Products of somatic hybrids and cybrids

Chromosome status of somatic hybrids

The chromosome numbers of the somatic hybrids successfully obtained through protoplast fusion indicate that only few have exact number expected in an amphiploid. Hence selection will also need to be applied at the cytological level, if true amphiploids need to be obtained. The variability in chromosome number in hybrids could be due to any one of the following reasons:

(i) Multiple fusions give a higher chromosome number. In PEG-induced and electro-induced fusions between more than two protoplasts.
(ii) Asymmetric hybrids result from fusion of protoplasts isolated from actively dividing tissue of one parent and quiescent tissue of the other parent.
(iii) Unequal rates of DNA replication in two fusing partners may also give asymmetric hybrids.
(iv) Somaclonal variation in cultured cells used for protoplast isolation may also lead to variation in chromosome number.

Practical applications of somatic hybridisation and cybridisation

1. Means of genetic recombination in asexual or sterile plants

Somatic cell fusion appears to be the only approach through which two different parental genomes can be recombined among plants that cannot reproduce sexually. Further, protoplasts of sexually sterile (haploid, triploid and aneuploid) plants can be fused to produce fertile diploids and polyploids. There are several reports describing the amphidiploid and hexaploid plants produced from fusion of haploid protoplasts of tobacco. Protoplasts isolated from dihaploid potato clones have been fused with isolated protoplasts of *Solanum brevidens* to produce hybrids of practical breeding value. Haploid protoplasts from an anther-derived callus of rice cultivars, upon fusion also produce fertile diploid and triploid hybrids.

2. Overcoming barriers of sexual incompatibility

In plant breeding programmes, sexual crossings at interspecific or intergeneric levels often fail to produce hybrids due to incompatibility barriers. The bottlenecks in sexual hybridisation may therefore, be overcome by somatic cell fusion. In some cases somatic hybrids between two incompatible plants have also found application in industry or agriculture.

Schieder (1978) obtained amphidiploid *Datura innoxia* (+) *D. discolor* and *D. innoxia* (+) *D.*

stramonium, by fusing their diploid mesophyll protoplasts. These hybrids did not exist in nature as conventional breeding procedures proved unsuccessful. Somatically produced amphidiploids of these combinations of *Datura* species are propagated for industrial uses as they demonstrate heterosis and higher (20-25%) scopolamine content than in the parental forms.

Nicotiana repanda, *N. nesophila* and *N. stockonii* are resistant to a number of diseases but are not sexually crossable with tobacco (*N. tabacum*). However, fertile hybrids have been reported in combination *N. tabacum* (+) *N. nesophila* and *N. tabacum* (+) *N. stocktonii* by protoplast fusion. Somatic hybridisation of dihaploid and tetraploid potato protoplasts with isolated protoplasts of *Solanum brevidens*, *S. phureja* and *S. pennelii* resulted in the synthesis of fertile, partially **amphieuploid** plants possessing important agricultural traits, e.g., resistance to potato leaf virus, potato virus Y and *Erwinia* soft rot. Using this approach, tomato (*Lycopersicon esculentum*) hybridised somatically with a number of wild species has resulted in the synthesis of hybrids which are fertile and used in breeding programmes. Interspecific somatic hybridisation involving species that are sexually incompatible with egg-plant (*Solanum melongena*) has also resulted in the production of amphidiploids with traits resistant to verticillium wilt.

Rapeseed (*Brassica napus*) is a natural amphidiploid of *B. oleracea* and *B. campestris*. Schenk (1982) was the first to resynthesise rapeseed *in vitro* using protoplast fusion. Somatic hybridisation between *B. napus* and *B. nigra* cultivar, possessing the gene for resistance to *Phoma lingam*, yielded amphidiploid plants carrying this gene. These hybrids possess all the three *Brassica* genomes (A, B and C) and are now incorporated in breeding programmes. Recently, hybrids have been produced parasexually by protoplast fusion, between *Brassica juncea* (a major oilseed crop of the tropical world) and the sexually incompatible species *Diplotaxis muralis* and *Erica sativa*.

The potential of somatic hybridisation in perennial tree breeding is best illustrated by interspecific and intergeneric somatic hybridisation among citrus species. Somatic hybrids produced through these experiments are amphidiploids featuring characteristics for scion improvement and increased rootstock potential.

Somatic hybrids for cytoplasmic male sterility

Methods were also developed to substitute the nucleus of one species into the cytoplasm of another species, whose mitochondria were inactivated. This type of substitution in some cases, led to generation of cytoplasmic male sterility.

For this purpose, the two types of protoplasts, used for the production of somatic hybrids, were treated differently, as follows:

(i) mesophyll protoplasts of tomato (*Lycopersicon esculentum*) were treated with iodoacetamide (IOA) to inactivate mitochondria and

(ii) (ii) mesophyll protoplast of *Solanum acaule* (or S. *tuberosum*) were irradiated with g or x-rays to inactivate nuclei.

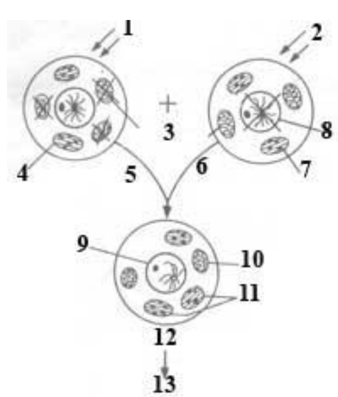
The protoplasts were mixed in 1:1 ratio and induced to fuse using Ca^{2+} and PEG, leading to the production of heterologous or alloplasmic hybrids. Among the fusion products, some hybrid tomato plants were indistinguishable from the original cultivars, with respect to morphology, physiology and chromosome number (2n = 24), but exhibited various degrees of male sterility. In five tomato cultivars, male sterility induced in this manner was inherited maternally over several generations. Therefore, it was obviously cytoplasmic male sterility. The mitochondrial DNA of these CMS hybrids did not resemble mtDNA of either parent, and was instead recombinant type, representing a hybrid mitochondrial genome. Therefore, protoplast fusion can be effectively used for production of CMS lines and has the following advantages:

(i) Only one step is required;

(ii) The nuclear genotype of the cultivar remains unaffected,

(iii) There are prospects that 100% of the progenies of somatic hybrids will be CMS. The restorer lines for these CMS lines have also been shown to be available in tomato, so that hybrid seed can be produced without manual emasculation.

Generation of cytoplasmic male sterility by fusion



1. IOA (damages mitochondria), 2. γ rays or x-rays (inactivate nuclei), 3. Mitochondria, 4. chloroplast (tomato protoplasts), 5.Ca^{++}+ PEG, 6. Protoplast fusion, 7. chloroplast (potato or S.*acaule* protoplasts), 8. Nucleus, 9. tomato nucleus, 10. recombinant mitochondira, 11. chloroplasts (mixture), 12. fused protoplasts, 13. somatic hybrid plants (CMS)-resemble tomato

Cytoplasm transfer

Power *et al*. (1975) fused mesophyll protoplasts of *Petunia* with cultured cell protoplasts of the crown gall of *Parthenocissus* and selected a line which contained the chromosomes of only *Parthenocissus* but exhibited some of the cytoplasmic properties of *Petunia* for some time. This was followed by direct application of cybridisation in agricultural biotechnology by transfer of cytoplasmic male sterility from *Nicotiana techne* to *N. tabacum*, *N. tabacum* to *N. sylvestris* and *Petunia hybrida* to *P. axillaris*. Besides cytoplasmic male sterility, the genophore of the cytoplasm codes for a number of practically important traits, such as the rate of photosynthesis, low or high temperature tolerance and resistance to diseases or herbicides. Recent experiments on cybridisation have resulted in plants with reconstructed cytoplasm combining mitochondrial DNA (mt DNA) and cp DNA encoded traits from both parents.

The best example illustrating the potential for protoplast fusion in reconstructing cytoplasm for practical purposes is the genus *Brassica*. Two desirable traits coded by cytoplasmic genes have been genetically manipulated through interspecific cybridisation between different species of *Brassica*. These traits include cytoplasmic male sterility (cms) and resistance to atrazine herbicides. The cms gene in *Brassica* plants, *Diplotaxis muralis* and *Raphanus sativus* is of alloplasmic (the nucleus of one species into a foreign cytoplasm) origin. Raphanus sativus is of interest because it leads to complete male sterility. Cms restorer genes have been introduced into rapeseed (*Brassica napus*) from this plant. Mutants resistant to atrazine herbicide have also been discovered both in *Brassica napus* and *B. campesteris*. Protoplast fusion experiments (conducted in various laboratories) have resulted in the synthesis of cybrid plants with reconstructed cytoplasm combining both cms (coded by *Raphanus* mt DNA) and low temperature tolerance or atrazine resistance (coded by *Brassica* cp DNA). Similarly, cytoplasmic genes coding for atrazine resistance and cms have been transferred into cabbage, rice and potato.

In somatic hybridisation and cybridisation, the essential pre requisite is that parental protoplasts and their fusion products regenerate to whole plants. Research in the past decade has shown that plants can be raised *in vitro* from isolated protoplasts of species belonging to a range of angiosperm families. Somatic hybrids have been produced between sexually compatible as well as incompatible species. It could be possible to overcome prezygotic embryo/endosperm (*Petunia parodii* (+) *P. inflate*) and postzygotic (*Datura innoxia* (+) *D. stramonium*; *Petunia parodii* (+) *P. parviflora*), incompatibility barriers by protoplast fusion. Experiments on intergeneric somatic hybridisation have also been successful in some cases such as potato (+) tomato somatic hybrids and synthesis of 'Arabidobrassica'. With these initial successes, and subsequent advancements in protoplast technology it is desirable that efforts be concentrated on important plant species which have potential in industry or for food production. Crops which have not yielded satisfactory results through conventional methods of genetic manipulation need to be aided by non-conventional *in vitro* techniques such as somatic hybridisation/cybridisation, embryo culture, etc. to manifest their full potential.

Interspecific hybrids produced through protoplast fusion

Parent species and their chromosome numbers	Chromosome number of hybrid
Brassica oleracea (2n = 18) + B. Campestris (2n = 20) B. napus (2n = 38) + B. oleracea (2a = 18) B. napus (2a = 38) + B. nigra (2n = 16) B. napus (2n = 38) + B. carinata (2n = 34) B. napus (2n = 38) + B juncea (2n = 36)	Wide variation
Nicotiana glauca (2n = 24) + N. longsdorfii (2n = 18)	56-64
N. tabacum (2n = 48) + N. alata (2n = 18)	66-71
N. tabacum (2n = 48) + N. glauca (2n = 24)	72
N. tabacum (2n = 48) + N. glutinosa (2n = 24)	50-88
N. tabacum (2n = 48) + N. Knightiana (2n = 24)	44-137
N. tabacum (2n = 48) + N. mesophile (2n = 48)	96
N. tabacum (2n = 48) + N. octophora (2n = 24)	48
N. tabacum (2n = 48) + N. rustica (2n = 48)	60-91
N. tabacum (2n = 48) + N. stocktonii (2n = 48)	96
N. tabacum (2n = 48) + N. sylvestris (2n = 24)	72
N. tabacum (2n = 48) + N. phumbaginifolia (2n = 20)	-

Petunia parodii (2n = -48) + P. hybrida (2n = 20)	44-48
P. parodii (2n = 14) + P. hybrida (2n = 14)	46
P. parodii (2n = 48) + P. parviflora (2n = 18)	31-40
Solnum tuberosun (2n = 24, 48) + S. chapcoense (2n = 14)	60
S. tuberosum (2n = 24, 48) + S. brevidens (2n = 24)	-
Lycopersicon esculentum (2n = 24) + L. Peruvianum (2n = 14)	72
Daucua carota (2n = 18) + D. capillifolius (2n = 18)	36, 38
Datura innoxia (2n = 24) + D. capillifolius (2n = 24)	46, 48, 72
D. innoxia (2n = 24) + d. sanguinea (2n = 24)	46, 72, 96
D. innoxia (2n = 24) + D. candida (2n = 24)	-

Intergeneric hybrids produced through protoplast fusion

Plant species and their chromosome numbers	New genus
Raphanus sativus (2n = 18) + B. oberacea (2n = 18)	Raphanobrassica
Moricandia arvensis (2n = 24, 28) + B. oleracea (2n = 18)	Moricandiobrassica
Eruca sativa (2n = 22) + B. napus (2n = 38)	Erucobrassica
E. sativa (2n = 22) + B. juncea (2n = 36)	Erussica
Diplotaxis muralis (2n = 42) + B. napus (2n = 38)	Diplotaxobrassica
D. muralis (2n = 42) + B. juncea (2n = 36)	Diplotaxojuncea
Sinapis alba (2n = 24) + B. napus (2n = 38)	Sinapobrassica
S. alba (2n = 24) + B. oleracea (2n = 18)	Sinapo-oleracea
Nicotiana tabacum (2n = 24) + Lycopersicon esculentum (2n = 24)	Nicotiopersicon
N. tabacum (2n = 24) + Petunia inflorata (2n = 14)	Nicotiopetunia
Solanum tuberosum (2n = 24) + Lycopersicon esculentum (2n = 24)	Solanopersicon
Daucus carota (2n = 18) + Petroselinum hortense (2n = 22)	Daucoselenium
Datura innoxia (2n = 48) + Atropa belladonna (2n = 24)	Daturotropa
Oryza sativa (2n = 24) + Echinochloa oryzicola (2n = 24)	Oryzochloa

Intertribal somatic hybrids produced within the family Brassicaceae

Arabidopsis thaliana (Tribe Sisymbrieae)	(2n = 10) +	B. Campestris (2n = 20) (Tribe Brassiceae)	Arabidobrassica
Thlaspi perfoliatum (Tribe Lepideae)	(2n = 14) +	B. napus (2n = 38) (Tribe Brassiceae)	Thlaspobrassica
Barbarea vulgaris (Tribe Arabideae)	(2n = 16) +	B. napus (2n = 38) (Tribe Brassiceae)	Barbareobrassica

Even somatic hybrids of sexually compatible plants may exhibit new variations as a result of interactions between plastomes donated by parental species during protoplast fusion. The technique of cybridisation, besides transfer of male sterility, can be adopted for the introduction of genes for resistance into the new species. The modification of plants with respect to nitrogen fixation can also be contemplated through transformation of protoplasts by uptake of exogenous DNA, or organelles, carrying this trait. Further, genetically heterogenous clones can be derived from protoplast culture and fusion which display a high frequency of variations for several agronomic traits.

The above developments suggest an immense potential for somatic cell genetics in crop improvement. However, the genetic diversity that can be generated *via* somatic cell fusion is still poorly understood. This is because only a very limited number of the synthesised somatic hybrids or cybrids have been fertile or amphiploids. Induction and control over the degree of species-specific chromosome elimination in wide or distant somatic hybridisation requires to be mastered in order to understand the mechanism of producing desirable asymmetric nuclear hybrids.

Questions

1. The variability in chromosome number in somatic hybrids could be due to …………

a). Multiple fusions give a higher chromosome number	b). Asymmetric hybrids
c). Somaclonal variation in cultured cells	**d). All the above**

2. The somatic hybridisation is used …………

a). Genetic recombination in asexual or sterile plants	b). Overcoming barriers of sexual incompatibility
c). Both a and b	d). None of the above

3. The first somatic hybrids are produced in …………

a). *Datura innoxia*	b). *Nicotiana repanda*
c). *N. nesophila*	d). *N. stockonii*

4. The first somatic hybrids are produced by …………

a). Schieder	b). Cocking
c). Schenk	d). None of the above

5. …………….. was the first to resynthesise rapeseed *in vitro* using protoplast fusion.

a). Schieder	b). Cocking
c). Schenk	d). None of the above