Theoretical and Applied Genetics 47, 299-302 (1976)

Genetic Transformation and "Graft-Hybridization" in Flowering Plants

K.K. Pandey

Genetics Unit, Grasslands Division, D.S.I.R., Palmerston North (New Zealand)

Summary. Recent pollination experiments with highly irradiated (100,000 r) pollen in *Nicotiana* have shown that radiation-"pulverized" pollen chromatin can cause genetic transformation of the egg. A new model is proposed here for integration of chromatin fragments into host chromosomes. It is also proposed that heterochromatin may be involved in the process of gene transfer, and in the phenomena of meiotic drive associated with gene transfer.

It is suggested that this discovery throws new light on the phenomenon of "graft-hybridization". In spite of many reports to the contrary, "graft-hybrids" have so far been explained only on the basis of their being chimaeras. A mechanism is suggested here by which they may result from genetic transformation.

Introduction

Recent pollination experiments with *Nicotiana* have shown that specific genes from pollen, which had been exposed to a high dose of radiation (100,000 r), could be transferred to the egg nucleus without actual gametic fusion (Pandey 1975). The highly irradiated pollen is still able to germinate and complete pollen tube growth but cannot effect normal fertilization. It was suggested that the radiation-"pulverised" pollen chromatin is discharged into the egg, and this acts as "pseudo-fertilization". The block to cell division in the egg is lifted as usual and the chromosomes are replicated, but the presence of disorganized pollen chromatin prevents a normal mitotic division. This results in a diploid egg with the "induced" physiology of a zygote. Presumably, at least in certain cells, the free chromatin fragments are rapidly degraded or lost, and their disorganizing effects soon overcome to permit subsequent divisions to occur normally. The resulting embryo is a parthenogenetic diploid.

1. Model of gene transfer

It was further suggested that the fragments of pollen chromatin may occasionally associate with their homologues in the egg chromatin, and this may lead to substitution or addition during replication. However, in the previous model explaining these results (Pandey 1975), no details were given regarding the possible molecular events leading to addition and substitution states. Fig. 1 provides a more precise model of gene transfer in which a single molecular mechanism accounts for both addition and substitution. Addition is the primary event, and the substituted state is secondarily derived by loss of a displaced strand of the original DNA.

One of the intriguing characteristics revealed in the testcrossed progeny of the transformed seedlings of *Nicotiana* occurred in triallelic plants. Here the pollen gene, in addition to the maternal gene, was believed to have been incorporated into the chromosome. However, it was the original maternal allele, and not the additional pollen allele, which tended to be lost. The present model (Fig. 1) explains why it should be so.

2. Proposed role of heterochromatin

Another intriguing property revealed by the testcrossed progeny concerned a general selection in favour of gametes containing the transferred gene. It is suggested here that a gene transfer is usually associated with a "heterochromatization effect" in the genomes which carry it. Firstly, the structural genes which are transferred may themselves be associated with heterochromatic regions, thus implying that there is a selection of genes which may be transferred. Secondly, chromatin fragmentation may possibly produce a heterochromatization effect at the breakpoints, as pointed out by Brink (1964). There are reported effects of heterochromatin on chromosome movement in maize and other plants (Rhoades and Dempsey 1966; Grant 1975). Genomes carrying a transfer (or a significant number of transfers) may therefore show accelerated movement on the spindle at meiosis, which

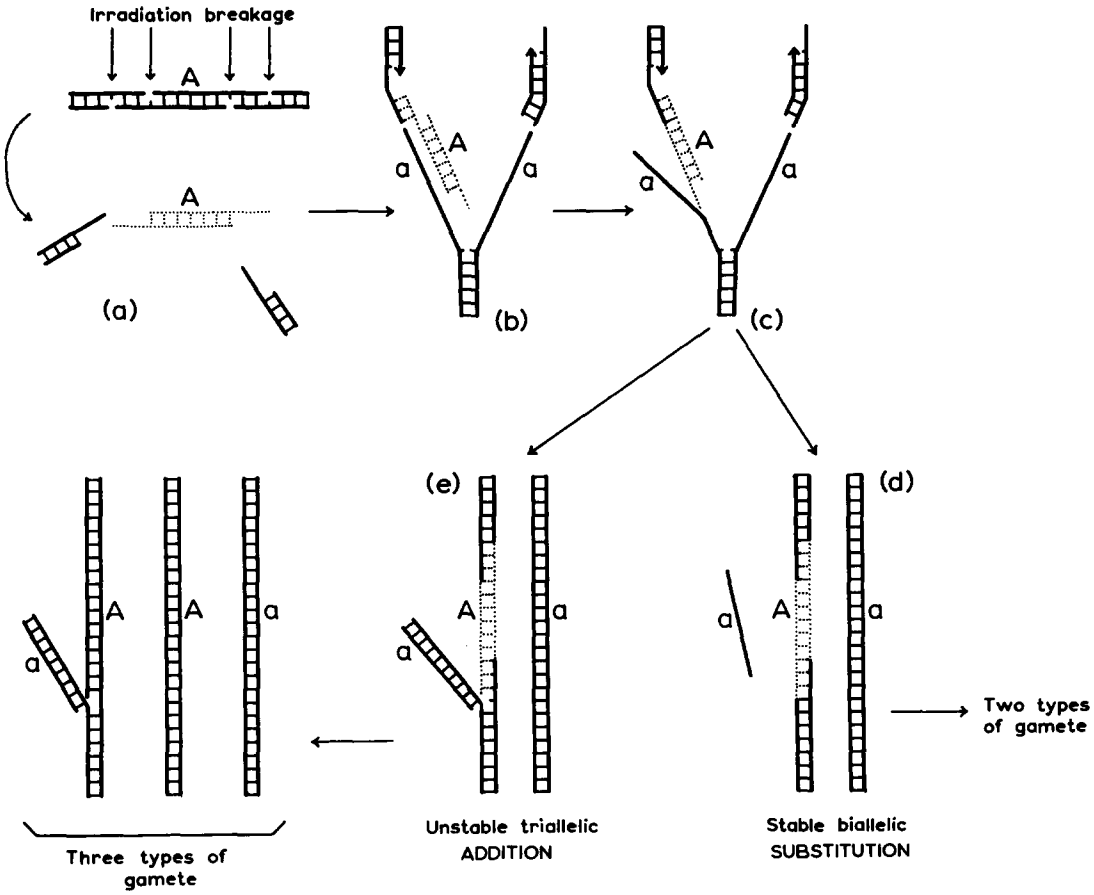

Fig. 1. Suggested series of events leading to gene transfer (Dotted lines indicate the original pollen-derived chromatin fragment). (a) Double-stranded DNA fragment with single-stranded tails generated by irradiation (or other form of pulverization). (b) Fragment makes initial chance, or directed, contact with homologous segment undergoing replication, and is held in register by pairing of single-stranded tail. (c) Fragment inserts into nick associated with replication, displacing original DNA strand. When a new DNA chain is assembled behind the replication fork the foreign fragment is incorporated *in situ* in its double-stranded form. (d) If the original displaced DNA segment is lost, the cell will have a biallelic substitution state. (e) If the original displaced segment remains attached as an "exosome" the transformed cell will show a triallelic addition state. An addition may ultimately give tissue producing three types of gametes - normal, substitution and addition. A substitution chromatid is derived from the addition chromatid by replication (of the right-hand strand containing no addition), or by loss of the "exosome"

may lead to precocious maturity of the resulting nuclei. This may lead in turn to competition within the common cytoplasm of the spore mother cell and inviability of the two later maturing spores which do not carry the transferred gene. If this were to occur in both male and female sex organs the progeny would be homozygous for the transferred gene, as is found regularly in DNA induced transformations in *Arabidopsis* (Ledoux, Huart and Jacobs 1971, 1974) and *Petunia* (Hess 1969, 1973), but only occasionally in the case of *Nicotiana* transformations.

In the *Nicotiana* transformations the suggested meiotic drive in favour of the substituted gene, the usual loss of the original gene rather than the new gene in a plant with an addition, and the possibility that there is a selection for heterozygosity at the replication level in the egg, would all seem to be peculiarly advantageous characteristics in plant improvement.

3. "Graft-hybrids"

There is a striking similarity between genetic transformation in *Nicotiana* and certain cases of "graft-hybridization" reported by Soviet and Eastern bloc geneticists and horticulturists. These workers persistently claimed that heritable characters could be trans-

mitted by grafting. To take one example, in 1955, the Belgrade scientist, Glavinic, claimed to have heritably transmitted three single gene Mendelian characters in tomato (cut leaf rather than potato leaf, yellow fruit rather than red and short fruit rather than long) from the variety 'Kartofelisni' used as stock to the variety 'Golden Trophy' used as scion. Scions were grafted as young seedlings at the cotyledonary stage. Significantly, it was the first generation seedlings, produced from selfed fruits of the scion, and not the scion itself, that produced various combinations of stock and scion characters. When one plant of each combination was selfed, there was a general tendency to breed true, although considerable segregation also occurred. These results rule out chimaerism, the only explanation so far considered, as being the basis of graft-hybrids (Baur 1919; Jorgensen and Crane 1927; Darlington and Mather 1949; Cramer 1954). The following working hypothesis is proposed to account for graft-hybridization phenomena:

When tissues are cut for grafting some cells may be weakened or have parts of their walls removed. This may occasionally lead to fusion of stock and scion protoplasts at the graft interface. If such a fusion involves a stock cell at the S phase and a scion cell at the M phase, then by analogy to the phenomenon of premature chromatin condensation in fused animal cells (Boyd and Harris 1973; Mazia 1974), the chromatin of the stock cell may be pulverised. Such fragments may remain in the cell in which they were formed or move into neighbouring cells through cytoplasmic connections. They may also move through the vascular tissue of the scion to nearby meristematic tissue. If fragments of stock chromatin are generated in, or taken up by, a dividing cell of the scion they may be integrated into the scion chromosomes in the same way as has been proposed here for gene transfer in *Nicotiana*. If integration occurs in a bud meristem, the size of the meristem, the position of the transformed cell, and the behaviour of its descendents in subsequent cell cycles, together with the developmental phase at which the expression of the particular gene occurs, will determine the extent to which the transformed character is expressed in the scion and transmitted to its progeny. The experimental system used by Glavinic would appear to have made effective use of transforming fragments since the scion was a seedling with an extremely simple tissue system, comprising only the hypocotyl, cotyledons and, most significantly, only a single small apical bud. Nevertheless, the possibility of sufficient pulverised chromatin thus being available would normally be extremely rare, hence the great difficulty of repeatability of results.

If transformation occurs at the graft interface under conditions which favour callus or bud formation at the graft union (e.g. mature scion on mature stock), then the expression of the transformed character in adventitious shoots will depend, as above, among other factors, on the fate of the original cell and its descendents and on the developmental phase at which the expression of the transformed gene(s) occurs. In certain cases "chimaeral" shoots may develop from the graft union. Some of the unusual characteristics of graft-hybrids and graft-chimaeras reported in the past may be better understood in this context.

If a "heterochromatization effect" accompanies transformation in the graft system, as suggested here to account for preferential recovery of gametes carrying gene transfers in *Nicotiana*, a change in cell cycle dynamics might favour a transformed cell in competition with neighbouring normal meristematic cells.

The above hypothesis suggests certain refinements which might enable the breeder to use graft-hybridization as a standard technique in plant improvement programs. For example, somatic cell hybridization methodology (Gamborg et al. 1974), may be applicable *"in vivo"* in the graft system. The following refinements might be tested: -

(1) Enhancement of numbers of cell divisions in the immediate vicinity of the graft by choice of graft site or chemical pretreatments of stock and scion.

(2) Treatment of cut surfaces with cellulases to remove cell end walls from intact protoplasts.

(3) Treatment of cut surfaces with polyethylene glycol (or other agent) to stimulate protoplast fusions.

(4) Use of conditions, e.g. mature scion on mature stock, which favour growth of buds directly from the graft union or growth of wound callus around the graft site.

In the field of plant improvement, techniques of gene transfer from irradiated pollen, and graft "transformation" may provide simpler alternatives to *in vitro* transformation, mutagenesis, somatic cell hybridization and regeneration in tissue culture. These two sim-

ple techniques not only may reduce the time needed to produce a new variety, but also, by breaking down linkage relationships, may allow selective transfer of desirable genes alone from relatively distantly related species.

Extract from the Guest Lecture (Transformation, Politics and Plant Improvement) given at the Annual Conference of European Association of Atomic Energy in Agriculture on the 10th September, 1975, in Cadarache, France. Since then the author's attention has been drawn to a recent paper "Hereditary changes in *Capsicum annuum* L. I. Induced by ordinary grafting" by Y. Ohta and P.V. Chuong (Euphytica 24, 355-368, 1975). To the author's knowledge, this is the first report on "graft-hybrids" by scientists from non-Eastern bloc countries. The results reported are extraordinarily parallel to those obtained by Glavinic through grafting in tomato and by the present author through egg transformation in *Nicotiana*, and furnishes further proof, if one was needed, of the genuineness of the phenomenon of "graft-hybrids", thus strengthening the possibility discussed here that genetic transformation may be involved.

Acknowledgement

I thank Dr. Elizabeth Williams for fruitful discussions and for her help in preparing the figure.

Literature

Baur, E.: Einführung in die experimentelle Vererbungslehre. Ed. 3 and 4. Berlin: Borntraeger 1919

Boyd, Y.L.; Harris, H.J.: Correction of genetic defects in mammalian cells by the input of small amounts of foreign genetic material. J. Cell Sci. 13, 216-227 (1973)

Brink, R.A.: Genetic repression in multicellular organisms. Amer. Natur. 98, 193-211 (1964)

Cramer, P.J.S.: Chimeras. Bibliogr. Genet. 16, 193-381 (1954)

Darlington, C.D.; Mather, K.: The elements of genetics. London: Allen and Unwin 1949

Gamborg, O.L.; Constabel, F.; Fowke, L.; Kao, K.N.; Ohyama, K.; Kartha, K.; Pelcher, L.: Protoplast and cell culture methods in somatic hybridization in higher plants. Can. J. Genet. Cytol. 16, 737-750 (1974)

Glavinic, R.: L'hybridation végétative comme méthode dans la selection des tomates. Proc. 14th Internat. Hort. Congr., I, 440-445, Netherlands (1955)

Grant, V.: Genetics of flowering plants. New York and London: Columbia Univ. Press 1975

Hess, D.: Versuche zur Transformation an höheren Pflanzen: Induktion und konstante Weitergabe der Anthocyansynthese bei *Petunia hybrida*. Z. Pflanzenphysiol. 60, 348-358 (1969)

Hess, D.: Transformationsversuche an höheren Pflanzen: Untersuchungen zur Realisation des Exosomen-Modells der Transformation bei *Petunia hybrida*. Z. Pflanzenphysiol. 68, 432-440 (1973)

Jorgensen, C.A.; Crane, M.B.: Formation and morphology of Solanum chimeras. Jour. Genet. 18, 247-272 (1927)

Ledoux, L.; Huart, R.; Jacobs, M.: Fate of exogenous DNA in *Arabidopsis thaliana*. II. Evidence for replication and preliminary results at the biological level. In: Informative molecules in biological systems, pp. 159-175, ed. by Ledoux, L. Amsterdam-London: North Holland Publ. Co. 1971

Ledoux, L.; Huart, R.; Jacobs, M.: DNA-mediated genetic correction of thiamineless *Arabidopsis thaliana*. Nature 249, 17-21 (1974)

Mazia, D.: The cell cycle. Sci. Amer. 230, 54-64 (1974)

Pandey, K.K.: Sexual transfer of specific genes without gametic fusion. Nature 256, 310-313 (1975)

Rhoades, M.M.; Dempsey, E.: The effect of abnormal chromosome 10 on preferential segregation and crossing over in maize. Genetics 53, 989-1020 (1966)

Received November 25, 1975
Communicated by H.F. Linskens

Dr. Kamla K. Pandey
Genetics Unit
Grasslands Division
D.S.I.R.
Palmerston North
(New Zealand)