



**FACULTY OF AGRICULTURAL SCIENCES AND ALLIED INDUSTRIES**

## Ploidy breeding

The mitotic and meiotic divisions are very precise as a result of which the chromosome numbers of different species are highly stable. But a low frequency of irregularities do occur both during mitotic and meiotic divisions. These irregularities give rise to individuals with chromosome numbers different from the normal somatic chromosome number of the concerned species. Changes in chromosome number (some types) have contributed greatly to crop evolution, and (all the types) are of much use in plant breeding. In this chapter, we shall discuss in some detail the types of changes in chromosome number, their characteristics, production and applications in crop improvement.

### TYPES OF CHANGES IN CHROMOSOME NUMBER

The Somatic chromosome number of any species, whether diploid or polyploid, is designated as  $2n$ , and the chromosome number of gametes is denoted as  $n$ .

An individual carrying the gametic chromosome number,  $n$ , is known as haploid.

A monoploid, on the other hand, has the basic chromosome number,  $x$ . In a diploid species,  $n = x$ ; one  $x$  constitutes a genome or chromosome complement.

The different chromosomes of a single genome are distinct from each other in morphology and/or gene content and homology; members of a single genome do not show a tendency of pairing with each other.

Thus a diploid species has two, a triploid has 3 and a tetraploid has 4 genomes and so on. Individuals carrying chromosome numbers other than the diploid ( $2x$ , and not  $2n$ ) number are known as heteroploids, and the situation- is known as heteroploidy.

The terminology of heteroploidy is summarised in Table. The change in chromosome number may involve one or a few chromosomes of the genome; this is known as aneuploidy. The aneuploid changes are determined in relation to the somatic chromosome number ( $2n$  and not  $2x$ ) of the species in question. Therefore, the terminology for aneuploid individuals arising from diploid and polyploid species is the same.

Heteroploidy that involves one or more complete genomes is known as euploidy. By definition, therefore, the chromosome numbers of euploids are an exact multiple of the basic chromosome number of the concerned species, while those of aneuploids are not.

Aneuploid individuals from which one chromosome pair is missing ( $2n - 2$ ) are termed as nullisomic, while those lacking a single chromosome ( $2n - 1$ ) are known as monosomic.

A double monosomic individual has two chromosomes missing, but the two chromosomes belong to two different chromosome pairs ( $2n-1-1$ ).

An individual having one extra chromosome ( $2n + 1$ ) is known as trisomic, and that having two extra chromosomes each belonging to a different chromosome pair is called double trisomic ( $2n + 1 + 1$ ).

When an individual has an extra pair of chromosomes, it is known as tetrasomic ( $2n + 2$ ).

In euploids, the chromosome number is an exact multiple of the basic or genomic number.

Euploidy is more commonly known as polyploidy. When all the genomes present in a polyploid species are identical, it is known as autopolyploid and the situation is termed as autopolyploidy.

But in case of allopolyploids, two or more distinct genomes are present. Euploids may have 3 (triploid), 4 (tetraploid), 5 (pentaploid), 6 (hexaploid), 7 (heptaploid), 8 (octaploid) or more genomes making up their somatic chromosome number.

In case of autopolyploidy, they are known as autotriploid, autotetraploid, autopentaploid, autohexaploid, autoheptaploid, auto - octaploid and so on, while in the case of allopolyploidy they are termed as allotriploid, allotetraploid, allopentaploid, allohexaploid, alloheptaploid, allooctaploid, etc.

Amphidiploid is an allopolyploid that has two copies of each genome present in it and, as a consequence, behaves as a diploid during meiosis. A segmental allopolyploid contains two or more genomes, which are identical with each other, except for some minor differences.

### **ANEUPLOIDY**

Of the various aneuploids, monosomics (in polyploid species, such as, tobacco, wheat and oats) and trisomics [in diploid species, e.g., *Datura*, maize, bajra, tomato (*L. esculentum*), rye (*S. cereale*), pea (*P. sativum*), spinach (*S. oleracea*), etc.] are the most commonly used in genetic studies.

Nullisomics are viable in a few highly polyploid species only, e.g., wheat and oats; they are not viable even in tobacco, which is an allotetraploid.

A trisomic is known as primary trisomic if the extra chromosome is the same as one of the haploid genome, that is, it is not modified. In a secondary trisomic, the additional chromosome is an isochromosome.

In an isochromosome, the two arms of the chromosome are identical.

A tertiary trisomic has a translocated chromosome as the extra chromosome. For the present, we shall confine ourselves to primary trisomics.

### **Applications in Crop Improvement**

Aneuploids are useful in the studies on effects of loss or gain of an entire chromosome or a chromosome arm on the phenotype of an individual. Their study has clearly demonstrated that character expression is governed by a balance between a large number of genes

present in the genome, that is, a loss or a gain of chromatin upsets the normal development. Aneuploids are useful in locating a linkage group and a gene to a particular chromosome.

B) using a secondary or tertiary trisomic, the gene may be located to one of the two arms of a chromosome, or even to a part of the chromosome arm. The most important application of aneuploids is in locating genes on particular chromosomes; this will be considered in some detail.

Study of aneuploids has shown the homoeology between A, B and D genomes of wheat (*T. aestivum*), since a chromosome of A genome does compensate for the loss of the corresponding chromosome from genome B or D. For example, tetrasomic condition of 2B compensates for the nullisomic condition of 2A or 2D so that a tetra- 2A nulli-2B or 2D appears normal.

Aneuploids are useful in identifying the chromosomes involved in translocations. They are useful in the production of substitution lines. Chromosome substitution may be desirable for studying the effects of individual chromosomes of a variety or for the transfer of the genes carried by specific chromosomes or a variety into another one.

#### **Limitations of Aneuploid**

It is necessary to produce and maintain a complete set of aneuploids. Production, identification and maintenance of aneuploids require elaborate cytogenetic analysis, which is difficult, time consuming and requires considerable skill. Maintenance of aneuploids is complicated by the phenomenon of univalent shift. Univalent shift denotes that some of the progeny of an aneuploid plant would become aneuploid for a different chromosome as compared to the parent plant. Univalent shift generally occurs in monosomic lines and is a result of univalent formation in a chromosome other than that for which they are monosomic. Therefore, cytological analysis and testing must form an integral part of the aneuploid programmes.

During aneuploid analysis and chromosome substitution, cytological analysis must be carried out for accuracy. This involves a considerable cytological work and makes aneuploid analysis a time consuming and tedious task.

#### **MONOPOLOIDS AND HAPLOIDS**

Monoploids and haploids are weaker than diploids and are of little agricultural value directly. But they are of great interest because they offer certain unique opportunities in crop improvement.

(1) They are used for developing homozygous diploid lines, following chromosome doubling in two years. This greatly reduces the time and labor required for the isolation of inbreds and purelines.

(2) They may be useful in the isolation of mutants because the mutant allele (even if it is recessive) expresses itself in M, due to a single dose of the gene in somatic tissues.

Chromosome number of mutants may be doubled to produce homozygous mutant lines in a single generation.

(3) Since desirable gametes are more frequent ( $=p$ , that is, the frequency of desirable allele in the population) than desirable zygotes ( $=p^2$ ), selection based on haploids or doubled haploids may be expected to be more efficient than that based on diploid (zygote-derived) plants. There is some evidence that this may be so. And

(4) in autotetraploids like potato, breeding is relatively much easier at the haploid ( $2x$ ) level than at the tetraploid level ( $4x$ ). For comparison, consider segregation in an autotetraploid and in a diploid. There is an increasing tendency to breed potato varieties at the haploid level and then double their chromosome number to obtain tetraploid varieties. In monoploids and most haploids, the chromosomes do not pair and their distribution at anaphase I is random leading to an almost complete sterility. Some functional gametes with  $n$  chromosomes may be produced, which may give rise to  $2n$  progeny. Monoploids and haploids occur spontaneously in low frequencies, may be induced from pollen grains or haploid cells of unfertilized ovaries through callus formation or embryo production and by chromosome elimination in certain interspecific crosses, e.g., *Hordeum bulbosum* X *H. vulgare*.

In the first method, the recovery of haploids is generally very low (1 in 1,000 plants or lower). But the latter two methods produce a relatively high frequency of haploids in case of those species for which appropriate techniques are available. It may be pointed out that the latter two methods are not applicable to many crop species as yet.

### **AUTOPOLYPLOIDY**

In autopolyploidy are included triploidy, tetraploidy and higher levels of ploidy. Autopolyploids are produced directly or indirectly through chromosome doubling.

#### **Origin and Production of Doubled Chromosome Numbers**

Cells/individuals having doubled chromosome numbers may originate in one of the following several ways:

- (1) spontaneous,
- (2) due to treatment with physical agents,
- (3) regeneration in vitro,
- (4) colchicine treatment, and
- (5) other chemical agents.

#### **Spontaneous**

Chromosome doubling occurs occasionally in somatic tissues and unreduced gametes are also produced in low frequencies. Production of unreduced gametes is promoted by certain genes, e.g., genes causing complete asynapsis or desynapsis and, more particularly, Such mutant genes as those producing parallel spindle ( $ps$ ) and omission of second division ( $os$ ) in potato. Production of Adventitious Buds Decapitation in some plants leads to callus

development at the cut end of stem. Such a callus has some polyploid cells, and some of the Shoot buds regenerated from the callus may be polyploid. This is of common occurrence in Solanaceae where 6-36 per cent of adventitious shoot buds are reported to be tetraploid. The frequency of polyploidy buds may be increased by the application of 1% IAA at the cut ends as it promotes callus development.

### **Physical Agents**

Heat or cold treatments, centrifugation and X-ray or gamma ray irradiation may produce polyploids in low frequencies. Tetraploid branches were produced in *Datura* in response to cold treatment. Exposure of maize plants or ears to a temperature of 38-45°C at the time of the first division of zygote produces 2-5 per cent, tetraploid progeny. Heat treatment has been successfully used in barley, wheat, rye and some other crop species. Regeneration in Vitro Polyploidy is a common feature of the cells cultured in vitro. Some of the plants regenerated from callus and suspension cultures may be polyploids. Plants of various ploidy have been regenerated from callus cultures of *Nicotiana*, *Datura*, rice (*D. sativa*) and several other species.

### **Colchicine Treatment**

Colchicine treatment is the most effective and the most widely used treatment for chromosome doubling. It has been used with great success in a large number of crop species belonging to both dicot and monocot groups. Colchicine is a poisonous chemical isolated from seeds (0.2-0.8%) and bulbs (0.1-0.5%) of autumn crocus (*Colchicum autumnale*). It is readily soluble in alcohol, chloroform or cold water, but is relatively less soluble in hot water. Pure colchicine is  $C_{22}H_{25}O_6N$ . It blocks spindle formation and thus inhibits the movement of sister chromatids to the opposite poles. The resulting restitution nucleus includes all the chromatids; as a result, the chromosome number of the cell is doubled. Since colchicine affects only dividing cells, it should be applied to a shoot-tip meristem only when its cells are actively dividing. At any given time, only a small proportion of the cells would be in division; therefore, repeated treatments should be given at brief intervals to double the chromosome number in a large number of cells of the shoot apex. The polyploid and diploid cells present in a shoot-tip compete with each other and diploid cells may often out compete the polyploid ones. The degree of competition varies from species to species and even among varieties within species.

### **Applications of Autopolyploidy in Crop Improvement**

Autopolyploidy has found some valuable applications in crop improvement. These are briefly summarised below.

#### **Triploids**

Triploids are produced by hybridization between tetraploid and diploid strains. They are generally highly sterile, except in a few cases. This feature is useful in the production of seedless watermelons. In certain species, they may be more vigorous than the normal diploids, e.g., in sugarbeets. These two examples are described in some detail. Seedless watermelons are grown commercially in Japan. They are produced by crossing tetraploid (4x, used as female) and diploid (2x, used as male) lines, since the reciprocal cross (2x x 4x) is not successful. The triploid plants do not produce true seeds; almost all the seeds are small, white rudimentary structures like cucumber (*Cucumis sativus*) seeds. But a few normal sized seeds may occur, which are generally empty. For good fruit setting, pollination is essential. For this purpose, diploid lines are planted in the ratio 1 diploid: 5 triploid plants. There are several, problems, viz., genetic instability of 4x lines, irregular fruit shape, a tendency towards hollowness of fruits, production of empty seeds and the labour involved in triploid seed production (by handpollination). Recently, some diploid hybrids of watermelon ('ice-box type') have been developed that produce seedless fruits (all their seeds are like cucumber seeds).

Triploid sugarbeet produce larger roots and more sugar per unit area than do diploids, while tetraploids produce smaller roots and lower yields than diploids. Apparently, 3x is the optimum level of ploidy in sugarbeets. Triploid sugarbeet varieties have been grown commercially in Europe and Japan, but their popularity is declining. rapidly. The triploid varieties are mixtures of triploid, diploid and other ploidy level plants. Seed production of triploid sugarbeet is difficult because the beet flower is small.

Triploid seed may be produced in one of the following two ways:

- (1) using 4x plants as female and 2x as male or
- (2) using 4x as male and 2x as female. The first combination gives lower seed yield but a higher proportion of triploids, while the second gives a higher seed yield but a lower proportion of triploids. Commercial triploid sugarbeet seed is produced by interplanting 4x and 2x lines in the ratio 3: 1, and seeds from both 4x and 2x plants are harvested. This seed consists of about 75% triploid (3x) seeds. Triploid sugarbeet may give 10-15 per cent higher yields than diploids.

**Tetraploids : Autotetraploids** have been produced in a large number of crop species and have been extensively studied in several cases. Tetraploids may be useful in one of the following ways:

- (1) in breeding,
- (2) improving quality,
- (3) overcoming self incompatibility,
- (4) making distant crosses and
- (5) used directly as varieties.

In banana (*M. sapientum*), autotetraploids are inferior to triploids in that they have weaker leaves and increased fertility. But they offer the only available chance of adding disease resistance to commercially successful varieties. In banana, autotetraploids are produced by chance fertilization of an unreduced triploid egg (AAA) by a haploid pollen from a disease resistant diploid parent. A large number of such tetraploids have been produced, but they have not yet gained any commercial success.

Varieties of forage crops have been considerably successful. The most successful examples are, tetraploid red clover (*Trifolium pratense*) and ryegrass (*Lolium perenne*). Other examples are tetraploids of alsika clover (*Trifolium hybridum*, Variety Tetra) and berseem (*Trifolium alexandrinum*, variety Pusa Giant Berseem). Autotetraploid red clover and ryegrass are more vigorous, more digestible and palatable, and have greater resistance to nematodes as compared to the diploids.

Autotetraploid turnips (*B. rapa*) and cabbage (*B. oleracea*) are larger in size, but they also have more water content than the diploids; thus they are not commercially attractive. Many ornamentals are autotetraploids. In cases of ornamentals, increased flower size, and longer flowering duration of the tetraploids are desirable. Pusa Giant Berseem is the first autopolyploid variety released for general cultivation in India. It yielded 20–30 per cent more green fodder than the diploid berseem varieties.

Some autotetraploid varieties of medicinal plants have been released and adopted. Variety HMT-I of *Hyoscyamus niger* is an autotetraploid; it gives 15% more biomass and 36% greater crude drug yield than the diploid parent. Similarly, Sugandha is an autotetraploid variety of vetiver (*Vetiveria zizanioides*); it gives 11 % more oil yield than the control. In case of crops where seed is the commercial product, autotetraploidy has been much less successful.

The chief difficulty is the high sterility and genetic instability of autotetraploids. Fertility can be improved through breeding and selection, but the progress is slow.

Autotetraploids have been explored in several crop species but the most successful case that of rye (*S. cereale*) where tetraploid varieties have been released for cultivation (e.g., Double Steel, Tetra Petkus).

Other extensive programmes on autotetraploidy are on crops like barley (*H. vulgare*) and jowar (*S. bicolor*) where larger grains, increased protein content and larger yields are the objective. After many years of extensive breeding, some success in achieving these goals has been realized.

**ALLOPOLYPLOIDY:** Allopolyploids have genomes from two or more species. Several of our crop plants are allopolyploids. Production of allopolyploids has attracted considerable attention; the aim almost always was the creation of new species. Some success has been obtained as is evident from the emergence of Triticale as a new crop species in some areas, and the promise shown by some other allopolyploids, e.g., Raphanobrassica and some allopolyploids of forage grasses.



## **Applications of Allopolyploidy in Crop Improvement**

Allopolyploidy has three major applications in crop improvement:

- (1) as bridging species in the transfer of character from one species into another.
- (2) In the production of new crop species, and
- (3) for widening the genetic base of existing allopolyploid crop species.

### **Utilization as a Bridging Species**

Amphidiploids serve as a bridge in the transfer of characters from one species to a related species, generally from wild species to cultivated species. The use of an amphidiploid as a bridging species becomes necessary when the hybrid between the cultivated species (recipient species) and the wild species (donor species) is sterile. The sterility of F<sub>1</sub> hybrid makes it impossible to cross the recipient species with the F<sub>1</sub> and this does not permit the transfer of characters from the donor to the recipient species. In such cases, chromosome number of the F<sub>1</sub> interspecific hybrid is doubled to produce an amphidiploid, which is a novel species; it will be generally reasonably fertile and can be crossed to the recipient species. Progeny from the cross between the recipient species and the amphidiploid would have the somatic (2n) chromosome complement of the recipient species and one genome from the donor species. As a result, they would be sufficiently fertile to be used in backcross with the recipient species. From such a programme, alien addition and alien substitution lines are recovered, which are used in the transfer of genes, groups of genes or of small chromosome segments to the recipient species.

An example of the use of an amphidiploid as a bridging species is the use of synthetic *N. digluta* (allohexaploid) for the transfer of resistance to tobacco mosaic virus from *N. sylvestris* to *N. tabacum*.

The F<sub>1</sub> obtained from the cross *N. tabacum* × *N. sylvestris* is sterile. Chromosome doubling of the F<sub>1</sub> hybrid produces the synthetic allohexaploid *N. digluta*, which is reasonably fertile. *N. digluta* is backcrossed to the recipient species (*N. tabacum*) to produce a pentaploid having the complete somatic (2n) chromosome complement of *N. tabacum* and one genome of *N. sylvestris*.

The pentaploid is sufficiently fertile to be backcrossed to *N. tabacum*. In the progeny, *N. tabacum* like plants resistant to tobacco mosaic are, reselected and cytologically analysed. From among the backcross progeny, both alien addition and alien substitution lines can be recovered.

Other examples of the use of an amphidiploid as a bridging species are in the cases of transfer of genes from *G. thurberi* to *G. hirsutum* and of chromosomes from *Haynaldia villosa* to *T. aestivum*. Creation of New crop species It was once hoped that allopolyploidy would enable man to create new species at will, and that these species would be superior to the existing crop species. This hope was based on the fact that some of the present-day important crop species are allopolyploids, and that the existing as well as new allopolyploids

can be, synthesized in the same manner as they would have been produced in the nature. Thus it was expected that a duplication of the nature's own methods would lead to the creation of new and superior crop species as it had occurred in the nature. Allopolyploidy itself has not enabled a species to become successful as a crop; in fact, many allopolyploids are weedy wild species, e.g., *S. spontaneum* and *S. robustum* are noxious weeds. The natural allopolyploids have evolved over a long period to achieve their present-day forms. Newly synthesized allopolyploids, therefore, could hardly be expected to become successful as crops.

An allopolyploid that would be superior to the existing diploid species would have already been produced and refined by the natural forces. Consequently, the allopolyploids that are not already existing may be expected to be inferior to the diploid species. These present a discouraging picture of the possibilities of using new allopolyploids as crop species, which seems to have been confirmed by the experience with synthetic allopolyploids.

Triticale is the most successful synthetic allopolyploid produced by crossing wheat (tetraploid or hexaploid) with rye. Triticales derived from tetraploid wheats have been the most successful, but those from hexaploid wheats may also become a successful crop species. At present, triticales are being grown commercially in some parts of the world, e.g., in Canada, and the yields of triticales are comparable to those of the best wheat varieties. The desirable features of triticales are that they combine the yielding ability and grain qualities of wheat with the hardiness (tolerance to adverse environment) of rye. But the development of such superior lines of triticales has taken 50 years of intensive research. The newly synthesized triticales were of low yielding ability due to high sterility, poor seed set and poor and variable development of grains.

Triticales also show cytogenetic and genetic instability due to meiotic irregularities and produce some aneuploid progeny. In Sweden, the raw triticales yielded about 50 per cent of the standard varieties of wheat. The yielding ability of triticales increased under selection to about 90 per cent of the yield of wheat varieties in 15 years. Extensive breeding work on triticales is going on at CIMMYT, Mexico.

The breeding strategy involves

- (1) production of a large number of triticale strains using different combinations (varieties as well as species) of wheat and rye,
- (2) hybridization of these triticale strains among themselves, and
- (3) improvement of the defects of the triticales through selection.

The results from such breeding programmes have been spectacular and have led to the release of several commercial varieties of triticale, which yield as much as the best varieties of common wheat. Triticale varieties are being cultivated mainly in Poland (the largest area), Germany and France; the area of cultivation is around 2.6 million hectares with an

annual production of -8 million tons. In India, three varieties of Triticales have been released; these are TL419, TL1210 and DT46 (amber color grains).

The chief drawbacks of triticales is their deep grain colour. As a result, TL1210 is mainly grown as a fodder crop in Punjab although its grain yield is comparable to that of the best wheat varieties. Indian breeders have been successful in developing amber coloured triticales by using white-seeded rye as one of the parents of the triticales.

Some other promising allopolyploids are Raphanobrassica, the triploid (AAC) obtained by crossing *B. napus* (AACC) with *B. campestris* (AA), allopolyploid clovers, *Festuca-Lolium* hybrids and some species hybrids in *Rubus* and *Jute* (*Corchorus* sp.).

In Raphanobrassica, the breeding objectives are to combine the hardiness of *B. oleracea* with quick growth and disease resistance of fodder radish. The problems of Raphanobrassica are the same as those of triticales, i.e., low fertility, cytogenetic and genetic instability and leafy rape-like plants that do not produce bulbs. There is evidence that hybridization and selection at the polyploid level would be effective in improving Raphanobrassica.

The amphidiploid *B. napus* (AACC) crosses very easily with *B. campestris* (AA) to produce the triploid (AAC), which has some desirable features. The triploid is produced so easily that it may be used as a hybrid variety, a special case of hybrid varieties produced by crossing two different species.

Varalakshmi, a hybrid variety of cotton is also an interspecific hybrid between *G. hirsutum* (American cotton) and *G. barbadense* (Egyptian cotton); several other such hybrid varieties have been released in cotton.

### **Widening the genetic base of existing allopolyploids**

The genetic base of some natural allopolyploids may be narrow, and it may be useful to introduce variability in such cases by producing the allopolyploids afresh from their parental species. *B. napus* is a case in point; the genetic variability of this species is narrow and the only recourse available is to synthesize new allopolyploid *B. napus* to widen its genetic base. This is being done by crossing *B. campestris* ( $n = 10$ , AA) with *B. oleracea* ( $n = 9$ , CC), the parental diploid species, to produce the amphidiploid *B. napus* ( $n = 19$ , AACC). The two species, *B. campestris* and *B. oleracea*, have to be crossed as autotetraploids the cross is very difficult and embryo culture has to be used; somatic hybridization is being used to get around these problems.

### **Limitations of Allopolyploidy**

- The effects of allopolyploidy cannot be predicted. The allopolyploids have some features
  - from both the parental species, but these features may be the undesirable ones, e.g., Raphanobrassica, or the desirable ones, e.g., Triticale.

- Newly synthesized allopolyploids have many defects, low fertility, cytogenetic and genetic instability, other undesirable features, etc.
- The synthetic allopolyploids have to be improved through extensive breeding at the polyploid level. This involves considerable time, labour and other resources.
- Only a small proportion of allopolyploids are promising; a vast majority of them are valueless for agricultural purposes (except for their use as a bridging species). Thus a costly trial and error has to be done before one is likely to come across a promising allopolyploid combination that can be improved through breeding to yield a new crop species.