THEORY AND PRACTICE OF INDUCED ANDROGENESIS

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SUMMARY

The recently discovered technique of producing haploid plants from the culture of immature anthers of flowering plants (androgenesis) is potentially of great significance in plant breeding and genetics. Although rare haploid plants have been obtained by this method in a variety of species thus demonstrating the wide applicability of the technique, the method so far has been successful for only a very few species in producing a large number of haploid plants with ease and certainty. New variations of the technique involving, in general, modifications in the nutrient media and other environmental factors of anther culture, are being tried in different laboratories. Here, some of the theoretical aspects of anther culture are discussed with a view to contributing towards a better understanding of the phenomenon, and thus possibly to help in expediting practical androgenesis.

INTRODUCTION

One of the significant developments in the field of botany in recent years is the production of haploid plants through pollen culture. Since Guha and Maheshwari (1964, 1966) in Datura innoxia Mill. and Bourgin and Nitsch (1967) in Nicotiana tabacum L. and N. sylvestris Spagazzini & Comes, obtained haploid plants from the pollen of anthers cultured in vitro, there has been a worldwide interest in developing this method for a wide variety of crop plants. Haploids, owing to the fact that they have only one set of genes, are extremely valuable in the induction of mutations for theoretical and practical purposes. Haploids can easily be treated with colchicine to give homozygous true-breeding cultivars in a single generation. By contrast, the approach to homozygosity by conventional methods is infinitely slower. Homozygous diploids obtained by this method can eliminate long delays in producing inbred lines required to exploit hybrid vigour, which is increasingly becoming an important method of plant breeding. Thus the potential implication of this technique in plant breeding and genetic research is far-reaching (Clapham, 1971; Melchers, 1972; Nitsch, 1972; Sunderland, 1970).

Immature anthers, mostly at the stage of uninucleate microspores, are sown on a defined agar nutrient medium in culture tubes which are placed in growth chambers giving suitable light and temperature conditions. Under this artificial environment the young microspores, instead of growing into mature pollen grains, either (1) grow directly into embryoids and eventually into plants, or (2) produce undifferentiated calluses which, if conditions are right, may in turn give rise to shoots and roots and eventually entire plants.

To date, only limited success, involving not more than eight genera, has been achieved in producing, from pollen, callus that gave rise to plants (Abo El-Nil and Hilderbrandt,
ANDROGENESIS

Most natural haploids obtained in flowering plants are derived from the cells of the embryo-sac, and hence are maternal in origin. Androgenetic haploids, produced from the nucleus of the male gametophyte only, are relatively rare and have been recorded only in four genera—Nicotiana, Crepis, Antirrhinum and Zea, mostly in the genus Nicotiana (Kimber and Riley, 1963; Tulecke, 1965). In these cases it is suggested by the authors concerned that the paternal haploid forms resulted from the development of a male gamete in the female cytoplasm, in the embryo-sac of an ovule. It is proposed here that this type of androgenesis be called (i) ‘Ovule Androgenesis’ (OA) to distinguish it from others, ‘Anther Androgenesis’ (AA), in which there is no participation of the female cytoplasm. The latter again can be classified into two forms: (a) ‘Spore Androgenesis’ (SA), in which the sporophytes are initiated from the spores directly as embryoids either inside or outside the anther, without any intervening growth phase (Datura (Guha and Maheshwari, 1964, 1966), Nicotiana (Nitsch and Nitsch, 1969; Sunderland, 1970), Atropa (Zenkteler, 1971)); and (b) ‘Callus Androgenesis’ (CA), in which the sporophytes are initiated as plantlets, or shoots and roots, from the callus usually grown outside the anther (Oryza (Niizeki and Oono, 1968), Setaria (Ban et al., 1970), Brassica (Kameya and Hinata, 1970), Lolium and Hordeum (Clapham, 1971), Pelargonium (Abo El-Nil and Hilderbrandt, 1971), Lilium (Sharp et al., 1972)). In certain cases the intervening callus phase may be very short and not easily discernible without a closer cytological examination of the embryogenetic and organogenetic processes, and of the resulting plantlets. Such a condition is believed to occur in certain cultures of Nicotiana (K. K. Pandey, unpublished) and Datura (Narayananswamy and Chandy, 1971) and may be called ‘Spore-Callus Androgenesis’ (SPA).

SEX DIFFERENTIATION

There is now considerable developmental and embryological evidence in plants and animals which demonstrates that the origin of separate sexes in the higher organisms is in hermaphroditism. In animals the gonads of both birds and mammals pass through a bipotential stage in the embryo; and consecutive hermaphroditism is the normal route in the development of many species of fish. There are many examples of sex expression being controlled by environmental factors. In plants, the investigations of Nagai (1915),
Schaffner (1929, 1930), Heslop-Harrison (1957, 1961) and others (Tothill and Knox, 1968) have shown that sex reversals can be brought about by appropriate alterations in the environmental factors such as light intensity, photoperiodicity, temperature, moisture and nutrition. The divergence of the sexes must, therefore, be regarded as an act of specialization, in which one potential is increasingly developed at the expense of the other. ‘Maleness and femaleness can represent an induced state and a non-induced state of one and the same regulatory system and are in no way comparable to contrasting characters produced by a simple difference in a Mendelian gene’ (Mittwoch, 1971).

Nemec, as far back as 1898, reported the presence of embryo-sac-like giant pollen grains—female gametophytes—in the partial petaloid anthers of *Hyacinthus orientalis* L., and De Mol (1921) observed this phenomenon in a number of varieties. Naithani (1937) found the embryo-sac-like grains in the normal anthers of the variety ‘Yellow Hammer’; and Stow (1930) was able to induce such grains in the normal anthers of the variety ‘La Victoire’ by submitting the bulbs to high temperatures (30° C) at about the stage of reduction division. Stow demonstrated that the formation of such embryo-sac-like giant pollen grains depended on the stage of pollen development and the temperature at which the bulbs were grown in the glasshouse subsequent to heat treatment (moderate temperature in the glasshouse 17–25° C, as opposed to normally freezing or near freezing temperature outside).

Stow and Naithani both showed that the eight nuclei of the embryo-sac arose through successive divisions from the first nucleus and that this type of development occurred only in the pollen grains in which the generative cell was not yet formed. This suggested that the proper stage for the induction of a female gametophyte type growth was somewhere between the meiotic division and the first mitotic division in the microspore. More recently, pollen-embryo-sacs have also been observed in *Ornithogalum nutans* Linn. (Geitler, 1941) and *Leptomeria billardierii* R.Br. (Ram, 1959).

**Stage of the anther**

In all the experimental materials closely examined so far, a change in the course of development occurred only in anthers in which reduction division was completed normally and the young microspores were released from the tetrad sac (Nitsch and Nitsch, 1969). Any physiological disturbance which affected the meiotic process appeared to be detrimental to the growth and differentiation of the microspore in general, including the normal development into the male gametophyte.

The above observation may be relevant to the discovery of Mackenzie, Heslop-Harrison and Dickinson (1967) that a drastic reduction occurs in the ribosome population of the pollen mother cell during the mid-prophase of meiosis. This reduction meant that the mother cell is almost wholly deprived of protein-synthetic machinery from zygotene at the latest, restoration not occurring until the meiotic mitoses have begun. Good evidence exists that de novo nucleus-directed syntheses are required for pollen mitosis from observations on aneuploid spores of *Uvularia* (Barber, 1941). Here deficient spores fail to reach pollen mitosis but complementation occurs when neighbouring grains with mutually compensating chromosome numbers remain in cytoplasmic contact.

Thus, a normal meiotic phase probably sets the early limit to the ability of the microspore for growth and differentiation. One function of normal meiosis is to purge the cytoplasm of some of the effects of the diploid nucleus and adjust it to the expression of the haploid nucleus. This transformation in the meiotic cytoplasm appears to be necessary...
to provide the appropriate environment for the later expression of gametophytic potential, and may have been significant in the evolution of alternation of generations in plants (Heslop-Harrison, 1971).

Observations on induced growth of anthers in several species seem to suggest that the most responsive (inducible) phase when an anther is most likely to produce embryos or undifferentiated tissue is when it contains uninucleate microspores (Nitsch, 1969; Sunderland and Wicks, 1969). In Nicotiana tabacum L., the microspore appears to be at the height of its sensitivity during the later phase of the uninucleate stage, just before, during or after the first mitotic division, and this sensitive phase virtually ends with the completion of the differentiation of the daughter nuclei (Nitsch, 1969, Niizeki and Grant, 1971; Nöth and Abel, 1971; Sunderland and Wicks, 1969, 1971). However, in other species the peak period within the uninucleate phase is not so well defined (Clapham, 1971; Niizeki and Ono, 1968). Much of the variability in the response of the anthers grown at the uninucleate stage of the microspore may be due to the fact that this stage of the microspore occurs for a relatively prolonged period; and in plants belonging to cold regions there is often a resting stage lasting from a few days to several weeks (Maheshwari, 1950). Furthermore, freed from the unifying force of the sporophytic cytoplasm (Mather, 1948, 1965) the young microspores, when not grouped together as in tetrads or in pollinia, already behave as independent units under the genetic influence of their own haploid nuclei, and mitoses in them are usually not synchronized as are meioses in pollen mother cells.

The sensitive stage may be related to the low metabolite content of the microspore, for the peak sensitivity coincides with the considerable nuclear activity associated with the first mitotic division, e.g. DNA duplication, spindle formation, chromosome movement, etc., which possibly occurs without the simultaneous initiation of the processes producing metabolites. This view is supported by the observation that the microspores in which starch accumulation has occurred after mitosis, do not produce embryos (Nitsch and Nitsch, 1969). If this hypothesis is correct, the species having trinucleate pollen grains where two mitoses occur before the pollen is mature, might be poor in metabolite till late in the pollen development. In some of these species, therefore, depending upon the stage when starch formation and accumulation occurs in the microspore—and this may vary between species—the anthers may remain inducible till a considerably later stage of development. This has been found to be the case in Brassica oleracea (Kameya and Hinata, 1970) and Festuca–Lolium hybrids (Nitsche, 1970). In these plants, which have trinucleate pollen grains, callus tissues were obtained from almost mature anthers. Thus, in many species an apparent freedom from starch in the cytoplasm, rather than its nuclear state, may be a better criterion for recognizing the most advanced, inducible stage of the microspore. It is interesting to note that the ability of the microspores to hydrolyse starch, and the mutation of starchy to waxy pollen, can be influenced by irradiation when it is applied before the first pollen mitosis has occurred (Nettancourt and Eriksson, 1968) whereas irradiation at later stages has no effect. Thus, there may be a physiological relationship between starch accumulation, pollen mitoses, time of activation of certain set of genes and sensitivity for haploid induction.

**The process of induction**

There is evidence from Lolium and Hordeum which suggests that the stimulation to undifferentiated growth (callus) is associated with varying degrees of dedifferentiation at the
first pollen mitosis resulting, in extreme cases, in the production of two daughter nuclei of similar size and staining properties (Clapham, 1971). At the other extreme diffuse and compact nuclei resembling the normal vegetative and generative nuclei in vitro are formed. The production of similar nuclei is, in turn, related to the position of the mitotic spindle which, instead of being oriented towards one side, as is normal, is located more centrally in these cells.

These observations, in conjunction with those of Stow (1930) and Naithani (1937) on *Hyacinthus*, suggest the potential sequence of sex differentiation in the young microspore of a flowering plant. Commitment to female gametophytic development occurs relatively early, for it is the microspore nucleus itself, rather than the vegetative or the generative nucleus, which gives rise to the pollen-embryo-sacs. Once the generative cells have been differentiated, further development is quite normal and no pollen-embryo-sacs are formed. In contrast, however, it would appear that differentiated growth directly from the microspore, either to the male gametophyte or to the primary sporophyte (embryoid), is possible only if the daughter nuclei are fully differentiated at the first mitotic division. If this fails, only undifferentiated (callus) growth occurs. (This, however, does not preclude the possibility that the callus may give rise to 'secondary' sporophytic development through the production of plantlets, or sequentially of roots and shoots.)

**Origin of the embryoids**

Of the two differentiated nuclei it is the normally quiescent vegetative nucleus which produces the new type of growth (Sunderland, 1970). The generative nucleus does not participate; sometimes it may divide to produce two to six cells but then it stops. Even in the case of ‘Ovule Androgenesis’ where it has been generally assumed, without supporting evidence, that these haploids arise from divisions of the male gamete, it is possible that the origin is actually from the vegetative nucleus. It is not ruled out, however, that, as a very rare event, a male gamete may give rise to a haploid. But in that case the possibility is strong that the resultant haploid may be vitally deficient, e.g. albino (Devreux et al., 1971).

The first division in the microspore is the critical transitional point for plantlet formation for it segregates the two faculties of the cell—the sporophytic in the vegetative cell and the gametophytic in the generative cell. The potentially sporophytic vegetative cell receives the bulk of cytoplasm at cytokinesis and shows intense synthetic activity; protein and RNA synthesis occurs rapidly and the cell increases in size to fill the space previously occupied by the pollen vacuole (La Cour, 1949; Sunderland, 1971; Woodward, 1958). The DNA content in the nucleus may remain at 1C (e.g. *Petunia* (Hosemann, 1971)) or 2C (e.g. tobacco (D’amato, Devreux and Scarsoia Magnozza, 1965)) level. The vegetative cell, therefore, although normally quiescent, is fully equipped with the synthetic machinery for growth and differentiation. The generative nucleus, on the other hand, which is highly specialized and gives rise to the male gametes, is cut off with only a small portion of cytoplasm at cytokinesis. This nucleus, which is in a highly condensed state, though retaining the capacity to undergo one further DNA replication, is devoid of the synthetic machinery for DNA:RNA transcription (Sunderland, 1971).

The significance of the first pollen mitosis in the developmental physiology of the cell is also suggested from the behaviour of B-chromosomes in certain plants, particularly of the family Gramineae. In these plants preferential segregation of B-chromosomes occurs at the first pollen mitosis. The B-chromosomes undergo non-disjunction and the two
adherent chromatids are preferentially segregated to the generative nucleus (Battaglia, 1964), indicating that the generative nucleus is potentially the principal centre of the limited activity in these cells. The use of the B-chromosome-carrying plants in induced androgenesis would be another way of confirming the origin of the haploids from the vegetative nuclei. The haploids so raised should lack B-chromosomes.

In the cultured anthers which produce embryoids the first mitotic division remains discriminatory, producing the two distinct types of cells; what the culture shock does is to produce the environment which principally induces the vegetative rather than the generative cell for division and growth. The generative cell even when induced to grow is incapable of producing more than a few cells, the resulting nuclei still maintaining a condensed, presumably predominantly inactive, state (Sauter, 1969). The vegetative cell when induced, on the other hand, owing to its fuller endowment with the synthetic machinery, is capable of sustained growth and differentiation.

**Potencies of the Young Microspore**

From the above discussion it is clear that, in contrast to an animal gonad which lacks the totipotency of plant cells and so cannot develop into a sporophyte, a young microspore has all the four possible kinds of potencies, as shown in Fig. 1. It may develop into (1) a male gametophyte, (2) a female gametophyte, (3) a sporophyte, or (4) an undifferentiated tissue, callus.

Under normal conditions the balance is strongly towards maleness and so a male gametophyte is produced. But, with a change in the environment, either naturally occurring or artificially generated, the dominance of the male potency may be weakened or even abolished. There is a combination of two main factors underlying induced anther androgenesis: (1) neutralizing the normal male potency of the young microspore; and (2) providing appropriate environmental conditions (nutrients, growth hormones, light, temperature, etc.) which trigger the development of the microspore into a new direction.
In the 'activated phase' of the cell, freed from the male potency, there are the three remaining courses open for its growth, all of which have been realized experimentally. In the cited case of *Hyacinthus* the environmental conditions favoured the course of female-ness, and so a female gametophyte was produced. In the species *Nicotiana tabacum*, where induced anther androgenesis has been found to be relatively easy owing to its genetic make-up, the young microspore stage appears naturally to be close to the threshold of neutrality. In this species, and in *Datura innoxia* Mill., the mere shock of the transfer of the anthers from the parental environment to an artificial one appears to be sufficient to neutralize male potency in the young spore. Indeed, in *Nicotiana tabacum*, an immature anther of the right stage of development, if only severed from the bud and kept in a moist chamber without any medium, may show preliminary growth of the spore into embryos, up to 100-200 cells in 20 days (Pelletier, 1972). The initial stimulation of a neutralized young microspore to develop into a sporophyte is not species specific but is of a more basic, general nature. As shown by Pelletier (1972), it can be reproduced in association with anther tissue, or even with cultivated tissue such as callus grown from petals of *Petunia hybrida* Vilm., a species of a related genus, as well as with the self-anther tissue of *Nicotiana tabacum*.

When the switch to sporophytic development is complete, the growth in the microspore leads directly to the development of embryoids that give rise to plantlets (*Nicotiana, Datura* and *Atropa*). However, when the male potency is neutralized but not replaced by another potency, the microspore may give rise to callus. Such callus, under appropriate cultural conditions, may produce plantlets, or may give rise sequentially to roots and shoots and eventually to full plantlets. Thus plantlets may be produced without having passed through the typical embryoid phase (*Oryza, Brassica, Lolium, Hordeum, Pelargonium* and *Lilium*).

In certain cases the switch to sporophytic phase may be incomplete and there may be an undetermined or mixed state of varying durations after the microspore growth is initiated. In *Datura metel* L. (Iyer and Raina, 1972; Narayanaswamy and Chandy, 1971) and *Nicotiana tabacum* (K. K. Pandey, unpublished; Sharp et al., 1972) certain observations suggest the occurrence of a very short-lived callus state. In *Datura* a spindle-shaped structure is produced, which is unlike an embryo, but has meristematic loci at opposite ends destined to develop into the root and shoot primordia. In general, the regeneration of plants from usual cell cultures is more frequently through plant formation (organogenesis) than through embryogenesis (Gambourg, Constabel and Miller, 1970). In all cases where a callus phase intervenes, as in some materials between the microspore and the plantlet formation, the resultant plants, owing to instability in calluses, may not be cytologically identical.

A relatively complex case of callus intervention has been found in *Petunia* (Raquin, 1972; Raquin and Pilet, 1972). In these plants, when anthers were cultured in media containing growth substances, 90% of the plantlets produced were triploids, the remainder comprising haploids, tetraploids and hexaploids (a total of fifty-two plants). All ten plants produced from cultures in medium containing no growth substances were triploids. The mature binucleate pollen of *Petunia* has the generative nucleus in the doubled-DNA (2C) state and the vegetative nucleus in the single-DNA (1C) state (Hesemann, 1971). This is in contrast to the situation in *Nicotiana tabacum* where both the vegetative and the generative nucleus are in doubled-DNA (2C) state (D'amato et al., 1965). It is suggested by Raquin (1972) that in *Petunia* the 1C vegetative nucleus is incapable of DNA synthesis without uniting with the 2C generative nucleus, thus giving
initially $3n$, activated nucleus in the microspore. Another possibility, not considered by Raquin, is that in *Petunia* the switch to the sporophytic development is incomplete and there is a slight initial tendency towards callus formation, with its attendant property of endomitosis and nuclear fusion. If microspores in which there has been a fusion between the vegetative and the generative nucleus have a growth advantage over those in which there has been no fusion, there would be a selection for the $3n$ proembryos. Rare endomitosis, when initiated in the vegetative nucleus alone, may give rise to $2n$ and $4n$ proembryos, and when initiated in the fused $3n$ nucleus may give rise to $6n$ proembryos. Other forms of ploidy could arise through fusion between different types of cells arising from one or both sources. An apparently somewhat similar situation prevails in experiments with *Datura metel L.*, except that in this species diploids and triploids were the predominant group, and there were no hexaploids (Narayanaswamy and Chandy, 1971). Thus the genetic characteristic of the plant regarding the DNA status of the vegetative nucleus, and the relative completeness of the switch from the male gametophytic to the sporophytic phase of growth in the young microspore, may both be significant in determining the quality of the resulting androgenetic product. It is likely that the relative ease with which pollen haploids are produced in *Nicotiana tabacum* may be at least partly related with the normally precocious DNA duplication in its vegetative nucleus.

In *Oryza* (Guha et al., 1970) after the shock of anther severance from the bud the microspore first gives rise to a normal sporophytic development producing embryonal mass, but as this is released from the pollen wall the growth recedes to callus growth. This recession can be avoided by removing anthers from the influence of hormones at an early stage of the culture. Similarly, in *Nicotiana tabacum* the recession can be induced by inclusion of an auxin in the medium (Sunderland and Wicks, 1971). The induction to a particular phase of development is apparently a function of critical balance between specific hormones during a particular period of growth. Hence, it is possible to induce a desired change in the growth phase through inclusion or exclusion of certain growth substance(s) in the medium at an appropriate time.

In species in which the attempts to produce haploids from young microspores have so far entirely failed, or success has been insignificant (Niizeki and Grant, 1971; Sunderland, 1970, 1971), the genetic balance is so strongly towards maleness that the reaction is undisturbed in any of the environmental and nutritional conditions yet tried. Nevertheless, it is possible that the pollen of many other plants might be induced to form a sporophyte, directly through embryoids or indirectly through callus, by subjecting the young floral bud or the immature anther to an environment quite different from that which it normally encounters. The variability in the response of microspores between different varieties of the same species as exemplified by *Hyacinthus* and to some extent also by *Nicotiana tabacum* (Devreux et al., 1971; Nitsch and Nitsch, 1969; Sharp, Dougall and Paddock, 1971) gives cause for optimism.

**Neutralizing the male potency**

**Temperature shock**

Thus the question arises, are there any particular artificial means of neutralizing the more powerful male potency normally present in the young microspores of the majority of flowering plants? One means, suggested by the observations in *Hyacinthus orientalis*, already mentioned above, is temperature shock. In this species high temperature shock to the bulbs at about the time of reduction division in the microspore mother cell led to
the switch in the developmental path from the male to the female gametophyte. In *Hyacinthus*, where microsporogenesis occurs in winter, a relatively mild heat shock (30° C) changed the course of development of the microspore. In plants where microsporogenesis occurs in the spring or summer, it is possible that a cold shock or a relatively severe heat shock, might be more effective. Temperature treatments, hot or cold, or a combination of both, may have different responses in different species. Prevailing temperature subsequent to temperature treatment is also important, as suggested by the study of *Hyacinthus* where optimum temperature, within the range 20–25° C, was necessary.

So far all successful attempts to produce haploids from anther culture have involved incubating the cultures at high temperatures ranging from 23° to 28° C (Sunderland, 1971).

**Age and growing condition of the parent plant**

Age of the plant has a significant effect upon induction. Anthers from flower buds arising early on the onset of flowering are better than from buds arising later in the season (Narayanaswamy and Chandy, 1971; Sunderland, 1971). Although light during the incubation of anthers has not been found to have a critical effect on induction, the light condition under which the parent plant is grown appears to have a considerable effect. Plants grown under natural light, in season, have been usually found to be better than those grown under artificial light out of season (Pelletier, 1972; Raquin, 1972).

**Treatment of parent plants with hormone sprays**

The above effects of the age of the parent plant, the stage of flowering, and the position of the bud in the inflorescence, indicates that the content of growth hormones in the growing tissues of the young bud may be relevant in this connection. Thus hormone spraying of the parent plant, about 1 week before using its bud, with IAA, NAA or gibberellic acid, alone or in mixtures, may be beneficial for induction of pollen haploidy.

**Cytoplasmic male sterility**

A condition which occurs widely in flowering plants and has been found in many species which has been examined closely, is cytoplasmic male sterility. One of the most interesting features of the plants is that in the great majority of cases meiosis is normal, and pollen sterility sets in later, usually at the stage of the young uninucleate microspore (Edwardson, 1970). One possible reason for the failure of the young microspore in these plants to develop any further is a lack of the stimulus which ‘activates’ this cell and normally leads it to the path of male gametophytic development. The young microspore in such cytoplasmically male sterile plants may thus be naturally neutralized so that, devoid of any further direction, it dies. If such is the case, then certain cytoplasmically male sterile plants may be inherently better material for the induction of haploidy. Since it is likely that different mechanisms, involving genetic as well as cytoplasmic elements, are responsible for controlling cytoplasmic male sterility in angiosperms, only certain types of cytoplasmically male sterile strains, if any, may respond in the desired fashion. Furthermore, the nutrient medium may require certain special ingredients to ‘switch on’ the normally blocked growth of the young microspore.

It is possible that the use of cytoplasmically male sterile anthers may prove to be beneficial as a medium on which the pollen grains may be plated in the nurse culture technique recently developed by Sharp *et al.* (1972) for *Lycopersicon esculentum* Mill.
Mutagens

Ionizing radiations and colchicine have been reported to induce cytoplasmic changes which may be relevant in the induction of haploidy (Edwardson, 1970; Kimber and Riley, 1963; Pandey, 1968). Of these two mutagens, colchicine appears to be by far the more potent in inducing non-genetic changes in the reproductive behaviour. Seeds treated with colchicine at a concentration too low to induce polyploidy produced cytoplasmically mutant diploid individuals having an altered reproductive behaviour in a relatively large proportion of treated plants (Pandey, 1968).

Hybridization and polyploidy

Interspecific hybridization, or hybridization between two distinct strains having very different genetic backgrounds, with or without subsequent polyploidy, may be another practical means of disturbing the normal male potency of the young microspore. In this connection it is interesting to note that the most successful species so far in induced haploidy, *Nicotiana tabacum*, is an allotetraploid, and some success has been reported in *Festuca–Lolium* hybrids (Nitzsche, 1970). The hybrid between *Brassica oleracea* L. and *B. alboglabra* showed better results in the formation of callus tissues and subsequent shoots than did the parental species (Kameya and Hinata, 1970). Allopolyploids, which are genetically buffered and often show a slight physiological shift from the usual condition, may thus be a better material than a diploid.

An interesting demonstration of the physiological shift in hybrids affecting the reproductive process is provided by a number of artificial interspecific hybrids of *Citrus*, in which reproduction is facultatively or obligately agamospermous or pseudo-gamous through adventitious embryony from nucellar cells. These hybrids breed true to type from seed (Swingle, 1967).

Natural relative inbreeders may have a better chance of producing haploids in large numbers than the outbreeders, for haploidy in outbred, highly heterozygous plants would expose the normally hidden recessive deleterious genes. However, inbreeding in a normally outbreeding species may produce plants with a changed cytoplasmic condition (Mather, 1948). Where expression of recessive genes does not adversely affect the general competence of the reproductive process this may produce plants more amenable to induction of haploidy.

The fact that the only reasonable success in inducing androgenesis regularly has been achieved in two genera, *Datura* and *Nicotiana*, both belonging to the family Solanaceae and both showing 'Spore Androgenesis', has led Kameya and Hinata (1970) to suggest that species-specific differences are involved, the solanaceous species in general having a high ability for shoot formation. If this is true, 'Callus Androgenesis', which has been more widely observed, may be the only route possible for a large number of species. Species with trinucleate pollen, which is short-lived and known to be hard to germinate and grow in vitro (Gramineae, Cruciferae, Compositae, etc.), appear to need a much higher concentration of sugar in the medium, as is already shown from the work of Clapham (1971) in *Lolium* and *Hordeum* (Gramineae).

General comments

So far experience in induction of pollen haploidy suggests that there is probably no one single procedure which will be applicable to all flowering plants, or even to a large group of plants. Rather, each species, according to its peculiar developmental, evolutionary and
cytogenetic background, may be found to have its own combination of requirements for the successful induction of haploidy; and for some species this method may not be practicable at all. A quicker realization of the great potential of induced androgenesis in plant breeding and genetic research therefore depends upon elucidation, in a number of different species, of certain key factors that might be involved in this novel and exciting phenomenon in the flowering plants.

REFERENCES


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