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The Science of Hybridisation[®]

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INTRODUCTION

The future of all sectors of commercial horticulture is in part dependent on the production of new cultivars of plants that possess improved characteristics, such as growth habit; attributes of flowers, fruit, and foliage; and resistance to pests and diseases. The genetic improvement of plants by selection has been practised for centuries, going back to the beginnings of agriculture, and is still used today, for example in tree breeding programmes. In contrast, it is only in much more recent times that the potential of artificial hybridisation has been realised and used to improve specific traits of plants. In the late 1700s, crosses were made between many species of *Nicotiana* and to produce new fruit varieties. At this time, from studies on peas, it was concluded that male and female parents contribute equally to offspring in the first filial generation (F1) and that segregation occurs in the F2. Later, in 1866, Gregor Mendel, in crossing different varieties of peas and studying their progenies, established Laws of Inheritance based on individual heritable characters. However the importance of his work was not recognised until 35 years later, after which, in the early 1900s, rapid developments in plant breeding took place. Mendel determined that plant characters were inherited as pieces of information from each of the parents. We now know that these parts are genes, sequences of the deoxyribose nucleic acid (DNA) which makes up the chromosomes present in the nucleus of plant cells.

CHROMOSOMES AND HYBRIDISATION

In hybridising plants, either naturally or through man's activities, discrete units of very large molecules of DNA contained within the chromosomes in the nucleus of cells are passed to the next generation. Most of the genetic information in plant cells is contained within the nucleus. However, small amounts are also present in the mitochondria and chloroplasts. The number of chromosomes varies with plant species and each chromosome is comprised of a series of units (sequence of nucleotides), termed genes, that usually code for a single characteristic. Many

plants normally have two sets of chromosomes (the diploid number) which may carry identical or different forms (alleles) of a particular gene. The alleles may be dominant or recessive, and a plant is considered to be homozygous (true breeding genotype) if the alleles are identical, or heterozygous (do not breed true) if the alleles are different.

When a cell divides to produce another identical cell, the chromosomes are duplicated so that each new cell contains the same genetic information. When a cell in the ovary or the anther of a flower divides, it produces four new cells, each of which contains half of the original number of chromosomes (the haploid number). When a male gamete contained within the pollen fuses with a female gamete (egg cell), the resultant product (zygote) is diploid and contains genetic information from both parents. So, the inheritance of characters depends on the alleles of genes in paired chromosomes.

Mendel used pea plants that were homozygous for characters such as plant height. When he crossed homozygous dominant tall plants (TT) with homozygous recessive short plants (tt), he obtained all tall plants (Tt) which were heterozygous. So, crossing of two homozygous parents results in an F1 generation that is heterozygous and non-segregating. When this progeny was self fertilised, in the F2 generation he obtained genetic segregation with the plants in the ratio of 3 tall : 1 short. In terms of their genetic make-up, the plants were in the ratio 1 TT : 2 Tt : 1 tt.

Single gene or multi-gene traits may be inherited in this way, since genes on separate chromosomes segregate independently of each other. Thus, a plant heterozygous for two gene pairs (Aa and Bb) will produce four types of gametes in equal proportions (AB, Ab, aB, ab). Assuming complete dominance, the progeny from selfing will have the ratio of 9A_B_ : 3A_bb : 3aaB_ : 1aabb (in this notation the character _ indicates the presence of either the dominant or recessive allele as the second of the pair).

PLANT SEX

Initiating a breeding programme requires a knowledge of the mode of pollination and fertilisation in the plant species in question. Is the plant self- or cross-fertilised and is it capable of being crossed or selfed? It is also important to understand the behaviour of a plant when it is inbred or crossbred.

Many plants possess strategies to ensure cross-pollination, a result of the evolutionary advantage of the variability caused by reshuffling gene combinations, through which species may adapt to changes in the environment. Some species, e.g., holly, hops, and asparagus, possess single-sex flowers on different plants (dioecious), so cross-pollination is obligatory. Others, e.g., walnut and hazel, have single-sex flowers on the same plant (monoecious), which also favours cross pollination. Species with hermaphrodite flowers may have the male and female organs maturing at different times. Protandry occurs when the stamens mature before the stigma is receptive, e.g., in carrot, alliums and many compositae; while protogyny is when the stigma is receptive before the pollen is shed, e.g., in avocado and arums. The protandrous nature of small-flowered crops, such as carrot, makes hand emasculating of flowers unnecessary. Since the male organs wither before the stigma is receptive, bagging of the inflorescence causes a plant to function as a female.

Hermaphrodite flowers may also be modified to ensure that pollen is collected and delivered by insects to maximise cross-pollination, e.g., in the “pin” flower form of

primulas the style is prominent and the stamens are concealed below while in the "thrum" flowers the reverse arrangement exists.

With genetically controlled self-incompatibility a flower rejects its own male gametes, for example by blocking growth of the pollen tube in the style. Other mechanisms include male or female sterility that may be caused by genetic and cytoplasmic factors or induced by chemicals. Genetic male sterility is a recessive trait and undergoes Mendelian inheritance. Cytoplasmic male sterility is controlled by factors in the cytoplasm of the maternal parent and allows for the reproduction of the female by fertilisation with fertile pollen. In breeding programmes, male sterility has been used very effectively in the production of F1 hybrids in crops such as corn and sunflowers.

In contrast to cross-fertilisation, some plants may ensure self-fertilisation quite simply by reaching anthesis before the anthers or styles are exposed, i.e., pollination is achieved in the flower bud. This is termed cleistogamy and occurs in a few species of grasses. The flowers may open after the stigma has been pollinated and, if no fertilisation has occurred due to the lack of viable pollen, cross-pollination may subsequently take place.

HYBRID VIGOUR

The vigour (heterosis) seen in F1 hybrids is thought to relate to the "covering up" of the effects of recessive alleles by dominant alleles. Such hybrids are produced by crossing inbred parent lines, which, through successive inbreeding (selfing), have become homozygous with a concomitant reduction in vigour (inbreeding depression). So, the F1 hybrids are a homogenous population of heterozygous plants, and the advantages to the grower of such plants are their uniformity and their enhanced vigour. However, plants produced from seeds collected from these F1 hybrids will lack this uniformity due to segregation in the F2 generation.

Plants which naturally inbreed, such as peas and lettuce, show little or no inbreeding depression. It is possible to obtain varieties that are vigorous and possess genetic uniformity, however they do run the risk of little or no genetic variation to protect them from new forms of pests and diseases. In contrast, in outbreeding plants genetic heterogeneity contributes to vigour, yield, and other important plant traits, so inbreeding of them causes deterioration in size, vigour, and yield.

PLOIDY

Plants with cells containing more than the diploid number (2 copies) of chromosomes are known as polyploids and can range from triploid (3 × haploid) to octaploid (8 × haploid). In many species, polyploidy is associated with an increase in cell size and enhanced vigour, so the plants may be bigger, brighter, and more attractive. The increased number of copies of each chromosome may be based on the haploid genome sets all being the same (autopolyploid as in potato) or different (allopolyploid as in many brassicas). Allopolyploids arise naturally, or can be created, from a wide cross between two species followed by a doubling of the chromosomes. After chromosome doubling the plant is known as an amphidiploid since it possesses two of each chromosome of each of the parent species and is functionally diploid.

Triploids may have value as seedless fruits, e.g., watermelons, and perpetual flowering ornamentals with larger flowers. They may result from the crossing of a diploid and a tetraploid and are commonly sterile. Polyploids do occur spontane-

ously but they may also be induced by the alkaloid colchicine, which doubles the chromosome number.

UNEXPECTED OUTCOMES OF HYBRIDISATION

Sometimes the inheritance of characters is not as expected. Gene interaction or epistasis can occur if two genes affect the same process and either gene, as homozygous recessive, blocks some essential step, e.g., in tomato fruits recessive genes *rr* deplete all pigment and *atat* deplete lycopene. So to obtain red fruit colour in tomato, dominant genes *R* and *At* are both required. Likewise, linkage between genes on the same chromosome may make it difficult to breed for a specific characteristic if the desired gene is linked to one for an undesirable trait.

MUTATION BREEDING

Changes to the nucleotides of the DNA change the genetic information stored. Mutations may therefore result from the replacement, loss, or addition of nucleotides—whether as individual genes, or as parts of chromosomes, whole chromosomes, and whole genomes. Some mutations arise spontaneously in plant populations, however in breeding programmes they may be induced by chemical “mutagens”, e.g., alkylating agents, and physical agents, e.g., high energy radiations. This is a very random approach to increasing genetic variation, which requires the use of a large amount of starting material and a screening procedure after treatment to identify progeny with desired traits. Once a desirable mutant has been isolated, back-crossing can be used to transfer the trait into other cultivars.

BACK-CROSSING OR INTROGRESSION

In order to achieve the transfer of the gene of interest to the crop species and to eliminate undesired genes, hybridisation is followed by back-crossing with the parental crop species. If back-crossing to the same homozygous parent is continued for 6 to 10 generations, more than 95% of genes of the hybrid will be identical to those of the parent and will be homozygous. If the gene is dominant, selfing after the last back-cross will make the gene homozygous. If the gene is recessive, each generation must be tested to ensure that the plant selected for back-crossing is heterozygous and contains the desired recessive gene. This is a valuable technique for transferring single characters from related species into crop plants, particularly in self-pollinated crops, and in improving inbred lines of cross-pollinated plants. The main advantage of the method is its predictability and the improved cultivar is often not distinguishable from the old one except for the added character.

CONCLUSION

During the past century, our understanding and the application of conventional hybridisation has contributed greatly to the genetic improvement of plants of economic importance to man. However, since hybridisation involves the recombination of large numbers of genes, many traits may be influenced and the outcome may be difficult to predict. For many plants, e.g., hardy ornamental nursery stock and trees, improvements will continue to be made using selection and traditional hybridisation. However, some desired plant improvements, e.g., resistance to grey mould caused by *Botrytis cinerea*, may require more precise procedures for the transfer of specific genes from a number of sources, possibly plants that are not closely

related or even different organisms. The application of plant biotechnology to complement traditional hybridisation offers the opportunity to meet such challenges.

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Latest Developments in Plant Breeding: Application of Biotechnology[®]

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INTRODUCTION

The generation of new plant varieties is an on-going process that provides material for the commercial market. Conventional breeding involving self and cross-pollinations, fertilisation, and seed production followed by rigorous selection of plants with desirable traits, is a long-established and reliable procedure for generating novel germplasm. In addition to this traditional approach, techniques involving the culture of plant cells, tissues, and organs in the laboratory provide a means for direct and indirect plant improvement. A unique characteristic of plant cells is their so-called totipotency—individual viable cells of almost any origin carry the genetic information needed to develop into complete fertile plants. This developmental pathway can be induced in the laboratory. Major advances have been made in plant biotechnology during the last two decades, several of which exploit cell totipotency, the basis of plant tissue culture, and which can underpin conventional breeding and propagation.