Discussion
DISCUSSION

Nullisomics – Cytology and its variations

Origin of nullisomics

Progenies of monosomics, obtained either by selfing or intercrossing, are generally considered to be the best source of nullisomics. In such cases there are maximum chances of production and fusion of two gametes lacking the same chromosome of a particular chromosome pair. Nullisomics for different chromosomes in the complement have been obtained in the selfed progenies of *Avena sativa* (Nishiyama, 1933; McGinnis, 1962; McGinnis and Andrews, 1962; McGinnis and Linn, 1966; Gauthier and McGinnis, 1965; Lafever and Patterson, 1964; Chang and Sadanaga, 1964; and Hacker, 1965) and *Avena byzantina* (Singh and Wallace, 1967b). Sears (1954) while obtaining the nullisomics from the progenies of monosomics of wheat, also isolated them from mono-telosomics and monoisosomics. Random assortment of univalents at anaphase I in a pentaploid wheat hybrid (cross between hexaploid and tetraploid wheat with AABBD genomes) were reported to produce several nullisomics through the formation and fusion of abnormal gametes (Kihara and Wakakuwa, 1930, 1935). Matsumura (1957) was able to isolate nullisomics for all the seven chromosomes of D genome from this population of pentaploid wheat. Wheat nullisomics were also obtained in the ratio of 9:1 (9 normal : 1 nullisomic). In a mutant of *Avena sativa*, Griffiths and Thomas (1957) are of the opinion that recessive gene in the homozygous
condition brings about loss of a particular bivalent during mitosis at the time of
differentiation of embryo, but before its maturation. Varietal populations of
*Triticum aestivum* (Love, 1940), *Avena sativa* (Huskins, 1929; Tagenkamp and
Finkner, 1954; McGinnis and Taylor, 1961; McGinnis, 1962; Hacker and Riley,
1963) and *Avena byzantina* (Ramage and Suneson, 1958) have produced
nullisomics probably as a result of spontaneous origin of monosomics in these
varietal populations.

Although the present population of nullisomics of *Coix gigantea* might
have originated from monosomics, possibility of their origin directly from diploids
have been proved by Mashalkar (1983). Some diploids from the wild population
were recorded to show complete nondisjunction of the largest bivalent at anaphase
1 in a large number of PMCs. The phenomenon of non-disjunction, considered
usually to be accidental was fairly common in these diploids. This resulted in
formation of hyperploid (*n* + 1) and hypoploid (*n* − 1) gametes in a fairly large
number. In addition to that, univalency or misbehaviour of this bivalent further
increased the frequency of *n* − 1 (*n* = 9) and *n* + 1 (*n* = 11) gametes in the
population. Presuming that in these diploids similar events occurred during
meiosis in the megaspore mother cell, chance mating of gametes with *n* − 1 is very
high giving nullisomics in the progeny directly. Also it is this largest bivalent
from the diploid complement that is seen missing in the nullisomic complement,
supporting the above convention.
Although nullisomics in the varietal populations of *Triticum aestivum*, *Avena sativa* and *Avena byzantina* are believed to have originated in a spontaneous manner in the population, and while monosomics are certainly a rich source of n – 1 gametes to form nullisomics, their direct origin from diploids through complete non-disjunction and / or lag of a bivalent is more likely as seen in *C. gigantea*.

The results obtained after screening the natural progeny and the progeny raised from the seeds collected from variants for four years showed predominance of nullisomics with 2n = 18 chromosomes. Irrespective of tolerating the loss of the largest bivalent the plants appeared normal, vigorous and outnumbered the diploids. It is generally believed that whenever a population with chromosomal variations is screened, the segregation ratio theoretically expected from the normal crosses involving the diploids and aneuploids are seldom obtained because the balance is largely tilted in favour of diploids. This is understandable in light of selection pressure operating at various levels (gametic, zygotic, post zygotic, seedling etc., Khush, 1973). In *Plantago lagopus*, triploid offsprings have been reported to outnumber the diploids. They also overtake diploids in germination and flowering (Sharma and Koul, 1984). In present studies also maximum variants recorded from the progeny were nullisomics. They not only outnumbered any other chromosomal variants, but exhibited normal growth, cytology and were involved in natural hybridization with *Caix aquatica* producing quite a high number of hybrid plants with 2n = 14, indicating the superiority of n – 1 gamete over n gamete as insignificant number of 2n = 15 hybrid were recorded in the
progeny. Thus in this species with \(2n = 20\) chromosome number, no selection against hypoploid gamete \((n = 9)\) was observed. What is more important is that these plants are not only surviving but also set sufficient seeds. Fertility of these plants revealed that loss of a bivalent does not impair the fertility.

**Ploidy level of *C. gigantea***

Nullisomic plants, where both the members of one particular pair of homologous chromosomes are missing from the complement and generally expressed as \(2n\) minus a bivalent \((2n - 2)\) were first reported and studied by Blakeslee (1921a), Sears (1941). Normally in dibasic diploid, loss of a complete bivalent is lethal and nullisomics therefore do not survive in such basically diploid species. However from among the populations of polyploids, nullisomics have been frequently isolated. Loss of chromosomes being easily tolerated in such plants. It is therefore often inferred that appearance of individuals with less than the diploid chromosome number in a species population is an indirect proof of the species being a polyploid.

There is every reason to believe that *C. gigantea* with \(2n = 20\) is at a tetraploid level, since complete aneuploid series from nullisomy to hexasomy has been isolated from the natural population of these diploids (Mashalkar, 1983).

In the tribe Maydeae, it appears from the literature (Darlington and Janaki – Ammal, 1945; Nirodi, 1955 and Bor. 1960) that there are two basic chromosomal number, \(x = 5\) and \(x = 9\). All the genera included in the tribe Maydeae have chromosome numbers in multiples of 5. *Tripsacum* is the only exception to this.
and has 9 as its basic chromosome number. Within the genus *Coix*, the species *C. aquatica* has \( n = x = 5 \). Considering therefore 5 as the basic chromosome number for the genus *Coix*, the present species, *C. gigantea* with \( 2n = 20 \) can be numerically considered as at a tetraploid level. Considering the high frequency of nullisomics in the population, easily tolerating the loss of a bivalent and efficiently competing with diploids, it seems that 9 as a basic chromosome number in the tribe Maydeae might have been secondarily evolved and established. 5 as the basic chromosome number for the genus *Coix* is further supported by the fact that nullisomics (\( 2n = 18 \)) cross very freely in nature with *C. aquatica* (\( 2n = 10 \)) producing hybrids with \( 2n = 14 \). These hybrids produce trivalents in large number of PMCs (Deshpande, 1986). Although segregation of chromosomes is irregular, certain \( 2n = 14 \) hybrids produced sufficient seeds and exhibited genomic affinity between the two parents. It may be concluded that although *Coix gigantea* is functionally diploid showing perfect bivalents and no multivalents, it is at a tetraploid level.

**Sources of nullisomics**

Diploids, monosomics, nullisomics, \( 2n = 14 \) hybrid are the main sources of nullisomics.

The reason for getting maximum nullisomics in the population is to some extent attributed to \( 2n = 14 \) hybrids also. It was recorded that there is emergence of pure parental plants from \( F_1 \) hybrids and hybrid derivatives (Deshpande, 1986).
Besides typical nullisomics, few nullisomics when observed critically turned out to be hybrid 2n = 18 plants carrying one chromosome of *aquatica* (17G + A), forming 8 perfect and one heteromorphic bivalent. Morphologically the plants appeared normal with normal seed setting carrying a chromosome of *aquatica* Producing gametes of n = 9 with one *aquatica* chromosome in its complements.

Earlier, several workers carried out cytological studies on nullisomics of wheat and oat. In most of the nullisomics, some or the other meiotic disturbances through irregular behaviour of chromosome was noted and very few nullisomics were reported to be normal, forming n – 1 bivalents regularly. Normal meiosis in the nullisomics of *Avena sativa* (Lafever and Patterson, 1964) and in *A. byzantina* (Ramage and Suneson, 1958) was reported. The behaviour of chromosomes during meiosis of nullisomics also seemed to vary with the kind of bivalent missing from the complement. Ohta and Matsumura (1961) gave an account of meiosis in seven different nullisomics of the genome wheat. They reported five among these plants to be with more or less normal meiosis, about 90 % of the PMCs produced n – 1 bivalents. On the other hand, remaining two plants showed only a few normal PMCs, while a majority of them show 19 bivalents and two univalents, along with some other PMCs with 18 bivalents plus either 4 univalents, a trivalent and a univalent or a quadrivalent. Cells with 16 and 17 bivalents also occurred but rarely. Somewhat similar observations were reported by Ray and Swaminathan (1959) in a few nullisomics of Chinese spring variety of wheat and
also by Mochizuki and Shigenaga (1964) in the 20 nullisomic plants of the same variety of wheat.

In addition to univalency and multivalency, some nullisomics were found to show a variable number of asynapsis / desynapsis. Sears (1944) working on 2A and 5B chromosomal nullisomics of wheat and Costa Rodriguez (1954) and Vonder Schulenburg (1965) working on Oat nullisomics, recorded partial asynapsis. More or less fully asynaptic nullisomics of *Avena sativa* were reported by Huskins (1927), Huskins and Hearne (1933) and Nishiyama (1931, 1933). Similarly nullisomics of *Avena byzantina* studied by Singh and Wallace (1967a) were also reported to be asynaptic / desynaptic.

In majority of the nullisomics of *C. gigantea* in the present study, fully normal and regular meiosis was noted. However few plants showed desynapsis of smaller bivalent and their misbehaviour through meiosis I and II. It appeared as if nullisomic population is now well established in the natural population producing large number of n – 1 gametes.

**Origin of *C. gigantea* from *C. aquatica**

**Taxonomy**

Taxonomically *C. aquatica* and *C. gigantea* are ill defined, although chromosome number and karyotype is different. The ability of these two species to hybridize producing a range of hybrid derivatives and chromosomal associations, and behaviour of hybrid and hybrid derivatives indicate the close relationship between these two species. It seems that *C. gigantea* is in a state of
flux as many aneuploids and hybrids were reported indicating that probably it is in the process of speciation.

Three species of the genus *Coix* are separated taxonomically by Bor (1960), Naik (1998) on the basis of floral characters like nature of lower glume, whether it is narrowly or broadly winged and certain other characters like nature of surface of leaves or leaf sheath etc. However in the mixed population of these species, the distinguishing characters of *C. aquatica* and *C. gigantea* did not appear to be qualitative. A range of mixture of was characters were observed. Different authors have given different treatments to the species of the genus *Coix*. Bor (1960) observed intergradations of morphological characters among the herbarium specimen suggested that *C. aquatica* could be a habitat of *C. gigantea*. Backer and Brink (1968) however described *C. aquatica* as a variety of *C. lacryma-jobi*. Naik in his flora of Marathwada has said that: *C. gigantea* and *C. aquatica* are two cyto-form with $2n = 20$ and $2n = 10$ and those growing in drier areas have $2n = 20$.

*C. aquatica* seems to inhabit two morphological forms. Plants with 30 feet long, floating, spongy stems growing around Assam and may be Orissa and the same species growing erect, slender and tough near the ponds, marshy places or rice fields of the Deccan (Patunkar, 1980), showing wide adaptations to these two ecological habitats, that only on morphological basis, it is difficult to believe them to be the same species.

In the laboratory, it is experienced that when grown in isolation, the distinguishing characters of these two species can be noted. However in mixed
population, these characters seem to be lost and the species could be identified only on the basis of chromosome number. Such similarities indicate close relationships between these species.

Cytology

*C. aquatica* (2n = 10) with its large nearly metacentric chromosomes and *C. gigantea* (2n = 20) with three classes of chromosomes two largest pairs, four medium and four smaller pairs differ from each other distinctly. The basic chromosome number is the primitive or original number from which polyploid numbers have been derived (Swanson, 1953). Actually the basic number of a genus is calculated from the lowest diploid chromosome number known in the group. However if the diploid, in some cases may no longer exist, in such cases theoretical basic number has to be inferred. This can be well explained with the example of the genus *Coix* in the tribe Maydeae. The basic chromosome number of all the genera in this tribe is 5 except *Tripsacum* and *C. aquatica* with 2n = 10 being the only diploid species. All other genera of the tribe with known chromosome number shows 2n = 20 including the maize. The three species of *Coix* may represent a polyploid series in the multiples of 5. However *C. gigantea* and other genera of Maydeae with 2n = 20 chromosome number behave as perfect diploids and no multivalency was recorded in them to suggest that these are at a tetraploid level.

Many cytotaxonomist believe in keeping the diploids and polyploids apart from each other although morphologically the species resemble each other. There
are many others who consider that chromosome number should not be given much importance for plant classification. It should be treated only as an additional evidence. However in many taxonomic problems, chromosome number and morphology have played a role to sort it out as both chromosome number and morphology is constant for a given species.

In the present study also *C. gigantea* with 2n = 20 show a different karyotype from *C. aquatica* and a normal meiosis without exhibiting multivalency and is still treated at the tetraploid level on two criteria. As loss of one or two chromosomes are easily tolerated by the species without showing much change on its morphology and also the size of the chromosomes. Compared to *aquatica*, although chromosome number of *gigantea* is double, the size of the chromosomes is reduced.

In nature, the three species of *Coix* do not naturally exist together. Characteristic geographical distribution is noted from the floras and the studies in this laboratory indicate that geographical isolation might have kept them reproductively isolated also. Successful interspecific hybridization have been recorded between these species, indicating the closeness between the species and their taxonomic status. The relationship between the species is established on the basis of their ability to hybridize and produce hybrids. The species status is determined on that. Based on the nature of hybrid the closeness or distantness of the species is established. However inability to cross is also controlled by physical, physiological or genetical factors. Similarly diploids and polyploids of
the some species have different breeding potentials. However when species are morphologically similar then breeding is used as criteria to separate them. The unrelated species do not hybridize. Even if they hybridize, they produce sterile seeds. The degree of seed fertility can be correlated with the affinities of the parent species. Generally seed and pollen sterility suggests hybridity in the population. *C. aquatica* (2n = 10) and *C. gigantea* (2n = 18) breed naturally in the population producing many semisterile hybrids (2n = 14) through cross-pollination. Cytological studies of these hybrids especially chromosome pairing, clearly indicated that these two species are closely related. In a hybrids of 2n = 17 and 2n = 19, one *gigantea* chromosome forms a heteromorphic bivalent with a single *aquatica* chromosome in all the cells at diakinesis of all such hybrids recorded. Even in 2n = 18 hybrid, one perfect heteromorphic bivalent is present showing one *aquatica* and one *gigantea* chromosome. Two separate univalents of this bivalent are rare. Thus in many natural hybrids *aquatica* and *gigantea* chromosomes show pairing in such a way that except size, it is difficult to identify these genomes. Whatever range of aneuploids and hybrids recorded in these species, they did not show much morphological variations, as the two parental species themselves are ill defined.

**Establishment of certain hybrids involving two species**

Many interspecific hybrids like 2n = 14, 2n = 17, 2n = 18, 2n = 19 etc. are trying to get established in the natural population. When the two species are grown together i.e. if the geographical distance between the two is reduced, every
year only these types of hybrids are observed. These hybrids are semifertile producing hybrid derivatives further. For successive four years same types of aneuploids and hybrids, and parental type ranging from $2n = 10$ to $2n = 19$ were observed with same combination of *aquatica – gigantea* chromosome, showing close relationship between these species. In nature such hybrids having chromosomes of both the species and producing sufficient amount of fertile seeds, maintaining the genic balance are rare.

**Chromosomal behaviour in hybrids**

In the meiosis of hybrids, chromosomal behaviour is used as a criteria to establish the affinities between the parental species. Number of chiasma and their distribution have been shown to be the indications of chromosomal and hence species affinity (Darlington, 1937). Many cytotaxonomists utilize this as a criteria for grouping the species. But this has got a limited significance as chiasma frequency varies widely between the sexes and between the closely related species with fluctuations in environmental conditions and genotype.

Much emphasis is given to the degree of chromosomal homology which is revealed by the paired associations of chromosomes during meiosis. Normally if the parents are closely related, regular chromosomal pairing is seen, but if they are distantly related, reduced homology and the pairing between the chromosomes is seen. The degree of pairing thus directly indicates the relationship between the two parents. However chromosome pairing has been shown to be under simple genic control as in *Triticum* (Riley, 1960).
Among the hybrids and their derivatives of *Coix*, fairly good amount of pairing is noted between *C. aquatica* and *C. gigantea* chromosomes, showing homology and affinity between them. As was suggested by Dr. Naik in flora of Marathwada, *C. gigantea* and *C. aquatica* are two cyto forms with $2n = 20$ and $2n = 10$ and those growing in drier areas have $2n = 20$. Although it was thought that *C. gigantea* is formed from *C. aquatica* species some phenomenon other than only doubling of chromosomes may be involved in this as intragenomic pairing was observed to be absent.

*C. gigantea* and *C. aquatica* genomes are in a state of flux. *C. aquatica* with $2n = 10$ large metacentric chromosomes is the only species showing basic chromosome number for the whole tribe, Maydeae ($x=5$, Darlington, 1956). Among the *Coix* species also, *C. aquatica* forms the basic species. Other two species are *C. gigantea* ($2n=20$ and 40) and *C. lacryma-jobi* ($2n=20$) (Bor, 1960). These species might have originated from *C. aquatica* by structural and numerical rearrangements and hybridization. Various meiotic irregularities recorded in these species earlier in this laboratory (Mashalkar, 1983, Deshpande D.S. 1986) indicate that the genomes are in the state flux. All these aberration within these species revealed that the genome is undergoing major changes towards repatterning of the genotype.

**Chromosomal instability in the species:**

Karyotype of a species is used as a character for classification of plants as chromosome number and structure are constant for the species and this is
maintained from one generation to other generation by cell division i.e. mitosis and meiosis. Natural deviations can occur in these processes that produce variations in structure and number of chromosomes. If these variability perpetuate, lead to origin and establishment of new chromosomal races and after a long period may eventually form a new species in evolution. Different species have been originated at different places i.e. at the centre of origin and from there, diversification of these species took place leading to gradual modification in their structure and number. Lot of genetic imbalance is observed in such natural population and the stability of the genome is lost.

Generally it is thought that the minor or major changes in the genetic constitution are lethal or sub-lethal and are not normally selected by nature. However this does not stand true for all the species Some species are hypersensitive to genetic changes and even minor changes affect few vulnerable genes. However it is not true for some other species like C. gigantea If the species does not change, the process of evolution may stop. Life is dynamic and ever-changing. Changes, whether minor or major, as a response to the changing environment, may be expressed or accumulated in organic evolution. There exist a constant interaction between the organisms and their surrounding environment. However such reactions may vary from organisms to organisms

In the population raised from seeds (product of sexual reproduction), every individual should be treated genetically as differently and should be studied individually Any cytological problem of the population can be resolved after
single plant cytological studies. Chromosome variations may also occur due to age of the plant. The two species of the genus *Coix-aquatica* and *gigantea* have shown lot of chromosomal variations. Apart from the chromosome number $2n = 10$, $2n = 18$, $2n = 20$ and $2n = 40$ reported earlier, some forms with $2n = 12$ and $2n = 32$ (Koul and Paliwal, 1964) have also been reported recently. All these variations are stable and breed true. Many natural hybrids between *C. aquatica* and *C. gigantea* and their open pollinated hybrid derivative (Deshpande, 1986, Naik 1991) have been studied earlier. In the present piece of work many such natural hybrids and aneuploids were recorded which are trying to get established in the population. Most of the aneuploids – especially $2n = 18$ are perfectly fertile as if whatever bivalent is lost from its complement was of no use to the plant. Many other hybrids like $2n = 17$, $2n = 18$, $2n = 19$ show much fertility of seeds and hybrids like $2n = 14$, $2n = 15$ and $2n = 16$ were partly fertile. Most interesting thing was plants showing odd chromosome numbers like $2n = 17$, $2n = 19$ and $2n = 14$ hybrids produced more individuals of same type maintaining the parental genetic constitution. In most of these hybrids, it appeared as if *aquatica* – *gigantea* chromosomes balance each other by forming heteromorphic bivalents (A – G) and segregate regularly producing gametes with *aquatica* and *gigantea* chromosomes. *C. aquatica* and *C. gigantea* although are two different species, their chromosomes show friendly gesture and can be complemented in to different hybrids naturally alongwith aneuploidy. May be the population is trying out new cytotypes. In most of the aneuploids and hybrids the parental type emerged and
behaved regularly showing similar fertility. A large number of hybrid seeds however did not germinate, or died in seedling stage or did not flower. Such conditions are attributed to under-developed embryos, may be due to incompatible combination of chromosomes from the two parents. Also the weak seeds may carry chromosome number less than 10. If all such seeds are grown in laboratory conditions (tissue culture) the deficiencies in these plants can be traced out.

Four years screening data of the natural progeny and also earlier data of this lab (Deshpande, 1986, Naik, 1991) have not shown any hybrid carrying less than 10 chromosomes. Any number above 10 or 10 is functional indicating that $2n = 10$ is the basic number. Morphologically any change in chromosome number from $2n = 16$ to $2n = 18$ (hybrids or aneuploids) did not show any remarkable difference. It is really difficult to distinguish these variants in the population. Permutations and combinations of *aquatica – gigantea* chromosomes do not seem to affect morphology of the plants, irrespective of its odd cytological constitution.

Thus again can be expressed in the terms of close relationship between these two species. May be the speciation of these is not yet complete