A model for transfer of nuclear and organelle genes from alien species into oilseed Brassicas by fusion of microspore protoplasts with somatic cell protoplasts

Deepak Pental, Arundhati Mukhopadhyay, Akshay K. Pradhan

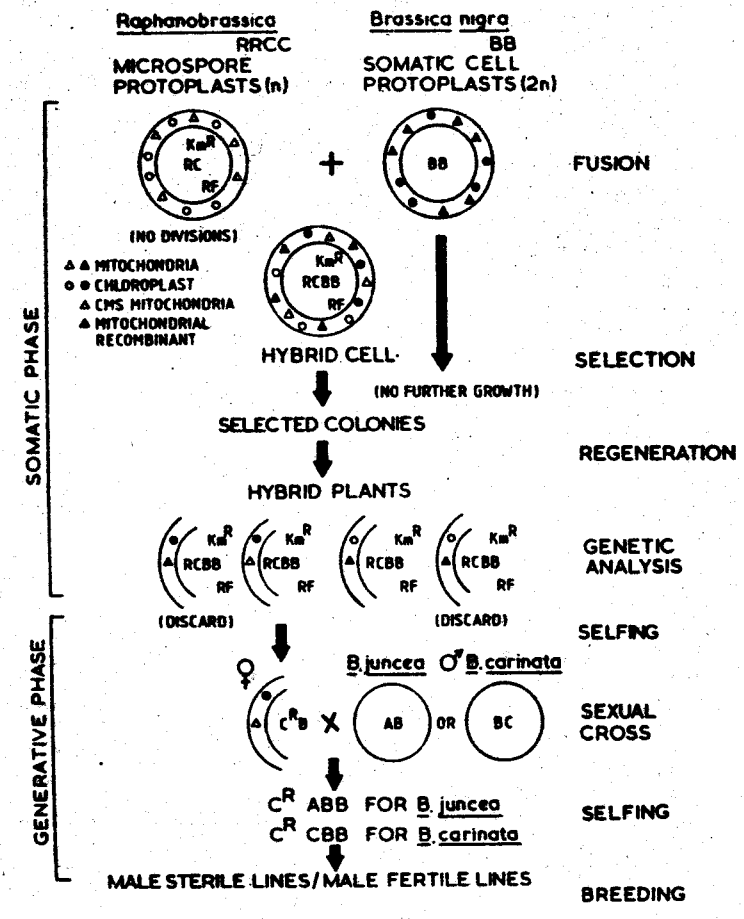
Biotechnology Programme, Tata Energy Research Institute, 90 Jor Bagh, New Delhi, India.

We propose a model for bringing about effective transfer of nuclear and organelle genes from alien species into digenomic oil seed Brassicas namely - B. napus AACC, B. juncea AABB and B. carinata BBCC. The model combines use of the techniques of somatic cell hybridization and sexual hybridization to bring about alien gene introgression.

The first step involves combining the alien species genome with the genome of a monogenomic species - B. campestris AA, B. nigra BB or B. oleracea CC by sexual crosses. Resulting hybrids would carry the cytoplasm of the alien parent and a hybrid nuclear genome with one set of chromosomes from both the parents. Protoplasts of such a plant could be fused with somatic cell protoplasts of a monogenomic species. Alternatively, hybrid plants of the alien parent and monogenomic species can be diploidized by colchicine treatment. Protoplasts isolated from microspores of the alien/monogenomic species hybrid could be fused with somatic cell protoplasts of a monogenomic species of the Brassica family.

Description of the Model (Fig.)

We explain the model by taking an example of Raphanus sativus RR as the alien parent. R. sativus carrying 'Ogura' type cytoplasmic male sterility and fertility restorer gene (Rf1/Rf1) can be hybridized with B. oleracea CC to synthesise Raphanobrassica RC. Hybrids can be diploidized with colchicine treatment to produce Raphanobrassica with RRCC genome. Microspore protoplasts isolated at the tetrad stage can be fused with somatic cell protoplasts of B. nigra BB to produce plants with genomic constitution RCBB.


Raphanobrassica
RRCC
MICROSPORE
PROTOPLASTS (n)
Brassica nigra
BB
SOMATIC CELL
PROTOPLASTS (2n)
KmR
RC
RF
BB
FUSION
(NO DIVISIONS)
△▲ MITOCHONDRIA
○● CHLOROPLAST
△ CMS MITOCHONDRIA
▲ MITOCHONDRIAL RECOMBINANT
KmR
RCBB
RF
HYBRID CELL
SELECTION
(NO FURTHER GROWTH)
SELECTED COLONIES
REGENERATION
HYBRID PLANTS
GENETIC ANALYSIS
(DISCARD)
(DISCARD)
SELFING
♀
B. juncea
♂ B. carinata
CRB
X
AB
OR
BC
SEXUAL CROSS
CR ABB FOR B. juncea
CR CBB FOR B. carinata
SELFING
MALE STERILE LINES / MALE FERTILE LINES
BREEDING
SOMATIC PHASE
GENERATIVE PHASE

Such gameto-somatic hybrids can be screened for their organelle constitution. Novel chloroplast and mitochondrial combinations and recombinants of mitochondrial genome can be identified at this stage.

In the hybrids with RCBB constitution, at the time of meiosis, pairing would occur between R and C genome chromosomes while BB genome will provide meiotic stability by perfect pairing. The extent of genetic exchange, however, will depend upon the extent of homology between R and C genome chromosomes. Hybrid plants with RCBB constitution are the bridging material for transfer of R. sativus nuclear and organelle traits into digenomic species - B. juncea AABB and B. carinata BBCC. Hybrids RCBB could be sexually crossed with B. juncea AABB or B. carinata BBCC to produce plants with genomic constitution of C^R ABB for crosses with B. juncea and plants with genomic constitution C^R CBB for crosses with B. carinata. Such plants could be selfed or back crossed for the transfer of R. sativus genes into B. juncea and B. carinata.

If the RCBB plants carried mitochondria of R. sativus or mitochondrial recombinants, one could effectively monitor the introgression of nuclear restorer gene by selecting for male fertile plants in the subsequent sexual crosses. No test cross will be needed. In those plants where no introgression of restorer genes has occurred male sterile lines of B. juncea and B. carinata can be isolated with desired orgranelle combinations and mitochondrial recombination.

Variations on the Model

The use of microspore protoplasts for the production of allotriploids has been outlined earlier (Pental and Cocking, 1984) and some selection schemes for recovering allotriploid hybrids from the parental protoplasts were discussed.

If a dominant genetic marker e.g. kanamycin resistance is introduced into the alien (e.g. RR) or monogenomic Brassica parent (e.g. CC) which is sexually crossed with the former, it would be sufficient for selecting a large number of RCBB plants as has been shown in the Figure. Microspore protoplasts do not divide

and somatic cell protoplasts of B. nigra could not divide in presence of kanamycin. As an alternative, protoplasts of RC plants carrying Km^R can be treated with a metabolic inhibitor e.g. indo acetic acid to stop them from dividing and select RCBB plants on a medium containing kanamycin.

No genetic markers would be needed if the techniques of single heterokaryon isolation, manual or automated, are used. However, we would prefer using dominant drug resistance markers as these can be introduced readily into plants by Agrobacterium based vectors using regenerating explants. For a large number of wide hybrid combinations, markers need to be introduced into only two of the monogenomic species - B. campestris AA and B. oleracea CC.

Discussion

In our initial model (Pental and Cocking, 1985) we had proposed synthesizing functional allotriploids by fusion of microspore derived protoplasts with somatic cell protoplasts. The importance of this genomic constitution for introgression of alien genes has been recently discussed (Rick et al, 1986). However, such a situation will lead to e.g. in case of R. sativus (n) + B. juncea (2n) a genomic constitution of RAABB. With A/A and B/B pairing preferentially, the scope for alien gene introgression will depend upon production of lines with alien chromosomes addition or alien chromosome substitution. The latter situation is not desirable, but could be rare and difficult to identify. Compared with this, RCBB constitution could afford better exchange between R and C chromosomes. For Brassicas the original model (Pental and Cocking, 1985) has been suitably modified and described here.

References

Pental, D and E.C. Cocking, 1985. Some theoretical and practical possibilities of plant genetic manipulation using protoplasts. Hereditas supp. Vol. 3: 83-93.

Rick,C.M., J.W.DeVerna, R.T.Chetlat and M.A.Stevens, 1986. Meiosis in sesquidiploid hybrids of Lycopersicon esculentum and Solanum lycopersicoides. Proc. Natl. Acad. Sci. USA 83: 3580-3583.