CHAPTER - 2

REVIEW OF EARLIER WORK

REVIEW OF EARLIER WORK

Though grain and vegetable amaranths were in use as edibles a long since in human history, scientific investigation in this group of economically important plants started rather very late. As Feine et al. (1979) write "Amaranth has received increased scientific attention in recent years as we have searched for 'by passed' crops having potential for broadening man's food base". Therefore Sauer (1950) rightly remarks the history of amaranth as neglected and obscure.

While survey and taxonomic studies in the family Amarnthaceae in general and in the genus Amaranthus L. in particular was initiated in the early part of this century, chromosomal studies in this group was taken up only in early 1930's. The progress initially was slow but round about 1960, when more detailed chromosomal studies were carried out. Attempts were soon made to apply some of the cytogenetic techniques for the improvement in grain and vegetable production. Advantage of Amaranthus L. as a tool for genetic research was soon realized because, it could grow in a small space due to it's plastic morphology, it's ability to produce as many as six generations per year and it's capacity for both self and cross pollination with no marked inbreeding depression or heterosis (Feine et al.,1979). For these reasons, relatively rich literatures could gather on amaranth genetics in recent years primarily on three subject areas such as (i) interspecific hybridization, (ii) Sex-expression and (iii) induced polyploidy. However, Sauer (1950, 1955, 1957, 1967) developed keen interest in this group and made very large scale survey which provided many basic information for cytogenetic investigation.

2:1. Taxonomic treatment

The genus Amaranthus L. belonging to the family Amaranthacea (order Caryophyllales) is taxonomically a difficult group. Sauer (1967) attributed the taxonomic problems to

> " the hopeless attempts to recognise by pigmentation which segregates within populations and growth which is extremely plastic under different day lengths and other environmental variables ".

However, attempts on taxonomic classification have been made by Thellung (1914), Standley (1917), Schinz (1934), Kowal (1954) and Sauer (1950, 1955, 1957, 1967) .

Basing on floral structure in various species of Amaranthus L., Thellung (1914) and Schinz (1934) had recognised two sections viz. Amaranthotypus Dumort and Blitopsis Dumort. From the study of morphology and anatomy of twenty one species, Kowal (1954) suggested the transfer of A.spinosus L. from section Amaranthotypus to the section Blitopsis and created a new section Puncticulatae.

However, Sauer (1967) replaced the section Amaranthotypus Dumort by section Amaranthus Sauer as per the present rule of nomenclature which states that the section must bear the same name as the genus since it includes the type species A.Caudatus L. (Sauer, 1967).

Although, all taxonomists recognised the two sections, there had been considerable disagreement regarding the number of species within the genus. Schinz , (1934) reported forty five species for the genus Amaranthus L. while Aellen (1961) recognised one hundred species. But Santapau and Henry (1973) and

Townsend (1974) reported about fifty species of the genus in the world flora.

2:2. Chromosomal analysis

Determination of chromosome numbers in various species of Amaranthus L. had been carried out since long. In view of the small chromosome size very limited attempt had been made for detailed Karyotypic analysis. While sporadic chromosome reports in few species were made by Kihara et al. (1931), Takagi (1933), Murray (1940a), Covas and Schnack (1946), Heiser and Whitaker (1948), Gardenas and Hawkes (1948) and Krishnaswamy and Raman (1949), more detailed account was given by Grant (1959 a) , Sharma and Banik (1965), Tandon and Tawakley (1970), Behera and Patnaik (1974) and Madhusoodanan and Pal (1981) involving many species of the genus Amaranthus L. .

Grant (1959 a) reported chromosome numbers in thirty species of Amaranthus L., of which chromosome numbers for eight species were recorded by him for the first time. He studied a number of biotypes in many species from different regions and obtained uniform results for each . Except A. dubius Mart. ex Thell. (2n = 4x = 64), he observed somatic chromosome numbers to be either 32 or 34 for different species of the genus. Grant also recorded that large number and small size of Amaranthus chromosomes coupled with relative uniformity in morphology did not make the species suitable for a karyological analysis. Therefore, it could not be possible to detect any particular chromosome which was added or lost from a species complement. However, a pair of satellited chromosomes could be detected for a number of species which he considered to be the characteristic of the genus. He further concluded that natural interspecific

hybridization and aneuploidy were major sosurces for species variation, speciation and taxonomic complexity in the genus.

Twenty species belonging to nine genera of Amaranthaceae were cytologically investigated by Sharma and Banik (1965), of which chromososme numbers of eight species and one variety were repeorted for the genus Amaraanthus L., all characterised by 2n = 34. They made detailed karyotypic analysis in these species and concluded that the species could be diferentiated from each other with details of karyotype such as number and type of secondary constrictions and satellites. Meiotic study was done in few species.

Tandon and Tawakley (1970) reported chromosome numbers in sixteen species of the genus. Some of their findings contradicted the previous reports.

Gill and Vasudeva (1970) reported chromosome numbers in ten species of the family Amaranthaceae from north-west region of India, out of which chromosome numbers of one species of the genus Amaranthus L. was reported. They found natural polyploidy, aneuploidy and B-chromosomes in some members of the family.

Morphological and cytological studies were carried out by Desai (1971) in both diploid and tetraploid forms of A. Polygamus L. along with Digera arvensis and Celosia argentea of the family Amaranthaceae. He found tetraploid plants to be quite distinct and diploids to be in two different morphological types. The meiotic analysis in both diploid and tetraploid forms revealed the chromosome number to be n = 17 and n = 34 respectively. By veryfying previous works, he concluded that in Amaranthus L. 8 or 17 is the basis chromososme set from which different series have been evolved.

Mitra (1971) reported chromososme numbers in ten species under six genera of the family Amaranthaceae, out of which three were of the species of Amaranthus L. She studied the Karyotypes and observed homogeneity in the chromososmes of various members.

Behera and Patniak (1974) cytologically analysed twenty three species belonging to nine genera of the family, while chromosome numbers of six species were their first report, the chromosome numbers in two species were different than those reported earlier. They recorded chromosome number of nine species in Amaranthus L. . The chromosome size was found to be smaller in the genus Amaranthus L. than other members of the family. They also found larger size of cells , chromosomes and nuclei in tetraploid species.

Madhusoodanan and Pal (1981) made morphological and cytological investigations in five species of vegetable amaranths (Section Blitopsis). They found these species to be uniformly diploid with either n = 16 or n = 17, the latter being more common. Cultivated species were found to have relatively lower chiasma frequency to that of the wild species.

Murray (1940a)studied the genetics of sex-determination in dioecious Amaranthus and from genetic evidences, he considered A. tamariscinus Nutt. as heterogametic in sex. He found out no change in sex even after doubling the chromosome numbers but in next generation the progeny was containing more abundance of males. The excess of males in second generation has been explained on the basis of high percentage of X-X and Y-Y synapsis of the XXYY males . The high percentage of XY gametes resulted in many XXXY plants, which were males. On the other hand, the tetraploids of two monoecious species were fertile and exhibited no change in sex ratio. Cytological aspect of sex-determination in dioecious

species of the genus was worked by Grant (1959b). He, through cytological analysis, observed the absence of heteromorphic chromosomes associated with sex in these dioecious species. As he found two different haploid chromosome numbers (i.e. n = 16, n = 17) in both monoecious and dioecious species, he emphasised that aneuploid condition arose early in Amaranthus, while dioecious habit had been a secondary phenomenon.

Pal (1971) found a polyhaploid plant of A. dubius in a population of A. dubius. It was completely sterile but resembled the red A. dubius in essential features. But the chromosome number of this plant was found to be 2n = 32. The occurrence of a diploid chromosome number in a plant of a tetraploid plant population suggested it to be a haploid. All vegetative and floral parts of the polyhaploid were found to be smaller in size than normal A. dubius tetraploid although there was no difference quantitatively. The average associations of bivalents and univalents per cell were found to be 2.24 II + 27.52 I.

Pal (1972) made morphological and cytological analysis in two sub species or varieties viz. A.graecizans sbsp.graecizans and A. graecizans sbsp. silvestris of the genus Amaranthus. He studied their morphology and cytology from large number of progenies raised from the seeds collected from different sources. He found these two sub species to be quite different in their morphology and chromosome number. Hence he suggested these two sub species to be two distinct species viz. A. graecizanes Linn. and A. silvestris Vill.

2:3. Inter specific hybridization

Many species of the genus Amaranthus have long been suspected to have arisen through interspecific hybridization in nature (Thellung, 1928 ; Priszter, 1949; Grant 1959C ; *Pal and*

Khoshoo, 1965, 1972, 1973, 1982; Behera and Patnaik, 1982) involving two or sometimes three species. Inter specific hybridization involving nearly half of the species of the genus has been attempted for genetic improvement in raising good agronomic types which met with failure.

Grant (1959c) studied the cytology of A.spinosus L., A.dubius Mart, ex Thell. and the plants which were highly sterile and somewhat relating to both the species. These turned out to be triploid hybrids between the two species. Due to frequent occurrence of the triploid hybrids in the vicinity of these species and typical pairing behaviour of chromosomes at metaphase-I in them, Grant conjectured A.spinosus to be one of the parents of A.dubius. However the other parent could not be determined with certainty.

While raising various species from open-pollinated seeds, Khanna et al. (1960) detected natural interspecific hybrids among a number of species of Amaranthus. However all these hybrids were found to be sterile and there was no seed setting in any of the hybrids though all hybrids exhibited distinct hybrid vigour. They suggested the sterile nature of these hybrids is due to independent phylogenetic relationship of the parent species. However, there was no cytological test as to the authentication of these hybrids.

Pal and Khoshoo (1965), by crossing diploid A.spinosus with tetraploid A.dubius raised F_1 triploids and subsequently converted them into hexaploids through colchicine treatment , tried to trace the origin of A.dubius. Since the natural hybrids of these two plants were formed whenever and wherever these two species existed in close proximity and the hybrids were also produced in articificial crosses, these two workers held the view

that the only naturally existing tetraploid, A.dubius might be originated through allopolyploidy based on 16+16 rather than 16+17 or 17+17 chromosomes followed by aneuploidy. They did not agree to the views of Grant (1959c).

Pal (1972 b), studying the chromosome associations in triploid hybrids and their hexaploids, did not consider a particular type of chromosome pairing in the triploid hybrids to be indicative enough for demonstrating the genetic homology between the two species. Rather lack of distinctive flower arrangement in A.spinosus and absence of dominant characters like spinosity in A.dubius were the evidences which were cited against the postulation that A.spinosus is one of the progenitors of A.dubius.

A natural population of interspecific hybrids between diploid A.spinosus and tetraploid A.dubius was studied by Tandon and Tawakley (1971). They observed cytologically a heterogeneous population of these hybrids ranging in chromosome numbers from 2n = 50 to 2n = 330. From morphological and cytological studies, they considered these hybrid populations not to represent the F_1 generation but the segregants of introgressive hybridization of the hybrid with A.spinosus.

Through the study of A.spinosus X A.dubius hybrids alongwith their parents, Behera and Patnaik (1982) made detailed genome analysis of A.dubius. They analysed cytologically triploid hybrids, hexaploids derived through colchicine treatment and certain deviant hexaploids obtained through subsequent generations. Various types of chromosomal associations ranging from 22 II + 5 I to 4 IV + 9 II + 15 I with high frequency of 17 II + 15 I were observed by them, from which they concluded that a

set of chromosomes genetically comparable to those of A.spinosus is present within A.dubius genome.

During extensive hybridization programme, Pal and Khoshoo (1972) raised ten experimental hybrids involving eight species of Amaranthus. They observed hybrid inviability, weakness and sterility ranging from probable endosperm malfunction, seedling mortality, stunted and deformed plants with tumorous stems and roots, virus like syndrome in leaves to deformed and malformed flowers and finally to pollen and ovule sterility. On the basis of these observations, they considered the belief that "there are no barriers to crossability in the genus and that hybridization is the major factor in initiating variation and promoting speciation" rather exaggeration.

For analysing cytogenetic relationships in grain amaranths, Pal and Koshoo (1973 a) studied four domesticated species, two ancestral weed species and eight of their hybrids. The parents were found to have normal meiosis and pollen and seed fertility. However, they found the parents with n = 16 and n = 17 failed totally to produce the hybrids. Some hybrids involving parents with n = 16 could not proceed beyond two leaf stage while others showed vigorous F_1 plants with good meiotic pairing associated with a reasonable amount of differentiation in chromosomes leading to 25-55 % pollen fertility and 49 - 66 % threshable seeds. They also found different degrees of recombination of characters in F_2. Amphidiploids from F_1 hybrids showed typical autoploid or segmental alloploid type of meiosis indicating that the parental chromosomes are quite homologous. From their experimental evidences and possible parallel mutations in the parent species, they held the view that it was not certain whether natural hybridization particularly introgressions could be taken as evidence for or against the two hypotheses proposed

by Sauer (1967). However they further stated categorically that A. caudatus had given rise to A. edulis and finally they had the view that what ever may be the origin of grain types, at present they exist only in cultivation.

For analysing cytogenetic relationships in vegetable amaranths, Pal and Khoshoo (1973 b) studied the cytology of three interspecific hybrids involving vegetable species. Meiotic studies revealed that the cytogenetic differentiation between the parents was chiefly due to interchanges and paracentric inversions. The interchange complexes involved four to fourteen chromosomes indicating that the parents differ from each other in one to six interchanges. Due to small size of the chromosome, the crossing over in interchanges was restricted. The preferential pairing and the restoration of fertility in amphidiploids confirmed that the interchanged segments are small and sterility in the hybrids is entirely chromosomal.

In view of analysing the cytogenetic relationship between the two basic chromosome numbers (n = 16 and n = 17) in the grain group of Amaranthus, Pal and Khoshoo (1982) made an interspecific reciprocal cross involving white seeded green A. hypochondriacus (n = 16) and black seeded wild A. hybridus (n = 17) . They got hybrid seeds when A. hybridus was taken as female. The F_1 hybrids exhibited heterotic effects in vegetative characters. Metaphase I of F_1 hybrids exhibited 98 % of pollen mother cells with 15 II + 1 III and remaining cells with 16 II + 1 I . The anaphase I showed an unequal segregation of 16 and 17 chromosomes. Chromosome counts in 55 hybrid plants revealed the distribution of plants with 2n = 32, 33 and 34 chromosomes in the ratio of 1:2:1 respectively. They further suggested the origin of n = 17 from n = 16 through Primary trisomy.

2:4. Induction of Polyploidy

Induction of Polyploidy has been attempted in very few species of Amaranthus L. . The first attempt of inducing tetraploidy was made by Murray (1940b) in two monoecious species viz. A.caudatus L. and A. hybridus L. and one dioecious species viz. A.tamariscinus Nutt.. He compared the morphology of raw tetraploid plants with those of the diploids in case of monoecious species. But in diocious species, his chief interest was to induce autotetraploidy to study sex-expression at higher chromosomal level of the male and female plants.

Tandon and Chinoy (1950) induced tetraploidy in A.blitum L. . They observed larger stomata, Less number of stomata per unit area, bigger pollen grains, late flowering and maturity, bigger seeds with delayed germination, Larger and darker green leaves, more number of leaves per plant and prolonged period of vegetative growth in tetraploid plants.

Through colchicine treatment, Pal and Khoshoo (1968) raised autotetraploids in A.edulis speg.. Studying the raw tetraploid plants of this species through C_o, C_1 and C_2 generations , they observed many features which may make this crop successful at the tetraploid level. The small chromosomes, low chiasma frequency, high degree of cross pollination and heterozygousity were visualised to be the causes of low quadrivalent frequency and high initial fertility in these tetraploids. The appearance of some predominantly male plants in C_1 and C_2 generations was considered by them due to disturbance in genic balance in sex-mechanism caused by autopolyploidy. They further suggested a rigorous programme of stabilizing this plant at tetraploid level for better agro-economic use.

Behera, et al. (1974) made histological analysis of colchicine induced deformities and cytochimeras in A. caudatus and A. dubius. Histological analysis of shoot apices and leaves of the induced plants revealed the inhibition of shoot growth with the loss of apical dominance and abnormal growth in mesophyll and epidermal cells of the leaves. They further suggested that colchicine either interferes with the normal auxin activity or acts as a repressor for the production of enzymes responsible for normal growth. They observed different types of cytochimeras in the germ layers of the meristems. Inducing polyploidy in diploid species, A. hypochondriacus and tetraploid species, A. dubius.

Behera and Patnaik (1975 a) tried to compare the morphology and cytology at tetraploid and octaploid levels. They found various morphological changes in colchicine treated plants. Meiotic analysis revealed varied proportions of hexavalents, quadrivalents, bivalents and univalents in polyploid cells with other cytological abnormalities like uneven separation of chromosomes and laggards. While the percentage of induction of polyploidy in A. dubius was found to be less than that of A. hypochondriacus, the higher frequency of multivalent association and greater meiotic irregularity were marked in polyploids of A. dubius, which accounted for greater sterility in octaploid.

Behera (1975) induced tetraploidy in three varieties viz. f.2 (red), f.3 (hort), f.4 of A. tricolor L. and made a comparative study of diploids and autotetraploids at morphological, biochemical and cytological levels up to C_1 generation. Morphological observation revealed pronounced enhancement in characters like lamina, stomata and Pollen. Similarly the protein and sugar contents of the leaves of autotetraploids were found to be significantly high compared to their diploid counterpart. From cytological analysis, she found highest percentage of polyploid induction in A. tricolor L. f.4. Meiotic analysis revealed varied

proportions of quadrivalents and bivalents at C_0 and C_1 generations. She studied this autotetraploid up to C_4 generation and observed gradual increase in bivalent formation.

Madhusoodanan and Pal (1983) induced tetraploidy in three varieties viz. AV-5, AV-12, AV-18 of A.tricolor L. and studied the morphology and cytology of the respective tetraploids at C_0, C_1 and C_2 generations. When compared to the diploids, the tetraploids of these varieties were dwarfer, sturdier with less branches. Leaves were broad and deep green in colour with larger size of stomata and guard cells. Inflorescences were shorter and there was late flowering and maturity. Meiotic analysis revealed varying number of quadrivalents, trivalents, bivalents and univalents with low frequency of trivalents and univalents. They further observed a tendency towards increase bivalency in C_1 and C_2 generations.

Madhusoodanan and Pal (1984) further raised autotriploids in two varieties viz. AV-5 and AV-12 of A.tricolor through reciprocal crosses between C_1 autotetraploids and their respective diploids. Triploids exhibited gigantism in their morphological characters. The meiotic analysis revealed the most frequent chromosome association of 11 III, 6 II and 6 I at metaphase-I. Similarly the anaphase-I was highly irregular with unequal distribution of chromosomes to the poles with lagging univalents which led to the reduction of fertility.

Pal and Pandey (1982) made morphological and cytological investigations in autotetraploids of A.caudatus and A.edulis for 10 generations. They found no significant difference in morphological characters in the plants of C_{10} generation from those of C_0 and C_1 generations. The plants maintained the same gigantism in determinate parts. However, they found the mean

quadrivalent frequency at metaphase-I varying from 1.600 to 3.266 in A.caudatus and 1.400 to 2.33 in A.edulis in C_{10} generation whereas the mean quadrivalent frequencies for C_0 generation in two species were 6.800± 0.29 and 6.00± 0.27 respectively. Thus they observed a reduction in the frequency of quadrivalents in the plants of C_{10} generation and 20 % of chromosomes were found to be involved in quadrivalent formation in C_{10} generation.

2:5. Induction of mutation and genetic analysis.

There has not been much work on mutation in the genus Amaranthus L. so there is very less information about induced mutation in the genus. Behera and Patnaik (1975) were probably the first to make initial attempt to induce mutation through EMS in A.tricolor (red). They studied the percentage of germination of seeds in different concentrations EMS and number of mutants from the treated seedlings. Out of five mutant plants, only four could survive at maturity which were completely green though red colour appeared later in the development. They found a gradual decrease in percentage of anthocyanin at M_1, M_2 and M_3 generations. Their cytological analysis revealed multivalents in all mutants with high frequency.

Behera and Patnaik (1979) induced mutation in A.hypochondriacus through physical and chemical mutagens. Using different concentrations of EMS and DES and various doses of gammarays, they studied the various morphological peculiarities starting from seedling stage to the stage of maturity of the mutants in M_1 and M_2 generations. They found remarkable fascinations in the inflorescence of these mutants. Behera and Patnaik (1982) made histological analysis of these mutants in M_3 and M_4 generations specifically to correlate the fasciated floral axis and the leaf curls in mutants. They found out flattening and

enlargement of the growing points. They also observed the bulging of leaf surface due to irregular development of lamellar vascular bundles and enlargement of adjacement tissues in curled region.

Behera and Patnaik (1981) made an attempt to induce mutation in tetraploid species, A.dubius using different concentrations of EMS and studied the morphological and cytological peculiarities at M_1, M_2 and M_3 generations. They observed a gradual chlorophyll deficiency in different generations. Meiotic analysis revealed a frequent occurrence of chromosome clumping and a single quadrivalent in M_1 generation. They further detected four trisomics in M_2 generation. All these trisomic revealed meiotically similar chromosome association of 32 II + 1 I in much higher frequency than those of 31 II + 1 III . The trivalents exhibited either chain, Y or frying Pan configuration. There was no occurrence of pentavalent or quadrivalent in any of the cells of these trisomics.

Walton (1968 a) emphasised the genus Amaranthus as one of the best plant materials for genetic studies which can overcome the difficulties arising in Drosophila melanogaster in genetic studies except the low chromosome number. It's easy culture, vigour of growth, small seeds, easy germination, good response to photoperiod, unisexual flowers simplifying the process of emasculation, monoecious and dioecious members of the genus facilitating the study of two types of breeding systems under identical genetic background and capability of both self and cross pollination without showing a marked degree of either heterosis or inbreeding depression were considered by him to be the important characters for the genetic studies. Walton (1968 b) further analysed the breeding behaviour in commercial Gossypium sp and A.caudatus and reported the similar breeding behaviour of these two groups of plants.

Basing on the studies of cytogenetic mechanism, the breeding system and morphological characters in sixteen species, thirteen interspecific hybrids and four amphidiploids of the genus Amaranthus, Koshoo and Pal (1972) pointed out that in section Amaranthus species differentiation was mainly due to gene differences and cryptic structural hybridity resulting in bivalent pairing and varying degree of sterility in the interspecific hybrids whose amphidiploids showed autoploid or segmental alloploid characters where as in section Blitopsis interspecific hybrids showed interchange heterozygousity involving long segments of 4 to 14 chromosomes with total sterility but restoration of fertility in amphidiploids. They justified the two sections to be natural from these patterns and the lack of hybrids between them.

For phenotypic and genotypic correlations in Amaranthus, Pandey (1981) studied six varieties and their F_1 and F_2 hybrids in A.hypochondriacus. He found grain yield showing positive association with number of panicles per plant, length of panicle and plant height in all generations. Among yield components, number of panicles/ plant X plant height , number of panicles / plant x panicle length and panicle length x plant height exhibited positive associations. Thus he suggested the yield in A.hypochondriacus should be through the selection for these traits.

Jain et al. (1982) estimated crossing rate in several collections of grain amaranths using three sets of data. The first involved a red-green seedling colour locus (R,r). Progenies of recessive mothers (rr) grown at Davis gave a wide range of values (mean rate of outcrossing 31 %, SE = 25 %). Plants collected from India showed 3.5 % to 14 % outcrossing at locus R/r. Using two allozyme loci, plants collected from India and South America gave 3 % to 25 % outcrossing rate with

significant interpopulation variation. They further emphasised that this level of variation in breeding system of grain amaranths will help in further studies.

Kulakow et al. (1985) made Mendelian analysis of six morphological traits in three grain species viz. A.cruentus, A.hypochondriacus and A.caudatus. They found seed coat colour in A.hypochondriacus and A.caudatus controlled by two loci giving a 12:3:1 segregation ratio for black-yellow pale and blackbrown-pale colour classes respectively. A leaf spot trait segregated as a single dominant gene while leaf 'V"-mark trait segregated by two dominant complementary epistatic genes. Three loci were described by them for pigments on plant parts, red seedling colour by diallelic locus and betacyanin by single dominant gene. The multiple homologous loci controlling seed coat colour supported monophyletic evolution of pale seededness in the domesticated grain amaranths.

Peters and Jain (1987) discovered the male sterility in four Indian accessions of A.hypochondriacus. Analysing the segregation patterns in F_2 generation of numerous crosses involving male sterile plants, they suggested gene-cytoplasmic mode of inheritance of male sterility. Segregation ratios provided evidence for one or two restorer nuclear genes in different populations. Cytological studies showed male sterility to be associated with abnormal tapetal cell functioning and microsporogenesis failure prior to the first metaphase leading to abortive anthers.

Kulakow (1987) studied the genetic control of four developmental characters in A.caudatus. He found one recessive gene responsible for determinant panicle growth, two major genes

governing panicle orientation with erect panicles incompletely dominant to drooping panicles. He further detected additional modifier genes altering the orientation of panicle, a single recessive gene determining dwarfism and two complementary epistatic genes for pink embryo colour.

2:6. Tissue and protoplast culture.

Plant tissue and protoplast culture technique is a new development but it's application is already a reality. Besides being successful in micropropagation of ornamentals, fruit trees, crops and forest species, it provides shortcuts to the time consuming efforts of screening species populations for traits. A lot of plants have been introduced to *in vitro* studies for different aspects. Unfortunately available information of *in virto* studies in the genus *Amaranthus* L. is very little.

As the grain and vegetable amaranths offer more genetic variability than most major cereal crops and the germplasm collection represents a vast source of material for *in vitro* studies, Flores and Teutonico (1986) emphasized that complementation of tissue culture technique with conventional breeding practice might result in a powerful combination that could enhance the reintroduction of this under developed crop in tropical areas. Thus they carried out *in vitro* studies in *A cruentus* L., *A.hypochondriacus* L. and *A. tricolor* L.. Explants like primary leaves and hypocotyl were tried in B5 solid medium with various combinations of 2,4-D, N A A, B A, Kinetin and Zeatin, Embryoids like structures were formed which on further growth resulted in the formation of friable callus. They further attempted to grow these embryoids in liquid medium with various

combinations of N A A, 2,4-D and coconut water and observed no further development. However they obtained fast growing cell suspensions of grain amaranths.

In an attempt to regenerate, they observed while cytokinin in the B5 solid medium was necessary for callus formation, presence of N A A and zeatin resulted in the formation of shoot from the callus surface. These on subsequent transfer to B5 or MS produced roots and were grown to maturity producing few seeds in both A.cruentus L. and A.hypochondriacus L.

They also made a preliminary attempt for protoplast isolation and culture and encountered low yield of protoplast with usual enzymes available. They observed cell wall regeneration and few cell divisions in protoplast cultures in both vegetable and grain species.

Reviewing the biochemical works of various authors on vegetable amaranths, they indicated that the oxalate content in the leaves which has direct corelation to nitrate accumulation resulting in low palatability, could be altered through cell suspension and screening and therefore, oxalate free plants could be obtained through in vitro culture.

Rina Das et al. (1987) carried out in vitro studies in A.paniculatus L. for finding out the biochemistry of cell prolifiration in callus and regeneration of plantlets. They obnserved enzyme glyoxalase I involved in cell prolifiration and also tested the response of inhibitors on the callus growth. They established callus cultures in A.caudatus L. and A.hybridus L. and regenerated plants upto flowering in B5 medium from the callus obtained from hypocotyl segment of A.paniculatus L. and suggested that Amaranthus could be made amenable to culture condition.