photoperiodic requirement of different *Amaranthus* species

J. Oxalate and nitrate content in *Amaranthus* species

A. Morphological description of *Amaranthus* spp.

1. *Amaranthus tricolor* L.

Erect annual, usually branched, upto 1 m high; leaves broad ovate, apex acute, base truncate; flowers in polychasial scorpioid cymes condensed in globose axillary clusters or reduced terminal panicle, monoecious and trimerous; bract slightly shorter than perianth lobes, has membraneous lower half and ridged upper half, sepals 3, lanceolate, equal to or longer than the utricle; style branches 3, slightly pubescent, long and reflexed; utricle circumscissile, its cap narrowing into tower. Pericarp membraneous, almost concealed by perianth parts, seeds black (Plate 1a, Fig. 3)

The axillary cymose clusters contain 23% male flowers while the terminal panicle has about 35% male flowers. Cultivated as vegetable.

2. *A. lividus* L.

Decumbent annual herb, up to 75 cm high, profusely branched; leaves small, rhomboid, apex emarginate, base cuneate; flowers in dichasial or polychasial scorpioid cymes condensed in dense axillary clusters or reduced terminal panicle, monoecious and trimerous; bracts shorter than perinath, spatulate; tepals 3, ovate-oblong with acute tips, utricle biconvex, smooth and indehiscent with short style branches. The large utricle is almost exposed being subtended by narrow scale like perianth lobes; seeds black (Plate 1b, Fig. 4)

The terminal inflorescence has 14% male flowers while the axillary clusters have only 7.5%. Cultivated as vegetable.

PLATE 1

a. Plant morphology of *A. tricolor*

b. Plant morphology of *A. lividus*

PLATE 1

a

b

3. *A. viridis* L.

Annual herb of 80 cms high erect or ascending, branching from base or higher, leaves deltoid to rhombic ovate, apex retuse/obtuse, base subacute flowers in dichasial or polychasial scorpioid cymes mostly clustered on slender branched terminal panicles, a few axillary female cymes also present; bracts minute,persistent on inflorescence axil even after shedding of the utriculi; tepals 3, linear, apex acute, much shorter than the utricle; style branches 2, short and recurved, thick indehescent and strongly rugose; seeds black (Plate 2a, Fig. 5)

Axillary clusters contain only female flowers; terminal panicle has about 10% male flowers. Distributed as a cosmopolitan weed.

A. spinosus L

An erect or ascending spinous herb with hand stem up to 1 m high; every node has a pair of sharp spines 1 cm long; leaves ovate - lanceolate apex obtuse/retuse, base cuneate, margin wavy; spacial separation of male and female flowers found only in this species in the genus *Amaranthus;* clusters of dichasial or polychasial scorpioid cymes of monoecious and pentamerous flowers are borne on slender terminal lax panicles and leaf axils, male flowers on upper side and female flowers on lower side; the small axillary cymes are exclusively pistillate; each cluster of flowers (glomerule) is subtended by a large spinous bract; individual bracts subtending the flowers are equalling perianth lobes and bristle tipped; tepals 5, ovate-oblong, apex acute or slightly acuminate and incurred against the utricle; style branches 2-3, hairy and straight, utricle circumscissile and seeds are black (Plate 2b. Fig. 6)

PLATE 2

a. Plant morphology of *A. viridis*

b. Plant morphology of *A. spinosus*

PLATE 2

a

b

Distributed as a cosmopolitan weed.

5. *A. dubius* Mart.ex.Thell.

Erect annual herb up to 2 m high; stem soft and fleshy, often branched; leaves large, rhombic ovate, apex obtuse, base rounded; the polychasial scorpioid cymes of monoecious pentamerous flowers are borne on huge terminal branched panicles and also in small axillary clusters. In each flower cluster (glomerule) the initial flower is staminate and the remainder pistillate, clusters of the axillary cymes are all pistillate; tepals 5, ovate-lanceolate with acute tips; equal in length and incurved against the utricle, the bracts are shorter than tepals, and are persistent on the inflorescence axis upon shedding of the utriculi; the flowers are deciduous, the utriculi are also shattered as and when they mature. The style branches are long, recurved and hairy (Plate 3a, Fig.7)

The terminal panicles have 16% male flowers.

A. dubius is cultivated as vegetable.

6. *A. hypochondriacus* L

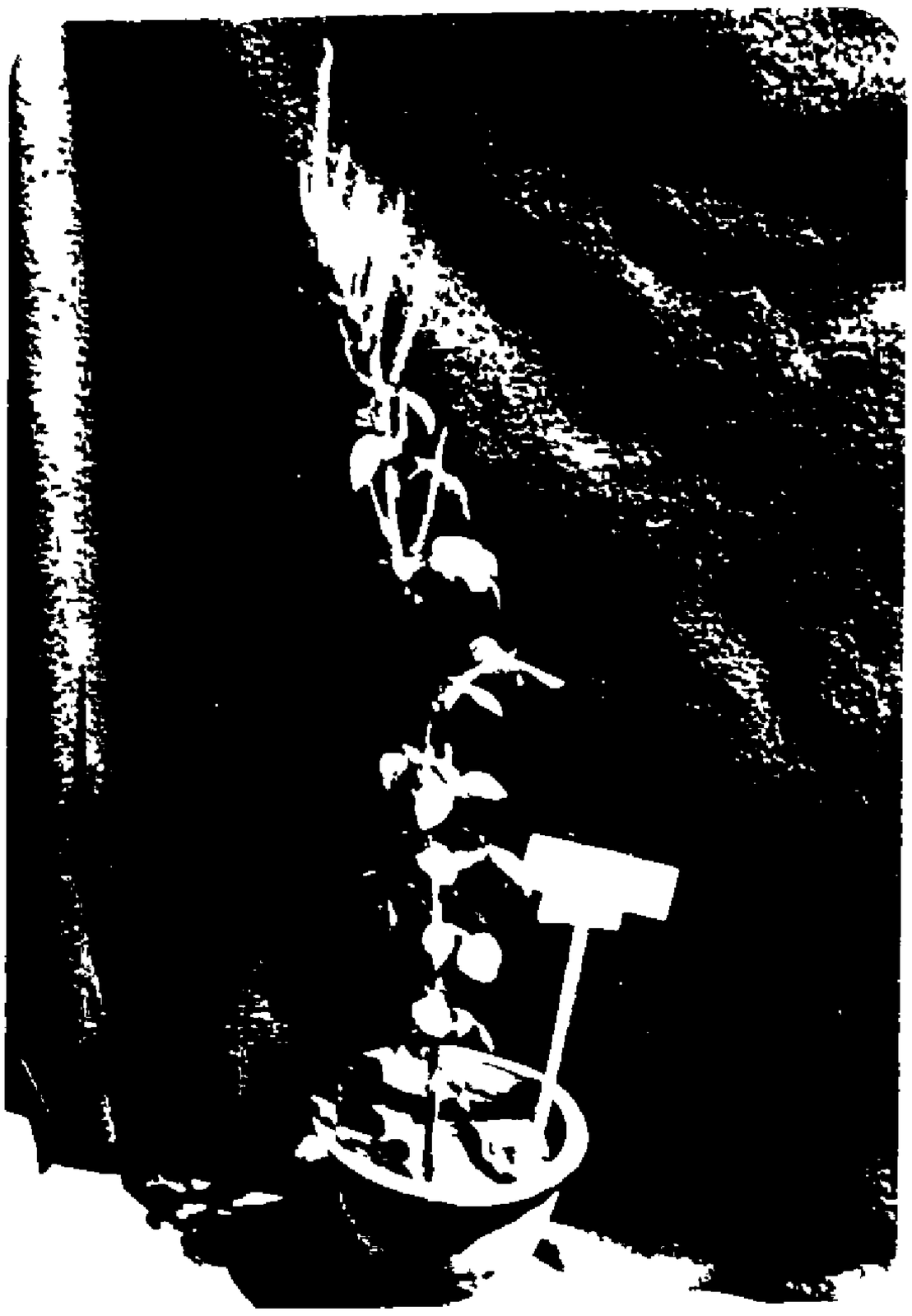
Erect annual herb, upto 1 m high, usually unbranched, leaves elliptic, apex acute, base cuneate/acute. The polychasial scorpioid cymose flower clusters are borne on huge terminal panicle inflorescence characterised by its stiff appearance. The lateral branches of the terminal inflorescence are erect/ascending. The bracts substending the pentamerous unisexual flowers are characteristically large and long pointed giving the inflorescence a prickly appearance. The tepals also are longer than in other species; slightly recurved and acuminate. The 3-4 style branches have thick bases and are small and recurved. the utricle is circumscissile and the utricle cap is very large. The seeds are creamy white (Plate 3b, Fig. 8)

PLATE 3

a. Plant morphology of *A. dubius*

b. Plant morphology of *A. hypochondriacus*

PLATE 3



a

b

The panicle has about 19% male flowers. The species is cultivated as grain.

7. *A. cruentus* L.

Erect annual herb, up to 2 m high, usually unbranched; leaves ovate lanceolate and the terminal leaves reflexed in orientation; leaf bases of subterminal leaves often lyrate; leaf apex acute, bases cuneate or lyrate. The unisexual and pentamerous flowers are borne on polychasial scorpioid cymose clusters in large terminal panicles. The inflorescence is moderately stiff and the lateral branches are divergent. In each glomerule, only the initial flower is staminate and all the rest are pistillate. The bracts are acuminate and as long as the style branches (not smaller as described by Feine, 1980) giving the inflorescence a prickly look, but are soft to feel. The anthers are often hooded. The 3-4 style branches are hairy, slender and erect. The utricle is circumscissile and its neck is elongated than in other species. The seeds are brownish black (Plate 4a, Fig. 9)

The panicle has about 7% male flowers. The species is cultivated as grain and vegetable.

8. *A. caudatus* L.

Erect annual herb up to 2 m high, usually unbranched, stem fleshy and brittle; leaves ovate lanceolate with acute tip and cuneate bases. The specific name 'caudatus' is derived from the long drooping tail like inflorescence, characteristic of the species. The monoecious and pentamerous flowers are produced in polychasial scorpioid cymose clusters on the extremely long and drooping panicles. The inflorescence often has distinct

PLATE 4

a. Plant morphology of *A. cruentus*

b. Plant morphology of *A. caudatus*

PLATE 4

a

b

knobby appearance due to large glomerules, spaced relatively far apart. The bracts are shorter than utricle, lower part membraneous and hairy and the upper half ridged. Tepals 5, broad, often overlapping, tips acute. The three style branches are spreading/reflexed. The glomerules contain more male flowers than all other species because the central flower in each cymose subunit is staminate. Utricle is circumscissile and seeds are pinkish (Plate 4b, Fig. 10)

The inflorescence has about 41% male flowers, the highest number than any other species used in the study. The species is cultivated as grain or as ornamental.

B. Development and arrangement of flower clusters

The eight *Amaranthus* species have monoecious sex habit with a definite growth pattern. Main inflorescence axis is usually branched and the length, number, position and orientation of these branches determined the overall shape/appearance of the inflorescence. Individual flower clusters developed along these branches in an alternate fashion, while within each flower cluster individual flowers are produced in the manner illustrated in Fig.2. The unit of inflorescence is a dichasial or polychasial scorpioid cymose cluster. The pattern of development of flowers is modified (Fig.2)

The developmental pattern appeared to be rather complex and confusing. Apparently the flower development appears to be symmetrical with the first flower terminal on its branch and two branches developing at its base ending in second and third florets. On close observation, it is found that the floral development is in a dichasial or polychasial scorpioid cymose fashion. The initial flower is always terminal and at its base, two branches developed ending in second and third florets. From the basal

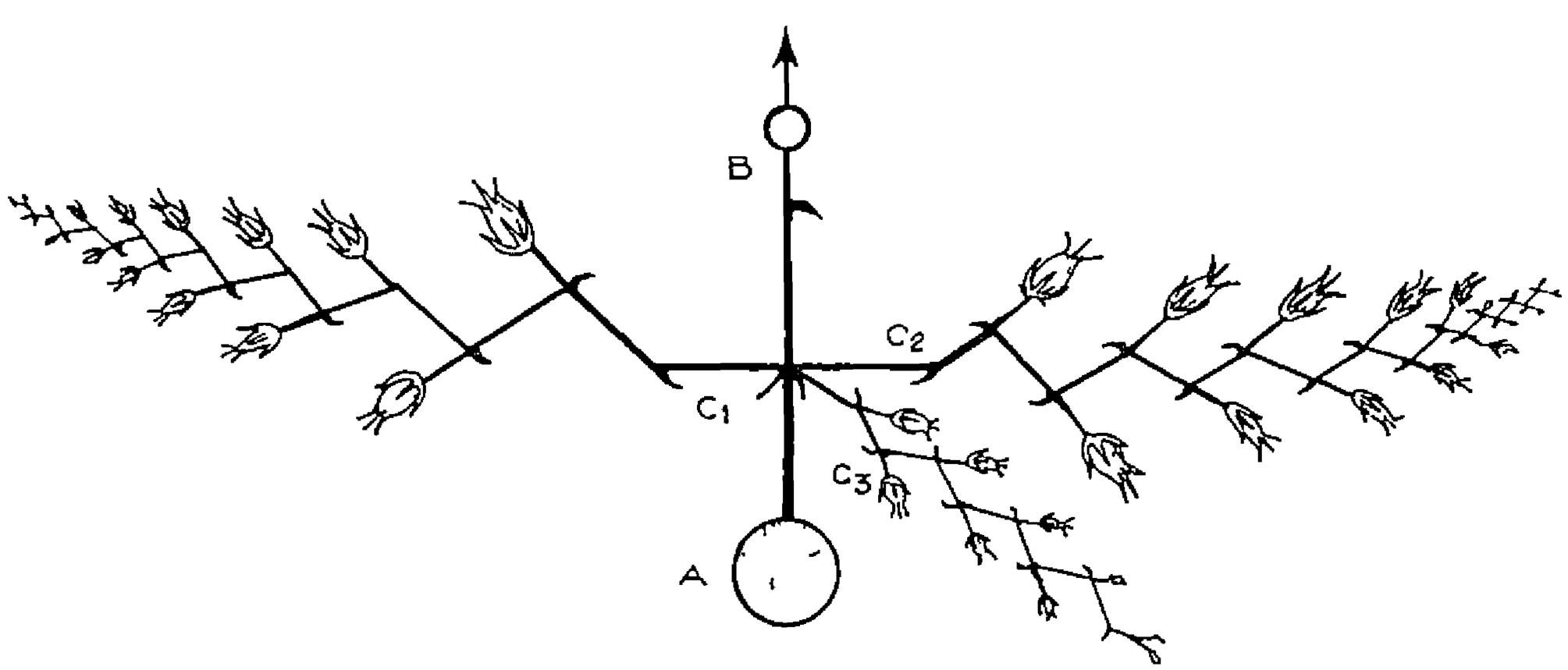


FIG 2

DIAGRAMATIC REPRESENTATION OF THE ARRANGEMENT AND DEVELOPMENT OF A FLOWER CLUSTER IN *Amaranthus* THE NUMBER OF BRANCHES IN THE CYME MAY BE TWO OR MORE

A THE SHADED CIRCLE SHOWS THE MAIN AXIS OF THE INFLORESCENCE

B THE INITIAL STAMINATE FLOWER

C_1 - C_2 - C_3 SCORPIOID CYMOSE BRANCHES OF THE POLYCHASIAL CYME MAKING THE FLOWER CLUSTER

region (axils of bractcoles) of these florets, two or more branches are formed each of which developed into a monochasial scorpioid cyme. The zig - zag pattern of the scorpioid cymes becomes evident, only upon shedding of the utriculi or in cymes with sterile flowers as those of interspecific hybrids. The number of branches in a polychasial cyme varies depending upon the space available, the nutritional status of the soil, fertility of the plant, etc. The fertility of the plant was negatively correlated with number of flowers as there is prolific production of female flowers in the sterile/semifertile interspecific hybrids.

C. Modification of the existing key

The eight species are described in detail for their vegetative and floral characters (Tables 4, 5). The identification of the different species is rather difficult using the existing keys of Sauer (1967) and Feine (1980) because the primary key characters are minute floral details. The key is hence simplified using gross morphological characters like nature of the inflorescence (Table 6). The amaranth species have three different types of inflorescences ranging from dense axillary clusters to slender terminal panicles and then to huge terminal panicles. Considering this feature as the primary character, the eight different species are grouped into three major classes. These classes are further subdivided based on other floral and leaf characters. The floral details of the eight species are also given in Figs. 3 to 10.

Table 4 Vegetative characters in eight *Amaranthus* species

	A tricolor	*A lividus*	*A viridis*	*A spinosus*	*A dubius*	*A hypochondriacus*	*A cruentus*	*A. caudatus*
Habit	Erect and branched	Prostrate, well branched	Erect and branched	Erect and branched	Erect often branched	Erect unbranched	Erect unbranched	Erect unbranched
Plant height (cm)	100	40	50	80	120	65	150	120
Nature of the stem	Medium hard	Fleshy	Medium hard	Hard	Fleshy	Medium hard	Medium hard	Fleshy
Number of branches	4	5	5	12	Nil	Nil	Nil	Nil
Stem colour	Green	Pale green	Green	Dull red	Pale green	Purplish green	Red/Green	Pale green (White)
Stem girth (cm)	4	2 5	2	3	4	4	4.5	4.5
Petiole length (cm)	6	3	4	6	8	7	6 5	6
Petiole colour	Green	Pale green	Green	Dull red	Light green	Purplish green	Red/Green	White
Number of leaves	60	60	50	150	15	25	30	25
Leaf size (Length & Breadth) (cm)	9 x 7 5	3 x 2 5	6 x 4	8 x 4	15 x 10	14 x 8	16 x 7	14 x 6
Leaf shape	Broad ovate	Rhomboid	Deltoid	Ovate lanceolate	Rhombic ovate	Elliptic	Ovate – lanceolate	Ovate-lanceolate
Leaf apex	Acute	Emarginate	Retuse/ obtuse	Obtuse/ retuse	Obtuse	Acute	Acute	Acute
Leaf base	Acute	Cuneate	Sub acute	Cuneate	Rounded	Cuneate	Cuneate + lyrate	Cuneate
Leaf margine	Entire	Entire	Entire	Wavy	Entire	Entire	Entire	Entire
Leaf colour	Green with deep purple centre	Pale green	Green	Green	Green	Purplish green	Red/Green	Pale green
Days from sowing to full blossom	70	45	60	80	75	65	125	95
Days from sowing to harvest	115	75	90	120	110	95	165	125

Table 5. Floral characters in the eight *Amaranthus* species

Floral characters	*A tricolor*	*A lividus*	*A viridis*	*A spinosus*	*A dubius*	*A. hypochondriacus*	*A cruentus*	*A caudatus*
Inflorescence position	Axillary and small terminal panicle	Axillary and small terminal panicle	Slender terminal and small axillary panicle	Slender terminal & axillary panicle	Huge terminal and small axillary panicle	Huge terminal panicle	Huge terminal panicle	Huge terminal panicle
Inflorescence orientation	Erect, unbranched	Erect, unbranched	Erect branched	Erect branched	Erect branched	Erect branched	Erect branched	Drooping branched
Inflorescence colour	Green	Green	Green	Green	Green	Deep pruple	Deep pruple	Pink
'Feel of the inflorescence	Bristle like but not stiff	Rough	Rough	Rough	Smooth	Bristle like and stiff	Bristle like and stiff	Smooth
Mean panicle length	6 5 ± 0 46	3 5 ± 0 32	12.5 ± 0 69	30.5 ± 1 03	14 0 ± 0 68	19 5 ± 0 83	20 0 ± 0 71	52 5 ± 1 61
Mean number of lateral branches	Nil	Nil	9 5 ± 0 46	6.5 ± 0 41	18 5 ± 1 36	11 5 ± 0 60	53 0 ± 2.11	58 5 ± 2 78
Mean laterals length	-		6 0 ± 0 37	8.5 ± 0 34	11 3 ± 0 55	15 5 ± 0 44	7 25 ± 0 59	18 4 ± 0 68
Density of florets	Dense	Dense	Lax	Lax	Dense	Dense	Dense	Lax
Number of glomerules/cm of terminal panicle	11	12	14	9	14	16	14	12
Number of florets/glomerule Range	6-13	6-10	3 10	4 8	6 12	3 12	10-18	4 11
Mean	8 8	7 4	5 6	6.7	9 2	7 0	12 9	7 1
% Male flowers/panicle	24 94	7.5	10 2	100/0	16 30	18 86	7 70	40 84
Bract length (mm)	4 5	1 5	1	2	2	3	3	3 5
Nature of the bract	Lower part membraneous upper part ridged	Spatulate	Lower part membraneous upper part ridged	Lower part membraneous upper part ridged	Lower part membraneous upper part ridged	Lower part membraneous upper part ridged	Lower part membraneous upper part ridged	Lower part membraneous upper part acuminate
Tip of the bract	Acuminate but not sharp	Obtuse	Acute	Spinosus	Acute	Acuminate & Bristle tipped	Acuminate & bristle tipped	Acuminate but not sharp

(contd.)

Table 5 (contd.)

Floral characters	*A tricolor*	*A lividus*	*A. viridis*	*A. spinosus*	*A dubius*	*A. hypochondriacus*	*A cruentus*	*A caudatus*
Male flower size (mm) (Mature and unopened)	4 5 x 3.0	1 5 x 1 0	1 0 x 0.5	2.0 x 1 5	2.5 x 1.5	3.0 x 2.0	3.0 x 1 5	3 0 x 2.0
Filament length at anthesis (mm)	5 0	1 3	1 0	2.5	2.0	3.0	1 5	3 0
Anther length (mm)	2.0	1 0	1 0	1 5	2.0	2 0	1 0	1 5
Perianth lobe length (mm)	5 0	1 5	1 5	2.0	1 5	4 0	3 0	3 0
Number of style branches	3	3	3	2	2 or 3	3 or 4	2 or 3	3
Nature of style/stigma	Pubescent	Pubescent with short hairs	Pubescent with very short hairs	Pubescent	Hairy	Pubescent with short hairs	Pubescent	Pubescent
Utricle size (mm)	4 0 x 2.0	3.0 x 2 6	1 5 x 1 3	2.0 x 1 5	2.0 x 1 5	2.5 x 1 2	2.0 x 1 0	1 8 x 1 6
Utricle nature	Circumscissile	Indehiscent smooth	Indehiscent and rugose	Circumscissile	Circumsci-ssile	Circumscissile	Circumscissile	Circumscissile
Seed colour	Black	Black	Black	Black	Black	Cream coloured	Browhish black	Pinkish
Seed size (mm) Length xBreadth	1 33 x 1 33	1 47 x 1 45	1 13 x 1 05	0 83 x 0 80	0.88 x 0 84	1 22 x 1 17	0 96 x 0 96	1 16 x 1 18
1000 seed weight (g)	1 15 ± 0 02	1 16 ± 0 02	0.80 ± 0 001	0.21 ± 0 001	0 22 ± 0 003	0.66 ± 0.007	0 41 ± 0 009	0 64 ± 0 011

FIG 3 *Amaranthus tricolor* L

FIG 4 *Amaranthus lividus*

utricle

male flower

female flower

seed

FIG 5 *Amaranthus viridis* L

male flower

female flower

utricle

seed

FIG 6 *Amaranthus spinosus. L*

male flower

female flower

utricle

seed

FIG 7 *Amaranthus dubius* Mart ex Thell

male flower

utricle

female flower

seed

FIG 8 *Amaranthus hypochondriacus , L*

FIG 9 *Amaranthus cruentus* L

FIG 10 *Amaranthus caudatus* L

Table 6 A simplified provisional key to a few *Amaranthus* spp.

A. Flowers in cymes mostly clustered in leaf axils, stamens and perianth parts-3.

B. Utricle, circumscissle, concealed by long and bristle like perianth lobes ..... *A. tricolor*

BB. Utricle biconvex, indehiscent, partially covered by short and narrow perianth lobes, leaves emarginate ..... *A. lividus*

AA. Flowers in cymes mostly clustered on slender terminal panicles; a few axillary clusters also present. Stamens as many as perinath lobes.

B. Plants unarmed, leaves deltoid-ovate, utricle rugose and indehiscent; stamens 3 ..... *A. viridis*

BB. Plants armed, bracts bristle tipped, distal cymes staminate, and basal cymes pistillate, stamens 5 ..... *A. spinosus*

AAA. Flowers in cymes mostly clustered on large terminal panicles, stamens and perianth parts-5.

B. Each cyme with initial staminate flower and remainder pistillate

C. Inflorescence smooth, lateral branches divergent, often with axillary female flowers ..... *A. dubius*

CC. Inflorescence stiff, lateral branches ascending, style branches thick at the base, leaves elliptic ..... *A. hypochondriacus*

CCC. Inflorescence moderately stiff, lateral branches divergent, utricle neck elongated, anthers often hooded, leaf base often lyrate ..... *A. cruentus*

BB. Each cyme with more staminate flowers

Inflorescence smooth and drooping, lateral branches ascending, female flowers cup shaped, perinath lobes overlapping, style branches spreading ..... *A. caudatus*

In the simplified provisional key, based on the inflorescence type, *A. tricolor* and *A. lividus* from the first group (A), *A. viridis* and *A. spinosus* from the second group (AA) and *A. dubius*, *A. hypochondriacus* *A. cruentus* and *A. caudatus* from the third group (AAA). The species *A. viridis* and *A. spinosus* brought under the second group belong to section Blitopsis and Amaranthus respectively. The individual flower characters are similar in *A. dubius* and *A. spinosus*. However there is no similarity between these two species in the nature of inflorescence, pattern of arrangement of florets, nature of leaves, spines etc. Further *A. dubius* universally treated as a vegetable amaranth is now grouped along with the three grain amaranth species in the third category. These four species resemble each other in the pattern of arrangement of male and female flowers and the huge terminal inflorescence. In the third group AAA, *A. dubius*, *A. hypochondriacus* and *A. cruentus* have their cymes with initial staminate flower and remainder pistillate and together they constitute a subgroup (B). On the other hand; *A. caudatus* has the cymes with more staminate flowers and forms a separate subgroup (BB).

D. Meiotic studies in Amaranth species

Meiosis is almost normal in all species with regular bivalent formation. The bivalents are fairly differentiated and chiasma distribution can easily be studied at diakinesis and metaphase I. In all the species one bivalent is always associated with the nucleous indicating presence of a pair of nucleolar organizers. Of the eight species, 7 are diploids with n = 16 or 17 and one species *A. dubius* is a polyploid with n = 32. A preponderance of chromosome number, n = 17 is observed among all the species investigated. The different species characterised by this number include all the species under section Blitopsis viz., *A. tricolor*, *A. lividus*

and *A. viridis* and also *A. spinosus* and *A. cruentus* of the section Amaranthus. Only *A. caudatus* and *A. hypochondriacus* have n = 16. An interesting feature observed at metaphase I in *A. tricolor* is the precocious disjunction of one bivalent, usually the smallest bivalent in the complement. The chromosome complement of *A. tricolor* is also the biggest among the different species and *A. spinosus*, the smallest upon visual observations. Anaphase I stage is normal in all the species with equal distribution of chromosomes to the poles. Second meiotic division is also normal in all the species culminating into a higher percentage of pollen fertility.

Chromosome associations at metaphase I in different species are presented (Plate 5 and 6, Table 8). Usually one or two chiasmata were formed randomly in each bivalent. In the polyploid *A. dubius*, 32 bivalents were found consistently at metaphase I. As in other species, bivalents in *A. dubius* also had one or two chiasmata and the mean number of ring bivalents ranged from 21 to 25. The mean number of ring bivalents/PMC varied between 12-14 in *A. tricolor*, 10-13 in *A. lividus*, 9-12 in *A. viridis*, 5-8 in *A. spinosus*, 10-13 in *A. hypochondriacus*, 10-12 in *A. cruentus* and 9-12 in *A. caudatus*.

1. Section Blitopsis

A regular meiotic division with 17 bivalents was observed in all the three species of this section. The mean number of ring bivalents was the highest in *A. tricolor* (12.92) followed by *A. lividus* (10.88) and *A. viridis* (10.44). Consequently *A. viridis* had the highest number of rod bivalents (6.56). Mean number of chiasmata/PMC was also the highest in *A. tricolor* *A. lividus* and *A. viridis* did not differ significantly in number of chiasmata/bivalent, but *A. tricolor* was significantly different from others for this character.

PLATE 5

Meiotic chromosomes of different *Amaranthus* spp at metaphase I (x1500)

A. *A. tricolor*

B. *A. lividus*

C. *A. viridis*

D. *A. spinosus*

E. *A. dubius*

PLATE 5

A

C

D

E

PLATE 6

Meiotic chromosomes of different *Amaranthus* species at metaphase I (x1500)

A. *A. dubius* B. *A. caudatus*

C. *A. hypochondriacus* D. *A. cruentus*

E. *A. cruentus*

(metaphase II)

PLATE 6

B

C

D

Table 8 Chromosome pairing and chiasma frequency in *Amaranthus* species

SPECIES	No. of cells analysed	Mean number of bivalents/cell				Mean number of chiasmata	
		Rings		Rods		per PMC	Per Bivalent
		Range	Mean	Range	Mean		
SECTION BLITOPSIS							
A. tricolor	25	12-14	12.92	3-5	4.08	29.92 ± 0.16	1.75 ± 0.01[d]
A. lividus	25	10-13	10.88	4-7	6.12	27.88 ± 0.18	1.64 ± 0.01[b]
A. viridis	25	9-12	10.44	5-8	6.56	27.44 ± 0.22	1.61 ± 0.01[b]
SECTION AMARANTHUS							
A. spinosus	25	5-8	6.64	9-12	10.36	23.64 ± 0.25	1.38 ± 0.02[a]
A. dubius	25	21-25	22.92	7-11	9.12	54.92 ± 0.27	1.71 ± 0.008[c]
A. hypochondriacus	25	10-13	11.16	3-6	4.84	27.32 ±0.18	1.69 ± 0.01[c]
A. cruentus	25	10-12	10.92	5-7	6.08	27.92 ± 0.19	1.64 ± 0.002[b]
A. caudatus	25	9-12	10.20	4-7	5.80	25.24 ± 0.19	1.64 ± 0.01[b]

a, b, c and d p = 0.01 CD = 0.0318

a) Natural polyploidy in *A. lividus*

During the course of this study, a natural tetraploid in *A. lividus* was observed. This polyploid exhibited gigantism in morphological features, the leaves were much longer, thicker, leathery and deep green as compared to the diploid. There was general increase in stomatal size. Morphological features of the diploid and tetraploid *A. lividus* are given in Table 7 and Plate 7a.

Table 7 Morphological features of the diploid and tetraploid of *A. lividus*

Plant characters	Diploid	Tetraploid
Plant height (cm)	30	46
Number of primary branches	4	6
Number of leaves	50	68
Stem girth (cm)	2.5	3
Internodal length (cm)	2.5	3
Size of the 5th leaf (lxb cm)	3x2.5	5.5 x 4.8
Stomatal size	24 µm x 16 µm	28 µm x 16 µm

Meiosis in the autotetraploid was typical allopolyploid in nature. The cytogenetical observations of the PMCs revealed that the 68 chromosomes formed only bivalents at diakinesis (Plate 7b). The pollen was observed to be fully sterile (Plate 7c)

II. Section Amaranthus

In this section *A. spinosus* and *A. cruentus* have 17 regular bivalents at metaphase I stage. Also normal meiosis with 16 bivalents was observed in *A. hypochondriacus* and *A. cruentus*. The number of ring bivalents was the highest in *A hypochondriacus* (11.16) followed by *A. cruentus* (10.92), *A. caudatus* (10.20) and *A. spinosus* (6.64). Consequently the mean number of rod bivalents was the highest in *A. spinosus* (10.36) and the lowest in

PLATE 7

A. Plant morphology of diploid and tetraploid *A. lividus*

B. Chromosomes at diakinesis in the tetraploid plant (x600)

C. Pollen grains of the tetraploid plant (x600)

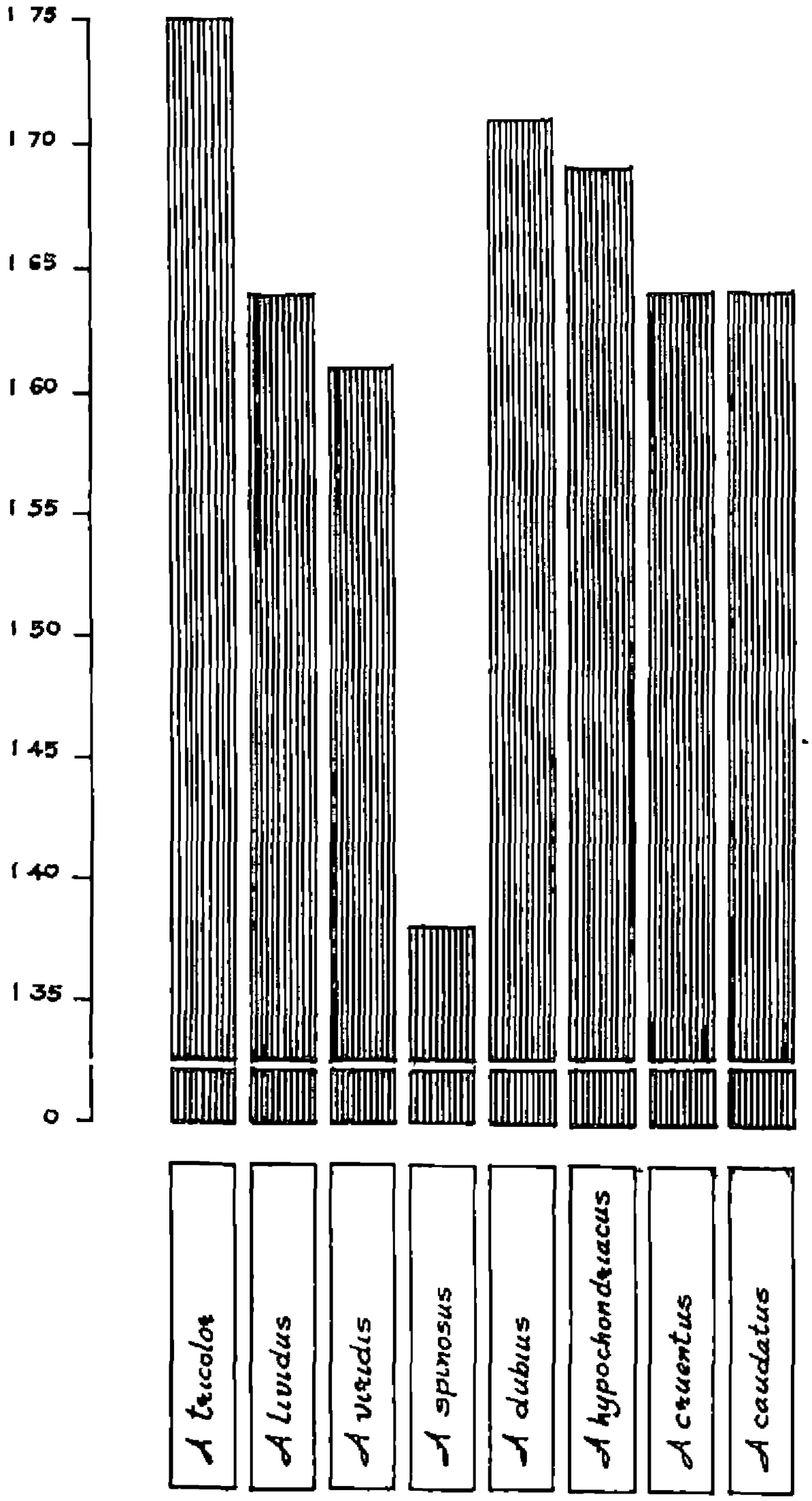
PLATE 7

A

B

C

FIG 11 MEAN CHIASMATA / BIVALENT IN THE DIFFERENT SPECIES OF *Amaranthus*



A. hypochondriacus (4.84). The chiasma frequency/PMC was the lowest in *A. spinosus* (23.64), followed by *A. caudatus* (25.24). The mean chaismata also exhibited a narrow range between the other two grain species, *A. hypochondriacus* (27.32) and *A. cruentus* (27.92).

In the polyploid species *A. dubius*, no multivalents were found and 32 bivalents were always counted at metaphase I. The mean chiasmata/PMC was 54.92 and all the bivalents had only one or two chiasmata as in diploid species. Among the members of section Amaranthus, the mean chiasmata/bivalent was the highest in this polyploid (1.71) and the lowest in the weedy type *A. spinosus* (1.38). No significant difference was observed between the two important grain species *A. cruentus* and *A. caudatus* for chiasmata/bivalent. Number of chasmata/bivalent in *A. hypochondriacus* was significantly higher than the other two grain species (1.69) (Table 8, Fig.11).

E. Pollen morphology

In each species of *Amaranthus*, pollen sizes was not uniform. For convenience, pollen grains of each species were classified as macropollen and medium pollen, the former characterised by lower grains of more than 24 µm diameter and the latter by 12 - 24 µm diameter. The percentage of each type of pollen varied in the different species. The biggest sized pollen grains (31.5 µm) were found in *A. tricolor* closely followed by *A. hypochondriacus*. These two species also had the maximum percentage of bigger sized macropollens. Higher percentages of smaller grains were observed in *A. spinosus* and *A. caudatus*. (Table 9, Plate 8)

Pollen fertility was fairly high in all the species ranging from 83.4% in *A. viridis* to 93.8 per cent in *A. lividus* (Table 9). The percentage fertility was not a rigid character and the same varied from plant to plant as well as from season to season.

Table 9 Pollen types of different *Amaranthus* species

Species	Pollen types (%)		Mean size (μm)		Pollen fertility (%)
	Medium sized pollen (12-24 μm)	Macro pollen (>24 μm)	Medium sized pollen	Macropollen	
A. tricolor	16.65	83.35	23.1 ± 0.26	31.4 ± 0.44	90.2
A. lividus	37.23	62.77	22.1 ± 0.35	27.6 ± 0.29	93.8
A. viridis	28.45	71.55	21.8 ±0.24	26.3 ± 0.23	83.4
A. spinosus	45.17	54.83	20.0 ± 0.29	25.5 ± 0.17	89.2
A. dubius	28.50	71.50	19.0 ± 0.58	27.1 ± 0.58	91.0
A. hypochondriacus	15.72	84.28	23.6 ± 0.23	30.0 ± 0.40	93.4
A. cruentus	35.67	64.33	22.0 ± 0.28	26.6 ± 0.33	88.6
A. caudatus	43.25	56.75	18.7 ± 0.22	25.3 ± 0.16	91.6

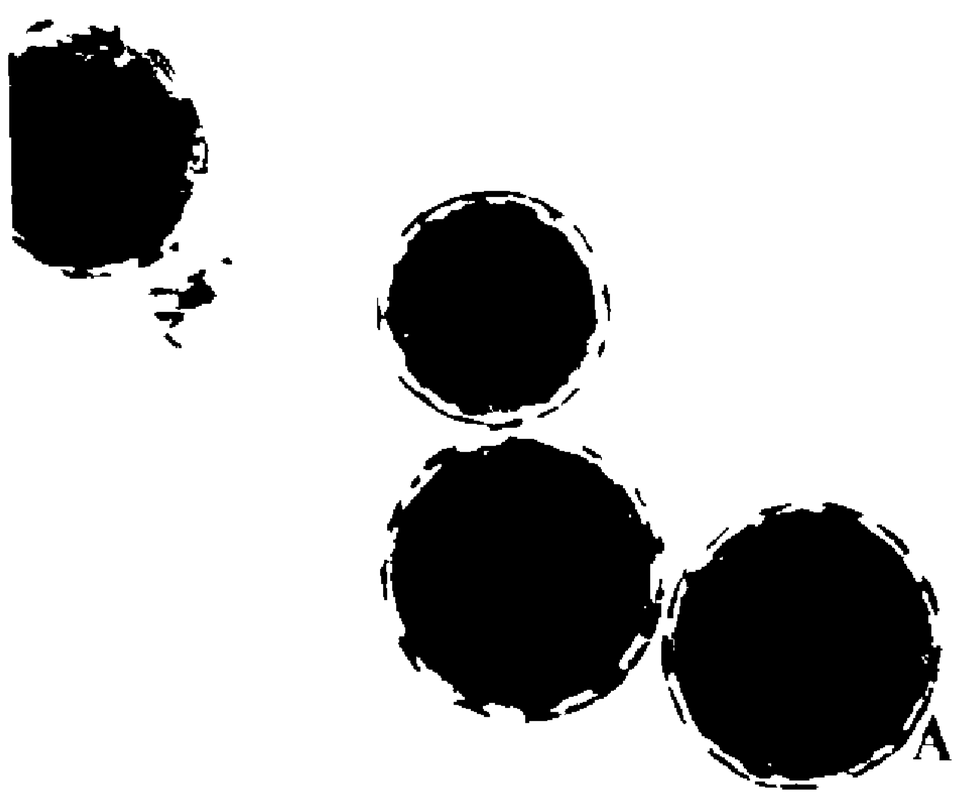
PLATE 8

Pollen grains in different species of *Amaranthus*.

Note the pollen grains of varying diameter.

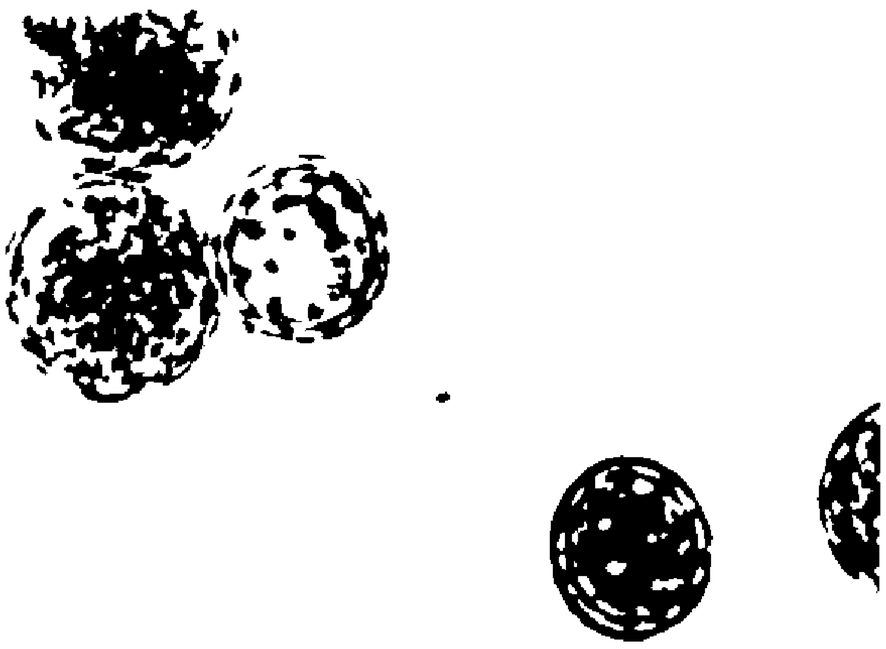
A. *A. tricolor* (x1200)
B. *A. lividus* (x600)
C. *A. dubius* (x600)
D. *A. spinosus* (x600)
E. *A. hypochondriacus* (x1200)
F. *A. cruentus* (x1200)

PLATE 8

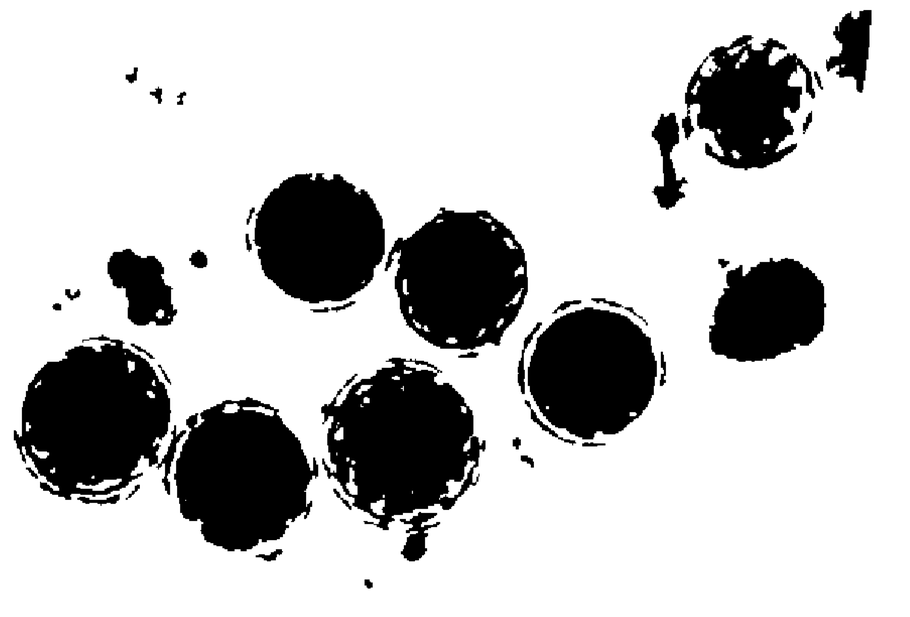


A

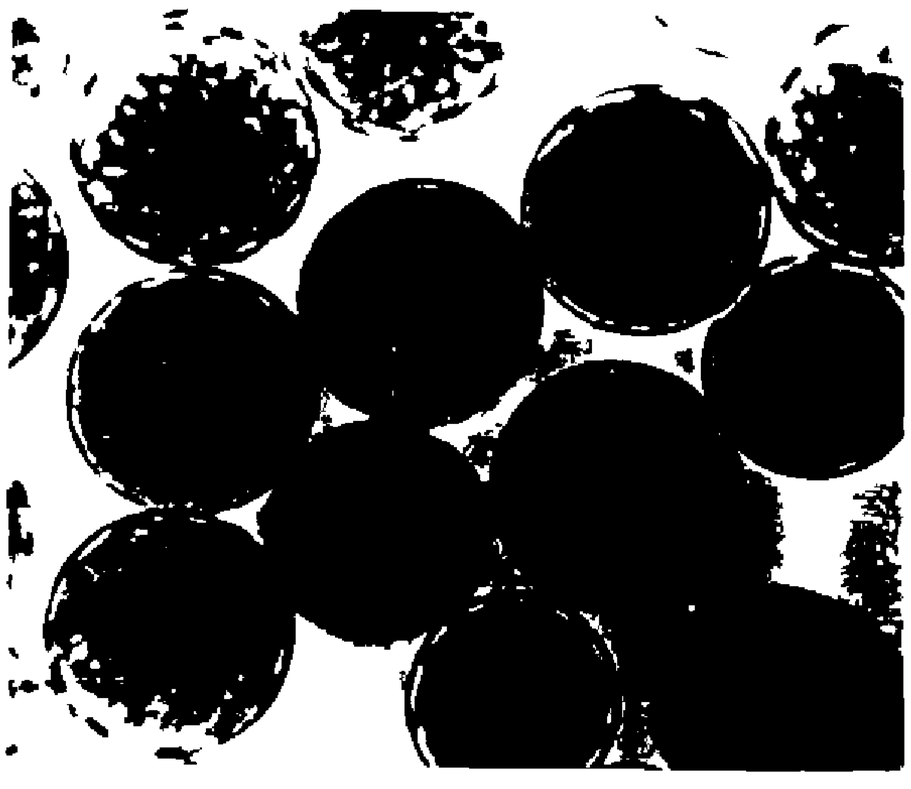
B



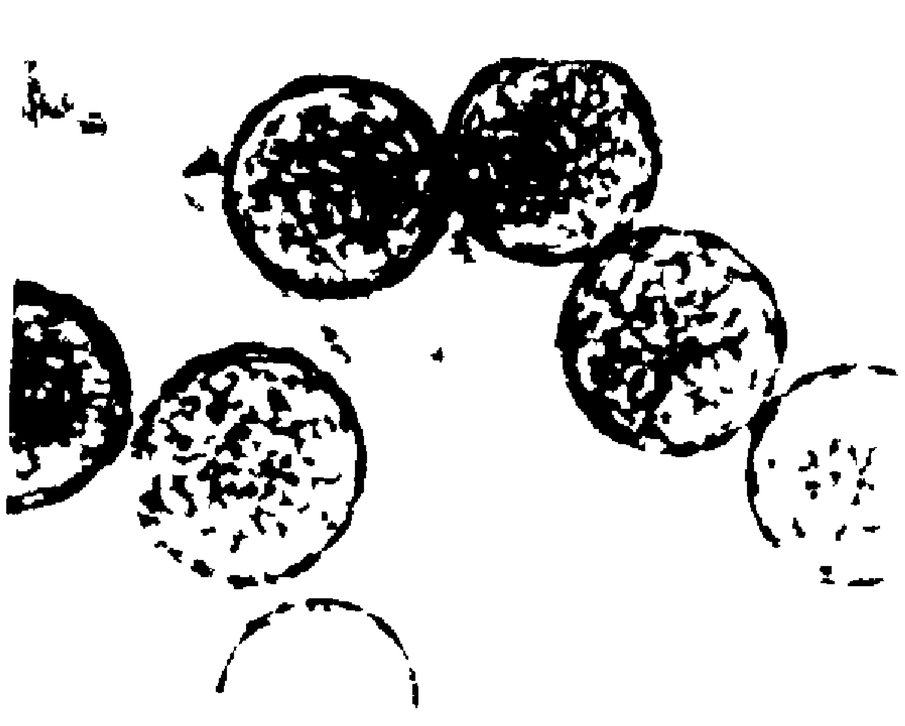
C



D



E



F

F. Interspecific hybridization in *Amaranthus*

Interspecific hybridization was attempted in all possible combinations including the reciprocals, but many of the crosses were unsuccessful. Only 7 interspecific hybrids were obtained which exhibited almost normal growth and flowering. In many of the unsuccessful crosses, mortality of the hybrid seedlings was observed at 2-3 leaf stage. The growth was highly arrested in these seedlings. When seedlings of the parental species of the same age reached a height of 25-30 cms, these hybrid seedlings were hardly more than 5 cms (Plate 9 & 10). Further, growth was arrested due to the dissolution of terminal buds. Such hybrids were obtained between (1) species of the section Blitopsis (2) between Blitopsis and Amaranthus (3) between Amaranthus and Blitopsis and (4) between Amaranthus and Amaranthus. These are *Amaranthus lividus x A. viridis, A. lividus x A. spinosus, A. tricolor x A. spinosus, A. caudatus x A. lividus, A. dubius x A. tricolor, A. caudatus x A. spinosus, A. caudatus x A. cruentus, A. cruentus x A. dubius* and *A. cruentus x A. spinosus.*

In case of *A. caudatus x A. spinosus* and *A. caudatus x A. cruentus;* this condition was not observed in the reciprocal hybrids and both the reciprocals had nearly normal seedlings which grew into adult plants and flowered. They exhibited sterility at later stages.

1) Morphological studies

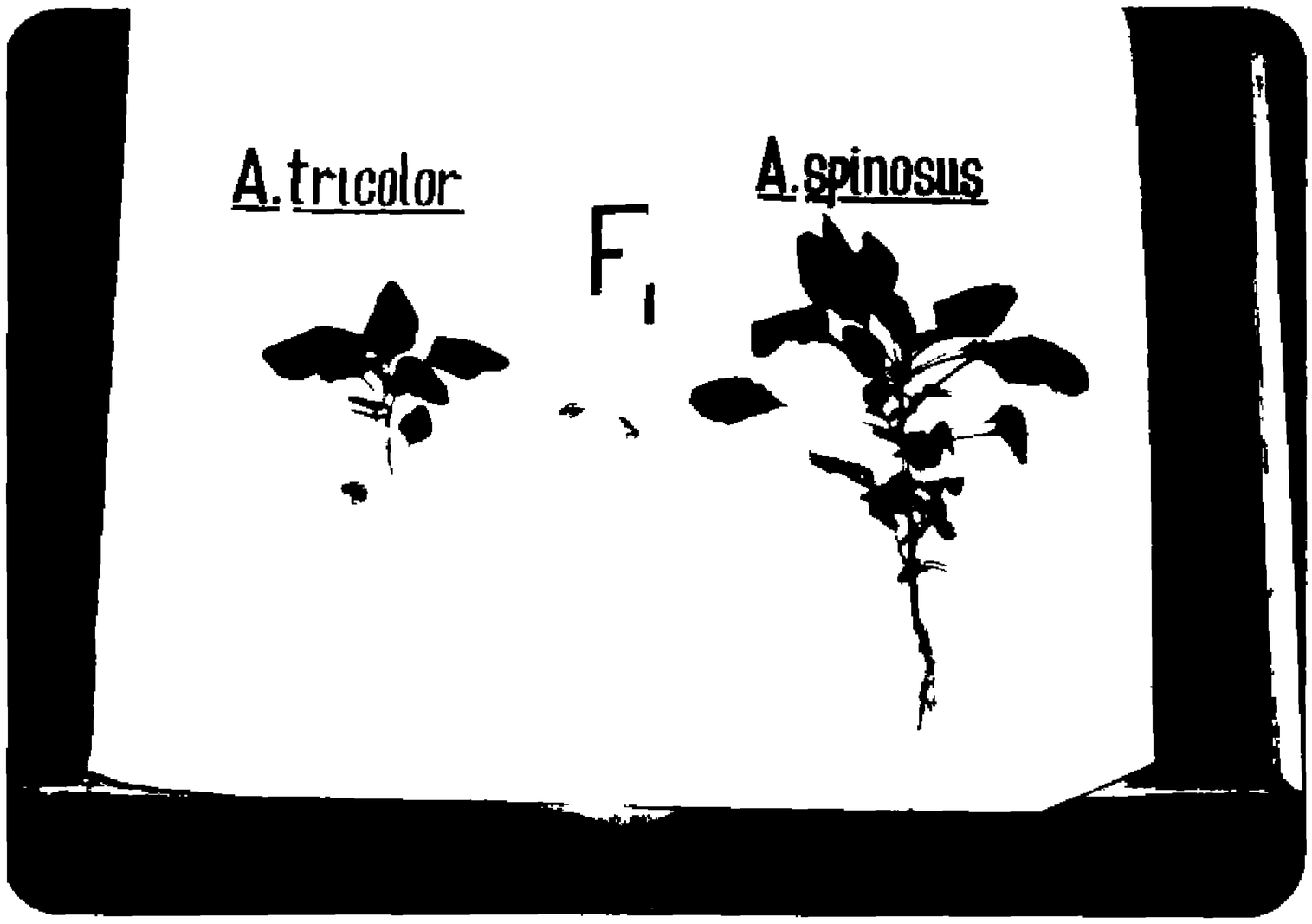
The seven interspecific hybrids which exhibited normal growth and flowering were critically examined for vegetative and floral characters (Table 10). In section Blitopsis, only one hybrid *A. lividus x A. tricolor* was obtained and in section Amaranthus, five hybrids were successful. Only one inrterspecific hybrid, *A. spinosus x A. viridis* was obtained in cross between sections Amaranthus and Blitopsis.

PLATE 9

a. Seedling abnormality of the interspecific hybrid *A. tricolor x A. spinosus* in comparison with normal parental seedlings.

b. Seedling abnormality of the interspecific hybrid *A. lividus x A. viridis* in comparison with normal parental seedlings.

PLATE 9



a

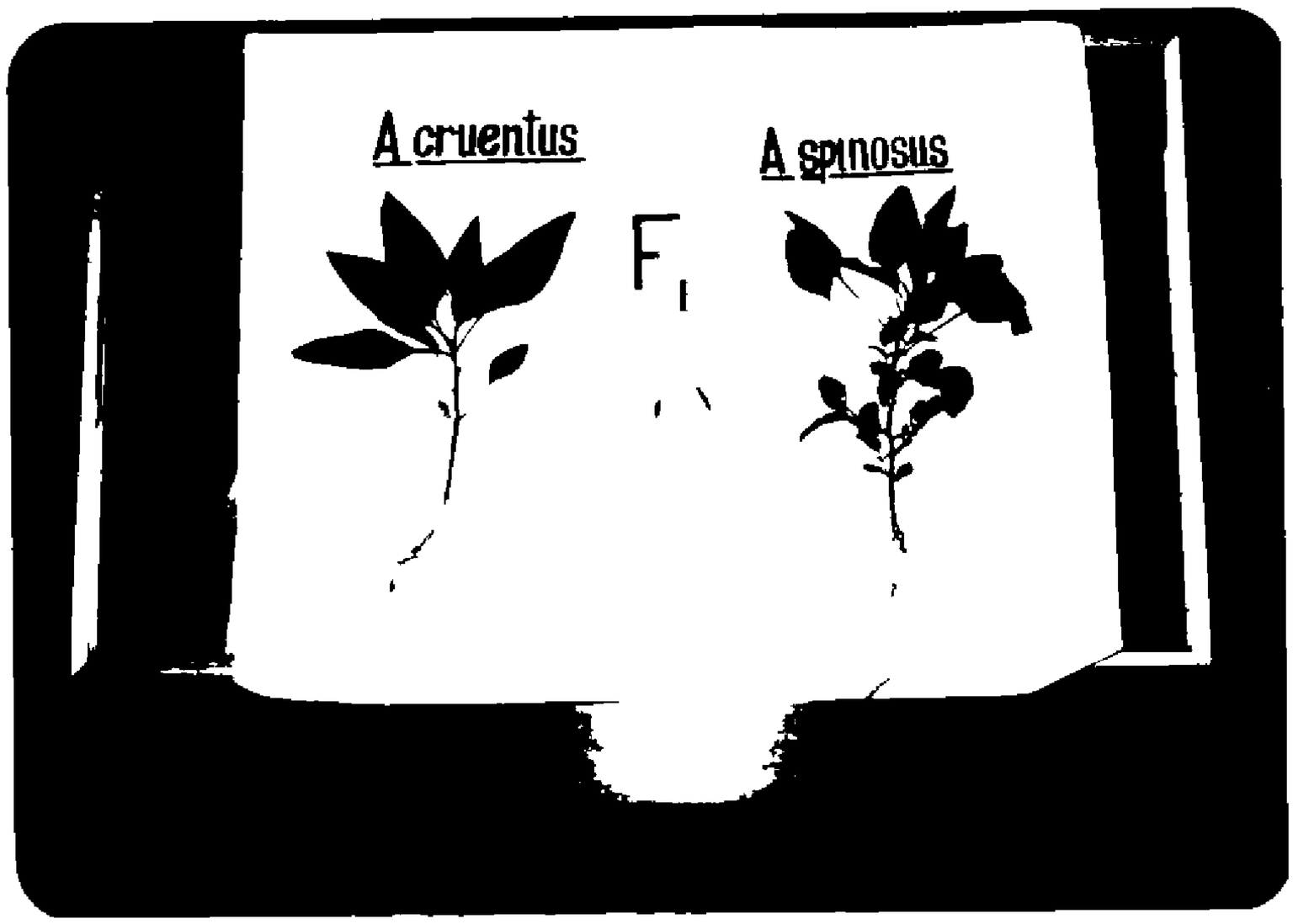
b

PLATE 10

a. Seedling abnormality of interspecific hybrid *A. cuentus x A. spinosus* in comparison with normal parental seedlings

b. Seedling abnormality of interspecific hybrid *A. caudatus x A. lividus* in comparison with normal parental seedlings

PLATE .10



a

b

Table 10. Morphological characters of interspecific hybrids of *Amaranthus*

Characters	*A lividus* x *A tricolor*	*A spinosus* x *A dubius*	*A spinosus* x *A hypochondriacus*	*A spinosus* x *A cruentus*	*A spinosus* x *A caudatus*	*A. cruentus* x *A. caudatus*	*A spinosus* x *A viridis*
Plant height (cm)	90	125	50	80	70	90	45
Number of branches	15	8	16	20	25	16	17
Stem texture	Soft & fleshy	Fleshy	Semihard	Hard and woody	Semihard	Semihard	Very hard and sturdy
Stem colour	Pale green	Pale green with pinkish streaks	Purplish green	Dull pink	White/ pale green	Deep purple	Green
Stem girth (cm)	5	3	4 5	4 0	4 5	4 0	5 0
Spines on the stem	Nil	Soft spines	Semihard spines	Semihard spines	Hard & sharp spines	Nil	Short, broad & sharp spines
Internodal length (cm)	4	1 5	6	3 5	3	3 5	2
Length of branches of stem (cm)	30	50	20	30	35	50	15
Total leaf number	190	130	500	360	480	240	230
Leaf size (LxB) (cm)	6 0 x 4 5	12.0 x 9 0	7 0 x 4 0	4,5 x 2 5	5 0 x 2.5	7 0 x 3 0	5 5 x 3.3
Leaf shape	Triangular ovate	Ovate	Rhombic ovate	Ovate-lanceolate	Rhombic ovate	Ovate-lanceolate	Ovate-lanceolate
Leaf colour	Green with purple centre	Green	Purplish green	Green	Pale green	Pale green	Dark green
Leaf apex	Emarginate	Acute	Acute-Obtuse	Obtuse	Acute-obtuse	Acute	Retuse
Leaf base	Rounded	Cuneate	Cuneate	Cuneate	Cuneate	Cuneate	Cuneate
Leaf margin	Wavy	Entire	Wavy	Entire	Entire	Entire	Entire
Orientation and branching of inflorescence	Erect, unbranched	Erect, well branched	Erect unbranched	Erect, unbranched	Erect, unbranched	Semierect, well branched	Erect, branched
Inflorescence type	Terminal and axillary	Terminal & axillary	Terminal & axillary	Terminal & Axillary	Terminal & axillary	Terminal only	Terminal & axillary
Inflorescence colour	Green	Whitish green	Whitish green	Whitish green	Pale green	Deep purple	Green

(contd.)

Table 10 (Contd.)

Characters	*A lividus* x *A tricolor*	*A. spinosus* x *A. dubius*	*A spinosus* x *A hypochondriacus*	*A spinosus* x *A cruentus*	*A spinosus* x *A caudatus*	*A cruentus* x *A caudatus*	*A spinosus* x *A viridis*
Inflorescence texture	Rough	Velvetty	Soft	Spongy	Rough	Soft but stiff	Stiff
Number of branches of Inflorescence	Nil	13	Nil	Nil	Nil	40	5
Inflorescence length (cm)	12	35	15	10	6	30	8
Position of male flower in panicle	Central floret in few cymes	Central floret in cymes and also at distal ends	Distal ends of panicle	Distal ends of panicle	Distal ends of panicle	No male flowers	Distal ends of panicle
Proportion of male flowers	Normal	Extremely low	Extremely low	Extremely low	Normal	Normal	Very few
Anthesis of male flowers	Normal	Normal	No anthesis	No enthesis	Normal	-	No anthesis
Nature of axillary clusters	Prominent	Slightly prominent	Extremely prominent round the node	Prominent	Few female cymes	Nil	Few female cymes
Anther colour	Creamy	Creamy	Creamy	Creamy	Yellowish	-	Whitish
Seed colour and set	No seed set	No seed set	Few black seeds	Very few black seeds	Enough black seeds	No seed set	No seed set
Duration of the plant (days)	110	120	100	Perennial	120	110	Perennial

a) Morphology of interspecific hybrids within section Blitopsis

1) *A. lividus x A. tricolor*

Morphologically the hybrid showed an overall dominance of the male parent in habit and floral characters. The purple blotch of the leaf inherited from the male parent helped to spot out easily the hybrids even when they were one week old. The only character of the female parent expressed in the hybrid morphology was the leaf tip nature and tenderness of the stem. The hybrid had more vigorous growth with longer primary leaves than both the parents (15 x 12 cm). The hybrid also flowered earlier than both the parents. In respect of plant height, leaf number of branches and secondary leaf size, the hybrid showed similarity with the male parent. The stem was tender and fleshy as in female parent but the thickness was more as in *A. tricolor*. (Plate 11a)

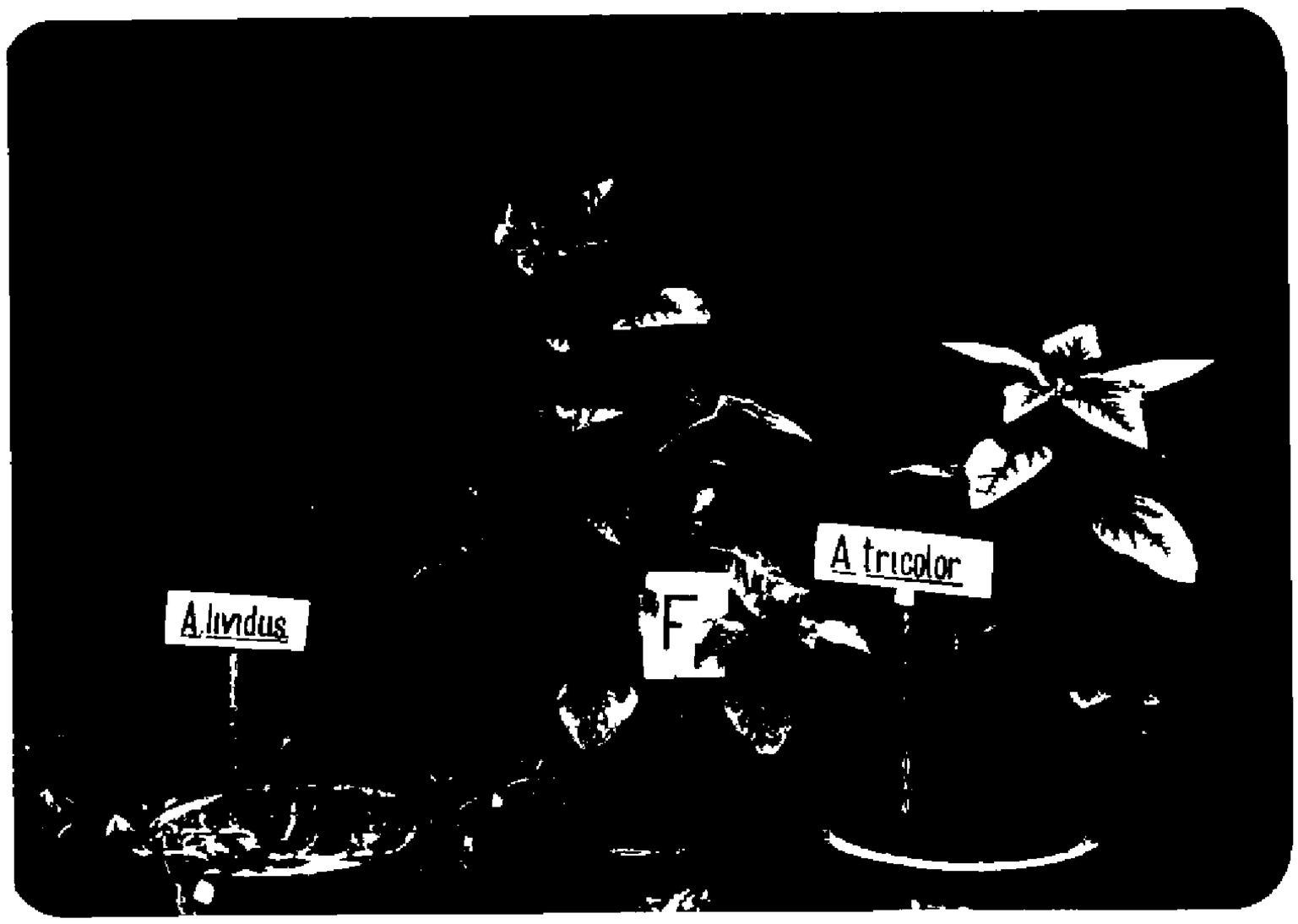
The flowers were borne on terminal panicles at the end of every branch and also in axillary clusters at most of nodes. The male flowers were borne as central flowers in a few cymes and they had normal anthesis eventhough the anthers were shrivelled. Female flowers were produced profusely. Flowers were condensed on long scorpioid cymose branches of polychasial cymes in every glomerule. The hybrid had maximum length (0.8 cm) for each scorpioid cymose branch within a flower cluster. The long and densely crowded sterile scorploid cymes imparted a fasciated appearance to the inflorescence (Plate 11b). Anthesis of male flowers were normal but no seed set was observed in the hybrid. This vigorous hybrid had a life span of about 110 days.

PLATE 11

a. Plant morphology of *A. lividus x A. tricolor* hybrid

b. Late flowering stage of *A. lividus x A. tricolor* showing fasciation of the inflorescence.

PLATE 11



a

b

b) Morphology of interspecific hybrids within section Amaranthus

i) *A. spinosus x A. dubius*

This hybrid was obtained with better success by hybridization than all other interspecific hybrids. No reciprocal difference was observed when *A. spinosus* was used either as male or female parent. In morphology, the hybrid resembled the tetraploid parent *A. dubius*, but was quite distinct by the presence of soft axillary spines and velvetty inflorescence (Plate 12b) The hybrid was very vigorous and exhibited heterosis for height, number of branches, leaves and flowers. The leaves were rhombic-ovate as in *A. dubius* but were slightly narrower than *A. dubius* leaves and had acute leaf tips. The floral characters were almost like *A. dubius* except for their velvetty appearance imparted by enlarged stigmas of a large number of unfertilized female flowers. As in other interspecific hybrids, involving *A. spinosus*, the distinct placement of male and female flowers was not observed in this hybrid. Floral arrangement was exactly like that of *A. dubius* but the terminal panicle was larger than the same. Anthesis was normal but the plant was completely sterile without any seed formation (Plate 12a & b)

ii) *A. spinosus x A. cruentus*

This was another hybrid, with a tendeancy for perennial growth habit The hybrid had an overall dominance of the female parent as observed by presence of spines, and distrinct arrangement of male and female flowers. The hybrid exhibited heterosis for number of branches, leaves and flowers but there was more reduction in leaf size than both the parents. None of the peculiarities of *A. cruentus* leaves were observed in the hybrid. The flowers were highly condensed in the terminal panicle and upper leaf axils and the prolific production of flowers imparted a spongy appearance to the

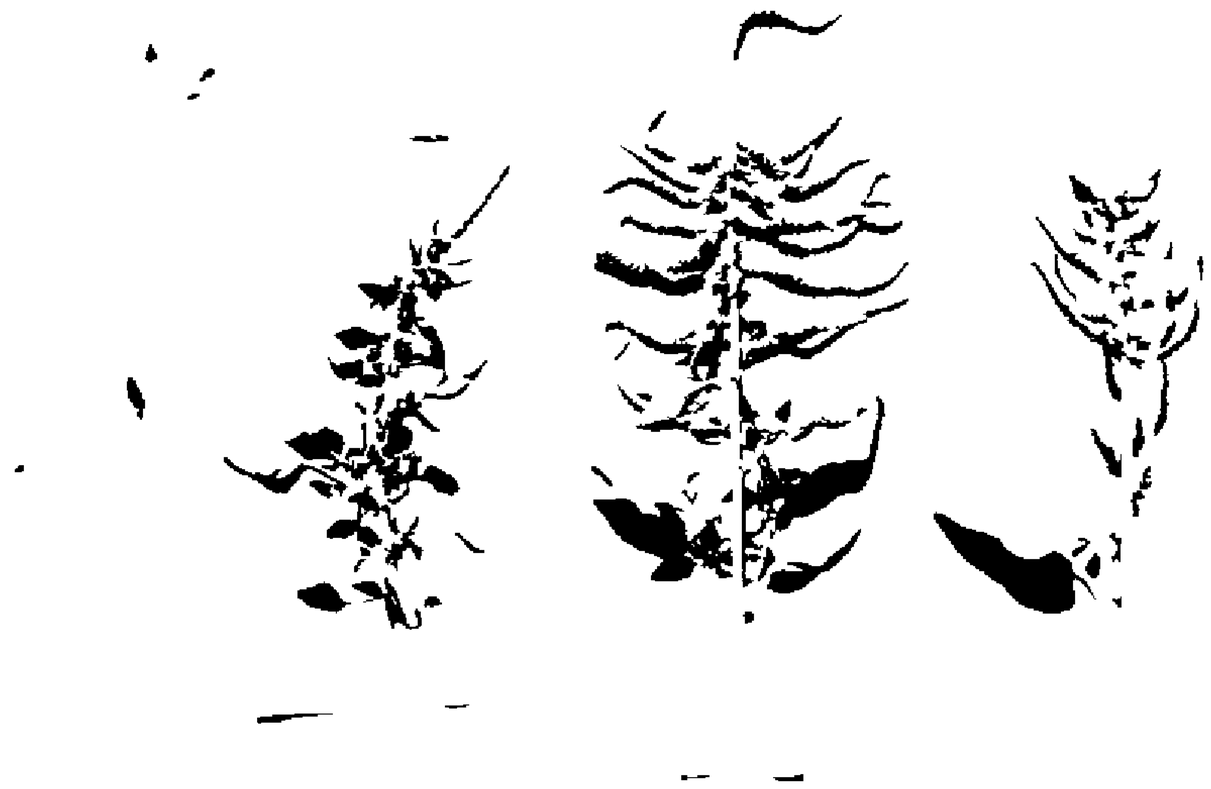
PLATE 12

a. Plant morphology of *A. spinosus x A. dubius* hybrid and parents.

b. Flowering twigs of the hybrid and parents in *A. spinosus x A. dubius* cross

PLATE 12

a



b

PLATE 13

a. Plant morphology of *A. spinosus x A. cruentus* hybrid and parents

b. Flowering twigs of the hybrid and parents in *A. spinosus x A. cruentus* cross

PLATE 13

a

b

inflorescence (Plate 13 a & b). The number of male flowers were very much limited, being confined to the distal ends of panicle and they did not open to shed their pollen. Many of the male flowers were barren and a few had only 1-2 stamens (Staminodes were observed only in this inter-specific hybrid). The stem was hard and woody with lenticels on them and seemed extremely resistant to drought conditions.

In the reciprocal cross of these two species, the hybrids were abnormal growing only to two leaf stage. These leaves were leathery and further growth was arrested by the decay of the terminal bud.

iii) *A. spinosus x A. caudatus*

This interspecific hybrid is identified from among the selfed seedlings by its highly arrested growth and the pale colour of the stem, petiole and leaves inherited from the male parent. The reciprocal cross (*A. caudatus x A. spinosus)* was also successful but the seedling failed to grow beyond the third leaf stage. When the parent seedlings attained a height of 20 cm, interspecific hybrid seedlings were hardly more than 5 cm. After two months of highly arrested and stunted growth, the hybrid grew and flowered as in a normal spinosus plant. The F_1 hybrid exhibited heterosis for leaf number, branches and flowers and there was an overall dominance of *A. spinosus* characters marked by the presence of sharp spines, leaf shape (though reduced in size), nature of flowers, distinct placement of male and female florets etc. (Plate 14a). The anthers were yellowish as in the male parent and anthesis was fully normal. There was copious seed formation on selfing. Profuse flowering was noticed in this plant as terminal panicles were produced at the ends of every branch in this highly branched hybrid. (Plate 14 a & b).

PLATE 14

a. Plant morphology of *A. spinosus x A. caudatus* nybrid and parents

b. Flowering twigs of the hybrid and parents in *A. spinosus x A. caudatus* cross

PLATE 14

a

b

iv) *A. spinosus x A. hypochondriacus*

This hybrid inherited the purple leaf colour of male parent and could be easily spotted out in the seedling stage. The hybrid was very vigorous even from the seedling stage and had a preponderance of *A. spinosus* characters. Eventhough slightly longer, the shape, margin and apex of the leaves were like that of *A. spinosus*. It resembled *A spinosus* for presence of spines at nodes (though not very sharp) and arrangement of staminate and pistillate flowers. A very promising feature of this hybrid was the profuse production of axillary female cymes commonly seen in *A. tricolor* of section Blitopsis. These axillary clusters produced round the node, all along the plant imparted a knobby appearence to the hybrid (Plate 15b). The proportion of male flowers were comparatively very few and were confined to the distal ends of panicle. Even these flowers failed to open and were dried off without pollen dehiscence. Unlike other interspecific hybrids, this plant had a shorter duration and withered off in 100 days.

v) *A. cruentus x A. caudatus*

This interspecific hybrid had distinct sex habit and nature of growth. The hybrid could not be identified in the seedling stage since it was almost similar to the female parent *A. cruentus*. After about 50 days of growth the plant showed a peculiar twining of the stem and growth of the terminal bud was arrested showing virus like symptoms in the top most branches. Flowering was normal in the lower branches while the upper branches were stunted and damaged with deformed panicles. The stem showed prominent ridges here and there and also had splitting of the cortical region at many places. The lyrate leaf base as seen in *A. cruentus* leaf seemed to more or less merge with the blade and the hybrid leaves showed more similarity to *A. caudatus* leaves. The reflexed type of terminal leaves of *A. curentus*

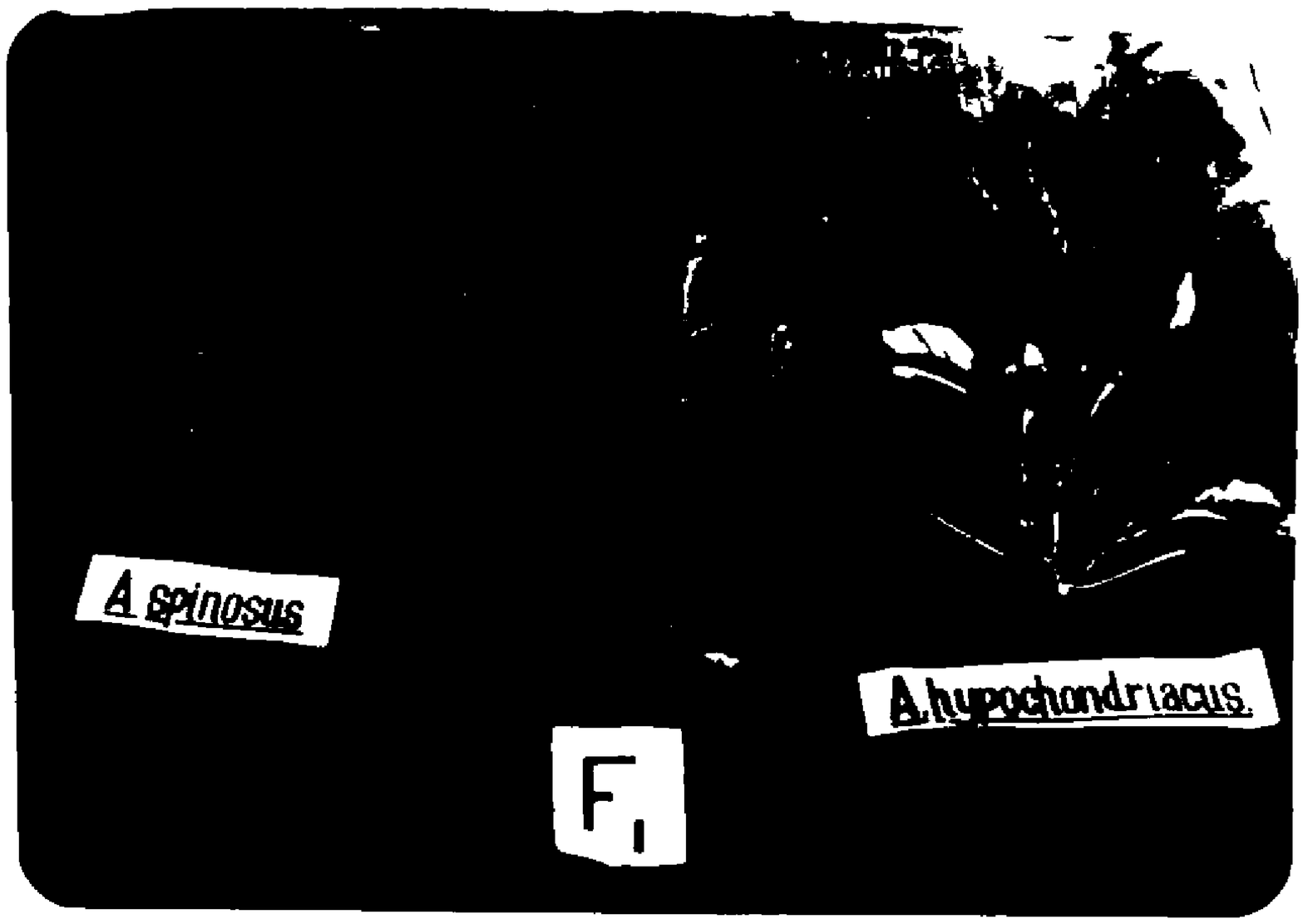
PLATE 15

a. Plant morphology of *A. cruentus x A. caudatus* hybrid

b. Flowering twigs of the hybrid and parents of *A. spinosus x A. hypochondriacus*

PLATE 15

a



b

was not observed in the hybrid. The most peculiar feature of this hybrid was the unisexual habit marked by the production of female flowers alone. Not even a single male flower was produced by this plant eventhough most of the floral characters were exactly like *A. cruentus* (Plate 15a). Hence the meiosis of this plant could not be studied using PMCs. The abnormalities observed in the hybrid did not appear to be associated with any recognizable pathogenic organism.

The reciprocal cross of these two species resulted in abnormal hybrid plants growing only upto two leaf stage. A forked appearence was noted in the seedlings and growth was very much arrested and these seedlings failed to grow further.

c) Morphology of interspecific hybrid between sections Amaranthus and Blitopsis

i) *A. spinosus x A. viridis*

This intersection hybrid had a distinct morphology and perennial growth habit unlike both the parents. The hybrid seedling could be spotted out from among the selfed *A. spinosus* seedlings by broader and thicker leaves and stunted growth. In the early stages, the plant exhibited a rosette form of growth without any stem elongation and for the first two months the growth was highly arrested. Later a large number of leaves typically ovate and leathery were produced. The stem was very hard and sturdy with very short internodes and had a pair or short and sharp spines at each node. The hybrid flowered after 100 days producing terminal panicle and axillary pistillate cymes as in the parents. There was distinct placement of male and female flowers in the panicle, the former restricted to terminal ends only. Eventhough the hybrid had a distinct morphology, there was an overall dominance of the female parent marked by the presence of spines at nodes, size and symmetry of flowers and also in the

PLATE 16

a. Plant morphology of *A. spinosus x A. viridis* hybrid

b. Flowering twigs of the parents and hybrid in *A. spinosus x A. viridis* cross

PLATE . 16

a

b

distinct placement of flowers of different sexes. Unlike *A. spinosus* the bracts and tepals were not bristle tipped, though the flowers as in the female parent. Most of the pentamerous male flowers were barren without any stamens, but a few had all the five stamens well developed. There was no anthesis in any of the male flowers and all the anthers shrivelled off before dehiscence. No seed set was observed in this hybrid though the pollen stainability was fairly high. (Plate 16 a & b)

2. Cytogenetical studies of interspecific hybrids in Genus *Amaranthus*

Interspecific hybridization attempted between species belonging to Blitopsis, Amaranthus and also between these two sections. Hybridization was successful. Hybrids expressed varying degrees of sterility. Seven interspecific hybrids grew into adult plants and flowered, permitting their detailed cytomorphological observations. As the hybrid *A. curentus* x *A. caudatus* produced only pistillate flowers, meiotic studies could not be carried out using PMC squashing. Chromosome pairing and distribution during meiosis in the six interpsecific hybrids are presented in Table 11 and 12. Pollen morphology of hybrids are furnished in Table 13.

a) Cytology of interspecific hybrid within Blitopsis

i) *A. lividus* x *A tricolor*

Parents of this hybrid had 2n = 34. The hybrid also had 34 chromosomes in their somatic cells. At metaphase I, bivalent frequency ranged from 8 to 15 with a mean number of 11.64. The prominent feature during meiosis was the associations involving 3 or more chromosomes in all the examined cells in this hybrid. Trivalents and quadrivalents were the most common and cells with chromosome complexes involving 5 or 6 chromosomes were not infrequent. The number of univalents ranged from 0-3

Table 11. Chromosome Associations in the interspecific F_1 hybrids of the genus *Amaranthus* at Metaphase I

Hybrid	No. of cells analysed	MEIOTIC CONFIGURATIONS						Chiasma frequency range and mean
		I	II	III	IV	V	VI •	
		Range & Mean	Range & Mean	Range & Mean	Range & Mean	Range & Mean	Range & Mean	
Section Blitopsis								
A lividus x A tricolor	25	0-3 1 04 ± 0 168	8 15 11 64 ± 0 475	0-3 1 16 ± 0 213	0 2 1 192 ± 0 149	0-1 0 12 ± 0 06	0-1 0 08 ± 0 055	22 27 24 97 ± 0 336[d]
Section Amaranthus								
A spinosus x A caudatus	10	0-6 1 30 ± 0 615	11-15 12 40 ± 0 599	0-1 0 70 ± 0 152	0-2 1 20 ± 0 249	-	-	23-30 26 80 ± 0 628[a]
A spinosus x A. cruentus	16	0-7 3.66 ± 0 637	9 16 13 00 ± 0 55	0-1 0 20 ± 0 107	0-3 0 93 ± 0 23	-		22-32 26 6 ± 0 694[a]
A spinosus x A hypochondriacus	10	5-15 11 8 ± 0 94	6-11 7 5 ± 0 562	0-1 0.20 ± 0 133	0-2 1 50 ± 0 223	-		14-18 16.6 ± 0 426[c]
A spinosus x A dubius	25	13-17 14.84 ± 0 110	14-17 16 36 ± 0 215		0-2 0 36 ± 0 127	-	-	21-28 26 12 ± 0 352[a]
Amaranthus x Blitopsis								
A spinosus x A. viridis	16	4-8 5 25 ± 0 403	13 16 14 37 ± 0 201	-	-	-	-	20-28 24 125 ± 0 59[b]

a, b, c & d p = 0 01

Table 12 .Percentage of chromosomal distribution at Anaphase I and Telophase II in the interspecific hybrids

Hybrid	Chromosomal distribution at Anaphase I					Chromosomal distribution at Telophase II		
	Number of cells analysed	Equal	Unequal			Number of cells analysed	Number of nuclei at Telophase II	
			Bridges	Laggards	Total		4 nuclei	Less or more than 4 nuclei
Section Blitopsis								
A. lividus x A. tricolor	40	40	15	45	60	75	24	76
Section Amaranthus								
A. spinosus x A. caudatus	35	91	6	3	9	55	22	78
A. spinosus x A. cruentus	25	68	8	24	32	35	27	73
A. spinosus x A. hypochondriacus	32	29	12	59	71	40	20	80
A. spinosus x A. dubius	38	11	48	71	89	65	15	85
Amaranthus x Blitopsis								
A. spinosus x A. viridis	29	76	7	17	24	40	62	38

Table 13 Pollen characteristics of interspecific hybrids in genus *Amaranthus*

Interspecific hybrids	Pollen stainability %	Pollen size range (Stainable and unstainable) % of different types			Average size of stainable pollen (µm)	Average size of Micropollen (µm)
		Micro (6-12 µm)	Medium (12-24 µm)	Macro (>24 µm)	Mean ± S.E.	Mean ± S.E.
A. lividus x A. tricolor	10	5	95	-	16.97 ± 0.453	9.89 ± 0.395
A. spinosus x A. caudatus	18	-	100	-	13.77 ± 0.32	-
A. spinosus x A. cruentus	20	2	98	-	15.45 ± 0.27	7.50 ± 0.34
A. spinosus x A. hypochondriacus	8	12	88	-	17.50 ± 0.395	7.80 ± 0.50
A. spinosus x A. dubius	11	8	88	4	26.64 ± 0.47	9.18 ± 0.38
A. spinosus x A. viridis	38	15	85	-	15.87 ± 0.49	8.23 ± 0.53

PLATE 17

Meiosis in the hybrid *A. lividus x A. tricolor*

A. Metaphase I showing 1 V + 2 IV + 2 III + 7 II + 1 I (x1500)

B. Metaphase I showing associations involving 4, 5 and 6 chromosomes (x1500)

C & D Anaphase I indicating dicentric bridges and laggards

E Telophase II showing abnormal number of nuclei (x600)

F Pollen grains in the hybrid indicating high amount of sterile grains (x1200)

PLATE . 17

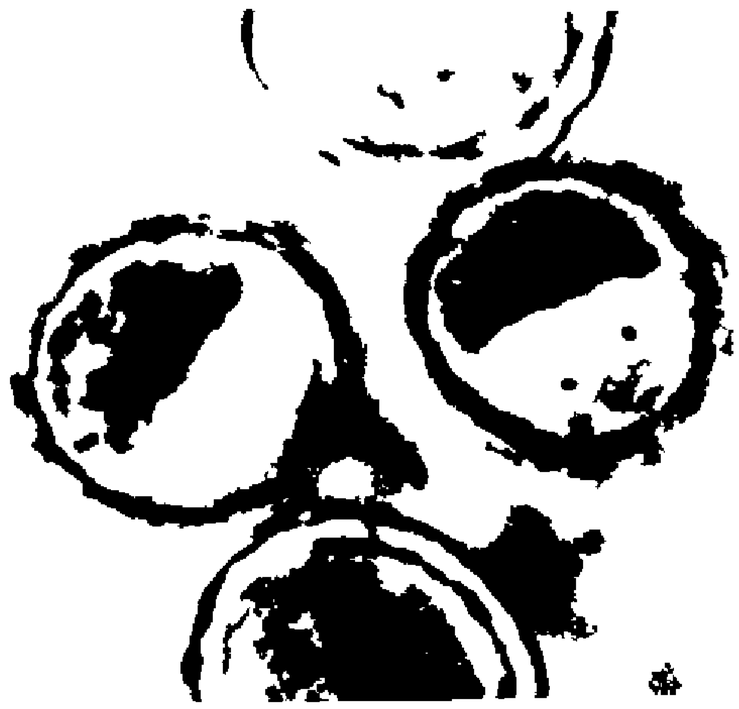
A

B

C

D

E



F

with a mean value of 1.04; and this hybrid exhibited the minimum number of univalents than all other hybrids involved in the present study (Plate 17).

Out of 40 cells analysed at anaphase I, 40% cells showed normal anaphase segregation and the remaining (60%) cells were characterised by unequal distribution. The cells having unequal distribution exhibited laggards in 45% cells and bridges in 15% cells. Often two bridges were observed in anaphase I. Second meiotic division was also abnormal with 75% of the cells showing unusual number of nuclei at telophase II. This hybrid plant showed 90% pollen sterility and the stained pollen grains were only medium sized (16.97 µm). The sterile micropollen in this hybrid constituted about 5% and they had an average size of 9.89 µm (Table 13)

b) Cytogenetical studies of interspecific hybrids within section Amaranthus

i) *A. spinosus x A. dubius*

Meiotic behaviour was highly irregular though the hybrid resembled the tetraploid parent phenotypically. The hybrid had the triploid chromosome number 2n = 49 and at metaphase I, 72% of the PMCs showed 17 II + 15 I. Eventhough the number of univalents ranged from 13-17, 15 was more frequent. About 36% cells had 1 or 2 quadrivalents also at metaphase I. Nearly 72% of the PMCs showed a configuration of 17 II + 15 I 20% had I IV + 15 II + 15 I and 8% had a 2 IV + 14 II + 13 I (Plate 18).

The bivalents had a normal orientation at metaphase plate, but most of the univalents failed to orient properly (Plate 17). During anaphase I, all the bivalents desymapsed normally and moved towards the poles while the univalents very often lagged behind. Only 11% of the PMCs at anaphase I showed a normal segregation of chromosome but unequal separation was observed in 89% of the PMCs. Of these 48% included cells

PLATE 18

Meiosis in the hybrid *A. spinosus x A. dubius* - First (x1500)

A, B & C Metaphase I showing 1 to 2 IV, 13-15 II and 13-15 I (Arrow indicates IV)

D & E Non-orientation of univalents at metaphse plate.

F Dicentric bridge at anaphase I

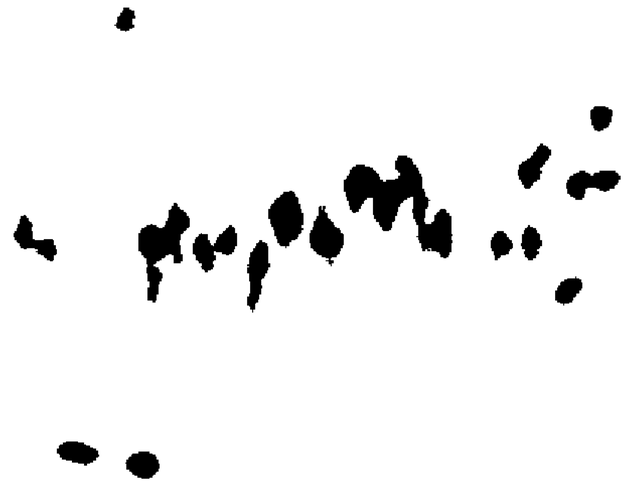
PLATE . 18

A

B

C

D



E

F

PLATE 19

Meiosis in the hybrid *A. spinosus x A. dubius*. Second Division

A. Non orientation of laggards at metaphase II (x1500)

B. Early metaphase II showing laggards (x1500)

C. Late anaphase II showing laggards (x1500)

D. Telophase II showing abnormal number of nuclei and micronuclei (x1500)

E. Pollengrains of varying sizes in the hybrid (x600)

F. Stained macropollen and sterile micropollen (x1500)

PLATE 19

A

B

C

D

E

with dicentric bridges and 71% exhibited lagging fragments and univalents, 2-7 laggards were usually observed at anaphase I (Table 12, Plate 18).

Second meiotic division was also highly abnormal with non-orientation at metaphase II, and laggards at anaphase II. All these abnormalities often led to the formation of abnormal number of nuclei at telophase II and 62% cells had 3, 5 or more nuclei at the end of the second division. These micronuclei led to smaller sized pollen ('Micropollen') grains and about 8% micropollen was observed in this hybrid. Pollen grains of different size ranges were observed in this hybrid but most of these were unstained. Only 11% stained pollen was obtained and these belonged to a mean size of 26.64 μm (Plate 19).

ii) *A. spinosus x A. caudatus*

This was one of the most fertile interspecific hybrids obtained in the present investigation. The 33 chromosomes of this dibasic hybrid formed a large number of bivalents, a few multivalents and univalents at metaphase I stage. The number of bivalents in PMCs varied from 11-15, and the mean number was 12.4. The range of univalents was 0-6 with an average of 1.3. The PMCs also showed either a quadrivalent, trivalent on both together in every cell examined. The mean number of quadrivalents was 1.2 and trivalents was 0.7. The chiasma frequency ranged from 23-30 with a mean value of 26.8; the highest value among all the hybrids studied.

Anaphase I was apparently normal and equal segregation of chromosomes was observed in 91% PMCs. Of the 9% abnormal cells, 6% cells carried dicentric bridges and 3% cells carried laggards (Plate 20). PMCs also showed abnormalities in the second meiotic division characterised by asynchronous orientation and disjunction at metaphase II and anaphase II

PLATE20

Meiosis in the hybrid *A. spinosus x A. caudatus*

A. Diakinesis showing 1 IV + 14 II + 2 I (x1500)

B. Metaphase I showing 1 IV + 1 III + 12 II + 3 1 (x1500)

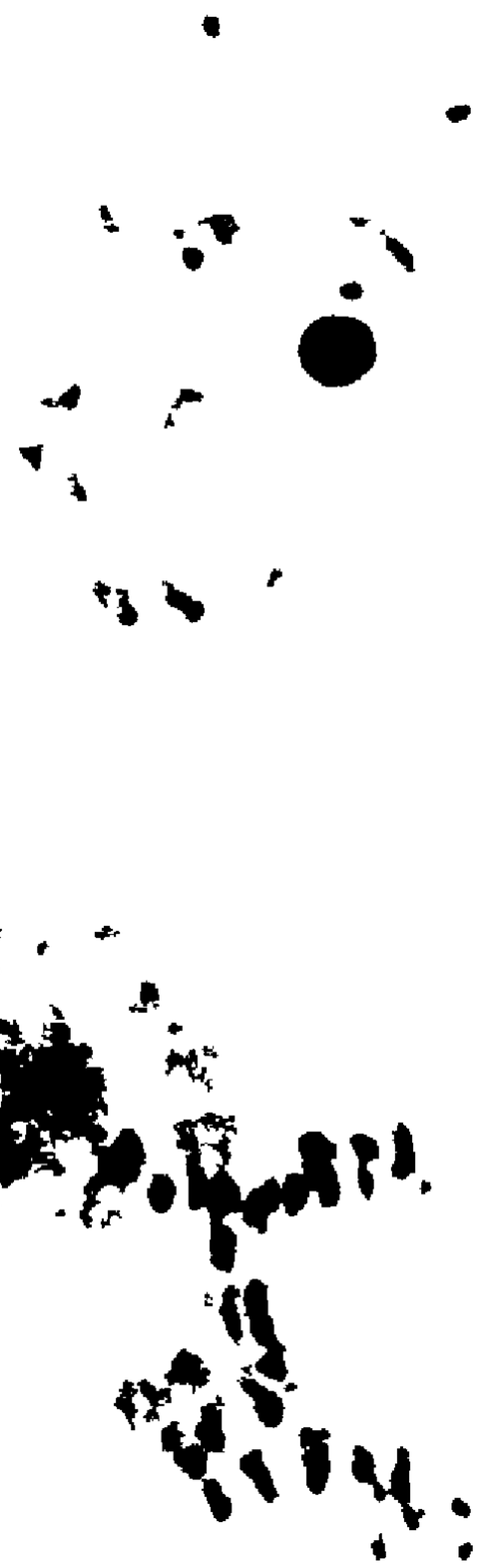
C. Anaphase I showing dicentric bridge and lagging bivalent (x1500)

D. Dicentric bridge at anaphase I (x1500)

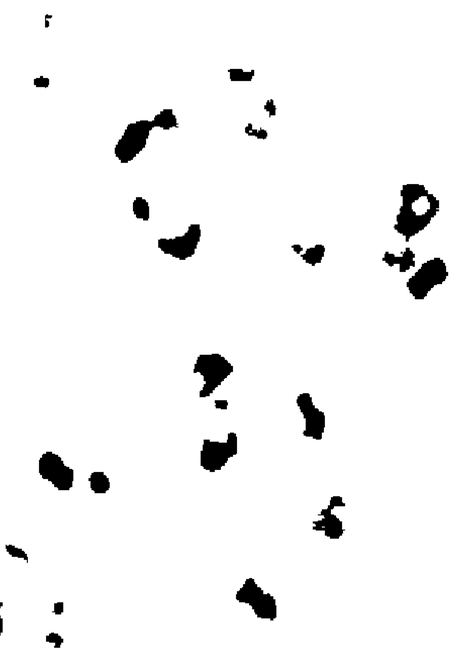
E. Asynchronous division with metaphase II and anaphase II stages in a PMC (x1500)

F. Abnormal number of nuclei at telophase II (x1000)

PLATE 20



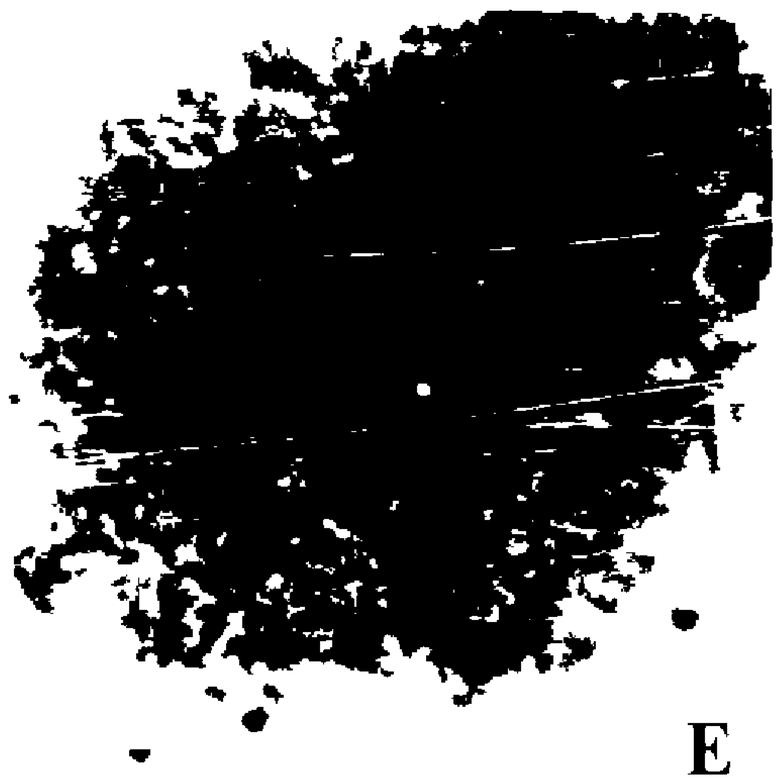
A



B

C

D



E

F

respectively. Abnormalities were observed in fairly high percentages during meiosis II and this led to 78% PMCs having unusual number of nuclei (above or below 4) at the end of the division. PMCs at telophase II usually carried 6, 7 and 8 nuclei. (Table 12)

All the pollen grains observed were medium sized. (Table 18) Micro-pollen (Pollen of <12 µm size) was not observed in the hybrid. Only 18% pollen was stainable and others were completely sterile (Plate 20 F). The average size of the stained pollen was 13.7 µm. In spite of the reduced fertility, there was copious seed formation on selfing in this hybrid.

iii) *A. spinosus x A. hypochondriacus*

Meiosis in this dibasic interspecific hybrid (2 n = 33, *A spinosus* n = 17 x *A. hypochondriacus* n = 16) was peculiar for the following features. Among all the hybrids studied, this had the highest number of univalents, the lowest number of bivalents, the highest number of quadrivalents and the lowest frequency of chiasmata in their PMCs. These observations indicate a comparatively lower affinity of chromosomes of the two species (Plate 21, Table 11).

On an average the number of univalents ranged from 5-15, with a high mean number of 11.8. Conseuqently bivalent frequency was also less ranging from 6-11 with a mean value of 7.5. Either one or two quadrivalents or trivalents were observed in all the cells studied. Mean number of quadrivalents was 1.5 in the PMCs at metaphase I. Most of the univalents failed to orient at metaphase I, and lagged at anaphase I; 71% of PMCs showed abnormal behaviour at anaphase I with dicentric bridges, fragments and laggards. Similarly at telophase II, 80% cells were abnormal with unusual number of nuclei at the end of cell division. (Table 11, 12).

PLATE 21

Meiosis in the hybrid *A. spinosus x A. hypochondriacus*

A. Metaphase I showing 2 IV + 6 II + 13 I (x1500)

B. PMC showing non orientation at metaphase plate (x1500)

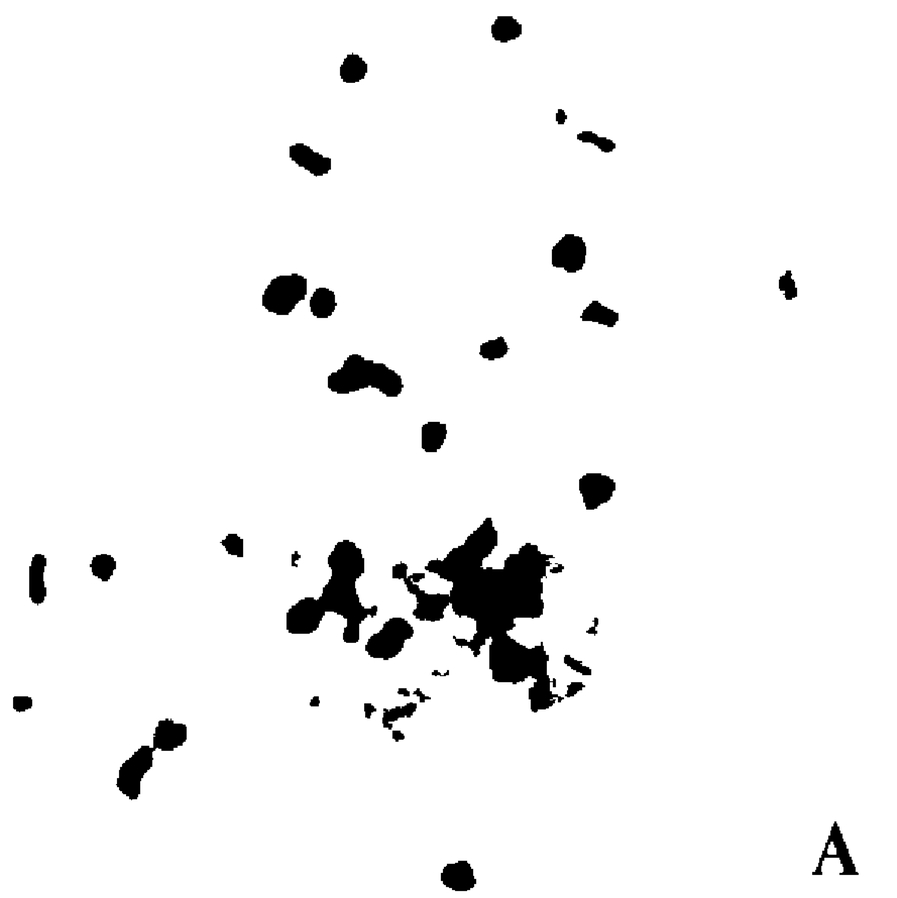
C. Anaphase I indicating asynchronous disjunction (x1500)

D. Laggards at anaphase I (x1500)

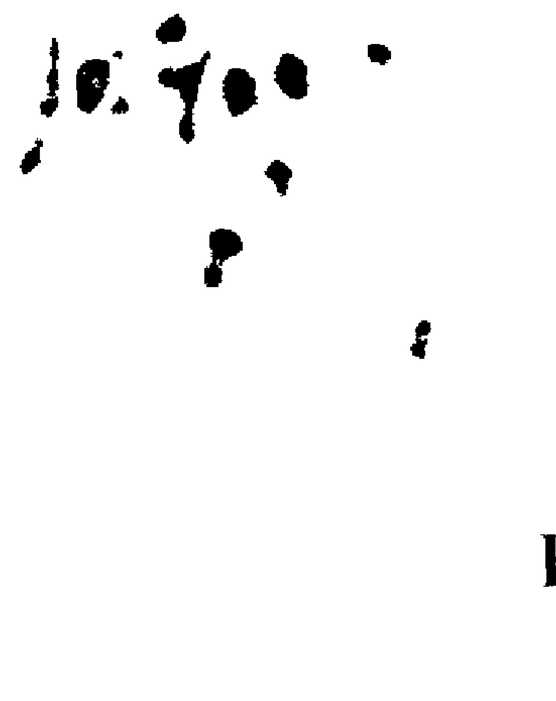
E. Micronuclei at telophase II (Arrows) x (1200)

F. Sterile pollen grains and micropollen (x600)

PLATE 21



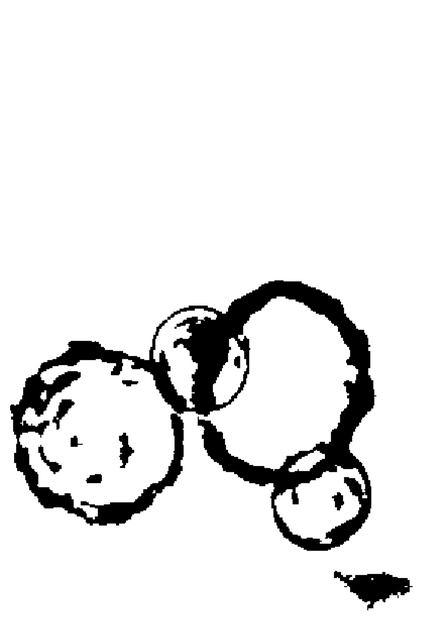
A



B

D

E



F

Pollen stainability was also minimum (8%) in this hybrid. (Table 13) The sterile micropollen amounted to 12% and the mean size of the stainable pollen was comparatively higher (17.5 µm) than other diploid hybrids (Plate 21).

iv) *A. spinosus x A. cruentus*

Both the parent species have diploid chromosome number of 2n = 34 and hence the hybrid had same number of 2n = 34. During metaphase I, the bivalent frequency ranged from 9-16, with a mean value of 13.0 (Table 11). Univalents were also observed in many of the PMCs, with a range of 0-7 and a mean of 3.66. Quadrivalents were also present in most of the cells and the mean number of quadrivalents was 0.93 (Plate 22). The mean chiasma frequency was 26.66.

Anaphase I separation was normal in 68% PMCs and the remaining 32% cells were characterised by bridges (8%) and laggards (24%). The univalents lagged at anaphase I and the bridge fragment associations also led to unequal segregation of chromosomes to poles. During telophase II 73% cells had abnormalities characterised by 3 to 5 nuclei at the time of tetrad formation. This contributed to reduction in pollen fertility and only 20% pollen was stainable. Mean size of the stained pollen was only 16.45 µm. The percentage of micropollen was only 2%. (Table 12, 13).

C. Cytology of interspecific hybrid between sections Amaranthus and Blitopsis

i) *A. spinosus x A. viridis*

PMCs in this interspecific hybrid had high frequency of bivalents at metaphase I. The 34 chromosomes of this hybrid did not form any multivalents and only bivalents and univalents were observed at metaphase I. The number of bivalents varied from 13-16 with a mean of 14.37. Univalents also failed to orient at metaphase plate (Plate 23, Table 11).

PLATE 22

Meiosis in the hybrid *A. spinosus x A. cruentus*

A. PMC at diakinesis showing I IV and 15 II (x1500)

B. Metaphase I showing non-orientation at metaphse plate (x1500)

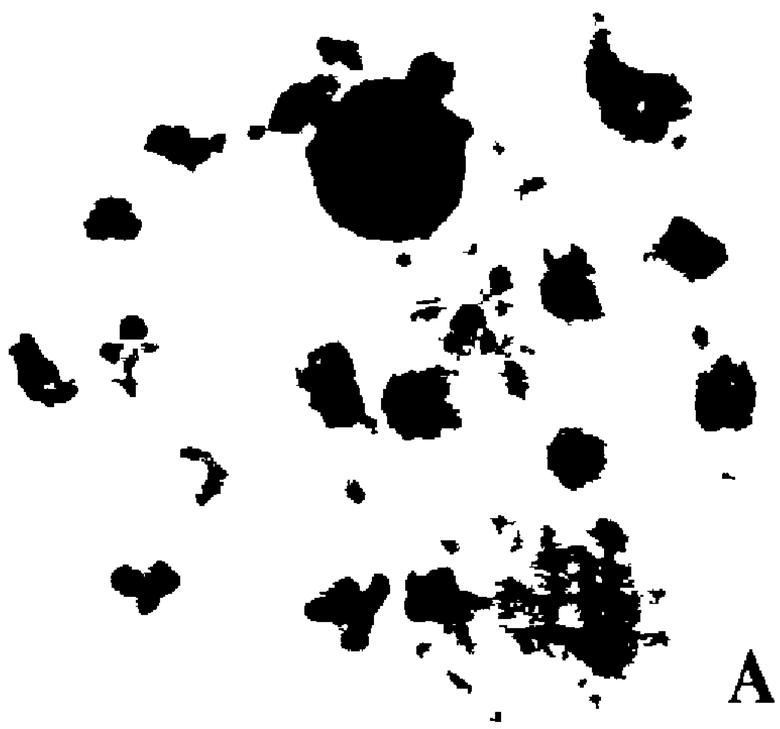
C. Anaphase I exhibiting dicentric bridge (x1500)

D. Late anaphase I showing laggards (x1500)

E. Abnormal number of nuclei at telophase II (x1200)

F. Pollen grains of the hybrid. Note the high amount of sterile grains (x1200)

PLATE 22



A

B

C

D

E

F

PLATE 23

Meiosis in the hybrid *A. spinosus x A. viridis*

A. Chromosomes at diakinesis showing 15 II and 4 I (x1500)

B. Metaphase I showing 14 II and 6 I (x1500)

C. Metaphase I showing 14 II and 6 I (x1500)

D. Dicentric bridge at anaphase I resulting from desynapsis of bivalents and lagging univalents (x1500)

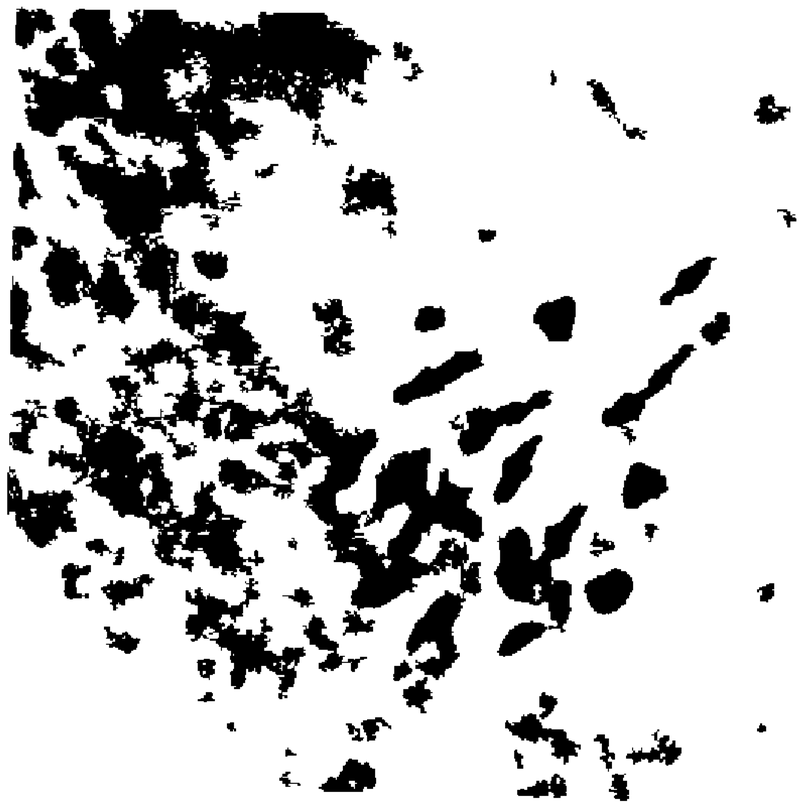
E. Late anaphase I indicating broken bridge, fragments and lagging univalents (x1500)

F. Abnormal telophase II exhibiting more than four nuclei (x1200)

PLATE . 23

A

B



C

D

E

F

Normal anaphase separation was noted in 76% of the PMCs and about 24% of the cells had abnormalities in the form of dicentric bridges and lagging univalents and fragments (Plate 23D, Table 12). These led to abnormalities in the second meiotic division also. PMCs at metaphase II showed asynchrony in division. While one set of chromosomes completed their anaphase II, other set was still in their metaphase II. As a result of these abnormalities many PMCs produced more than 4 nuclei at telophase II. About 38% also carried 5 or more nuclei towards the end of meiosis just before tetrad formation (Plate 23F). The hybrid showed 38% pollen stainability and the average size of the stained pollen was only 15.87 µm. The sterile micropollen amounted to 15% with an average diameter of 8.23 µm(Table 13).

d. D^2 Analysis of interspecific hybrids

Data on chromosome association at metaphase I in the interspecific hybrids were statistically analysed employing Mahalanobis D^2 statistics. The D^2 matrix is presented in Table 14.

Table 14. D^2 values between interspecific hybrids

Interspecific hybrids	1	2	3	4	5	6
1		1.53	2.72	10.99	6.74	5.32
2			0.68	9.69	6.62	3.62
3				7.75	5.65	1.64
4					11.80	8.65
5						6.89

1 = *A. lividus x A. tricolor*

2 = *A. spinosus x A. caudatus*

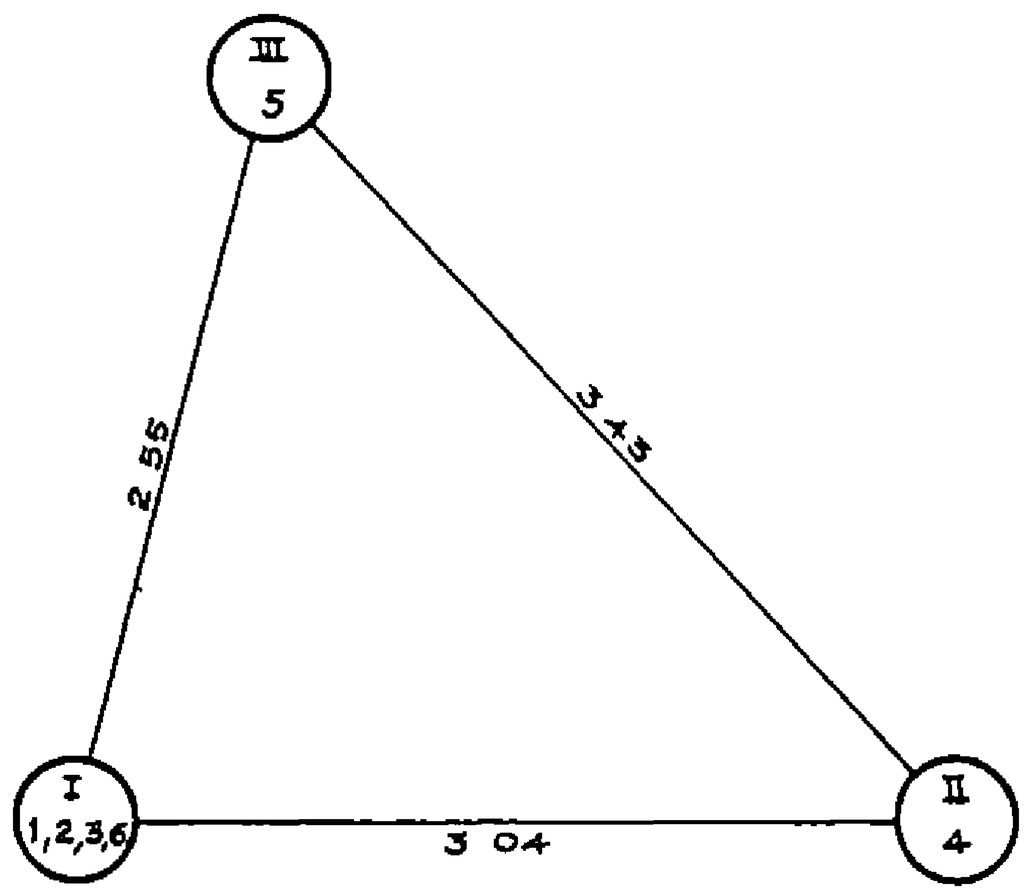
3 = *A. spinosus x A. cruentus*

4 = *A. spinosus x A. hypochondriacus*

5 = *A. spinosus x A. dubius*

6 = *A. spinosus x A. viridis*

FIG 12 DIAGRAMATIC REPRESENTATION OF CLUSTERING OF INTERSPECIFIC HYBRIDS



CLUSTER I - 1 *A lividus* x *A tricolor*
2 *A spinosus* x *A caudatus*
3 *A spinosus* x *A cruentus*
4 *A spinosus* x *A viridis*

CLUSTER II - 1 *A spinosus* x *A hypochondriacus*

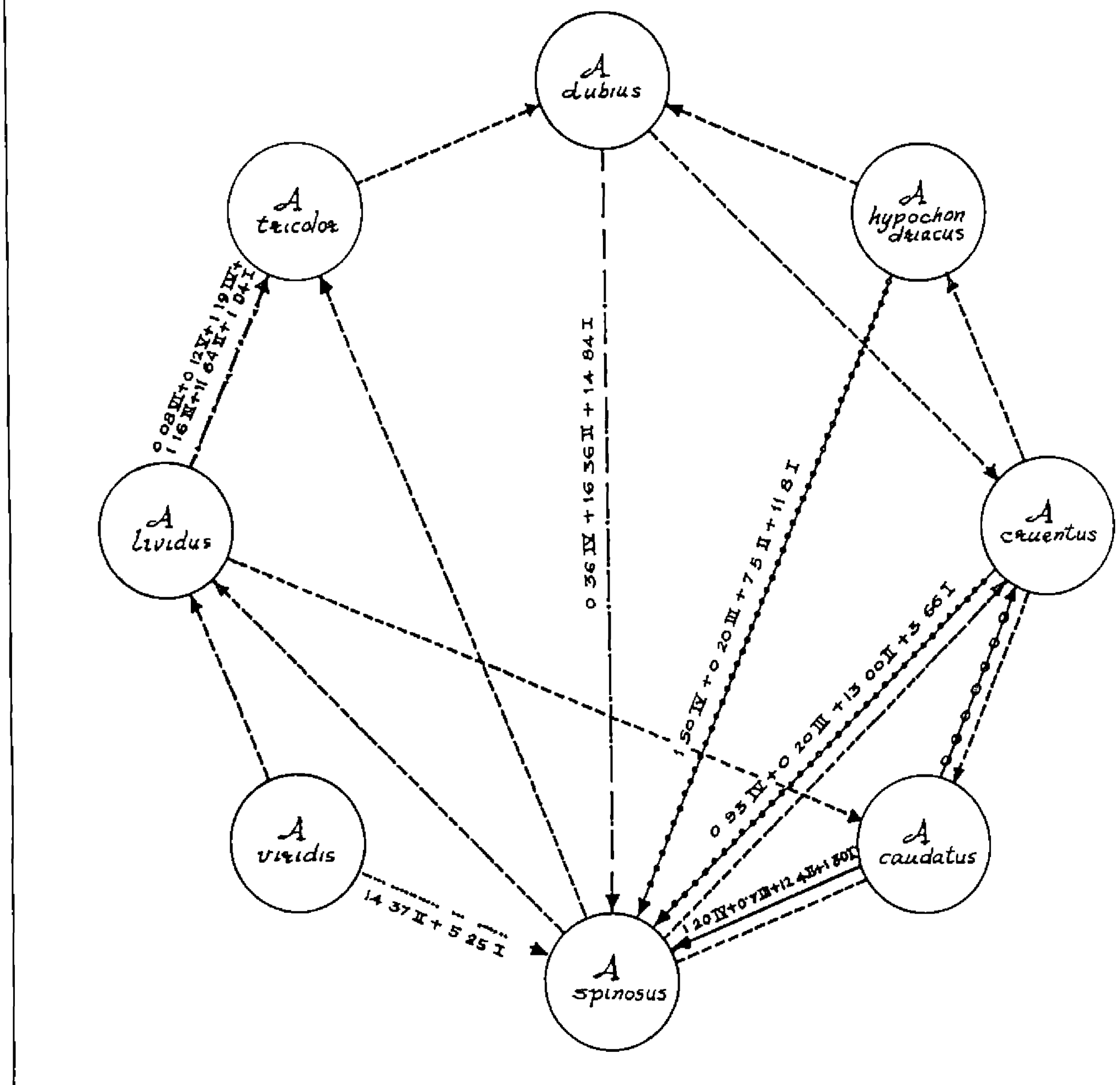
CLUSTER III - 1 *A spinosus* x *A dubius*

Based on D^2 values, the 6 hybrids were grouped into 3 clusters, I, II and III. Cluster I includes four hybrids 1, 2, 3 and 6 viz., *A. luridus x A. tricolor, A. spinosus x A. caudatus, A. spinosus x A. cruentus* and *A. spinosus x A. viridis.* Cluster II includes the hybrid 4 *(A. spinosus x A. hypochondriacus)* and cluster III includes 5 *(A. spinosus x A. dubius).* The average inter and intracluster D^2 values are given in Table 15 and Fig. 12.

Table 15. Average inter and intracluster D^2 values

	I	II	III
I	2.27	9.27	6.5
II		0	11.80
III			0

INTER RELATIONSHIP BETWEEN EIGHT *Amaranthus* SPECIES BASED ON CROSSABILITY OF SPECIES MEIOTIC CONFIGURATIONS AND NATURE OF MALE AND FEMALE FLOWERS IN THE INTER-SPECIFIC HYBRIDS



C) Interrelationship among the different species

Inter-relationship among the eight *Amaranthus* species based on crossability of species, meiotic configuration and the nature of male and female flowers in the interspecific hybrids are depicted in Fig. 13. The female sterility in the hybrids were judged from the proliferation of female flowers. Unopened (closed) nature of male flowers is considered as an index of male sterility. Male flowers with anthesis were normal. The varying degrees of male and female sterility in the hybrids are represented in Fig.13. Many of the crosses resulted in abnormal seedlings which failed to grow beyond the two leaf stage and these are also represented in the figure.

G. Classification of the existing germplasm into different species

The germplasm of 40 accessions was evaluated to identify the correct species status of each accession. Both morphological and cytological characters were studied in detail (Tables 16, 17 and Fig.14). The key for the identification of the species developed in this study was also employed. It was found that all the forty accessions belonged to three species in the following manner.

a) *A. tricolor* 21 accessions

b) *A. dubius* 4 accessions

c) *A. hypochondriacus* 15 accessions

The important morphological features of these 40 accessions are represented in a modified form of metroglyph in the Fig. 14.

Table 16. Source and morphological characters of Amaranth accessions in the germ plasm

Sl No	Accession No.	Source of Collection	Name of the species	Plant height (cm)	Stem colour	Branching habit	Petiole colour	Petiole length (cm)	Leaf			
									Size (cm)	Shape	Colour	Apex
1	A 2	Pant Nagar	*A tricolor*	80	Red	High	Red	4 5	14 x 8	Broad ovate	Greenish red	Acute
2	A 3	Tamilnadu (CO 1)	*A dubius*	177	Green	Medium	Green	6 0	12 x 9	Rhombic ovate	Green	Obtuse
3	A 4	Tamilnadu (CO-2)	*A tricolor*	122	Green	Low	Green	5 0	13 x 8	Obovate	Green	Acute retuse
4	A-6	Kannara	*A tricolor*	87	Deep red	High	Deep red	6 5	13 x 9	Triangular ovate	Deep red	Acute
5	A-12	Vellanikkara	*A tricolor*	98	Red	High	Deep red	3 0	10 x 7	Ovate	Reddish green	Acute
6	A-13	Nilambur	*A tricolor*	74	Deep red	Medium	Deep red	4 0	16 5 x 10	Triangular ovate	Deep red	Acute
7	A 16	Chellanum	*A tricolor*	64	Light red	Medium	Light red	5 5	15 x 8 5	Broadly ovate	Pinkish green	Acute
8	A-27	Trichur	*A tricolor*	79	Pale green	High	Pale green	4 0	18 x 10	Ovate	Green	Acute
9	A 30	Tripura	*A. tricolor*	81	Purple	High	Purple	4 5	16 x 8 5	Ovate	Purplish green	Retuse
10	A-39	West Bengal	*A tricolor*	84	Pale green	High	Pale green	3 5	9 x 6	Rhombic ovate	Green	Acute
11	A 43	Tamilnadu (CO-3)	*A dubius*	126	Green	Low	Green	5 0	15 x 6 5	Rhombic ovate	Green	Obtuse
12	A-44	Madurai	*A dubius*	155	Green	Medium	Green	4 5	17 x 8	Rhombic ovate	Green	Obtuse
13	A 45	West Bengal	*A dubius*	127	Green	Low	Green	5 5	13 x 8	Rhombic ovate	Green	Obtuse
14	A-47	Kasaragod	*A tricolor*	90	Greenish red	Low	Greenish red	5 0	16 x 5 5	Ovate with wavy margin	Reddish green	Acute
15	A-48	Ambalavayal	*A tricolor*	114	Red	High	Red	6 0	12 x 10	Ovate	Red	Acute
16	A 50	Malaysia	*A hypochondriacus*	68	Pale green	Low	Pale green	7 5	12 x 5	Elliptic/ lanceolate	Light green	Acute
17	A 54	Malaysia	*A hypochondriacus*	87	Pale green	Low	Pale green	9 0	14 x 5	Elliptic/ lanceolate	Light green	Acute
18	A 55	Malaysia	*A hypochondriacus*	89	Pale green	Low	Pale green	8 5	14 x 5	Elliptic/ lanceolate	Light green	Acute
19	A 56	Malaysia	*A hypochondriacus*	79	Pale green	Low	Pale green	8 0	12 x 5	Elliptic/ lanceolate	Light green	Acute

(contd.)

Table 16 (contd.)

Sl No	Accession No	Source of collection	Name of the species	Plant height (cm)	Stem colour	Branching habit	Petiole colour	Petiole length (cm)	Leaf			
									Size (cm)	Shape	Colour	Apex
20	A 57	Malaysia	*A hypochondriacus*	99	Pale green	Low	Pale green	8 0 9 5	14 x 6 5	Elliptic/ lanceolate	Light green	Acute
21	A-60	Malaysia	*A hypochondriacus*	85	Pale green	Low	Pale green	9 5	16 x 8	Elliptic/ lanceolate	Light green	Acute
22	A-61	Malaysia	*A hypochondriacus*	72	Pale green	Low	Pale green	9 0	15 x 7	Elliptic/ lanceolate	Light green	Acute
23	A-62	Malaysia	*A. hypochondriacus*	96	Pale green	Low	Pale green	8 5	15 x 8	Elliptic/ lanceolate	Light green	Acute
24	A-64	Malaysia	*A hypochondriacus*	78	Pale green	Low	Pale green	8 0	14 x 6	Elliptic/ lanceolate	Light green	Acute
25	A-66	Malaysia	*A hypochondriacus*	99	Pale green	Low	Pale green	8 0	11 x 7 5	Elliptic/ lanceolate	Light green	Acute
26	A-67	Malaysia	*A. hypochondriacus*	45	Pale green	Low	Pale green	7 0	8 x 5	Elliptic/ lanceolate	Light green	Acute
27	A-69	Malaysia	*A. hypochondriacus*	74	Pale green	Low	Pale green	8 0	13 x 6	Elliptic/ lanceolate	Light green	Acute
28	A-70	Malaysia	*A hypochondriacus*	52	Pale green	Low	Pale green	8 0	8 x 6	Elliptic/ lanceolate	Light green	Acute
29	A-72	Malaysia	*A hypochondriacus*	64	Pale green	Low	Pale green	8 5	14 x 6	Elliptic/ lanceolate	Light green	Acute
30	A-75	Malaysia	*A hypochondriacus*	85	Pale green	Low	Pale green	9 0	16 x 9	Elliptic/ lanceolate	Light green	Acute
31	A-76	Edappally	*A. tricolor*	97	Deep red	High	Reddish	3 5	12 x 9	Ovate	Red	Acute
32	A-77	Edappally	*A tricolor*	75	Deep red	Medium	Red	5 0	19 x 10	Ovate	Red	Acute
33	A-78	Edappally	*A tricolor*	78	Pinkish red	High	Pinkish red	3 5	12 x 7	Ovate	Pinkish green	Retuse
34	A 79	Edappally	*A tricolor*	46	Red	High	Red	5 5	22 x 12	Ovate	Reddish green	Obtuse
35	A-81	Edappally	*A tricolor*	62	Pinkish red	High	Pinkish red	5 0	14 x 8	Triangular ovate	Red	Obtuse
36	A-82	Edappally	*A tricolor*	86	Deed red	High	Red	5 0	18 x 11	Ovate	Purplish red	Acute
37	A-85	Edappally	*A tricolor*	61	Purplish red	High	Purplish red	4 0	9 x 5 5	Ovate	Purplish red	Acute

(contd.)

Table 16 (contd.)

Sl NO	Accession No.	Source of collection	Name of the species	Plant height (cm)	Stem colour	Branching habit	Petiole colour	Petiole length (cm)	Leaf			
									Size (cm) (L X B)	Shape	Colour	Apex
38	A 86	Edappally	*A. tricolor*	65	Purplish red	High	Purplish red	3 0	10 x 6	Rhombic ovate	Purplish red	Acute
39	A 91	Calicut	*A tricolor*	54	Purplish red	High	Purplish red	4 5	13 x 8	Ovate	Purplish red	Retuse
40	A 92	Mannuthy	*A tricolor*	89	Purplish red	Medium	Purplish red	4 5	12 x 6	Ovate	Purplish red	Acute

Table 16 (contd)

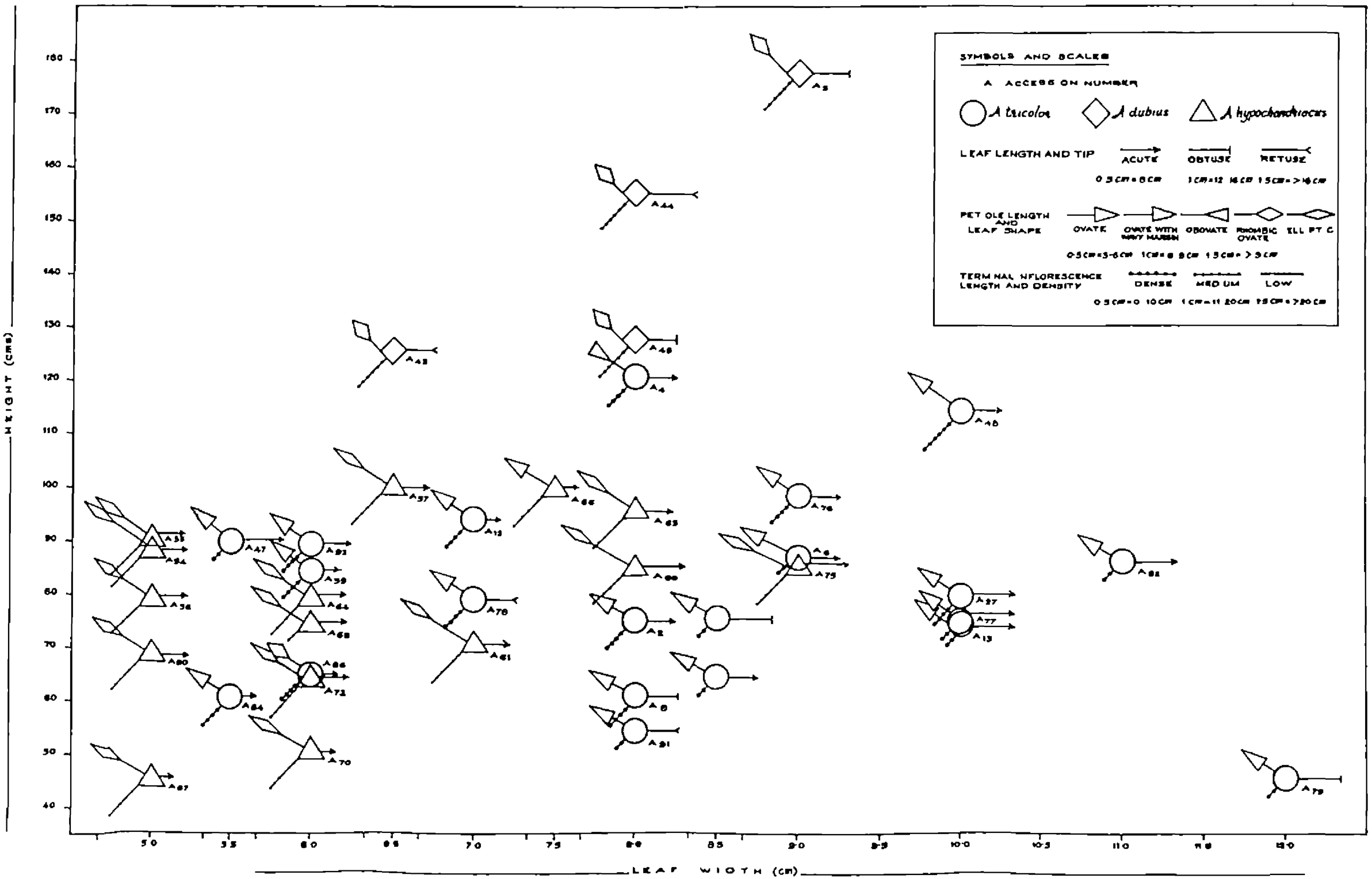
Sl No	Accession No.	Inflorescence		Inflorescence colour	Number and length of lateral branches of the panicle		Number of stamens & tepals	Bract length compared to style length	Colour of seed	Response to day length
		Density in axils	Length of terminal panicle (cm)		Number	Length (cm)				
1	A 2	Dense	16	Red	Nil		3, 3	Longer	Black	DN
2	A-3	Negligible	30	Green	-		5 5	Much shorter	Black	DN
3	A 4	Dense	12	Green	Nil		3, 3	Longer	Black	DN
4	A 6	Dense	8	Deep red	Nil		3 3	Longer	Black	SD
5	A 12	Dense	14	Red	Nil		3 3	Longer	Black	DN
6	A-13	Dense	7	Deep red	Nil	-	3 3	Longer	Black	DN
7	A 16	Dense	7	Greenish	Nil		3 3	Longer	Black	DN
8	A 27	Dense	21	Green	Nil		3, 3	Longer	Black	DN
9	A-30	Dense	10	Purplish green	Nil		3 3	Longer	Black	DN
10	A 39	Dense	16	Green	Nil		3 3	Longer	Black	DN
11	A 43	Negligible	29	Green	12	9	5 5	Much shorter	Black	DN
12	A-44	Negligible	22	Green	15	8	5 5	Much shorter	Black	DN
13	A 45	Negligible	30	Green	30	10	5, 5	Much shorter	Black	DN
14	A 47	Dense	2	Reddish	Nil		3, 3	Longer	Black	DN
15	A 48	Dense	29	Red	Nil	-	3 3	Longer	Black	DN
16	A-50	Nil	20	Pale green	20	6	5, 5	Equal	Cream	DN
17	A-54	Nil	29	Pale green	10	11	5, 5	Equal	Cream	DN
18	A 55	Nil	27	Pale green	13	24	5 5	Equal	Cream	DN
19	A 56	Nil	21	Pale green	13	20	5, 5	Equal	Cream	DN
20	A-57	Nil	30	Pale green	18	15	5 5	Equal	Cream	DN

(contd.)

Table 16 (contd.)

Sl No.	Accession No.	Inflorescence		Inflorescence colour	Number and length of lateral branches of the panicle		No. of stamens & tepals	Bract length compared to style length	Colour of seed	Response to day length
		Density in axils	Length of terminal panicle (cm)		Number	Length (cm)				
21	A 60	Nil	16	Pale green	19	7	5 5	Equal	Cream	DN
22	A 61	Nil	25	Pale green	22	12	5, 5	Fqual	Cream	DN
23	A 63	Nil	34	Pale green	20	23	5, 5	Equal	Cream	DN
24	A 64	Nil	36	Pale green	16	20	5, 5	Equal	Cream	DN
25	A-66	Nil	16	Pale green	25	14	5, 5	Equal	Cream	DN
26	A-67	Nil	31	Pale green	38	11	5, 5	Equal	Cream	DN
27	A 69	Nil	20	Pale green	15	19	5, 5	Equal	Cream	DN
28	A 70	Nil	29	Pale green	20	7	5, 5	Equal	Cream	DN
29	A-72	Nil	19	Pale green	22	15	5, 5	Equal	Cream	DN
30	A 75	Nil	20	Pale green	28	11	5, 5	Equal	Cream	DN
31	A-76	Dense	17	Purplish	Nil	-	3, 3	Longer	Black	DN
32	A-77	Dense	18	Reddish	Nil	-	3, 3	Longer	Black	DN
33	A 78	Dense	17	Reddish	Nil		3, 3	Longer	Black	DN
34	A 79	Dense	8	Red	Nil	-	3, 3	Longer	Black	DN
35	A-81	Dense	13	Red	Nil	-	3, 3	Longer	Black	DN
36	A 82	Dense	10	Red	Nil		3, 3	Longer	Black	DN
37	A-84	Dense	11	Red	Nil	-	3, 3	Longer	Black	DN
38	A 86	Dense	12	Red	Nil		3, 3	Longer	Black	DN
39	A 91	Dense	5	Purplish Red	Nil	-	3, 3	Longer	Black	DN
40	A 92	Dense	17	Reddish	Nil		3 3	Longer	Black	DN

FIG 14 A MODIFIED METROGLYPH APPROACH FOR THE DESCRIPTION OF AMARANTH ACCESSIONS IN THE GERMPLASM



All the 21 accessions of *A. tricolor* had monoecious flowers with trimerous symmetry and had circumscissile utricle / enclosed by long tepals and bracts in conformity with the key characters of Feine (1980). Many of these accessions exhibited variation for morphological features. The plants were erect with a height variation of 46-114 cm. Nature of branching also varied much and there were low, medium and highly branched types. Stem and foliage pigmentation varied from green, purple or with characteristic variegation. Majority of the accessions were purple while a few of them had green foliage and two had intermixed shades of both purple and green. A_{47} had wavy leaf margin while all others had entire margins. Leaves in general were ovate with acute tip, varying in size from 9 x6cms to 18 x 11 cms. In all accessions of *A. tricolor*, the monoecious flowers were borne on dense axillary clusters, and most of them had reduced terminal panicle of less than 10 cm length. Bracts as well as tepals were much longer than the circumscissile utricle.

Cytological studies revealed that all these 21 accessions had 2n = 34. The number of ring bivalents as well as chiasma frequency were reasonably high. All the accessions exhibited a reasonably high pollen fertility being more than 80%. Pollen diameter was not uniform and every accession had both macro and medium sized pollen. Occurrence of pollen grains of varying diameter within the same plant was a common feature. Most of the *A. tricolor* plants had a reasonably higher percentage of macropollen grains. The diameter of the macropollen ranged from 27-32 µm while that of the medium pollen ranged from 20-24 µm among the different *A. tricolor* accessions.

Table 17. Summary of meiosis and pollen fertility of Amaranth accessions in the germplasm

Sl No	Accession Number	Name of the species	Number of cells analysed	Chromosome number	Mean number of bivalents/Cell		Mean number of chiasmata per cell	Pollen fertility	Pollen types (%)		Mean pollen (µm) Diameter	
					Rings	Rods			Macro >24 µm	Medium 12-24 µm	Macro	Medium
1	A 2	*A tricolor*	25	2n = 34	11 92	5 08	28 92	90 56	57 95	42 05	28 5	22,6
2	A-3	*A dubius*	25	2n = 64	23 28	8 72	55 22	88 35	75 75	24 25	26 6	22 0
3	A-4	*A tricolor*	25	2n = 34	11 76	5 24	27 68	89 09	54 75	45 25	29 8	20 7
4	A-6	*A tricolor*	25	2n = 34	11 84	5 16	28 80	89 27	62 35	37 65	28 9	22 6
5	A 12	*A tricolor*	25	2n = 34	11 28	5 72	27 96	91 54	84 56	15 44	30 2	23 5
6	A-13	*A tricolor*	25	2nm = 34	10 20	6 80	26 16	89 00	67 67	32 33	27 3	23 8
7	A 16	*A tricolor*	25	2n = 34	9 64	7 36	26 64	86 77	17 92	82 08	27 6	23 5
8	A 27	*A tricolor*	25	2n = 34	11 80	5 20	28 80	70 23	77 67	22 33	26 3	23 8
9	A-30	*A tricolor*	25	2n = 34	9 16	7 84	26 16	91 11	68 26	31 74	25 8	20 6
10	A 39	*A tricolor*	25	2n = 34	12 00	5 00	26 68	88 28	64 44	35 56	26 3	23 4
11	A-43	*A dubius*	25	2n = 64	20 88	11 12	52 96	85 71	87 12	22.88	26 7	19 5
12	A-44	*A dubius*	25	2n = 64	23 48	8.52	53 48	86 86	90 15	9 85	26 2	20 4
13	A-45	*A dubius*	25	2n = 64	23 00	9 00	55 00	90 95	63.74	36 26	25 4	22.2
14	A-47	*A tricolor*	25	2n = 34	11 40	5 60	27 32	91 60	67 25	32.25	28 9	23 2
15	A-48	*A tricolor*	25	2n = 34	10 00	7 00	27 00	91.74	53.27	46 73	30 4	22 3
16	A 50	*A hypochondriacus*	25	2n = 32	10 64	5 36	26 64	88 02	47 25	52.75	28 5	21 1
17	A-54	*A hypochondriacus*	25	2n = 32	9 44	8 36	25 44	82 31	64 58	35 42	29 2	23 2
18	A-55	*A hypochondriacus*	25	2n = 32	11 44	4 56	27 48	85 10	77 16	22.84	28.0	22.7
19	A 56	*A. hypochondriacus*	25	2n = 32	9 56	6 44	25 56	77 64	64 28	35 72	27.4	21 6
20	A 57	*A hypochondriacus*	25	2n = 32	11 08	4 92	27 08	85 65	27 26	72 74	25 6	20 4

(contd.)

Table 17 (Contd.)

Sl No.	Accession Number	Name of the species	Number of cells analysed	Chromosome number	Mean number of bivalents/Cell		Mean number of chiasmata per cell	Pollen fertility	Pollen types (%)		Mean pollen (µm) Diameter	
					Rings	Rods			Macro >24 µm	Medium 12-24 µm	Macro	Medium
21	A 60	*A hypochondriacus*	25	2n = 32	10 88	5 12	26 88	82 81	53 27	46 73	28 4	22 3
22	A-61	*A. hypochondriacus*	25	2n = 32	9 52	6 48	25 52	79.11	30 00	70 00	27 6	21 5
23	A 63	*A hypochondriacus*	25	2n = 32	10 84	5 16	26 54	83 69	71.73	28 27	26 8	22 0
24	A-64	*A hypochondriacus*	25	2n = 32	10 44	5 56	26.44	78 43	51 70	48 30	27 7	21 5
25	A-66	*A. hypochondriacus*	25	2n = 32	11 44	4 16	27 44	89 48	76 38	23 62	28 4	22 8
26	A 67	*A hypochondriacus*	25	2n = 32	10 80	5 20	26 80	87 14	82 45	17 55	26 4	23 2
27	A-69	*A. hypochondriacus*	25	2n = 32	9 40	6 60	25 40	88.29	69 44	30 56	29 1	21 4
28	A-70	*A. hypochondriacus*	25	2n = 32	10 72	5 28	26 72	78 58	28 54	71 16	28 4	20 8
29	A 72	*A hypochondriacus*	25	2n = 32	10 76	5 24	26 76	82 63	63 36	36 64	24 5	20 6
30	a 75	*A hypochondriacus*	25	2n = 32	9 44	6 56	25 44	81 78	62.52	37 48	27.3	21 3
31	A-76	*A tricolor*	25	2n = 34	11 04	5 96	28 04	92.20	18.26	81 74	31 5	22 7
32	A 77	*A tricolor*	25	2n = 34	11 08	5 92	28 08	86 90	52 30	47 70	30 6	22 8
33	A-78	*A tricolor*	25	2n = 34	9 28	7.72	26.28	94 60	75 00	25 00	30 4	23 1
34	A-79	*A tricolor*	25	2n = 34	11 64	5 36	28 64	93 10	77 42	22.58	29 5	20 5
35	A-81	*A tricolor*	25	2n = 34	11 40	5 60	28.40	88 82	54 27	45 73	28 9	22.2
36	A-82	*A. tricolor*	25	2n = 34	9 84	7.16	26 84	90 69	62.16	37.84	30 2	22.8
37	A 84	*A. tricolor*	25	2n = 34	10 80	6 20	27.80	92.47	90 65	9 35	32 2	23 4
38	A-86	*A tricolor*	25	2n = 34	11.12	5 88	28 52	91.30	82.17	17.83	29 5	22.4
39	A 91	*A tricolor*	25	2n = 34	9 40	7 60	26 40	94.14	58 13	41 87	28 8	21 6
40	A-92	*A tricolor*	25	2n = 34	11 04	5 96	28.04	80 26	59 15	40 85	27 6	21 2

Four accessions belonged to *A. dubius*. These were typical gigantic plants with maximum height ranging from 126-177 cm. The stem and foliage were pigmented green. Their leaves were typically rhombic ovate with obtuse apex. They produced profusely branched terminal inflorescences 25-30 cm with spreading lateral branches. A few axillary pistillate flowers were also produced. The flowers had pentamerous symmetry and in every cyme, the initial flower was staminate and the remainder, pistillate. The interplant variation was comparatively low in this species.

Cytological studies revealed that all the four accessions were typical *A. dubius* plants with chromosome number 2n = 64, 32 bivalents could be regularly counted at meiotic metaphase I. Pollen grains of varying dimensions were observed in this species also, the macropollen being on an average about 26 µm and the medium pollen of about 19-22 µm diameter.

The remaining 15 accessions available in the germplasm and collected from Malayasia were grain types *A. hypochondriacus*. These plants did not show much variation in their morphological traits. Their height varied from 45 to 99 cms. All of them had stem and foliage pigmented light green. The branching was poor and the leaves were elliptic/lanceolate with length varying from 8-16 cm and width from 5-9 cm. All of them had profusely branched terminal panicles of 16-36 cm long with upright branches. The circumscisslle utriculi had cream coloured seeds in all the 15 types. The melotic metaphase showed 16 regular bivalents, predominantly ring bivalents as in *A. tricolor*. Pollen fertility was comparatively lower ranging from 77-89% and there were both types of pollen, macro and medium in every accession.

H. Investigations on seed yield in *A. tricolor* accessions

Both A_6 and A_{13} belonged to *A. tricolor* under section Blitopsis (Aellen, 1961), characterised by trimerous flowers and prominent axillary cymose inflorescences. The morphological observations of these accessions are given in the Table 18.

A_6 and A_{13} were erect annual herbaceous plants reaching a height of 75 cm (A_{13}) and 92 cm (A_6) respectively and maturing in 4 to 5 months (152 days in A_6 and 134 days in A_{13}). The stems, leaves and inflorescences of both the lines were pigmented deep red. The leaf size was higher in A_{13} (10.25 cm x 8.11 cm) while the total number of leaves was higher in A_6 (63). Flowering was early in A_{13} (65 days), when the mean height was 60 cm. A_6 flowered later (73 days) when it reached a height of 81 cm. The branching was more profuse in A_6 (3.1) than in A_{13} (2.6). The flowers were mainly borne in dense globose axillary cymes (Plate 24a).

Observations on the axillary cymes one week before harvest revealed that the number of glomerules (clusters of flowers)/leaf axil was more in A_{13} (36 Vs 27) and hence the cymes appeared more denser than A_6. The percentage of male flowers was 27% in A_{13} and 25% in A_6 within each glomerule. Female flowers dominated in the axillary cymes and they were at different stages of development. The utriculi of both the lines were dehiscent and circumscissile and hence some of the seeds were already shattered at the time of observation. Percentage of shattered seeds were more in A_6 (11.4%) than in A_{13} (7.37%). While 68% of the female flowers had fully on almost matured utriculi in A_{13}, only 53% had them in A_6. The rest of the female flowers were immature and underdeveloped (Table 19).

Table 18. Morphological characters of *Amaranthus tricolor* lines A_6 and A_{13}

Plant characters	A_6	A_{13}
Days to seed germination	5.1 ± 0.18	7.2 ± 0.25
Seedling height at transplanting (cm)	7.25 ± 0.19	7.18 ± 0.24
Height, one month after transplanting (cm)	18.30 ± 0.48	21.60 ± 0.48
Stem colour	Deep Red	Deep red
Leaf colour	Deep red	Deep red
Petiole colour	Deep red	Deep red
Leaf shape	Triangular ovate	Triangular ovate
Leaf apex	Acute	Obtuse
5th leaf length (cm)	9.60 ± 0.29	10.25 ± 0.30
5th leaf width (cm)	7.45 ± 0.26	8.11 ± 0.23
Petiole length (cm)	4.60 ±0.25	4.05 ± 0.13
Days to flower from transplanting	73.00 ± 0.73	65.70 ± 0.74
Height at flowering (cm)	81.40 ± 1.95	60.90 ± 1.56
Number of leaves at flowering	26.50 ± 0.93	19.10 ±0.45
Total leaf number	63.7 ± 2.17	44.6 ± 1.29
Stem girth (cm)	3.50 ± 0.11	2.88 ± 0.10
Number of fruiting nodes	53.5 ± 1.90	44.6 ± 1.29
Response to day length	Short day	Day neutral
Nature of the inflorescence	Dense axillary cymes and a reduced terminal panicle	
Density of flowers	Dense	Denser
Terminal inflorescence length (cm)	8.10 ± 0.25	7.35 ± 0.14

(contd.)

Table 18 (Contd.)

Plant Characters	A_6	A_{13}
Inflorescence colour	Deep red	Deep red
Bract colour	Straw coloured with pink ridge	Light purple with deep purple ridge
Perianth length (mm)	5 (3+2 mm tip)	5 (3+2 mm tip)
Perianth breadth (mm)	1.8	1.8
Tepal apex	Acuminate	Acuminate
Number of stamens and tepals	3, 3	3, 3
Bract length compared to style length	Longer than style	Longer than style
Number of styles/stigmas	3	3
Nature of the stigma	Slightly pubscent	Slightly pubscent
Height at harvest	92.10 $\pm$ 2.29	75.00 $\pm$ 2.12
Nature of utricle	Circumscissile	Circumscissile
Seed colour	Black	Black
Seed (length x breadth (mm))	1.65 x 1.53	1.71 x 1.62
Duration from seed to seed	152	134
Seed yield/plant (g)	7.36 $\pm$ 0.81	11.74 $\pm$ 0.75
1000 seed weight (g)	1.08 $\pm$ 0.05	1.15 $\pm$ 0.12
Chromosome number	2n = 34	2n = 34
Name of the species	*A. tricolor*	*A. tricolor*

Table 19 Floral characters of *A. tricolor* accessions A_6 and A_{13}

Accession No.	Mean number of glomerules/ leaf axil	Mean percentage of male flowers/ glomerule	Mean percentage of female flowers/ glomerule	Mean percentage of female flowers developing into utricle	Mean percentage of seeds shattered before harvest	Mean percentage of under developed and immature utriculi	Mean percentage of male flowers not effective in seed set
A_6	27.61 ± 0.92	24.27	75.72	53.11	11.44	35.40	38.80
A_{13}	36.65 ± 1.19	27.90	72.09	68.20	7.37	24.42	30.95

Table 20 Chromosome association during meiosis and pollen fertility in *Amaranthus tricolor* accessions A_6 and A_{13}

Amaranth Accessions	No. of cells analysed	Mean number of bivalents/cell		Mean number of chiasmata/cell	Pollen				
					Fertility (%)	Mean diameter (µm)		Pollen types (%)	
		Rods	Rings			Macro pollen (>24 µm)	Medium pollen (12-24 µm)	Macro	Medium
A_6	25	5.20	11.80	28.80 ± 0.21	86.27	28.91	22.60	62.35	37.65
A_{13}	25	6.80	10.20	26.16 ± 0.17	89.00	27.33	23.86	67.67	32.82

PLATE 24

a. *A. tricolor* accessions A_6 and A_{13}

b. Plants of *A. hypochondriacus* after exposure to photoperiods of 12h and 17h

PLATE 24

a

b

A major proportion of the total male flowers was not effective in causing seed set since these were borne towards the end of the season (30% in A_{13} and 38% in A_6). The pistillate flowers which received pollen from these flowers did not get enough time to develop into mature utricull. Both the lines had a reduced terminal panicle with contained predominantly male flowers.

Cytological studies

Meiosis was critically observed in both the lines. At diakinesis, the bivalents could be clearly observed and 17 bivalents were distinctly counted in both lines. One bivalent was always associated with the nucleolus. Metaphase I was regular with prominent ring bivalents. The mean number of ring bivalents was 11.8 and 10.2 in A_6 and A_{13} respectively. Usually one or two chiasmata were observed in each bivalent and the mean chiasma frequency/PMC is given in Table 20. Anaphase I was normal in all cases with equal distribution of chromosomes towards poles. Pollen fertility was slightly higher in A_{13} (89%) than in A_6 (86%) and the percentage of macropollen was also more in A_{13} (67.7) than in A_6 (62.3).

I Photoperiodic requirement of different *Amaranthus* species

Days to flower, plant height and number of leaves at flowering in eight species of *Amaranthus* exposed to varying length of photoperiod are presented in Tables 21, 22 and 23 and in Figs. 15, 16 and 17.

When the overall mean values were considered the different species except *A. tricolor* and *A. hypochondriacus* differed significantly for days to flower. Among different species *A. lividus* was the earliest (43 days) and *A. cruentus* (108 days) was the most late for flowering. The mean days to flower in other species were 57 days in *A. viridis*, 63 in *A. spinosus*, 63 in *A. hypochondriacus*, 66 in *A. tricolor*, 67 in *A. dubius* and 78 in *A. caudatus*.

Table 21 Days to flower in the eight species of *Amaranthus* exposed to varying length of photoperiod

Species	Treatment : Photoperiod (h)						
	12 h (control)	14 h	15 h	16 h	17 h	Grand mean	CD (P = 0.05)
A. tricolor	64.59 ± 0.40	65.00 ± 0.63	67.40 ± 0.80	68.19 ± 0.80	69.00 ± 0.89	66.84	
A. lividus	46.00 ± 1.00	42.00 ± 0.63	43.00 ± 0.45	43.40 ± 0.60	40.40 ± 0.40	42.96	1.09
A. viridis	57.00 ±0.95	57.20 ± 0.97	56.79 ± 0.97	57.20 ± 0.97	56.79 ± 0.92	57.00	
A. spinosus	65.40 ± 1.06	62.20 ± 0.80	63.59 ± 1.33	61.40 ± 0.40	60.20 ± 0.97	62.56	
A. dubius	74.19 ± 0.49	74.19 ± 1.02	72.19 ± 0.49	73.40 ± 0.75	66.59 ± 0.75	72.12	
A. hypochondriacus	71.80 ± 0.58	67.80 ± 0.48	65.80 ± 1.02	64.59 ± 0.75	62.20 ± 0.49	66.44	
A. cruentus	109.20 ± 0.80	107.80 ± 1.53	108.80 ± 1.34	108.00 ± 1.34	107.40 ± 1.75	108.24	
A. caudatus	75.00 ± 0.89	76.59 ± 0.98	78.19 ± 0.45	77.80 ± 0.80	82.19 ± 0.80	77.96	
Grand Mean	70.40	69.09	69.47	69.25	68.09		

CD (P = 0.05) 0.862

CD (P = 0.05) to compare any observation = 2.437

FIG 15 DAYS TO FLOWER IN EIGHT SPECIES OF *Amaranthus* EXPOSED TO VARYING PHOTOPERIODS

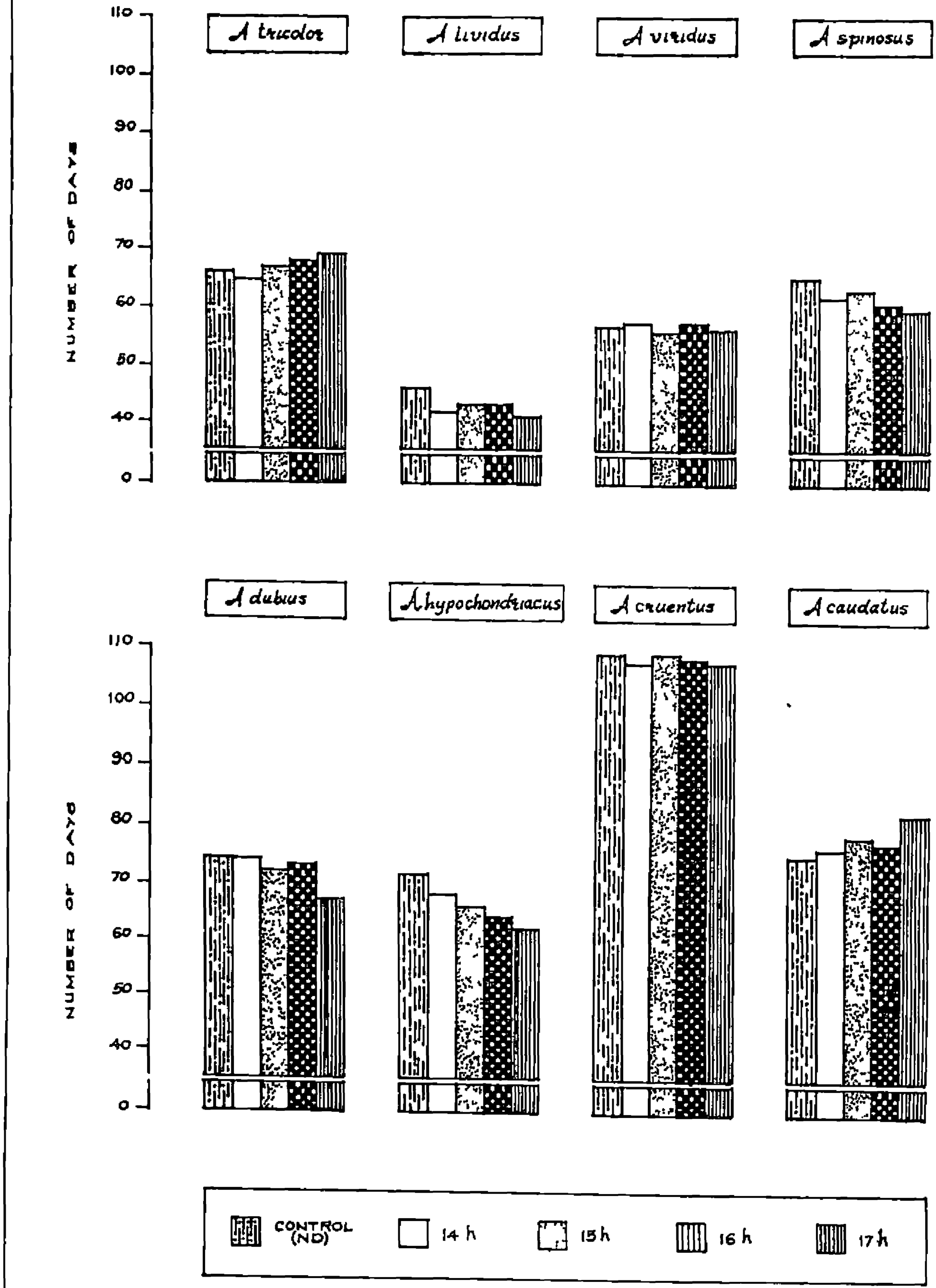


Table 22. Height at flowering in the eight species of *Amaranthus* exposed to varying length of photoperiod

Species	Treatment photoperiod (h)						
	12 h (control)	14 h	15 h	16 h	17 h	Grand mean	CD (P = 0.05)
A. tricolor	19.60 ± 0.75	22.40 ± 1.42	26.00 ± 1.42	28.20 ± 2.21	30.20 ± 3.73	25.28	
A. lividus	11.20 ± 0.85	8.80 ± 0.58	10.20 ± 0.37	11.00 ± 0.63	12.20 ± 1.01	10.68	
A. viridis	12.00 ± 1.91	9.19 ± 1.71	11.0 ±0.54	10.00 ± 2.01	11.00 ±0.70	10.64	
A. spinosus	46.20 ±1.56	40.00 ± 0.70	40.20 ±0.66	39.20 ± 2.17	37.40 ± 1.85	40.59	2.10
A. dubius	55.20 ± 1.01	53.20 ± 1.49	54.00 ± 1.41	54.40 ± 1.90	53.20 ± 1.62	54.00	
A. hypochondriacus	22.40 ± 1.46	22.20 ± 2.19	22.79 ± 1.58	23.20 ± 1.52	22.79 ± 2.47	23.28	
A. cruentus	68.59 ± 1.39	68.40 ± 0.67	66.80 ± 0.59	66.40 ± 2.49	67.80 ± 2.47	67.59	
A. caudatus	39.40 ± 1.16	43.59 ± 2.08	45.59 ± 0.50	45.20 ± 1.23	50.59 ± 2.08	44.88	
Grand Mean	34.32	33.47	34.57	34.70	36.02		

CD () = 0.05) 01.66

CD (P = 0.05) to compare any observation = 4.71

FIG 16 MEAN HEIGHT AT FLOWERING IN EIGHT SPECIES OF *Amaranthus* EXPOSED TO VARYING PHOTO PERIODS

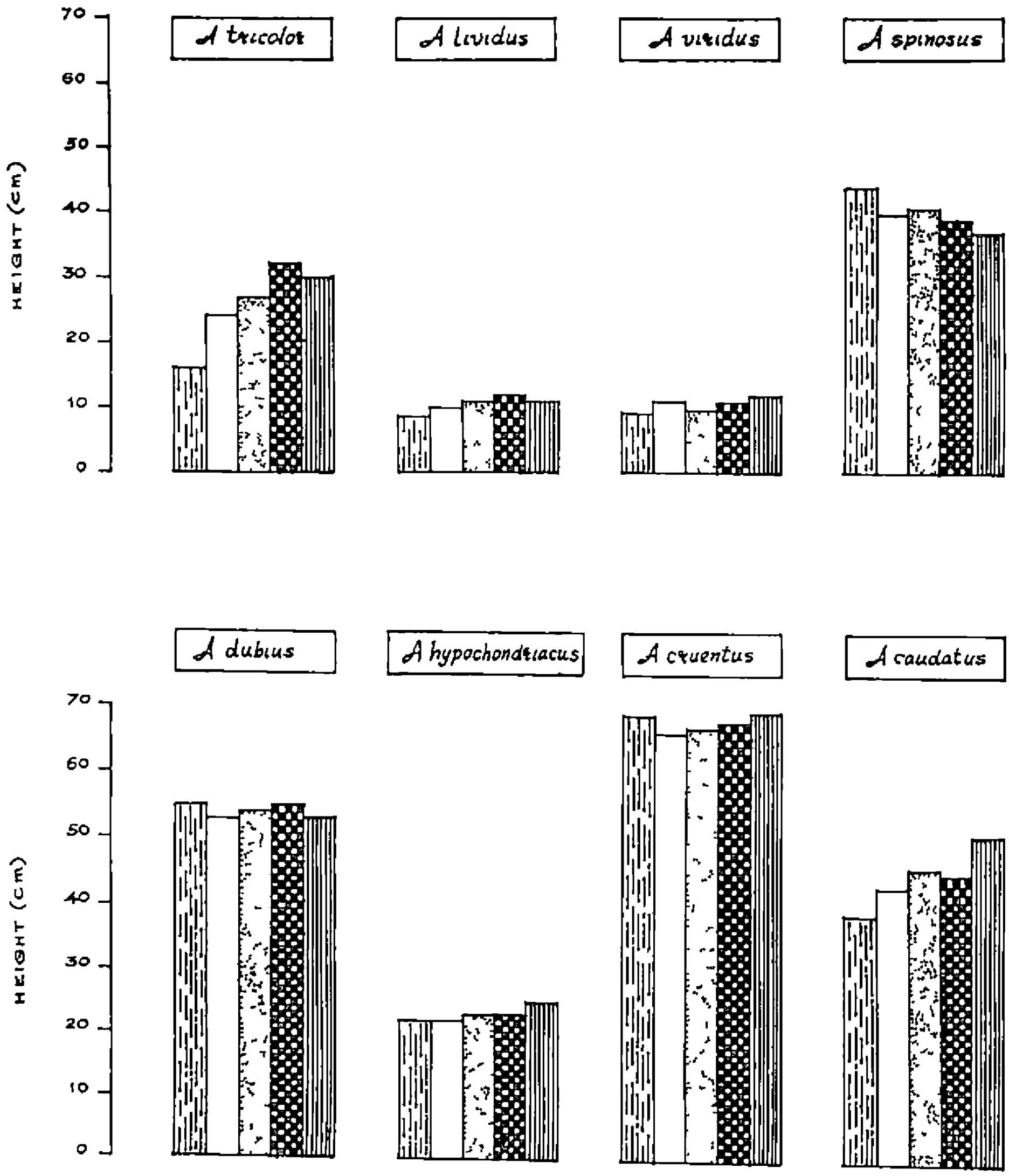


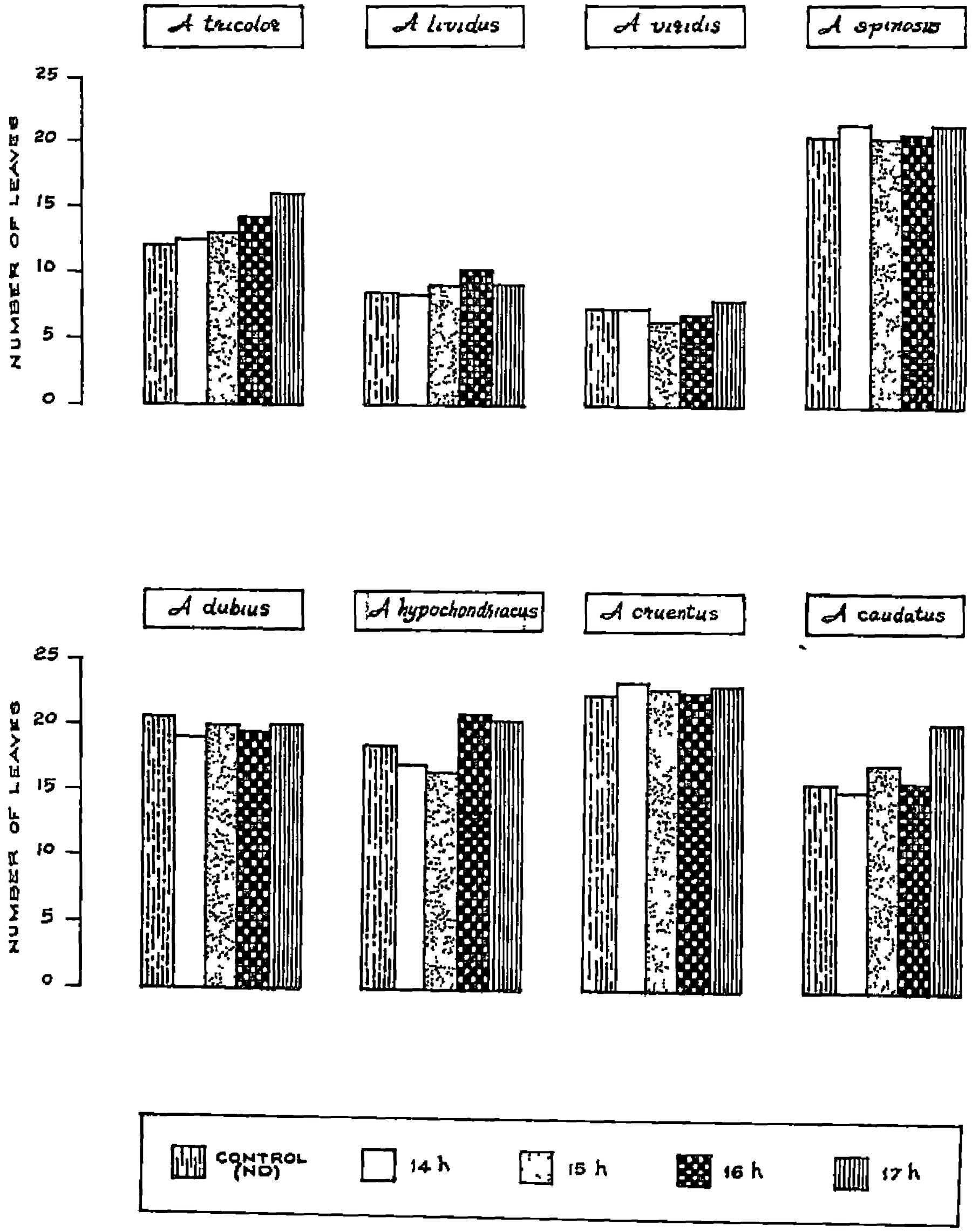
Table 23. Number of leaves at flowering in the eight species of *Amaranthus* exposed to varying length of photoperiod

Species	Treatment photoperiod (h)					Grand mean	CD (P = 0.05)
	12h	14 h	15 h	16 h	17 h		
A. tricolor	12.00 ± 0.63	12.40 ± 0.92	13.00 ± 0.89	14.40 ± 5.81	15.80 ± 0.70	13.52	
A. lividus	9.19 ± 0.37	8.39 ± 0.50	8.39 ± 0.39	9.12 ± 0.37	10.20 ± 0.73	9.07	
A. viridis	6.80 ± 0.37	7.40 ± 0.37	7.40 ± 0.24	6.40 ± 0.50	6.59 ± 0.50	6.92	
A. spinosus	20.60 ± 0.74	21.60 ± 0.67	20.79 ± 0.73	20.60 ± 0.59	22.79 ± 0.85	21.28	0.997
A. dubius	20.79 ± 0.48	19.20 ± 0.73	19.79 ± 1.15	19.60 ± 0.50	20.00 ±0.63	19.87	
A. hypochondriacus	18.60 ± 1.43	17.00 ± 0.63	16.79 ± 1.58	20.79 ± 1.87	20.60 ± 1.16	18.74	
A. cruentus	22.79 ± 0.85	22.20 ± 0.91	23.60 ± 0.59	23.00 ± 0.83	22.79 ± 0.79	22.87	
A. caudatus	15.82 ± 0.58	15.40 ± 0.92	17.40 ± 0.50	16.20 ± 0.48	20.40 ± 0.24	17.04	
Grand Mean	15.82	15.45	15.90	16.27	17.40		

CD (P = 0.05)

CD (P = 0.05) to compare any observation 2.229

FIG 17 MEAN NUMBER OF LEAVES AT FLORAL INITIATION IN EIGHT SPECIES OF *Amaranthus* EXPOSED TO VARYING PHOTO PERIODS



A definite effect of photoperiods on flowering was observed in the investigation. If all the species were considered cumulatively, the plants under natural day conditions flowered in 70 days and plants receiving illumination flowered significantly earlier. No significant difference was observed between 14, 15, and 16hphotoperiods but the 17h photoperiod was significantly effective to induce early flowering.

The general mean values for height at flowering and the leaves/nodes to flower also differed in the eight species, irrespective of photoperiods. *A. lividus* and *A. viridis* did not differ significantly and they flowered at a mean height of 10.68 cm and 10.64 cm respectively. *A. lividis* attained this height in 43 days from sowing while *A. viridis* took 57 days. Similarly *A. tricolor* and *A. hypochondriacus* flowered at apparently same heights. The height as flowering in other species were 40.59 cm in *A. spinosus* 44.88 cm In *A. caudatus*, 54 cm in *A. dubius* and 67.59cm in *A. cruentus*. The effect of photoperiod on height at flowering was not much pronounced except a significant effect between 14 h and 17 h photoperiods. The eight species showed significant differences for number of leaves at the time of flowering. *A. viridis* had 6.9 leaves, *A. lividus* 9.1, *A. tricolor* 13.5, *A. caudatus* 17.0, *A. hypochondriacus* 18.7 *A. dubius* 19.8 , *A. spinosus* 21.3 and *A. cruentus* 22.8.

With regard to flowering in individual species, *A. lividus*, *A. hypochondriacus*, *A. dubius* and *A. spinosus* preferred a 17h photoperiod to other photoperiods. In *A. caudatus* and *A. tricolor* more lengthy photoperiods delayed flowering. Earliness was observed under natural day length in the above two species. *A. viridis* and *A. cruentus* did not exhibit any significant differences between the treatments.

In *A. tricolor*, the most desirable photoperiod for early flowering was 12-14 h. There was delay in flowering with increase in photoperiod. A proportionate increase in plant height and number of leaves were observed with increase in day length simultaneous to delayed flowering. On the other hand *A. lividus* exhibited significantly early flowering when exposed to longer photoperiods plants under natural day length were late to flower. For number of leaves longer photoperiods were favourable but photoperiods did not influence the height at flowering. *A. viridis* was not sensitive to photoperiod treatments as all the day lengths produced similar effects. Vegetative growth also did not change with increase in photoperiod.

In *A. dubius* also, earliness was observed with increase in photoperiods. Vegetative growth was not affected by changes in day length. *A. spinosus* showed a proportionate earliness in flowering with increase in day length. Number of leaves produced were more under 17h day length but plant height showed a dwarfing trend. Plants under natural day length were the tallest, but flowered very late and had the maximum number of leaves. In *A. cruentus*, the different treatments did not show any marked difference for days to flower and vegetative growth. This species started flowering only after completion of flowering in all other species. *A. hypochondriacus* had a proportionate earliness in flowering with increase in the photoperiod (Plate 24b). *A. caudatus* showed increase in vegetative growth with increasing photoperiods. Precocious flowering was observed when plants were exposed to natural day length as compared to extended photoperiods.

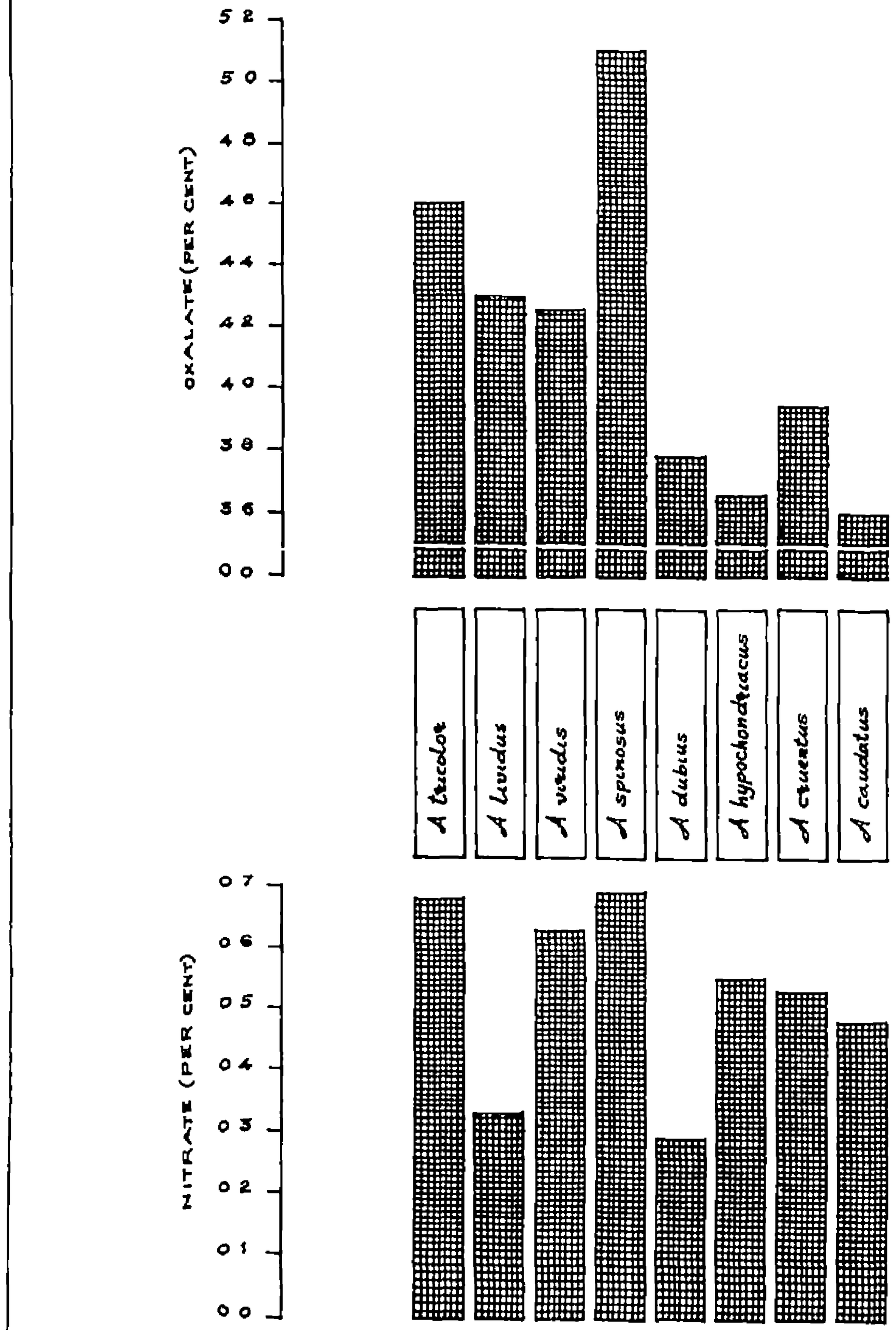
J. Oxalate and nitrate content in *Amaranthus* spp.

Mean oxalate and nitrate contents in the eight species are furnished in Table 24.

Table 24 Mean oxalate and nitrate contents in eight *Amaranthus* spp

Species	Oxalate (%)	Nitrate (%)
	Mean ± S.E.	Mean ± S.E.
A. tricolor	4.61 ± 0.09	0.68 ± 0.01
A. lividus	4.30 ± 0.04	0.33 ±0.01
A. viridis	4.27 ± 0.19	0.63 ± 0.02
A. spinosus	5.10 ± 0.10	0.69 ± 0.01
A. dubius	3.77 ± 0.13	0.20 ± 0.01
A. hypochondriacus	3.66 ± 0.08	0.55 ± 0.03
A. cruentus	3.94 ± 0.03	0.53 ± 0.01
A. caudatus	3.60 ± 0.15	0.48 ± 0.01
C.D. (P = 0.05)	0.350	0.057

FIG 18 OXALATE AND NITRATE CONTENT OF THE DIFFERENT SPECIES OF *Amaranthus*



Analysis of variance showed that the species were significantly different for nitrate and oxalate content (Table 25).

Table 25. General analysis of variance for oxalate and nitrate contents in the eight species of *Amaranthus*

Sources of variation	df	MS	
		Oxalates	Nitrates
Species	7	0.802**	0.067**
Error	23	0.041	0.001

** Significant at p = 0.05

The percentage of oxalates in the different species varied from 3.60 to 5.10% on dry weight basis. The highest content of oxalate was in the weedy species *A. spinosus* and it was not significantly different from *A. tricolor*. The grain species *A. hypochondriacus*, *A. caudatus* and *A. cruentus* had a lower content of oxalates and were not significantly different from one another.

The nitrate levels ranged from 0.295% to 0.659%. The widely accepted and high yielding vegetable type *A. dubius* was characterised by the lowest content of nitrates. (0.29%). The highest content of nitrate (0.69%) was in the weedy species *A. spinosus*. Among the grain types *A. caudatus* had the lowest content (0.48) and the other two grain species did not differ significantly.

The phenotypic coefficient of variation (pcv) and genotypic coefficient of variation (gcv) are also sizeable showing the variability in the expression of these characters. High heritability values indicate the dominant role of genetic effects in the expression of these characters (Table 26).

Table 26 Range, mean, gcv, pcv, h^2 and GA for oxalate and nitrate content

	Range (%)	Mean (%)	GCV	PCV	h^2	GA
Oxalates	3.60-5.10	4.16	0.25	0.29	0.86	0.52
Nitrates	0.29-0.69	0.52	0.02	0.23	0.95	0.04

Discussion

DISCUSSION

Amaranth is one of the ancient groups of crop plants, with tremendous potential for the present, because of its high content and quality of proteins, vitamins and minerals in leaves and grains. Cytogenetical investigations in many crop plants were successful to reveal the phylogeny, evolution and distribution of the species. The improvement of amaranths requires thorough elucidation of cytogenetical details and our present knowledge on this aspect is not complete. Interspecific relationship among *Amaranthus* species were worked out by many investigators (Sauer, 1967; Grant, 1959, Pal and Khoshoo, 1973 a & b). These studies were unsuccessful in establishing the relationship between the two sections of the genus, Blitopsis and Amaranthus. Previous workers also could not agree upon the centre of origin and progenitors of cultivated amaranths. Many of them proposed a Central or South American origin for grain amaranths. Regarding the origin and distribution of vegetable species, the information is practically very much scanty. Incidently amaranth is mainly grown for grain in the American continent, whereas in Asia especially in India, amaranth is predominantly cultivated for greens. The centre of origin, evolution and movement of vegetable species to Asia will be adding to our current knowledge on amaranths.

The existing key for the identification of *Amaranthus* species (Feine, 1980) is too complex to apply in the field. A simple provisional key for eight *Amaranthus* species is developed by using morphological features. The pattern and nature of development of flower cluster in amaranths were investigated thoroughly when a deviation was observed from the available reports.

Genome analysis in *Amaranthus* has to take into account, the role of weedy and wild species like *A. spinosus* towards the evolution of cultivated species. Eventhough there were conflicting surmises on the relationship between *A. spinosus* and *A. dubius* (Grant, 1959b; Pal and Khoshoo, 1965, Pal, 1972) such studies were not made between *A. spinosus* and other species excepting *A. dubius*. Since *A. spinosus* is a pantropical cosmopolitan weed in American and Asian continents, hybridization programmes involving *A. spinosus* will be very much rewarding in the elucidation of lineage. Therefore, the present investigations were undertaken to establish the interrelationship between sections, species and also between vegetable types and grain types. Attempts were also made to study the role of *A. spinosus* in the evolution of cultivated amaranths.

The existing germplasm in the College of Horticulture were classified into different species based on cytomorphological investigations in them. Finally the content of antimutritional factors such as oxalate and nitrate contents and photoperiodic requirement of different species are also discussed.

Development and arrangement of flower clusters

Murray (1940) described the pattern of arrangement and development of individual flowers within a flower cluster in a typical dichasial cymose pattern. On closer examination of glomerules it was observed that the initial development is as a typical dichasial cyme, but the branches in the cymose cluster developed in a scorpioid cymose pattern. Earlier workers did not report this type of development and arrangement of the flower cluster. The two types of arrangement of male and female flowers viz. the spinosus type and the "other type" (Murray, 1940) was found to be true in the present studies also.

Modification of the existing key

The identification of the different species was found rather difficult using the existing keys of Sauer (1967), or Feine (1980), because the primary key character was based on very minute floral details. Hence the existing key of Feine (1980) is modified in a simplified form using gross morphological features like nature of the inflorescence. In Feine's (1980) key, *A. viridis* was grouped along with *A. tricolor* and *A. lividus*, as all these species had trimerous flowers. In the modified key *A. viridis* is grouped along with *A. spinosus* just becuase of the similarity in the nature of their inflorescences. This grouping is later fully supported by cytological studies. The genomes of *A spinosus* and *A. viridis* were found very closely related since these two species differed only in 2-3 pairs of chromosomes as evidenced in the interspecific hybrid.

Genome analysis in Amaranth species

Meiotic studies in different species

Chromosome behaviour at meiosis determines the potentiality of recombination which in turn depends on number of chiasmata/bivalent. Rees (1961) pointed out that meiosis has played an indispensable role in the evolution of genetic systems and the chiasma frequency, which manifests the genetic recombination, is under genotypic control. Variation in chiasma frequency is also considered adaptive (Grant, 1958; Jones and Rees, 1966).

In the present study, all the species belonging to either Blitopsis or Amaranthus section showed regular meiosis. Regular meiosis indicates that the different species have acquired genomic stability without any relics of the process involved in speciation such as translocations, inversions etc. The number of ring bivalents was also high in almost all the

cultivated vegetable and grain types. Chiasma frequency/bivalent was the highest in *A. tricolor,* but not significantly different from *A. dubius* and *A. hypochondriacus*. *A. tricolor* and *A. dubius* are the widely types of vegetable amaranths in South India. Similarly *A. hypochondriacus* is the most widely cultivated and accepted grain species. High degree of domestication and selection pressure in these species are responsible for the increased recombination expressed in the form of high chiasmata/ bivalent (Stebbins, 1958; Das and Jain, 1972).

A. caudatus and *A. cruentus* did not differ significantly in number of chiasmata/bivalent and similar was the case with *A. viridis* and *A. lividus*. In the polyploid, *A. dubius* (2n = 64), 32 bivalents were regularly formed at metaphase I. Its chiasmata/bivalent was on par with *A. tricolor* (2n = 34). The formation of 32 regular bivalents in *A. dubius* is indicative of its allopolyploid origin (Grant, 1959b, Pal 1972). Hence polyploidization did not affect the chiasma formation in the chromosomes as it did not differ between *A. dubius* and *A. tricolor*.

During meiotic analysis, a preponderance of ring bivalents was observed in the PMCs of all the species except the semiwild type, *A. spinosus*. Also *A. spinosus* stood exceptionally separate with maximum number of rod bivalents and a very low chiasma frequency/bivalent or PMC. *A. spinosus* is a semiwild type with distinct morphological features like presence of a pair of sharp spines at node and distinct positional arrangement of male and female flowers. The primitive nature of this species is also indicated by its meiotic analysis expressed by the low chiasma frequency and more number of rod bivalents. According to Fayed *et al.* (1984) chiasma frequency is directly related to the degree of adaptibility of certain genotypes to a particular environment.

Madhusoodanan and Pal (1981) observed more gigantism in *A. tricolor* for all the phenotypic characters than the other four vegetable species studied. In the present investigation the bivalents in *A. tricolor* were comparatively bigger than in other species, and *A. spinosus* had the lowest size. The size of the bivalent and 2c DNA content in plant or animal species are positively correlated (Rees and Jones, 1972). The bigger size of bivalents in *A. tricolor* thus indicates the high DNA amount in its genome. Probably the evolution of *A. tricolor* and its domestication would have involved amplification or repetition of nuclear DNA thus resulting in the bigger size of complement (Rees and Jones, 1972; White and Rees, 1986).

The tetraploid plant of *A. lividus* investigated in the present study had desirable morphological features like increased plant height, more branching, longer leaf size and thicker leaf texture. Since the tetraploid plant has desirable vegetative features it can be exploited as a vegetable. The formation of exclusive bivalents or absence of multivalents in a natural polyploid species indicates allopolyploidy in its origin. The polyploids exhibiting exclusive bivalents at metaphase I stage are not uncommon in nature (Arora and Madhusoodanan, 1981). Their diploid like chromosome behaviour during meiosis is regulated due to the presence of multivalent suppressor system as the one found in wheat *(Triticum aestivum)*.

Inter relationship between species in the section Blitopsis.

A. lividus x A. tricolor was the only interspecific hybrid, formed in the section Blitopsis. Morphological and cytogenetical studies of the hybrid elucidated relationship between *A. lividus* and *A. tricolor*. Meiotic analysis in the parents showed that both this species are quite regular

with fertile pollen grains. On the other hand, hybrid resembled *A. tricolor* for most of the morphological features except for leaf tip, nature and tenderness of the stem. Pal and Khoshoo (1973b) observed an overall dominance of *A. tricolor* in *A. lividus* var. *lividus* x *A. trilocor* var. *viridis* hybrid. Interestingly in the present study, the hybrid had larger primary leaf size and showed earliness in flowering. Unlike small nonshowy inflorescences and prominent axillary glomerules in parental species, the hybrid had flowers in terminal panicles at the end of every branch and also in axillary clusters at most of the nodes. Number of male flowers was reduced and was restricted to the central position of a few cymes in the hybrid. Female flowers were produced profusely. The hybrid exhibited developmental abnormalities like another shrivelling and fasciation of the inflorescence. Probably this would explain the distance between genomes and the specific status of the parents (Khoshoo and Pal, 1972).

Cytogenetical analysis of the hybrid revealed the presence of two or more interchanges involving 3-6 chromosomes at metaphase-I in every PMC. In general, multiple association in hybrids are interpreted as due to interchange hybridity. Pal and Khoshoo (1973b) observed chromosome complexes involving 4-11 chromosomes in *A. lividus* x *A. tricolor* hybrid. Similar to their findings, PMCs in the hybrid at anaphase I was characterised by bridges and fragments. Contrary to their report on complete pollen and seed sterility, the hybrid plant in the present study produced about 10% stainable pollen, though they were medium sized. Chromosome bridges and fragments along with very high pollen sterility (90%) indicate that *A. lividus* and *A. tricolor* also differ by a paracentric inversion (Pal and Khoshoo, 1973b). The moderate frequency of bivalents in the hybrid suggests the homology of these chromosomes. A definite conclusion

in this aspect is possible only by pachytene analysis of the hybrid plant. Pal and Khoshoo (1973b) suggested that *A. tricolor* represents a more ancestral condition than *A. lividus* due to the presence of dominant features. Present findings also support this conclusion. In short, *A. lividus* and *A. tricolor* are related to a certain degree due to their crossability and bivalent formation, but multivalents, anaphase bridges and fragments, telophase II abnormalities, high amount of pollen and seed sterility indicate that translocations and paracentric inversions are involved during evolution of these two species. It seems that mechanism underlying interspecific cytogenetic differentiation and sterility within section Blitopsis is chromosomal rearrangements such as interchanges and inversions. The evidence for such a mechanism is available in *Secale* (Riley, 1955; Khush, 1962) *Avena* (Holden, 1966), *Linum* (Gill and Yermanos, 1967) *Amaranthus* (Pal and Khoshoo, 1973b) and *Solanum* (Rao and Kumar, 1983). Studies on all these genera showed that chromosomal reorganization is a frequent mode of speciation and often morphologically similar taxa differ by chromosomal rearrangements, detected through hybridization. This type of speciation is termed 'saltational speciation' by Lewis (1966).

Inter relationship between species of section Amaranthus

Interspecific hybridization is successful within section Amaranthus between *A. spinosus* x *A. caudatus*, *A. spinosus* x *A. cruentus*, *A. spinosus* x *A. hypochondriacus* and *A. spinosus* x *A. dubius*, where F_1 hybrids could be cytogenetically analysed. *A. cruentus* x *A. caudatus* produced only female flowers in the glomerules and cytogenetical analysis was not carried out due to absence of stamens. In two reciprocal crosses *A. caudatus* x *A. spinosus* and *A. caudatus* x *A. cruentus*, F_1 hybrids developed only up to two leaf stages and later post fertilization barriers were observed. The reason for this unilateral hybrid break down may be numerical differences in the chromosomes resulting in disturbance

of the endosperm ratios as observed by Pal *et al.* (1982) in *A. hypochondriacus* (n = 16) x *A. hybridus* (n = 17). Pal and Khoshoo (1972) stated that some form of endosperm malfunction in the interspecific hybrids affects adversely seed germination, and results in seedling abnormality.

Cytomorphological analysis of the hybrids of *A. spinosus* with *A. hypochondriacus*, *A. cruentus* and *A. caudatus* as well as the polyploid species *A. dubius*, revealed interrelationship of these species. In *A. cruentus x A. caudatus*, the hybrid grew and flowered but meiotic studies could not be carried out for want of male flowers. The stunted branches and leaves in the hybrid resembling viral infection simulated the symptoms in *A. edulis x A. hypochondriacus* hybrid as observed by Pal & Khoshoo (1972). Since no pathogens were found associated with these symptoms, these developmental defects have a genetical basis. Perhaps this situation reflects a highly disharmonious interaction of parental genomes as combined in the hybrid cell (genic disharmony). Although the exact nature of genetic control is not known, it probably creates irregularities in the concentration and distribution of the high content of free auxins as established in *Nicotiana*. (Kher and Smith, 1954; Ahuja and Hagen, 1967). According to Stebbins (1958) the time of degeneration generally coincides with some critical or maximal period of tissue differntiation, anywhere from the first division of the zygote to a situation where hybrids are produced without constiutional weakness of the plant body but are associated with hybrid sterility that expresses itself in the form of deformed flowers and/or totally sterile pollen and ovules.

Grant (1959a) pointed out that chances are more for the origin of dioecious species of *Amaranthus* from the monoecious species as a result

aneuploidy resulting from interspecific hybridization between species with n = 16 and 17. The situation in the *A. cruentus x A. caudatus* is akin to that of Grant's observation because the two grain species have chromosome numbers n = 17 and n = 16. The interspecific hybridization between these two, resulted in promoting a genic condition, necessary for the expression of female sex. In fact, the development of gynoecious type from two monoecious species of *Amaranthus* conforms very well with the evolution of sex (Correns, 1928).

In all the hybrids of *A. spinosus* with the three grain species, an overall dominance of the *spinosus* characters such as the presence of slender terminal inflorescences and a few axillary clusters, distinct placement of male and female flowers, presence of a pair of spines at nodes and comparatively smaller sized leaves was observed. The sharpness of the spines varied in different hybrids which indicates that the character is under the control of more than a single gene. Suppression of the male flowers was another interesting morphological feature observed in some of the hybrids. Male flowers were restricted to a few distal ones at the ends of panicle branches and these flowers dried off without any anthesis. Many of them were barren without any stamens. Staminodes were also observed in *A. spinosus x A. cruentus*. Such malformed flowers were also observed by Pal and Khoshoo (1972) in interspecific hybrids of *A. gracizans x A. lividus*, *A. tricolor* cv. 'purple leaf' x *A. tricolor* var. *salicifolius*. This situation was also similar to the 'neuter plants' observed by Murray (1940) in the F_1 hybrids between dioecious and monoecious species of *Amaranthus*. According to him, the neuter character was the result of a single dominant gene in the dioecious species. However a dioecious parent was absent in the present study. The normal production

of male flowers and their anthesis were observed in *A. spinosus* x *A. caudatus*. Pal and Khoshoo (1973a) suggested that among the successful hybrids, differentation between parents can be judged from the extent of sterility. Consequently *A. spinosus* and *A. caudatus* are the least differentiated since the hybrid between them was the most fertile in this group.

Contrary to the suppression of male flowers, there was abundant production of female flowers in most of these hybrids. The prolific production of female flowers often led to spongy or velvetty panicles of sterile female flowers. Probably lack of pollination may be factor underlying this. Murray (1940) also observed that new flowers were continuously produced in unpollinated inflorescence where seed set was poor and as a result, a large hybrid plant at the end of the season had as many as hundred thousand flowers. However, the hybrid *A. spinosus* x *A. caudatus* had normal production of female flowers. The production of female flowers was abundant in hybrid *A. spinosus* x *A. hypochondriacus*. The female flowers were confined not only to the terminal inflorescences but also to dense axillary clusters around most of the nodes. This observation points towards the plausible development of the section Blitopsis from the section Amaranthus as a result of the interspecific hybridization between members of the section Amaranthus. In this context, grain amaranths are considered one of the oldest crops, cultivated for more than 6000 years (Agogino, 1957). They originated in the new world and were used as 'cereals'. Since the section Amaranthus contains all the grain amaranths, the primitiveness of section Amaranthus is logical. On the other hand, members of the section Blitopsis are used as pot herbs and their selection seems to have taken place in the Indian subcontinent (Khoshoo and Pal, 1972).

In all the hybrids of the section Amaranthus, meiotic metaphase I was characterised by high frequency of bivalents, one or two multivalents involving 3-4 chromosomes and the rest univalents. None of the hybrids exhibited total bivalent pairing. Pal and Khoshoo (1973a) observed bivalent pairing to a great extent in all the interspecific hybrids of the section Amaranthus studied by them and multivalents were seldom observed. According to them, species differentiation in the section Amaranthus involved only gene differences and cryptic structural hybridity that resulted in bivalent pairing, while within Blitopsis translocations and inversions were involved. On the other hand, in the present investigation, multivalents involving 3-4 chromosomes were observed in the PMCs as a result of chromosomal interchanges like translocations. This shows that segmental interchanges of chromosomes are also involved during speciation in section Amaranthus.

There were also indications of structural changes like inversions in most of these hybrids corroborated by bridge configuration at anaphase. Anaphase I was also characterised by unequal distribution, lagging chromosomes, fragments etc. Many of the PMCs of the hybrids also showed abnormalities in the second meiotic division in the form of asynchronous orientation and disjunction at metaphase II and anaphase II respectively. Multivalents, anaphase bridges, fragments and unequal distribution of chromosomes, telophase II abnormalities etc. indicate that chromosome repatterning through inversions and translocations are also involved in the evolution of species of the section Amaranthus. In essence, the mechanism involved during the evolution of different species of *Amaranthus* do not differ very much between section Amaranthus and Blitopsis. The mechanisms were almost analogous and the distinctness reported by Pal amd Khoshoo (1973b) is not supported by the present investigation. Incidently,

Madhusoodanan (1976) reported the cytology of an interspecific hybrid between *A. graecizans x A. viridis* which exhibited complete bivalent pairing without any evidence of translocation. Eventhough both the species belonged to section Blitopsis their cytogenetic behaviour was similar to that of section Amaranthus.

High degree of chromosome pairing as indicated by bivalent associations was observed in the hybrids, *A. spinosus x A. caudatus*, as well as *A. spinosus x A. cruentus* while *A. spinosus x A. hypochondriacus* had only a few number of bivalents. Since genomic affinity is expressed by pairing, it is concluded that *A. hypochondriacus* is more distantly related to *A. spinosus* than the other two grain species. Chiasma frequency of the different species showed that *A. cruentus* and *A. caudatus* do not differ significantly in their number of chiasmata. The chiasma frequency was usually positively correlated with the frequency of ring bivalents/cell. The chiasma frequencies of hybrids were lower, intermediate or higher than those of parents. A much lower chiasma frequency than both the parents observed in *A. spinosus x A. hypochondriacus* indicates a measure of nonhomology and a limited recombination between the chromosome segments of the species. Magoon and Tayyab (1967) observed similar decrease in chiasma frequency in Sorghum hybrids. Conversely a higher value of chiasma frequency than both the parents was observed in *A. spinosus x A. caudatus* which indicates a higher degree of homology of these chromosomes. Incidently, this hybrid exhibited the highest pollen fertility and seed fertility among others.

The pollen fertility ranged from 0-20% in the interspecific hybrids. Meiotic irregularities like multivalent associations, bridges and fragments, laggards, asynchronous orientation etc., are involved in the production of

sterile grains in the hybrids (Dane *et al.*, 1980; Gopinathan *et al* 1986) *A. spinosus* x *A. hypochondriacus* showed maximum abnormalities due to the presence of a large number of univalents and exhibited the least fertility.

Genomic relationship between *A. spinosus* and *A. dubius*

The hybrid *A. spinosus* (n = 17) x *A. dubius* (n = 32) had 17 II + 15 I in most of the PMCs. The presence of 17 II in the hybrid results from pairing between *spinosus* genome (n = 17) and 17 chromosomes of the *dubius* complement, leaving the rest 15 chromosomes unpaired. Grant (1959b) recorded similar observations and suggested that *A. spinosus* is one of the progenitors of *A. dubius*. However, Pal (1972) disagreed with Grant based on his observations in artificial amphidiploid *A. spino-dubius* (n = 49). He observed preferential pairing of homologous chromosomes in the amphidiploid leading to a major reduction in the number of quadrivalents and suggested that the bivalents in the F_1 were the result of homeologous pairing. Present investigation can neither support nor disagree with Pal (1972) as artificial amphidiploids were not attempted here. However, from the literature, one can site the involvement of multivalent suppressor genes leading to complete or high frequency of bivalent formation in amphidiploids (Wagenaar, 1966; Arora and Madhusoodanan, 1981) and the role of such multivalent suppressor genes in *A. spino-dubius* (n = 49) may be suspected. In any case, the fact that *A. spinosus* contributed predominantly to one of the genomes of *A. dubius* is beyond doubt. The only part to be clarified is whether completely homologous or homeologous chromosomes exist between *A. spinosus* and *A. dubius*. If homeology is proved, the cytogenetic differentiation would have occurred through cryptic structural changes, gene mutation and genetic drift.

The origin of n = 15 genome in the *A. dubius* complement is not solved by the present study. Till today, the occurrence of an *Amaranthus* species with n = 15 is not reported. Hence only presumptions like the aneuploid hypothesis of Grant (1959b), are possible. According to him, the contribution of n = 15 in the *A. dubius* had either n = 16or n = 17 and by crossing with *A. spinosus* (n = 17) produced an amphidiploid with n = 33 or 34 which later stabilised as *A. dubius* (n = 32) by the loss of one or two chromosomes.

Both *A. dubius* and *A. spinosus* are easily crossable with each other. But the gene flow between them is very much restricted because of the high sterility of hybrids. The sterility in the hybrid was entirely chromosomal due to the high frequency of univalents, laggards, bridges etc., observed during meiosis.

Inter-relationship between sections Amaranthus and Blitopsis

The hybrid *A. spinosus x A. viridis* is the first success of hybridisation between the species belonging to two different sections in the genus *Amaranthus*. The short and sturdy hybrid plant inherited more *spinosus* characters such as presence of spines, distinct arrangement of male and feamle flowers, pentamerous symmetry etc. Meiotic analysis of the hybrid revealed certain valid inferences regarding phylogeny. The 17 pairs of chromosomes in the hybrid formed only bivalents and univalents at metaphase I indicating the absence of structural changes like inversions and translocations in the cytogenetic differentiation. On the other hand, cryptic differences and genetic drift are the factors responsible for cytogenetic differentiation. The high frequency of bivalents and the moderate fertility of the pollen in the hybrid suggested a high amount of genomic similarity and hence phylogenetic relationship between these two species. The presence of 4-6 univalents in the hybrid is due to the reduced homology between 2-3 pairs of chromosomes of these two species.

Based on bivalent association and pollen fertility in the hybrid, it is suggested that *A. viridis* and *A. spinosus* occupy nearby positions in the phylogenetic tree. However, *A. spinosus* distinguishes itself from the related species by presence of sharp spines at nodes and distinctly arranged pentamerous male and female flowers.

Cytogenetical and morphological studies in *A. spinosus x A. viridis* do not support the taxonomic treatment of these two genomically related species under two different sections. This is the first successful attempt on the relationship between these two species and hence between two sections. Kowal (1954) removed *A. spinosus* from section Amaranthotypus to section Blitopsis but the distance between the two species was further widened by including *A. viridis* (= *A. gracilis*) in the newly created section Puncticulatae. The existence of these different sections is not justified based on the present investigation. Madhusoodanan and Nazeer (1983) treated *A. spinosus* under section Amaranthus and *A. viridis* under section Blitopsis. The morphological and cytological observations of these two species and their hybrids question the validity of the treatment of these two species under different sections. From this point of view, the involvement of similar processes such as translocations and inversions were also observed in the evolution of different species in both sections. Most probably cryptic structural hybridity, genetic drift and gene mutation indicated by the presence of bivalents in different hybrids are also involved during speciation in the genus *Amaranthus*. (Pal and Khoshoo, 1973a and b). It would be worthwhile if pachytene studies are aimed in the interspecific hybrids between sections Amaranthus x Blitopsis, in order to identify the nonpairing segments and inversion loops. Also a large number of species hybrids can be developed using even embryo

culture techniques and their cytogenetical relationship worked out, which would be the future line of investigation in this direction.

In an attempt to modify the taxonomic key for identification of different species, *A. viridis* and *A. spinosus* were brought together based on similarity in gross morphological features. Cytological studies also supported their taxonomic closeness. Eventhough the two species differ in the symmetry of flowers, there are many other features which brought them together. It is suggested that either the sectional treatment may be avoided or *A. viridis* and *A. spinosus* may be treated under a new section, in between Blitopsis and Amaranthus.

The moderate pollen sterility in this hybrid seems to be entirely chromosomal resulting from lagging univalents which failed to be included in any of the nuclei at the end of the division. Eventhough pollen was moderately fertile, lack of anthesis of male flowers led practically to no seed set in the hybrid. Use of pollen from the male flowers was not feasible because the flowers usually do not open sufficiently to protrude the anthers and anthers fail to dehisce. Murray (1940) also observed similar type of male flowers in the intergeneric hybrids in *Amaranthaceae*.

Role of *A. spinosus* in the evolution of *Amaranthus* species

Sauer (1950, 1967) reported that most of the grain amaranth cultivars and their wild relatives belong to the American continent where they got firmly entrenched in the lives of the pre-historic people who cultivated them since time immemorial. *Amaranthus spinosus*, being a member of the section Amaranthus (Schinz, 1934) to which all grain types belong, is also likely to be a native of America. It has also been described as a cosmopolitan pantropical weedy species and was involved in natural hybridization even in the 19th century. A spontaneous hybrid

between *A. spinosus* x *A. dubius* was located as early as 1874 in the Berlin Botanic Garden (Thellung, 1914). Again, Sauer (1967) reported that *A. spinosus* was spreading, rapidly by 1700 AD through the warmer parts of the world from the New World both as a weed and as a sporadically planted pot herb. The primitiveness of *A. spinosus* was evident from the cytomorphological and chemical studies on the species as well as its hybrids undertaken in the present study. Cytological studies revealed that its chromosome complement was smaller in size than others, and the minimum number of ring bivalents and the lowest chiasma frequency, all indicative of its primitive wild nature not influenced by selection and cultivation. The species also had primitive dominant characters like presence of a pair of sharp spines, distinct positional arrangement of male and female florets along the slender panicle, stress tolerant hard stem etc. Spines at nodes would have helped the survival of this species and its distribution as a cosmopolitan weed.

Cross compatibility, chromosome pairing, pollen fertility and seed set in the interspecific hybrids between *A. spinosus* and the major grain species were studied in order to understand their phylogenetic relationship. Comparatively high chiasma frequency as well as pollen and seed fertility in *A. spinosus* x *A. caudatus* showed that *A. caudatus* is very closely related to *A. spinosus*. The genome of *A. cruentus* was also related to *A. spinosus* though to a less extent due to seed sterility of *A. spinosus* x *A. cruentus* hybrid. D^2 analysis indicated the clustering of these two hybrids together thereby showing relationship among each other. Interspecific hybridization between *A. spinosus* and *A. viridis*, a weed of the section Blitopsis revealed the relationship between the two species. In this cross, the homology between the two complements was very clear due to the formation of more number of bivalents.

Further, *A. spinosus* contributed completely or to a great extent to one of the genomes of the amphidiploid *A. dubius*. Therefore, based on the present investigation it is concluded that the cosmopolitan, pantropical weedy species *A. spinosus* played a major role in the evolution of other *Amaranthus* species especially *A. caudatus*, *A. cruentus*, *A. viridis* and *A. dubius*. It will be interesting if the natural diversity of *A. spinosus* in the American continent is surveyed is an attempt to confirm the place and mode of evolution of different *Amaranthus* species.

D^2 analysis in interspecific hybrids

Four interspecific hybrids viz. *A. lividus* x *A. tricolor*, *A. spinosus* x *A. caudatus*, *A. spinosus* x *A. cruentus* and *A. spinosus* x *A. viridis* were grouped in a single cluster indicating phylogenetic relationship among the six species. It is interesting to observe that the first hybrid is between two species of the section Blitopsis, second and third are between species of the section Amaranthus and the fourth one is between Amaranthus and Blitopsis. The clustering of these four hybrids together again questions the validity of the naturalness of the two sections supported by Khoshoo and Pal (1972). *A. spinosus* x *A. hypochondriacus* formed a separate cluster II and the highest value of intercluster distance indicated that *A. hypochondriacus* is phylogenetically distant from others. Incidently the hybrid had higher number of univalents than other species hybrids. Also higher values of pollen and seed sterility were observed in this hybrid. The hybrid *A. spinosus* x *A. dubius* also formed a separate cluster III and this is inferred from the polyploid nature of *A. dubius*. The results of Grant (1959b), Pal, (1972) and the present study indicated that *A. spinosus* (n = 17) contributed one of the genomes of *A. dubius*. However

the contributor of the other genome in the *A. dubius* complement is not known. Probably the unidentified genome in the *A. dubius* complement is responsible for the separate clustering of *A. spinosus* x *A. dubius* hybrid.

Clues to the origin of vegetable Amaranths

Origin of vegetable amaranths from grain amaranths is postulated in the present investigation. Archaeological excavations in South and Central America revealed that grain amaranths are associated with man since prehistoric times and are among the most ancient crops, cultivated as early as 4800 B.C. (Agogino, 1957). Ecogeographical, ethnobotanical, morphological and archaeological studies of Sauer (1959, 1967) showed that most of the grain amaranth cultivars and their wild relatives are indigenous to the American continent.

All the vegetable amaranths except *A. dubius* belong to section Blitopsis. The amphidiploid *A. dubius* though belonging to section Amaranthus originated at a later date and the weedy species *A. spinosus* contributed predominantly to one of the genomes of *A. dubius* (Grant, 1959b, Pal, 1972). According to Sauer (1967) *A. spinosus* and *A. dubius* are commonest and most widespread weedy amaranths of the New World tropical low lands where they had been presumably originated. The spread of *A. dubius* was slower than that
/of *A. spinosus* being found only at a few places before 1800. Hence the presumption is made on the evolution of vegetable amaranths from grain amaranths. In this event one prominent feature of the section Blitopsis, to which most vegetable amaranths belong, is the presence of flowers clustered densely at leaf axils. Earlier investigators (Pal and Khoshoo, 1973b) and the present study revealed that terminal inflorescence, characteristic of grain type, is dominant to axillary and the primitiveness of terminal habit is possible. Interspecific hybrids between *A. spinosus*

and *A. hypochondriacus* both belonging to section Amaranthus, produced axillary clusters of flowers simulating the dense nodal clusters of members of the section Blitopsis. Also the synthesis of hybrid between *A. spinosus* (section Amaranthus) x *A. viridis* (section Blitopsis) was possible in the present study and their close relationship was worked out. The dominance of major *spinosus* characters in the hybrid indicated its primitiveness over *A. viridis*. Furthermore, in all crosses of *A. spinosus* with grain types (*A. caudatus*, *A. cruentus* and *A. hypocondriacus*) the huge terminal inflorescence was completely suppressed by the slender panicle of *A. spinosus*. All the above aspects support the hypothesis of evolution of vegetable amaranths from grain species.

In the Americal continent, grain amaranths were grown for grains and use of amaranth leaves was seldom mentioned. But in Asia and Africa, leaves and stems of vegetable types are consumed. According to Pal and Khoshoo (1974) most of the grain amaranths reached Asia in the 18th or early 19th century and *A. hypochondriacus* is considered as the first domesticated grain crop in India. If the hypothesis of evolution of vegetable amaranths from grain amaranths is supported, the process might have occurred in the Indian or African continent in the case of species excepting *A. dubius*, where human selection played a major role in the domestication of species with large leaves and highly reduced terminal panicle. Comprehensive interspecific hybridization programmes between vegetable species and grain species and the cytomorphological analysis of the F_1 hybrids would be useful in checking further the hypothesis of origin of vegetable amaranths from grain types.

Classification of the existing gem plasm into different species

Forty accessions of Amaranth in the existing germplasm of the Department of Olericulture, College of Horticulture were evaluated for

their taxonomy, cytology and pollen characters. Based on key characters of Sauer (1967), Feine (1980) as well as the modified key developed in the present studies, and chromosome number, 21 accessions belonged to *A. tricolor*, 4 to *A. dubius* and 15 to *A. hypochondriacus*. *A. tricolor* exhibited variation in morphological features but the other two species did not show any pronounced variation.

As observed in the keys, the flowers of *A. dubius* had pentamerous symmetry. Accession No.2 is a released variety Co-1 from TNAU, Coimbatore, Tamil Nadu. Kamalanathan *et al.* (1973) released this cultivar for cultivation owing to its very high yield (7165 kg green/Ha), as a selection from the local type. They described the floral characters and indicated presence of 3 sepals and 3 stamens. Present study showed that its flowers have pentamerous symmetry with 5 tepals and stamens and not three as reported by the above authors.

Seed yield in *A. tricolor* Accessions, A_6 and A_{13}

The line A_6 with low seed yield has a long duration and more profuse vegetative growth characterised by more number of leaves and branches than A_{13}. Seed yield and vegetative growth are reported to be negatively correlated in *A. lividus* by Prasad *et al.* (1979) and Hauptli and Jain (1977). In *A. tricolor*, growth is indeterminate and the topmost axillary cymes are produced late in the season and they contain perdominantly male flowers. The utriculi of these cymes do not get time to reach full maturity. Eventhough the number of fruiting nodes is more in A_6 (53) than in A_{13} (44), the seed yield was low because of the following facts. The lower number of glomerules/leaf axil (A_6-27, A_{13}-36) has a direct relationship with low seed recovery. The prolonged flowering

period in the long duration type A_6 leads to shattering of the early matured seeds (A_6 - 11.4, A_{13} - 7.37) and immaturity of many later formed utriculi. A comparatively lower pollen fertility in A_6 (86%) also contributes to low seed recovery. Lower number of glomerules/leaf axil, lower percentage of female flowers developing into mature utriculi, higher percentage of shedding of seeds and the apparently lower pollen fertility and seed size are the possible reasons for low seed recovery in A_6.

Photoperiodic studies in *Amaranthus* spp.

The eight species of *Amaranthus* were subjected to varying photoperiods longer than the natural day length. All the plants flowered under each treatment thereby showing that the given photoperiods were not inhibitory for flowering.

Reduction in days to flower with increase in photoperiod was observed in *A. lividus*, *A. hypochondriacus*, *A. spinosus* and *A. dubius*. A decreasing photoperiod induced precocity in *A. tricolor* and *A. caudatus*. Earlier workers have described *A. hypochondriacus*, *A. caudatus* and *A. tricolor* as qualitatively short day species for flowering (Grubben, 1976, 1980; and Sawhney *et al.* 1980). It can be presumed that the critical day lengths for the above qualitative short day species is outside the range tested in this experiment. In the species *A. viridis* and *A. cruentus*, no definite relation between flowering and photoperiodic treatments were observed. Seth (1963) and Grubben (1976) reported that *A. cruentus* is day neutral.

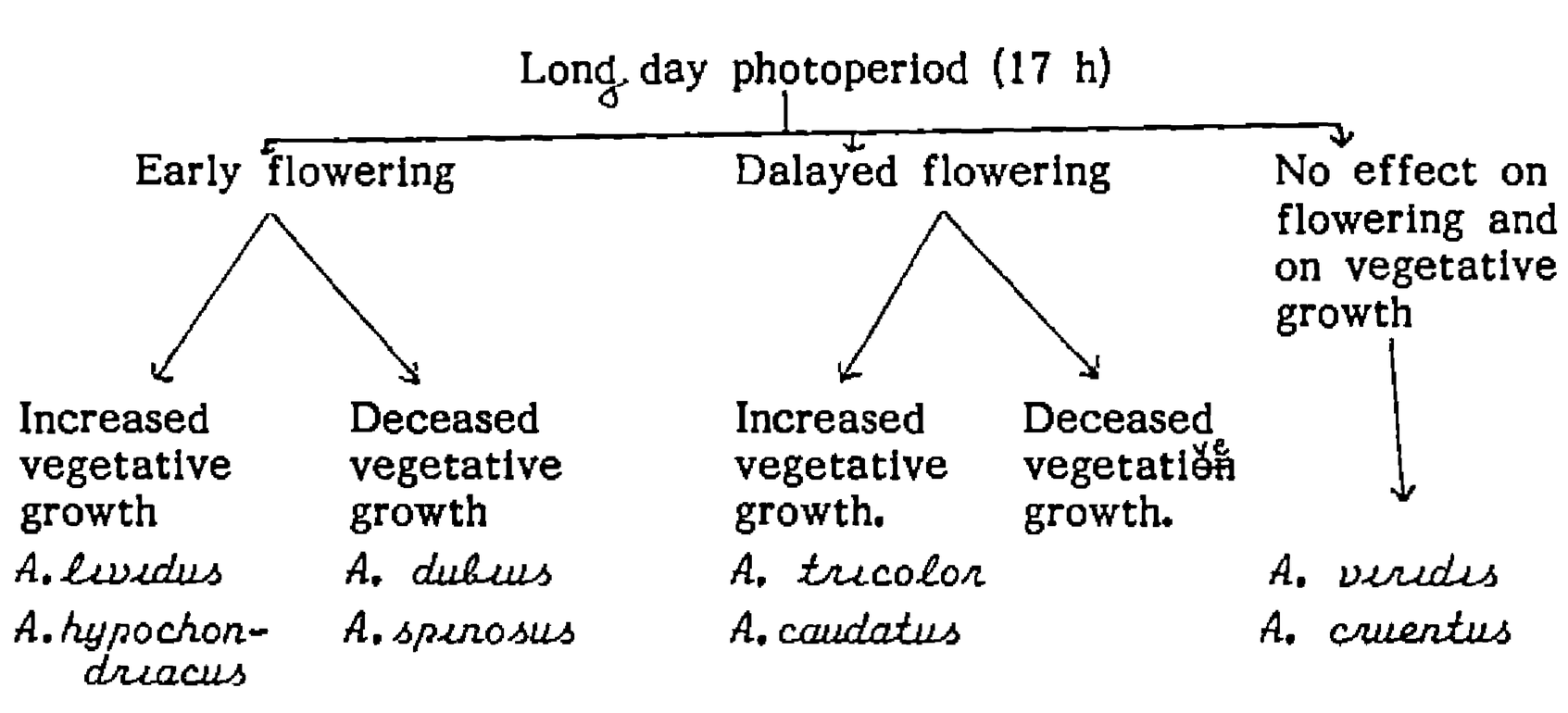
Higher photoperiod modulates floral bud morphogenesis and also interfere with vegetative growth and foliar differentiation in *A. lividus* and *A. hypochondriacus*. A negative influence of photoperiod on vegetative growth and positive effects on flowering were observed in *A. spinosus* and *A. dubius*. The reaction of the two species *A. cruentus* and *A. viridis*

to photoperiodic treatments were not significant. These two species appeared to be neutral for vegetative growth and flowering within the range of photoperiods used in the present study.

Photoperiodic response has nothing to do with their taxonomic identity. (Sawhney *et al.* 1980) The species *A. lividus* and *A. hypochondriacus* showed the same response to photoperiod. They did not have any taxonomic closeness as the former belonged to section *Blitopsis* (trimerous flowers) and the latter to section *Amaranthus* (pentamerous flowers). Similar were the cases between *A. tricolor* and *A. caudatus* and also between *A. viridis* and *A. cruentus*. Similarity in the photoperiodic requirement of these species supported the view that photoperiod requirement and taxonomic closeness were two independant aspects as observed by Sawhney *et al.* (1980).

Both *Amaranthus dubius* and *A. spinosus* belong to the section Amaranthus. They showed similar response to photoperiodic treatments. *A. dubius* is an allopolyploid into which *A. spinosus* is one of the progenitors. The similar response in these two species is attributed to their sharing of common genome.

Most of the species showed response to the long day photoperiod of 17 h. An attempt to classify the different species based on this treatment is given below.



Nitrate and Oxalate content in *Amaranthus* spp.

Amaranths contain antinutritional factors like oxalate and nitrate in varying amounts in different species. Oxalate contents in foods are of concern because free oxalates bind essential dietary divalent minerals primarily, calcium and makes them nutritionally unavailable (Marderosian *et al.* 1980). Even under normal dietic regimes, calcium deficiency is likely to occur in Indian diet especially among rice eating population (Srivastava and Krishnan, 1959). The oxalic acid levels in amaranth greens become high as the plants get older and when grown under dry conditions. The level of nitrate in the diet is of concern if it is converted to nitrate and nitrosamines. Nitrate is also absorbed into blood where it combine with haemoglobin to form methemoglobin. A few nitrosamines are mutagenic in experimental animals (Marderosian, *et al.* 1980).

The analysis of free oxalate and nitrate in the leaves and stem of eight *Amaranthus* species gave certain useful indications on the presence of these antinutritional factors in different species. *A. dubius* widely accepted as a vegetable amaranth was characterised by the lowest content of nitrate among the different species. It also has a low content of oxalate in leaves and stem. It is interesting to note that the taste of *A. dubius* was not accepted by the panel during the taste test comparison in USA (Kauffman & Gilbert, 1981). The lower content of nitrate in *A. dubius* and *A. lividus* are directly correlated with the smooth and soft texture of leaves in these two species. *A. lividus* is also an accepted green type in North India. *A. tricolor* is the most popular vegetable amaranth in Kerala, but it has very high amount of nitrate/oxalate. and The present study thus envisages the need to estimate the variability among the diverse types of this species for selection and popularisation of a type with lower content of nitrates and oxalates.

Members of the section *Amaranthus* in general are characterised by the lower content of oxalate and nitrate than section *Blitopsis*. The only exemption to this is *A. spinosus* which has the highest content of both oxalate and nitrate. This may be attributed to the semiwild nature of this species and less domestication than others. The phylogenetic relationship of members of both the sections to *A. spinosus* is of particular interest in this respect. The systematic grouping of *A. spinosus* under section Amaranthus has been questioned by Kowal (1954) and he grouped it under section Blitopsis. The content of oxalate and nitrate in the leaves and stem of *A. spinosus* failed to support the treatment of this semiwild species under section Amaranthus. *A. spinosus* excels all other vegetable amaranths in taste also. But the very high levels of both the antinutritional factors necessitate that its use as a pot herb should be discouraged.

Grain amaranth types have only lower levels of oxalate and medium levels of nitrate. Harwood (1979) expressed the view that cereal and vegetable amaranths should be treated as one unit since the young plants of cereal amaranths are frequently used as greens. This study shows that upon consumption of green of grain amaranths there is no need for any concern about their oxalate and nitrate. Altogether the results do not indicate any possible adverse nutritional effects from nitrate and oxalate content of amaranths when they are consumed in the normal dietary regimens. Although the present levels of nitrate and oxalate do not pose a nutritional problem under normal conditions of consumption, types which have lower level of these factors are certainly desirable. In this event, the most desirable type to be used as a vegetable type is *A. dubius*.

The high heritability estimates for oxalate and nitrate contents indicate that both these antinutritional factors are highly influenced by genes and comparatively less by environment. Hence phenotypic selection may be useful in eliminating the undesirable ones.

Summary

SUMMARY

Cytogenetical studies on eight species of *Amaranthus* and their hybrids were undertaken at the Department of Olericulture, College of Horticulture, Vellanikkara in order to understand their genome relationship. Initially these eight different species were analysed for their morphological and cytological features. This dissertation also embodies other aspects such as development of a provisional key for identification of *Amaranthus* species, classification of the forty accessions of *Amaranthus* available in the germplasm collection of the Department, analysis of reasons for low seed recovery in a promising vegetable type, studies on the photoperiodic response of the eight different species and studies on the content of antinutrient factors.

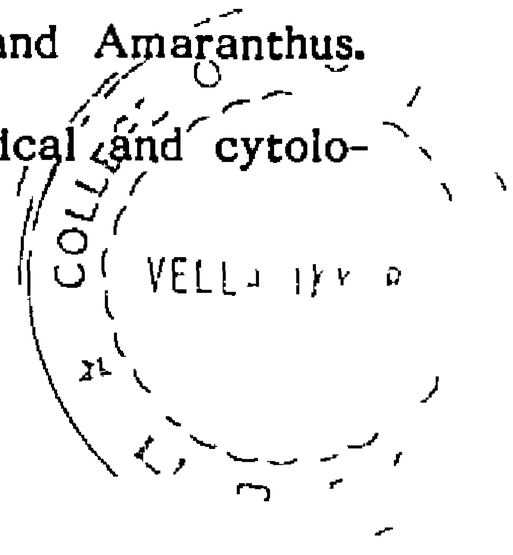
The eight species included in the present study are *A. tricolor*, *A. lividus*, *A. viridis*, *A. spinosus*, *A. dubius*, *A. hypochondriacus*, *A. cruentus* and *A. caudatus*, the former three belonging to the section Blitopsis and the latter five to section Amaranthus. These eight species were analysed for their morphological and cytological features. Microscopic examination of the floral characters resulted in identifying a deviation in the pattern of development of flower cluster in *Amaranthus*. The floral development was found to be in a dichasial or polychasial scorpioid cymose pattern and not in a typical cymose pattern reported earlier.

The identification of the different species was found difficult using the existing keys. Hence a simple provisional identification key for the eight species was developed using gross morphological features. Because of similarity in the nature of inflorescences, *A. spinosus* and *A. viridis*

belonging to two different sections were brought together, and *A. dubius* a vegetable type was grouped along with grain amaranths.

Meiosis in all the eight species were normal with regular formation of bivalents. Of the eight species, seven were diploids with n = 16 or 17 and one species *A. dubius* was a polyploid with n = 32. A preponderance of ring bivalents over rods was observed in all the species except the semiwild species *A. spinosus*. All the three species under section Blitopsis had n = 17 and maximum number of chiasmata was observed in *A. tricolor* the widely cultivated vegetable type. This species also had the largest chromosome in the complement. Under section Amaranthus, the polyploid species, *A. dubius* behaved as an allopolyploid with 32 bivalents. The lowest chiasma frequency/bivalent and per PMC and the highest number of rod bivalents was observed in the semiwild type *A. spinosus*, all indicating its primitive wild nature. Pollen fertility in all species was fairly high ranging from 83.4% in *A. viridis* to 93.8% in *A. lividus*. In all species pollen grains were not of the uniform size and included both macro (24 μm) and medium sized (12-24 μm) pollen grains. The percentage of each type of pollen differed in different species. Mean diameter of the pollen grains was the highest in *A. tricolor* among all species (31.4 μm)

Interspecific hybridization was attempted in all possible combinations including the reciprocals. In many of the unsuccessful crosses mortality of the hybrid seedlings were observed by the dissolution of the terminal buds. Seven interspecific hybrids were obtained which exhibited normal growth and flowering. These include hybrids within section Blitopsis, within section Amaranthus and also between sections Blitopsis and Amaranthus. These seven hybrids were subjected to detailed morphological and cytological studies.



The interspecific hybrid in the section Blitopsis *A. lividus x A. tricolor* resembled the male parent in most of the morphological features. Even-though the hybrid was vigorous, it exhibited developmental abnormalities like shrivelling of the anthers and fasciation of the inflorescence. Cytological studies revealed the presence of two or more interchanges including 3 - 6 chromosomes and moderate frequency of bivalents at metaphase I. Subsequent meiotic irregularities led only to about 10% stainable pollen and seed sterility was also noticed. The multivalent associations, bridges and fragments in this hybrid indicated that interchanges and inversions are involved in the evolution and speciation within section Blitopsis. The moderate frequency of bivalents suggests homology of these chromosomes.

The interspecific hybrids obtained in the section Amaranthus include *A. spinosus x A. dubius, A. spinosus x A. hypochondriacus, A. spinosus x A. cruentus, A. spinosus x A. caudatus* and *A. cruentus x A. caudatus*. The arrested growth, peculiar twining of the stem and inflorescence axis and the stunted leaves in *A. cruentus x A. caudatus* simulated viral infection. This situation reflected a highly disharmonious interaction of parental genomes as combined in the hybrid nucleus. Generally in all other hybrids, there was an overall dominance of the female parent *A. spinosus*, characterised by the presence of spines, reduced leaf size, presence of slender terminal and few axillary clusters of flowers and distinct placement of male and female flowers. However the distinct arrangement of male and female flowers was not observed in *A. spinosus x A. dubius*. An overall reduction of male flowers was noticed in most of the hybrids. Normal anthesis of male flowers was observed only in *A. spinosus x A. dubius* and *A. spinosus x A. caudatus* while the male flowers failed to

open in the other two hybrids *A. spinosus x A. hypochondriacus* and *A. spinosus x A. cruentus*. The presence of axillary clusters of flowers in the hybrid *A. spinosus x A. hypochondriacus* simulated the placement of axillary flowers in the *A. tricolor* species. Hence the plausible evolution of section Blitopsis from section Amaranthus by interspecific hybridization is suggested. The dominance of most of the *A. spinosus* characters in the hybrids indicated primitiveness of *A. spinosus* over other grain species.

Cytological studies in the hybrids of the section Amaranthus revealed that metaphase I was characterised by a high frequency of bivalents, one or two multivalents involving 3-4 chromosome and the rest univalents. The univalents failed to orient at metaphase plate, lagged at anaphase I and led to the formation of micronuclei at the end of the division. PMCs also showed abnormalities in the second meiotic division in the form of asynchronous orientation and disjunction at metaphase II and anaphase II respectively. These abnormalities often led to more nuclei than normal at telophase II and subsequently resulted in very high pollen sterility and micropollen. Melotic abnormalities indicated that chromosome repatterning through inversions and translocations were involved in the evolution of the species within section Amaranthus.

The hybrid *A. spinosus x A. viridis* was the first success of hybridization between the two sections Amaranthus and Blitopsis. This hybrid was short and sturdy and inherited more of *A. spinosus* characters as in other hybrids including pentamerous symmetry of flowers. Cytological studies revealed that PMCs at metaphase I had an average of 14.35 bivalents and 5.25 univalents. Eventhough 38% pollen stanlability was observed, the pollengrains were of medium size and the anthers failed to

dehisce. The complete absence of multivalents, and the presence of only bivalents and univalents in the hybrid indicated the absence of structural changes in the cytogenetic differentiation of these two species, only cryptic structural differences between 2-3 pairs of chromosome differentiated these two species.

D^2 analysis based on chromosomal association at metaphase I in 6 hybrids grouped them into three clusters. The unidentified genome in *A. dubius* complement may be responsible for the separate clustering of *A. spinosus* x *A. dubius*. *A. hypochondriacus* may be genetically distant from others. The clustering of the hybrid *A. lividus* x *A. tricolor* along with hybrids of section Amaranthus questions the validity of the naturalness of the two sections under the genus *Amaranthus*.

The forty accessions available in the amaranth germplasm were ascribed to different species based on morphology and cytology; 21 accessions were ascribed to *A. tricolor* 4 to *A. dubius* and 15 to *A. hypochondriacus* Cytological studies revealed the formation of regular bivalents in all the forty accessions with 17, 32 and 16 pairs of chromosomes in the species *A. tricolor*, *A. dubius* and *A. hypochondriacus* respectively. All the accessions exhibited a reasonably high pollen fertility (>77%) and both macro and medium type pollen grains were noticed in each. Plant to plant variation was comparatively less in the species *A. dubius* and *A. hypochondriacus* while *A. tricolor* exhibited much variation in morphological features. The analysis of reasons for low seed recovery in the *A. tricolor* accession A_6 revealed that the low seed recovery was due to long flowering span which leads to shattering of the earlier formed seeds, profuse vegetative growth which is negatively correlated with seed yield, the lower number of glomerules/leaf axil, the lower percentage of female flowers developing into mature utriculi and the apparently lower pollen fertility and seed size.

Investigation on photoperiodic response of eight species revealed that there is reduction in days to flower with increase in photoperiod in *A. lividus* *A. hypochondriacus*, *A. spinosus* and *A. dubius*. A decreasing photoperiod induced precocity in *A. tricolor* and *A. caudatus*. In the species *A. viridis* and *A. cruentus* no definite relationship between flowering and photoperiodic treatments was observed.

The content of two antinutrient factors, oxalate and nitrate present in the leaves and stems of eight *Amaranthus* species were analysed. The percentage of oxalate in the different species varied from 3.60 to 5.10% and that of nitrates from 0.295 to 0.695% on dry weight basis. Members of the section Amaranthus in general are characterised by lower content of oxalate and nitrate than section Blitopsis but *A. spinosus* the wild type was an exception having the highest content of both these factors. The three cultivated grain types did not show much variation in the content of these antinutrient factors.

The primitiveness of *A. spinosus* was evident from the cytomorphological and chemical studies on the species as well as its hybrids. Cytological behaviour as well as pollen and seed fertility of the hybrids indicated that *A. spinosus* is closely related to *A. caudatus* and *A. viridis*. *A. spinosus* also contributed predominantly to one of the genomes of *A. dubius*. Hence the cosmopolitan weed *A. spinosus* has played a major role in the evolution of *Amaranthus* spp. Suppression of huge terminal inflorescences in all crosses of *A. spinosus* with grain types and production of dense axillary flower clusters in *A. spinosus* x *A. hypochondriacus* resembling *A. tricolor* of section Blitopsis were observed. Based on these results and the historical data on domestication and spread of *Amaranthus* species, the plausible evolution of section Blitopsis from section Amaranthus and also the evolution of vegetable amaranthus from grain types are hypothesised.

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* Originals not seen

GENOME ANALYSIS IN THE GENUS *Amaranthus*

By

V K. MALLIKA

ABSTRACT OF THESIS

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ABSTRACT

Cytogenetical studies on eight *Amaranthus* species, viz., *A. tricolor*, *A. lividus*, *A. viridis*, *A. spinosus*, *A. dubius*, *A. hypochondriacus*, *A. cruentus* and *A. caudatus* and their hydrids were undertaken to understand their genome relationship, phylogeny and evolution. The development and arrangement of flower cluster in *Amaranthus* were analysed microscopically and discussed in detail. A simple provisional key was developed for identification of the eight species as the existing keys were complex and confusing. The eight species were also evaluated for their photoperiodic requirements and antinutritional factors.

Meiotic studies revealed that members of section Blitopsis had x = 17 while section Amaranthus had x = 16 and 17. *A. dubius* a polyploid with n = 32 behaved as an allopolyploid. Mean number of chiasmata/bivalent was maximum in the cultivated species and minimum in the semiwild species, *A. spinosus* which also had maximum number of rod bivalents. Pollen grains of varying sizes (Macro and medium) were observed in all the species. The cultivated species were characterised by bigger chromosomes and pollen grains.

Interspecific hybridization was attempted in all possible combinations but many of the crosses failed. A few failed crosses exhibited seedling mortality. Seven interspecific hybrids grew and flowered and these included hybrids within and between sections Blitopsis and Amaranthus. *A. lividus* x *A. tricolor* the only interspecific hybrid within Blitopsis resembled mostly the male parent but was highly sterile. Cytological studies revealed the presence of two or more interchanges involving 3-6 chromosomes

and subsequent meiotic abnormalities resulted in 90% pollen sterility. The interspecific hybrids within section Amaranthus included *A. spinosus* x *A. dubius* *A. spinosus* x *A. hypochondriacus*, *A. spinosus* x *A. caudatus*, *A. spinosus* x *A. cruentus*, and *A. cruentus* x *A. caudatus*. The hybrid *A. cruentus* x *A. caudatus* produced only female flowers in the stunted and deformed hybrid plant. Other hybrids exhibited a preponderance of *A. spinosus* characters indicated by presence of spines, reduction in inflorescence size, distinct placement of male and female flowers etc. Cytological studies revealed the presence of 1-2 multivalents including 3-4 chromosomes, moderate frequency of bivalents, and a low frequency of univalents in the hybrids. Chromosomal repatterning through translocations and inversions are also involved in speciation within both sections Blitopsis and Amaranthus.

A. spinosus x *A. viridis*, the first success of hybridization between the two sections resulted in a short and sturdy hybrid plant with dominating *A. spinosus* characters. Cytological studies revealed that PMCs had high frequency of bivalents and only low frequency of univalents. The complete absence of multivalents indicated that cryptic structural changes and genetic drift are only involved in the cytogenetic differentiation of the two species. D^2 analysis of data of chromosome associations in interspecific hybrids at metaphase I indicate the clustering of hybrids within and between sections under the same group. This as well as morphological studies questions the validity of the naturalness of the two sections in *Amaranthus*.

The 40 accessions available in the germplasm were classified into different species based on detailed cytomorphological studies. Twenty one species were ascribed to *A. tricolor*, four to *A. dubius* and 15 to *A. hypochondriacus*. The reasons for low seed recovery in the *A.tricolor* accession A_6 were also studied.

Investigations on photoperiodic response of the different species indicated that there is precocious flowering with increase in photoperiod in *A. hypochondriacus*, *A. dubius* and *A. spinosus*. Flowering was delayed with more light in *A. caudatus* and *A. tricolor* while *A. cruentus* and *A. viridis* were photoinsensitive. The content of antinutrient factors in the tender leaf and stem varied from 3.60 to 5.10% for oxalate and 0.295 to 0.695% for nitrate in the different species. In general Blitopsis had higher content of antinutrients than section Amaranthus, the only exception being *A. spinosus* of section Amaranthus.

The primitiveness of *A. spinosus* was evident from the present studies. This pantropical cosmopolitan weed has played a major role in the evolution of other *Amaranthus* spp. Evidences were also obtained on the origin of vegetable amaranths from grain amaranths.