75 years of *Brassica* cytogenetics in India

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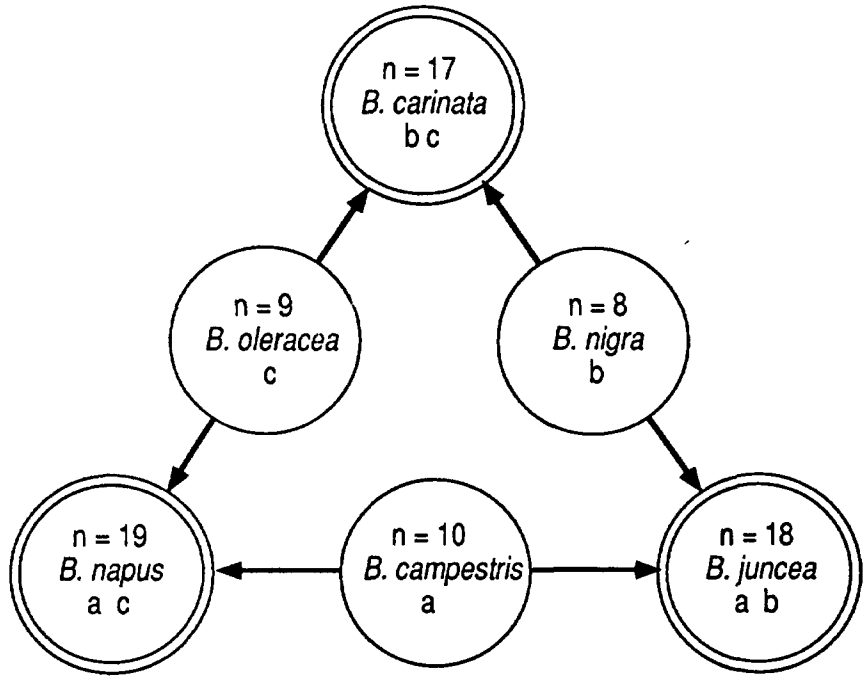
Systematic Brassica cytogenetics started with determination of chromosome number of Brassica campestris by the Japanese researcher, Takamine, in 1916. Although interspecific and intergeneric hybrids such as Raphanus sativus x B. oleracea (Augustin Sageret, 1826) and B.napus x B. campestris ssp. rapifera (William Herbert, 1834), were obtained much earlier, systematic hybridization and study of their cytology was initiated by Morinaga only in 1930s and was aimed at removing the prevailing confusion caused by the high degree of polymorphism in several Brassica species. Morinaga's (1934) genome analysis established that crop Brassicas consist of 6 species. Of these B. nigra (2n=16,BB) B.oleracea(2n=18, CC) and B.campestris (2n=20,AA) are diploids, and B.carinata (2n=34,BC), B.juncea(2n=36,AB) and B.napus (2n=38,AC) are naturally evolved allo-tetraploids. The scheme of cytogenetic relationship was presented by another Japanese scientist, Nagaharu U (1935), and is commonly referred to as U's triangle. During the 75 years since, Takamine's report, Brassica cytogenetics has come a long way. Centres of Brassica cytogenetics research have been in several countries of Europe, Japan, India, Canada and China. From classical cytogenetic manipulations in the past, the advent of techniques of somatic cell and molecular genetics has shifted emphasis to new approaches to introgression of desirable genes and cytoplasm, and construction of chromosome maps. Among the pioneers in Brassica cytogenetics research are Karpechanko, Morinaga, Nagaharu U, Mizushima, Olsson and Robbelen. In this report are summarized the highlights of achievements during the last 75 years.

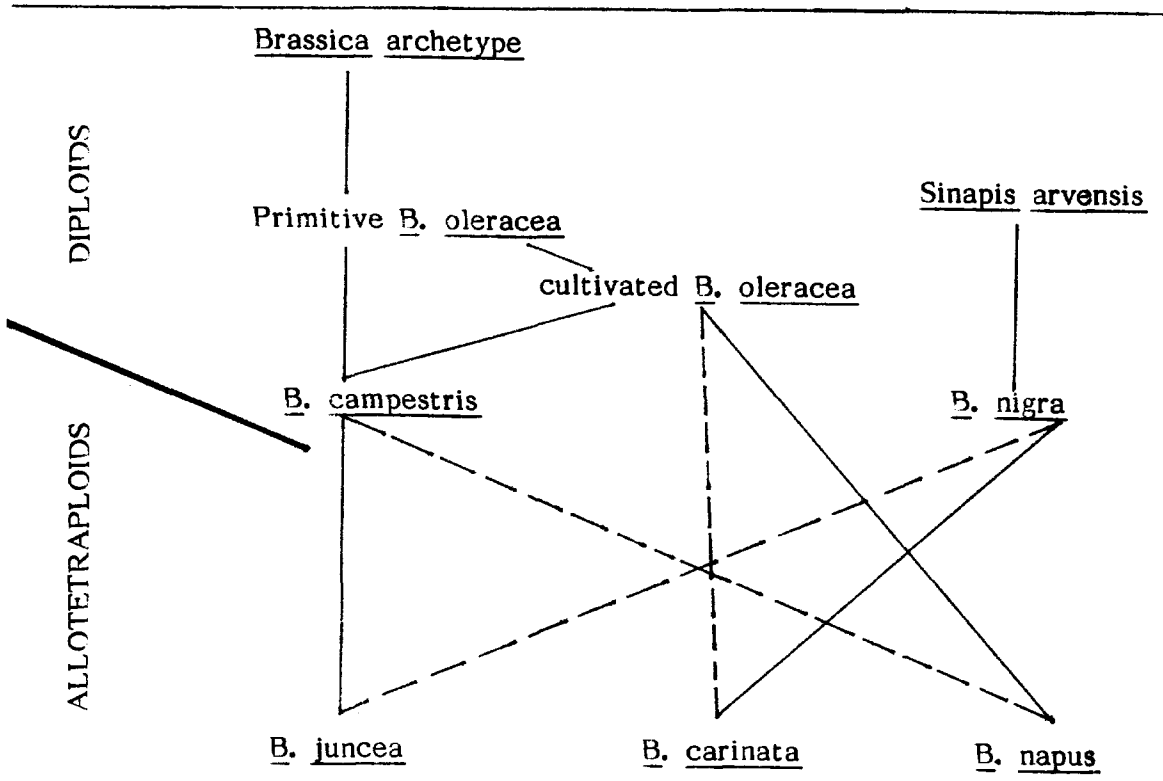
The Brassica genome

Diploid species form an ascending aneuploid series and are believed to have evolved from an archetype with x = 6 through secondary polyploidy. Evidence in support of this comes from secondary association among bivalents (Catcheside, 1934), chromosome association in haploids (Prakash, 1974; Armstrong & Keller, 1981, 1982), pachytene chromosome analysis (Robbelen, 1960), and rDNA genes (Quiros et al, 1987). Robbelen proposed the genetic constitution of diploid genomes as : AABCDDEFFF for B.campestris, ABCDDEFF for B.nigra and ABBCCDEEF for B.oleracea.

Alloploids are chromosomally balanced and form regular bivalents between homologous chromosomes at meiosis. Recent work on Rubisco and chloroplast and mitochondrial DNA established that B.nigra, B.oleracea and B.campestris are the cytoplasmic donors of B.carinata, B.napus and B.juncea respectively (Erickson et al, 1983; Palmer et al, 1983; Palmer 1988). It was also suggested that during the long course of evolution mt and ct genomes are coinherited. B.oleracea, B. campestris and B.juncea show enormous morphological diversity, and a high level of DNA polymorphism (Figdore et al, 1988).



Triangle of U



ORIGIN OF BRASSICA SPP.
(Solid lines represent female parents and dotted lines male)

Mizushima (1950, 1980) from a study of the amount of pairing in haploids and interspecific hybrids suggested close homology between the three basic genomes. He inferred that as many as 3,8 and 4 bivalents were of allosyndetic origin in AB, AC and BC hybrids. The intergeneric pairing is attributed to chromosome duplication, the latter having recently been established by RFLP (Figdore, 1988; Slocum, 1990).

Genome manipulation

Alloploidy

The validity of Morinaga's hypothesis on genome analysis (1934) was proved by experimental synthesis of naturally occurring alloploids viz. B.napus (U, 1935; Frandsen, 1947); B.juncea (Frandsen, 1943; Ramanujam & Srinivasachar, 1943), and B.carinata (Frandsen, 1947). Subsequently, the objective shifted to obtaining new genetic variants from utilization of the extensive variability in constituent diploid species and transfer of desirable genes. Important investigations include those on B.napus (Olsson, 1960; Nishi, 1959, Hosoda, 1961, Sarashima, 1973; Chen et al. 1988), B.juncea (Olsson, 1960; Prakash, 1973) and B.carinata (Prakash et al. 1983). These investigations revealed that (1) crosses between tetraploids yield more hybrids than between diploids (2) synthesis of B.carinata and B.juncea is easier than synthesis of B.napus (3) synthetics with reciprocal cytoplasms showed discernible effects on plant morphology. Examples are of B.carinata (Chopra & Prakash, 1991) and B.juncea (Craig et al 1991) (4) although synthetic alloploids resemble the natural forms in general, new usable variations are common (5) the synthetic alloploids are, to a large extent, pollen and seed sterile in the initial generations due to disturbed meiosis and genetic incompatabilities, and (6) the fertility gradually improved and was accompanied by reduction in frequencies of multivalents and univalents.

The yield of best synthetic was at par with, or inferior to, the existing cultivars. However, the synthetics constitute an important source of genetic variability that can be incorporated into agronomic types. In fact, it has been observed that far superior types arise when crosses are made between synthetics and cultivars. Svalof Panter, Norde, Brink and Jupiter (oilseed rape) in Sweden (Olsson, 1986) and 'Co' and Hakuran (fodder rape in Japan, Nishi, 1980) which have been marketed are from synthetic x cultivar crosses. Also synthetic genotypes of B.napus have been obtained from early indigenous constituent parents which has made cultivation of B.napus possible in India (Prakash, 1980). With the advancement of in vitro techniques, somatic hybrids of B.napus and B.juncea have also been obtained.

Several other alloploids which do not exist in nature have also been synthesized. B.napocampestris, a 58-chromosome auto-allohexaploid (AAAACC) was first obtained by Frandsen & Winge (1932) from the cross B.napus ssp. rapifera x B.campestris. Because of its genetical constitution, the meiosis is considerably disturbed with frequent higher associations (0-9 IV, 0-1 VI). An extensive range of B.napocampestris has been built by combining variations of B.campestris (Mc Naughton 1973; Olsson & Ellerstrom, 1980). The primary triploid hybrids(AAC, 2n=29) are vegetatively very vigorous and it has been suggested that they should be commercially exploited using self-incompatible or male sterile systems of B.napus for hybrid seed production. Raphanobrassica (2n=36,RRCC), the intergeneric alloploid between Raphanus sativus and B.oleracea was produced by Karpechenko (1926). Since then, numerous investigators have produced the intergeneric hybrid incorporating into

it the enormous diversity of B.oleracea. Reports on chromosome pairing behaviour have varied but a majority reported regular chromosome pairing (18II, 93%). However, seed fertility was very poor (1.3 seeds per pod) which has been attributed to genetic imbalance. Raphanobrassica will offer good prospects as a new fodder crop provided seed fertility can be improved (Olsson & Ellerstrom, 1980). Another intergeneric alloploid, Eruca-brassica (2n=42), has recently been produced through embryo rescue of the hybrid Eruca sativa (2n=22) x B.campestris (Agnihotri et al 1990). It showed 21 bivalents and has a high seed set (80%) in generation A1. The seed-set improved considerably in later generations. The seed yield was around 1800 kg/ha. It appears to be a promising strain and needs further improvement.

Haploidy

In the earlier years, haploids were either of spontaneous origin (the frequency being genotype dependent) or were recovered, as a result of parthenogenesis, from interspecific hybridization. Now pollen derived haploids can be obtained in large numbers as a reproducible method (Keller, 1984; 1990). Chromosome pairing in haploids of basic species (B.nigra, 2II + 4I; B.oleracea, 3II + 3I; B.campestris, 1III + 2II) has been used in interpreting the basic chromosome number of the archetype as x = 6. Haploids offer an opportunity of improving selection efficiency for recovering recombinants from F1 intervarietal hybrids and for obtaining homozygosis in plant improvement programmes. Doubled haploid of B.napus viz. Maris Haplona is under extensive cultivation in Europe.

Autoploidy

No natural autoploid is known to occur in crop Brassicas. Induced tetraploids have, however, been obtained in all species. Tetraploids of monogenomic species are vigorous as compared to those of digenomics and have lower quadivalent frequency (mean value of 0.8, 1.5-4.0 and 1.94-4.83 per cell in B.nigra, B.oleracea and B.campestris respectively). Meiosis in tetraploids of digenomics is highly irregular and quadrivalents are frequent. All the induced tetraploids have very poor seed set and are of little practical utility. However, tetraploids has been exploited successfully in fodder and forage types. Fodder forms of turnip(Svalof Siruis) and marrow-stem kale (Svalof Tema) have been marketed in Europe (Olsson, 1986).

Trigenomic hexaploids

Trigenomic hexaploids combining A,B and C genomes (2n=54) have been synthesized artificially by several workers. Plants have frequent multivalents and are weak.Takeda (1975) made a detailed study upto generation A10 and observed that plants were chromosomally unstable with chromosome number ranging from 42 to 54. They had poor seed fertility (50%) and were of no practical value.

Somatic hybrids

Synthetic alloploids obtained through sexual hybridization lack cytoplasmic diversity. Somatic hybrids, on the other hand, combine both the parental cytoplasms and generate new combinations of chloroplasts and mitochondrial genes. Gleba & Hoffman (1978) produced the first somatic hybrid in Cruciferae by fusion of Arabidopsis thaliana and B.campestris protoplasts. However, the first chromosomally balanced and fertile somatic hybrid was that of B.napus (Schenk & Robbelen, 1982). Subsequently, there has been a spurt and besides somatic synthesis of natural alloploids, a number of new interspecific and

intergeneric hybrids have been obtained (Table 1). An important feature of these somatic hybrids is the high degree of sterility inspite of normal meiosis. The observed sterility has been attributed to genetic incompatibilities. The hybrids have been produced primarily to generate variability (as in B.napus and B.juncea) and to obtain new interspecific/ intergeneric combinations in situations where strong hybridization barriers inhibit the production of sexual hybrids.

Non-homologous recombination and introgression of alien genes:

Non-homologous recombination between different genomes has been exploited to generate morphological and physiological variations in B.juncea (Prakash, 1973) and to transfer desirable attributes across interspecific barriers. The attributes transferred include club-root resistance (Plasmodiophora brassicae) from B.campestris to B.napus (Johnston, 1974) and from B. napus to B.oleracea (Chiang & Crete, 1983); increased fertility from Raphanus to Brassico-raphanus (Tokumasu, 1976); self-incompatibility alleles to forage rape (Namai et al 1980) and resistance to pod shatter from B.nigra to B.napus (Prakash & Chopra, 1990). Since allosyndesis frequently occurs between different genomes in the hybrids, it has wide application in breeding of crop Brassicas.

Production of alloplasmics:

A potential exploitable option of somatic hybridization is the development of pollen sterile alloplasmics (maternally inherited) for hybrid seed production. Elegant experiments involving restriction patterns of mitochondrial and chloroplast genomes have established that male sterility trait is encoded in chondriome DNA (Erickson et al 1986). Four alloplasmic systems have been developed in Brassica. Sterility inducing cytoplasm of Raphanus sativus (discovered by ogura in 1968 and often referred to as 'Ogura' cytoplasm) has been introgressed into B.oleracea, B.campestris and B.napus (Bannerot et al 1974). Subsequently alloplasmics (B.nigra) B.oleracea var. italica (Pearson, 1972); (Diplotaxis muralis) B.c. ssp. pekinensis (Hinata & Konno, 1979), and (B.oxyrrhina) B.campestris and B.juncea (Prakash & Chopra, 1988, 1990) were obtained. Raphanus and oxyrrhina cytoplasms also induce chlorosis (thermosensitive in the former) due to genetic incompatibilities between alien chloroplasts and nuclei. Chlorosis has been corrected in 2 ways;(1) by substituting the alien chloroplasts by those of nuclear chloroplasts through cytoplasmic hybridization as was accomplished in (Raphanus) B.napus (Pelletier et al 1983; Jarl & Bormann, 1988) and (Oxy) B.juncea (Kirti et al, 1991); and (2) by introgressing chlorosis correcting genes from the cytoplasmic donor genome chromosomes through conventional hybridization. This mechanism involved non-homologous recombination between Raphanus and B.napus chromosomes (Paulman & Robbelen, 1988) and B.oxyrrhina and B.juncea chromosomes (Prakash & Chopra, 1990). Fertility restoring genes for 'Ogura' CMS , which are available in many European radish cultivars, have been incorporated into B.napus (Rouselle & Dosba, 1985; Heyn 1979; Paulman & Robbelen 1988). Chromosome of B.oxyrrhina that carries genes for fertility restoration for 'oxy' CMS has been identified and is being transferred to B.juncea.

Cytogenetics of wild allies:

Many taxa which are sufficiently related to crop Brassicas and can be good donors of genetic material, are available in Brassica coenospecies. They are the source of many useful nuclear genes and cytoplasmic male sterility. Mizushima (1968) initiated cytogenetical work on wild species. Harberd (1976) made extensive studies on wild

Brassicas and their allies and classified them into cytodemes. Forty six cytodemes belonging to 10 genera have been identified and their distribution is as follows: Brassica (12); Raphanus (1); Diplotaxis (13); Enarthrocarpus (1); Eruca (1); Erucastrum (10); Hutera (1); Sinapidendron (1); Sinapis (5), and Hutera (1). The lowest chromosome number in the group is n=7. Half of the cytodemes have gametic chromosome number centered around 9 and 10. Polyploidy is not a dominant occurrence: Only 9 cytodemes are polyploids (6 auto- and 3 alloploids). Harberd (1976) and Harberd & McArthur (1980) have made numerous hybrids in the Brassica group. Extensive hybridizations, induction of alloploidy and its exploitation for introgression of desirable genes and cytoplasmic substitutions for generating male sterility are underway in our laboratory. Wide hybridization has resulted in many hybrids, both interspecific and intergeneric, (Mizushima, 1968; Harberd & McArthur, 1980; Takahata & Hinata, 1983; Prakash & Chopra 1990). The characteristic feature of meiosis in the hybrids is the virtual absence of multivalents and the occurrence of monochiasmate bivalents in varying numbers. It also appears that basic cytodemes are aneuploids, are highly differentiated and have independently evolved from an archetype. A number of synthetic alloploids involving such wild species as B.oxyrrhina, B.tournefortii, B.fruticulosa, B.spinescens, Sinapis arvensis, Diplotaxis muralis, D. settiana, D.harra, Erucastrum gallicum, Moncandia arvensis have been produced. They are characterized by occurrence of multivalents, irregular meiosis and poor seed set. These drawbacks and other undesirable characters hinder their direct applied use. However, they may serve as useful fertile bridges for gene transfer and cytoplasmic substitutions.

Alien chromosome addition lines:

Construction of alien chromosome addition lines to dissect the genome and assigning species-specific characters to particular chromosome is a recent development. Quiros et al (1987) generated 8 monosomic and desomic chromosome addition plants of B.oleracea in the background of B.campestris genome. These lines were characterized by genome specific markers. Jahier et al (1989) developed disomic addition lines of B.nigra in B.napus genome. McGarth & Quiros (1990) also obtained B.oleracea addition lines representing 7 of the synteny groups and reported that chromosome 7 had a marked influence on female fertility and was responsible for reproductive isolation of this species.

Future approaches: The following future approaches will prove useful:

1. Generation of chromosome addition lines to dissect basic genomes will provide information on the location of genes on specific chromosomes and genome characterization. Alien addition lines are most useful for transferring characters from one species to another.
2. Desirable genes are frequently scattered in wild allies. Also, their chondriome and plastome DNA sequence control many traits of breeding significance, i.e. photosynthetic efficiency, disease and herbicide resistance and cytoplasmic male sterility. Introgression of these characters across interspecific and intergeneric barriers may be facilitated by the advancements in vitro, somatic cell genetics and recombinant DNA techniques.
3. Enormous variability for practical application can be created in natural alloploid species by their resynthesis exploiting the vast variations of constituent diploid species through sexual and somatic hybridization.

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Table 1. Somatic hybrids in Cruciferae

Combinations	Chromosome number and behaviour at MI of meiosis	Pollen fertility (%)	Seed fertility (%)	Reference
Arabidopsis thaliana (2n=10) + B. campestris (2n=20)	2n=30, 40	Sterile	Sterile	Gleba & Hoffman, 1979
B. campestris (2n=20) + B. oleracea (2n=18)	2n=38	Low	Low	Schenck & Robbelen, 1982
		-	-	Robertson et al, 1985
		-	-	Taguchi and Kameya, 1986
		-	-	Jourdan et al, 1986
	2n=38, 48-86	5-70	1-40	Sunderberg et al, 1987
	2n=30-74	-	-	Rosen et al, 1988 Yamashita et al, 1989
Moricandia arvensis (2n=28) + B. oleracea (2n=18)	2n=46	Sterile	Sterile	Toriyama et al, 1987
Sinapis turgida (2n=18) + B. nigra (2n=16)	2n=34	-	-	Toriyama et al, 1987
Sinapis turgida (2n=18) + B. oleracea (2n=18)	2n=36	-	-	Toriyama et al, 1987
B. napus (2n=38) + Sinapis alba (2n=24)	2n=62; 1 IV + 29 II	20	Low	Primard et al, 1988
Diplotaxis muralis (2n=42) + B. juncea (2n=36)	2n=78, 3 IV + 33 II	-	-	Chatterjee et al, 1988
B. napus (2n=38) + Eruca sativa(2n=22)	-	14-97	7-29	Fahlson et al, 1988
B. napus(2n=38) + Diplotaxis harra (2n=26)	2n=64	Very low	Sterile	Klimaszewska & Keller, 1988
CMS B.napus(2n=38) + B. nigra(2n=16)	2n=54	Sterile	Sterile	Yamagishi et al, 1989
B.juncea (2n=36) + Eruca sativa	2n=58, 1 IV + 27 II	0-82	2-50	Sikdar et al, 1990
B. campestris (2n=20) + B. nigra (2n=16)	2n=36; 18 II	46	Low	Campbell et al, 1991
B. spinescens (2n=16) + B. juncea (2n=36)	2n=52; 26II	Sterile	Sterile	Kirti et al, 1991
Trachystoma balli (2n=16) + B. juncea (2n=36)	2n=52; 26II	Sterile	Sterile	Kirti et al, 1991