Induced Polyploidy in *Amaranthus hypochondriacus* L. and *Amaranthus dubius* Mart. ex Thell.

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Induction of polyploidy by experimental methods has been proved to be a handy tool in tampering with the genotype of the organisms and in widening the amplitude of variation in the gene pool. Though various methods of induction of polyploidy have been developed, induction through the application of colchicine has been met with remarkable success especially with many herbaceous plants.

As a part of the programme of induction of polyploidy in various species of *Amaranthaceae* two species viz. *Amaranthus hypochondriacus* L. and *Amaranthus dubius* Mart. ex Thell. were induced for polyploidy. As the vegetative parts such as the leaf and stem of various species of *Amaranthus* L. are edible and autopolyploidy very often magnifies the vegetative characters, this programme of work has been undertaken to evaluate whether such an effort will be of economic value and whether some improved varieties of this crop can be raised.

Attempts to induce polyploidy in some species of *Amaranthus* L. had been made in the past. Murray (1940) induced tetraploidy in some monoecious and dioecious species of *Amaranthus* L. Tandon and Chinoy (1950) also made effort to induce tetraploidy in *A. blitum* L. Recently Pal and Khoshoo (1968) induced tetraploidy in *A. edulis* Speg. and have made detail analysis of the tetraploids through C_0 and C_1 generations.

About 10 species of *Amaranthus* L. grow throughout India, almost all of which are edible. The reports of the induction of polyploidy in this genus seem to be fragmentary. With a view to have wide scale induction of polyploidy in this genus the present work is undertaken.

Materials and methods

The seeds of *Amaranthus hypochondriacus* were obtained from the state Agriculture Department and those of *A. dubius* were procured from the local gardens.

Two different types of colchicine treatments were adopted i.e. i) seed treatment and ii) seedling treatment. The different concentrations of colchicine used in both the treatments were 0.1, 0.2, 0.3, 0.4 percent of aqueous solution.

i) *Seed treatment*: The seeds were soaked in aqueous solutions of colchicine of different concentrations in petridishes for six hours. A set was maintained as control by soaking the seeds in distilled water. After soaking, the seeds were thoroughly washed in tap water and were sown in flat earthenware pots as well as in the field. The results of such treatment were not promising because very few seeds germinated.

Direct planting in the field was a total failure, where as planting in pots though resulted few seedlings, their growth were sufficiently checked for further plantation in the field.

ii) *Seedling treatment*: The treatment with colchicine in seedling stage was found to be very successful. The seeds were sown in the field in rows. After the seedlings came out, at four leaf stage (two cotyledonary leaves and two foliage leaves) little rearrangements in planting was made to maintain the seedlings in rows. The seedlings were thinned out by removing some of them. After 3–4 days of this rearrangements, when the seedlings were stout, colchicine treatments were given. Round cotton plugs were soaked in different concentrations of colchicine solution and were placed on the shoot apex where the two vegetative leaves supported the plug. The solutions were being dropped through glass jets at regular intervals. Some seedlings were given treatment for six hours continuously while others were given twelve continuous hours of treatment. After the duration of treatment the cotton plugs were removed.

As there were certain conspicuous morphological changes in the leaves and stems, the leaves and shoot apices from treated and controlled plants were fixed and processed for studying histological changes. The findings of this study is being analysed.¹

For the meiotic analysis, the flower buds were fixed in acetic-alcohol (1:3) generally during 9 A. M. to 12 noon and were kept for overnight which were transferred to 70% alcohol next day. During summer months cytoplasm was found to take intense staining which was reduced by maintaining the fixings in freeze.

Plant height and leaf measurements were taken at certain intervals. Pollen grains were stained in glycerine and aceto-carmin mixture (1:1) and were measured using micrometer scale. Stomata measurements were done taking smearings from the leaf surface with the help of the quickfix.

Observation

Plants after 5–6 days of treatment showed definite symptoms on the leaves. The treated plants of all concentrations were found to be characterised by mottled leaf appearance simulating the syndrome of certain viral diseases. Subsequently there are other abnormal features such as leaf rosette at the terminal region, elongation of the petiole and retardation in the height of the plants etc. in all concentrations. The flowering is also considerably delayed in treated plants than that of the control (Table 1). Very acute mottling and stunted growth of the plants were noticed in the treatment of higher concentrations of colchicine (Figs. 1 and 2).

There were, however, certain features of differences as regards these morphological changes in the initial phase in the two species. While in *A. hypochondriacus* the lamina growth was uniform with uniform mottling, in *A. dubius* the mottling was not uniform and the leaves were more or less shoe-tongue shaped. Another interesting feature was met with in *A. dubius* that while ordinarily it is characterised by a single terminal shoot, after treatment with colchicine, invariably a lateral shoot with

¹ The detail account has now been published in The Journal of Heredity 65: 179–184, 1974.

Table 1. Comparative morphological data of normal and colchicine treated plants of *A. hypochondriacus* and *A. dubius* after 15 days of treatment

Conc. in % aqueous solution	Duration of treatment in hours	<i>A. hypochondriacus</i>				<i>A. dubius</i>			
		$\frac{p}{p+1}$ in cm	% of flowering	Height in cm	Height at maturity in cm	$\frac{p}{p+1}$ in cm	% of flowering	Height in cm	Height at maturity in cm
0.1	6	4.3/9.6	33.3	14.6	68.5	5.4/12.8	—	13.5	103.0
	12	4.2/9.1	27.0	11.5	67.5	4.2/ 9.4	—	12.0	100.0
0.2	6	4.2/8.9	13.0	13.2	68.0	5.3/12.6	—	10.0	103.5
	12	2.5/5.4	—	5.4	69.0	6.1/14.5	—	5.5	99.5
0.3	6	4.0/9.2	9.6	11.3	67.0	3.0/ 9.1	—	7.0	101.0
	12	3.2/6.3	—	7.0	65.5	2.8/7.7	—	4.4	100.5
0.4	6	4.5/8.6	9.6	9.0	66.0	4.4/10.0	—	6.0	98.0
	12	4.0/7.6	1.9	8.0	65.5	3.8/ 9.4	—	4.5	98.0
Control	—	2.1/7.3	35.6	15.0	68.0	5.7/14.9	—	23.5	104.0

p: petiole length, l: lamina length.

Figs. 1-2. 1, control and treated plants of *A. dubius* after 15 days of treatment with different concentrations of colchicine. a. control, b. 0.1%, c. 0.2%, d. 0.3%. The right end. 0.4%. 2, treated and control plants of *A. hypochondriacus* after 15 days of treatment with different concentrations of colchicine. a. 0.1%, b. 0.2%, c. 0.3%, d. 0.4%, The right end, control.

Figs. 3-4. 3, plants of *A. hypochondriacus* at maturity. a, diploid, b, whole tetraploid. c, mixoploid with central tetraploid (arrow) branch and 3 lateral diploid branches. 4, plants of *A. dubius* at maturity. a, induced octoploid. b, normal tetraploid.

normal form of stem and leaf arises from the side consequent upon the retardation and deformation of the main shoot due to treatment. This lateral branch is more vigorous in growth and overtops the main deformed shoot (Fig. 1 c). This feature was not found in *A. hypochondriacus*. The most interesting feature of these deformities or mottling is that on subsequent growth towards the flowering phase there is a great deal of recovery in all aspects of deformities. The initial retardation or stunted growth was gained over and mottling to a very great extent disappeared towards the late phase. This is evident by the comparison of the diploid and polyploid plants at the mature stage (Figs. 3 and 4).

Fig. 5. Pollens, stomata and nucleoli of *A. hypochondriacus*. a, pollens of diploid. b, stomata of diploid. c, pollens of tetraploid. d, stomata of tetraploid. e, multinucleolate condition of a tetraploid cell. f, binucleolate condition of a tetraploid cell.

Cytological analysis were made of all the treated plants and the frequency of induction of polyploidy in the two species in C_0 generation in different concentrations are presented in Table 2. Very rarely plants were total polyploids and mixoploids were rather frequent. This was evident from the analysis of the fixings taken

Fig. 6. Meiotic analysis of the tetraploid (a-c) and the diploid (d) of *A. hypochondriacus*. a, metaphase showing $13^{IV} + 6^{II}$. $\times 2500$. b, metaphase showing 16^{IV} : arrow indicating two quadrivalents very closely overlapping. $\times 2500$. c, anaphase 32-32 separation. $\times 1700$. d, metaphase showing 16^{II} . $\times 2500$.

from the same plant revealing conditions of diploidy and tetraploidy in different branches (Fig. 3 c) and more so when seeds collected from the same plant resulted in larger number of diploid plants and few tetraploid plants in C_1 generation.

The cytological studies show clearly the increase in the cell size as well as nuclear volume. The meiotic analysis of the tetraploids revealed varied proportions of tetravalents and bivalents. Higher levels of multivalents have been recorded in few cases (Table 3).

Table 2. Differential effect of colchicine in inducing polyploidy in *A. hypochondriacus* and *A. dubius*

Conc. in % aqueous solution	<i>A. hypochondriacus</i>				
	No. of plants tested	whole	No. of tetraploids sectorial	total	Percentage
0.1	15	1	1	2	13.3
0.2	25	—	13	13	52.0
0.3	22	3	8	11	50.0
0.4	23	—	8	8	34.8

Conc. in % aqueous solution	<i>A. dubius</i>				
	No. of plants tested	whole	No. of octoploids sectorial	total	Percentage
0.1	11	—	—	—	—
0.2	11	1	—	1	9.09
0.3	10	2	—	2	20.0
0.4	10	1	—	1	10.0

Table 3. Range and mean of different chromosomal associations in *A. hypochondriacus* and *A. dubius*

Association	<i>A. hypochondriacus</i>				<i>A. dubius</i>	
	C_0 generation		C_1 generation		C_0 generation	
	range	mean	range	mean	range	mean
Hexavalent	—	—	—	—	2-4	1.63
Quadrivalent	4-16	9.42	6-13	9.91	6-19	12.45
Bivalent	0-24	12.95	6-20	12.16	14-48	34.20
Univalent	0-2	0.14	—	—	—	—

Besides these, various types of cytological abnormalities were noticed in the induced polyploids as contrast to the normal diploid *A. hypochondriacus* or tetraploid *A. dubius*. Though the anaphase is very often regular in *A. hypochondriacus* with equal separations, it is highly irregular in *A. dubius* due to large number of multivalent associations. In *A. hypochondriacus* sometimes the tetraploid cells showed two or more nucleoli per nucleus. (Fig. 5 e, f). This has, however, not been noticed in *A. dubius*.

Polyspory was found to be a conspicuous feature in autopolyploids with pentads, hexads etc. The range of spore formation from a single PMC has been recorded to be 5–10. There is, however, a considerable difference in the size of the spores

Fig. 7. Meiotic analysis of the octoploid (a–b) and the tetraploid (c) of *A. dubius*. a, metaphase showing $4^{VI} + 19^{IV} + 14^{II}$. $\times 2500$, b, anaphase: irregular separation with univalents and bivalents. $\times 1700$. c, metaphase showing 32^{II} . $\times 2500$.

from a PMC in a polysporic condition.

Pollens, stomata and seeds were found to be larger in size in induced polyploids as compared to those of the natural species (Table 4 and Fig. 5).

Discussion

The cytological analysis of various species of *Amaranthus* (Grant 1959, Behera and Patnaik 1974) revealed two basic numbers viz. $x=16$ and 17. Two species belonging to the basic number of $x=16$, one diploid *Amaranthus hypochondriacus* with $n=16$ and other tetraploid *Amaranthus dubius* with $n=32$ were selected for the present study with a view to have a comparative picture of induction of polyploidy in diploid and tetraploid level. The two species responded well to the treatment of colchicine and showed somewhat similar morphological changes following colchicine treatment, though the frequency of induction of polyploidy was found to be much higher in *A. hypochondriacus* than in *A. dubius*. The higher level

Table 4. Comparison of pollen, seed and stomata of natural and autopolyploid plants of *A. hypochondriacus* and *A. dubius*

<i>A. hypochondriacus</i>			<i>A. dubius</i>		
Character	Diploid	Tetraploid	Character	Tetraploid	Octoploid
Pollen			Pollen		
fertility (%)	93.0	76.5	fertility (%)	94.0	64.7
size (in μ)	8.8	10.3	size (in μ)	9.0	10.7
Seed			Seed		
fertility (%)	68.0	28.3	fertility (%)	89.8	24.0
Stomata			Stomata		
length (in μ)	11.0	16.5	length (in μ)	11.2	16.0
breadth (in μ)	3.5	6.5	breadth (in μ)	3.5	6.5

of polyploidy in *A. dubius* might be limiting for the process as Wettstein (1927) and Levan (1942) have observed that the chromosome number of a species can under no conditions be doubled indefinitely without deleterious results. Stebbins (1950) was also of the opinion that "although some autotetraploids are equal to or superior to their diploid progenitors in vigour or other desirable qualities, autooctoploids are nearly always so abnormal as to be sublethal". But the problem whether *A. dubius* is an autotetraploid or an amphidiploid is yet to be decided though conjectures have been made to suggest that *A. spinosus* might be one of the parents of *A. dubius* on the grounds of its naturally hybridizing with *A. spinosus* (Grant 1959). This view has however, been refused by Pal and Khoshoo (1972) through the cytological and morphological analysis of the naturally occurring species hybrids between *A. spinosus* and *A. dubius*.

The stunted growth with deformities and mottling of leaves at the initial phase were found to be repaired to a very great extent at the later stages of development. This shows that following treatment with colchicine there is probably serious hormon-

al unbalance resulting physiological disorder. This unbalance is gained over in the two species in two different ways. In *A. hypochondriacus* the mottling of the leaves is found to be disappearing in later stages probably due to the repatterning of the tissues in the leaves. But in *A. dubius* very often deformed terminal shoots with curled leaves persist and lateral growth of a normal branch invariably takes place in treated plants within few days. This overtops the deformed branch and ultimately takes the position of the main shoot. So evidently the morphogenetic regulation in both the species seems to be different and capacities for the proliferation of the vegetative shoot following colchicine treatment in *A. dubius* may be ascribed to its original tetraploid nature. This feature has not been noticed in *A. hypochondriacus*.

Induction of polyploidy in both the species has resulted in the reduction in the height of the plant as well as in the size of the leaves and length of the inflorescence. This was also true for the tetraploid plant of C_1 generation. Of the 30 plants of *A. hypochondriacus* tested in the C_1 generation one proved to be a tetraploid which was characterised by reduction in plant height, smaller leaf size, absence of lateral branches and reduction in the number of flowers. Singh and Roy (1971) have aptly remarked that "With increased instances of induced tetraploids, it has now become evident that gigantism is not always an attending feature of polyploidy". The most significant difference in both the induced plants was found to be in the nature of the inflorescence. While in *A. hypochondriacus* it was a distinctive feature that there is an inhibition of the terminal one, in *A. dubius* such feature has not been noticed, though there was a general reduction in the size of the plant and inflorescence. The general reduction in the size of the plant, leaf and inhibited growth of the terminal spike with a number of equally developed laterals were also found to be the features of C_1 plants in *A. hypochondriacus*. The inhibition of the terminal growth was due to the disorganisation of the apical meristem as evidenced from the histological changes.

The induction of polyploidy by colchicine resulted more of sectorial chimeras than whole polyploid plants. This could be visualised when the l. s and t. s of the shoot apices after colchicine treatment were examined which clearly show cytochimeras with mixed diploid and tetraploid cells. The tetraploid cells were distinctly marked out by their larger size with large sized nuclei. The frequency of polyploid cells, however, was much less in comparison to the diploid cell. These inevitably result in mixoploid tissues which give rise to sectorial chimeras. The fixings taken from the same plant in such cases show diploid and tetraploid cells from different branches.

The shorter and stockier plants with number of lateral spikes in bunches in tetraploids were found to be rather suitable characters for nonlodging due to heavy wind as contrast to the tall growth with long terminal spikes in diploids. The same effect has been noticed in induced tetraploids of *A. edulis* by Pal and Khoshoo.

Multivalent associations were recorded in both the species. These were found to be much more frequent in *A. dubius* than in *A. hypochondriacus*. Maximum level of association was found to be quadrivalents in *A. hypochondriacus* and highest range (16 quadrivalents) is noticed in some cases (Fig. 6). But higher levels of association (hexavalents) have been observed in *A. dubius* (Fig. 7). Thus the higher polyploid level (octoploid) with very high chromosome number may be responsible for greater amount of multivalent associations. This may also account for the

uneven separation and while univalents and bivalents move faster in anaphase I, multivalents are found as laggards in *A. dubius*. Thus abnormalities during meiosis are greater in the induced octoploid of *A. dubius* than that of the induced tetraploid of *A. hypochondriacus*. In this connection the observation of Singh and Roy (1971) is significant. They observed that in septaploid anthers of the mixoploid *Trigonella hamosa* L. the frequency of multivalents was considerably lower.

Both the induced polyploids were characterised by polyspory (5–10 spores from a single PMC). A considerable difference in the size of the spores are well marked out and this may be responsible for certain amount of pollen sterility. The uneven separation of the chromosomes and laggards may be responsible for this polysporic condition. Biswas and Bhattacharyya (1971) observed this polyspory in the diploid as well as in induced tetraploid plants of *Cyamopsis psoraloides* DC., though its frequency was found to be higher in the tetraploids. Here though polyspory is not found in normal plants, its frequency is much higher in induced polyploids.

Pollen sterility and seed sterility were found to be higher in octoploid *A. dubius* than tetraploid *A. hypochondriacus*. This may easily be accounted by the comparatively higher chromosomal irregularities seen in the octoploid than in the tetraploid. Reduced fertility in autotetraploids has been attributed by Darlington (1937) and Kostoff (1940) to be due to irregularities in chromosome behaviour at meiosis. Müntzing (1940) suggested tetraploids with small chromosomes and regular bivalent formation may show sterility where as tetraploids with multivalent association were highly fertile. Thus meiotic irregularities may not serve as the only explanation for sterility in autopolyploids. As the treatment of colchicine results in many physiological, morphological and histological changes at the initial stage it may also be responsible for the sterility in the later stages. It has, however, been maintained by Roy Tapadar (1963) and Stebbins (1950) that sterility may be due to the genetic as well as physiological disturbances.

Summary

As a part of the programme of induction of polyploidy in various species of *Amaranthaceae*, two species viz. *Amaranthus hypochondriacus* L., a natural diploid with haploid chromosome number of $n=16$ and *A. dubius* Mart. ex Thell., a natural tetraploid with $n=32$ were selected for the present study with a view to have comparative picture of induced polyploidy at tetraploid and octoploid levels.

Various types of morphological changes were noted in colchicine treated plants. Meiotic analysis revealed varied proportions of hexavalents, quadrivalents, bivalents and univalents in the polyploid cells with other cytological abnormalities such as the uneven separation of the chromosomes and the laggards. These result in the organisation of the micronuclei and polysporic condition which may account for high degree of pollen and seed sterility.

Comparatively percentage of induction of polyploidy in *A. dubius* is found to be less than that of *A. hypochondriacus*. On the other hand frequency and grade of multivalent association is rather higher in *A. dubius* than those of *A. hypochondriacus*. The meiotic irregularities in the induced polyploids of *A. dubius* are also greater than

those of *A. hypochondriacus* which may account for greater percentage of sterility in this induced octoploid.

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