ANTHER AND POLLEN CULTURE TO PRODUCE HAPLOIDS: PROGRESS AND APPLICATION FOR THE PLANT BREEDER[1]

K. C. Sink, Jr. and V. Padmanabhan[2]
Department of Horticulture, Michigan State University, East Lansing, MI 48824

Research efforts aimed at producing haploid plants by tissue culture of anthers or isolated pollen grains have increased recently. Likewise, the ability of the plant breeder to utilize haploid plants in the breeding program has added a new dimension and possible efficiency to the manner in which horticultural crops can be genetically improved. This review will discuss the recent progress that has been made in the art and science of anther and pollen culture to produce haploid plants and the potential for use by horticultural plant breeders. Previous reviews on this subject have been published by Bottino (12), DeBerg (24), Kimber and Riley (49), Melchers (57, 58), Smith (87), Sunderland (89) and the publication *Haploids in Higher Plants – Advances and Potential* from the first international symposium held in this area of plant biology, at Guelph, Ontario in 1974.

Tissue culture of pollen and/or anthers is only one means of obtaining haploids. However, this technique, when perfected, will offer the plant breeder the means, that may not always be available through some other system, to obtain haploids in large quantities. Other methods of obtaining haploid plants for genetic research include: spontaneous occurrence in relatively low frequencies (49, 55), ploidy crosses (44), induction by chemical or physical treatment (52), chromosome elimination following interspecific hybridization (47), delayed pollination and wide crosses. In some of these systems, the low frequency of occurrence and difficulties encountered in detecting the haploid individuals are limiting factors in the selective incorporation of haploids in the plant breeding program. On the other hand, semigamy, as observed in cotton (95) and the chromosomal elimination system reported in crosses between *Hordeum vulgare* and *H. bulbosum* (47) occur with such regularity that they offer the breeders of these species the opportunity to incorporate haploids into breeding programs at will.

Anther and pollen culture methods have aroused considerable interest among plant breeders due to the simplicity and ease of the operation. Since Guha and Maheshwari (38) first reported the production of haploid plants from pollen, the technique has been tried on numerous species of economic interest. The technique is straightforward and simple and involves plating anthers at the proper developmental stage on pre-sterilized, defined medium. Unopened flower buds are routinely surface sterilized using Chlorox (20%) or Roccal (1000 ppm). The anthers are carefully dissected out and plated immediately onto an appropriate agar medium. In species with minute flower buds, dissection is carried out with the aid of a dissecting microscope.

The culture medium and procedures must be adjusted so that only immature pollen grains are induced to divide. Under optimum cultural conditions, division results in:

1. Direct development of embryoids (28, 39, 68, 83, 104) when one or more haploid cells divide, extend through the pollen grain wall and develop in an organized manner as they pass through stages analogous to those of normal diploid seed embryos, finally, differentiating shoot and root meristems and growing into entire plantlets.
2. An undifferentiated callus which, when transferred to a regeneration medium will differentiate to form shoots and roots (1, 17, 23, 35-37, 46, 78, 85, 86, 98-99). The main drawback in this pathway is the variation in the ploidy of the resulting plants.

In many species, the plants regenerated often have exhibited variation in ploidy levels (29, 45, 63, 64, 67, 83, 91). Ploidy chimeras have also been observed in cereals (17, 18, 48, 73, 98, 99). Non-haploidy can, in general, result from:

1. Polyploidization in pollen callus (32, 64).
2. Nuclear fusion (91).
3. Induction of growth in abnormal non-haploid pollen (20).
4. Participation of anther wall and filament tissue in callus formation (73, 98, 99).

Direct development of embryoids from pollen grains theoretically insures a haploid plant population and is preferred to the callus pathway.

Embryoid pathways

Sunderland and Dunwell (91) have classified the development of embryoids from pollen grains into 3 groups: the A, B and the C pathways. Pathway A is the development of embryoids only from the vegetative cell, while the generative cell degenerates (7, 17, 26, 45, 51, 73). Pathway B bypasses the normal gametophytic development. The microspore is diverted from its normal routine, and undergoes symmetrical division resulting in two equal diffuse nuclei (51, 68, 69, 72, 91). Pathway C differs from the A pathway in that the generative nucleus is functional and takes part in embryo development (91, 92). While anther culture procedures are aimed at callus and/or plantlet induction, pollen culture is preferred when difficulties may occur in obtaining sufficient quantities of haploid plants. According to Nitsch (68) two basic steps are involved in the culture of isolated *Nicotiana* pollen grains: 1) Changing the fate of the microspore to favor an abnormal division – environmental factors act on this mechanism. 2) Formulating a suitable nutrient medium on which pretreated microspores can grow.

Role of anther wall

Based on the observation that wall tissue is believed to play a role in the induction of embryoids, Sharp et al. (85) stimulated colony formation of *Lycopersicon esculentum* microspores by employing the nurse-culture technique. Anthers of *L. esculentum* were slit and placed on the surface of an agar medium. A small disc of filter paper (pre-sterilized) was placed on top of the anther and a drop of pollen suspension with about 10 microspores was pipetted onto each disc. This method kept the anther wall in indirect contact with the microspores. Based on a series of transplant studies, Pelletier and Ilami (76) conceived of an anther wall factor (inducer). Stage 4 pollen from anthers of one tobacco cultivar were transferred to the anther of another cultivar, another species such as *N. glutinosa* or *Petunia hybrida* or callus derived from *Petunia* flower petals. In the latter case, the petal callus acted as the nurture tissue (75). Nurse culture has more recently been replaced by extracts from anthers cultured for a short duration (25).

Culture conditions

The nutrient requirements for anther and pollen culture vary widely from species to species. Some require a very simple medium. Tobacco grows on distilled water with sucrose and chelated iron (66); the needs of other species are more complex (Table 1). The base media generally used by most workers are: 1) modifications of Murashige and Skoog (MS) medium (60), 2) White's medium (100) and 3) Nitsch's medium (65). Sunderland (90), using *Paeonia hybrida* anthers, showed that media based on MS major salts are superior to others. However, the iron content in all media was based on formulations of Murashige and Skoog (60). While sucrose is usually used at a 2–3% level, higher levels have been used in some genera (18, 73, 83). Almost all species in the callus forming group require the presence of either auxin, kinetin or a combination of hormones, for callus induction and the anther response to the hormone medium differs between species. In general, the anther wall remains quiescent. However, there are species, *Triticum aestivum*, Ougung et al. (73) and *Triticale*, Wang et al. (98, 99), where both the pollen mother cells and the anther wall divide.

Extracts have also been used to induce pollen development (25, Sharp, personal communication). Amino acid analysis of these extracts led to their replacement with serine, glutamine and inositol (70). Activated charcoal has been added to an anther culture medium (2). Wagner and Hess (97) used para fluoro-phenylalanine as a selective agent for obtaining haploid plantlets in *Petunia* anther culture.

Anther stage. The stage of microsporogenesis which can be determined by acetocarmine smear at the time of inoculation is very critical for successful induction of pollen embryogenesis. Anthers

[1]Michigan Agricultural Experiment Station Journal Article No. 7501.
[2]Professor and Research Associate.

respond to culture procedures only at a specific time of the developmental cycle. This period usually lies between the tetrad stage (the beginning of haploid phase) and a stage just past the first pollen mitosis. However, according to Gresshoff and Doy (35, 36), earlier meiotic stages are essential for induction of callus in both *Arabidopsis* and *Lycopersicon* anthers. The optimal response of anthers varies considerably from species to species (Table 1). In barley, early microspore phase shows optimal response (17) while in wheat it is midway through microspore phase (73). Sunderland (90) suggested a standard nomenclature describing 6 meiotic stages between tetrad formation and binucleate formation. Such standardization is necessary as most observations relate plating stage to flower bud length, which may result in discrepancies between genotypes, age of plant material, season, etc. Ploidy level of embryoids produced also varies with stage (29). In testing different species for anther culture, one should use anthers of all ages.

Pre-treatments. Traumatic shocks promote induction of embryogenesis (cold treatment) and increased the number of pollen grains developing into embryoids by pre-treating the flower buds with cold temperature (69). Flower buds at first haploid mitosis were pretreated at 5°C for 72 hr or 3° for 48 hr, by placing their pedicels in water (13). The cold treated anthers produced a larger number of embryoids and responded sooner. According to Nitsch (71), the number of dead pollen grains in cold treated anthers is much lower and there is an increase in the number of symmetrical divisions at first pollen mitosis. Other shock treatments include cutting the tip of the inflorescence, used with wheat (79) and chemical treatment (5), although no embryogenesis has been observed.

Table 1 lists the horticultural crop species in which haploids have been successfully obtained to date by anther culture techniques. Success has been achieved with a wide variety of species, using diverse media, and plating anthers at various meiotic stages. In order for the plant breeder to utilize these haploid plants in the breeding program, the method of obtaining them should be reliable, applicable to a large number of varieties of a given species, and the yield, in terms of number of haploid plants produced or callus induced should be adequate, also, the technical procedures used for doubling to the diploid level should be available at a reasonable cost. For example, several *Nicotiana* species, including *tabacum*, readily produce a range of 20–32 plantlets per anther (62, 88) and it has been reported that from 75 to 100% of the anthers cultured yielded at least 1 plantlet (68). On the other hand, the conditions for haploid production in some other *Nicotiana* species have not been found using the simple media (68). Likewise, *Petunia* anthers still remain difficult to induce to form haploid plants (Sink, unpublished data). To date primarily triploid plants have resulted (30, 83) and among research reports where haploids were obtained (82, 97), only 2 were produced from a total number of 9400 anthers cultured.

Haploids can be obtained for a limited number of horticultural crops at the present time but are not readily obtained from the majority. Undoubtedly, future research work will unravel what appear to be primarily technical difficulties with obtaining haploids in those horticultural species not listed in Table 1.

The plant breeder's potential use of haploid plants in crop improvement is rather speculative. Breeding programs on tobacco (21), and potato (59) have utilized haploid plants and it is from these species that we draw inferences on the feasibility of haploid sporophytes in horticultural crop improvement. Only in a few other species have haploids been used in a breeding program and, then, only for a short time, except maize (16). Analysis of haploid derived breeding lines in comparison to inbred lines indicated that the former are generally equal to the latter for various traits (22). It appears then that the major advantage in utilizing haploids may be the saving of time prior to conducting combining ability tests for desirable heterotic genotypes and their evaluation under a broad regime of field or greenhouse environments.

Homozygous lines

Horticultural plant breeders will probably use haploids in the production of homozygous diploid or polyploid plants. The specific use will depend on the objective of the breeding program and whether one is working with a diploid, autoploid, or alloploid species, toward a true-breeding variety from seed, a heterotic product reproduced by seed or a species which is asexually propagated.

The incorporation of haploid sporophytes into a breeding program may be particularly applicable to self-pollinated diploid crops where the production of homozygous lines encompassing a broad range of meiotic recombinants obtained from an F_1 of a wide cross would be desired. The haploid approach would also shorten the lengthy period of time required to inbreed selections the necessary 6 to 7 generations before conducting combining tests for superior hybrid genotypes. An obvious advantage of the haploid technique is that individuals carrying lethal genes, in either the dominant or recessive state, would be eliminated from the population during the initial growth stages. It would be advantageous to the breeder if he could do selection at the haploid sporophyte level prior to doubling and line testing. This procedure would reduce considerably the number of lines carried in the program and lead to a more efficient system.

Horticultural crops where haploid breeding systems may be readily incorporated at the present time would be in the *Solanaceous* fruit crops (tomato, pepper and eggplant) in which F_1 hybrids are used for processing types. The tomato merits particular attention, but unfortunately all cultivars are not readily amenable to mass micropspore culture into haploid plants (35, 85).

The amount of time saved in further selection to bring the homozygous diploid lines derived from haploids into cultivar production is not known. Obviously, once the homozygous lines undergo a reproductive cycle, natural rates of mutation will occur which will necessitate selection for type in a similar manner as occurs with inbreds derived by self-pollination or sibbing.

Other horticultural species where it will be particularly interesting to evaluate the potential use of haploids in the breeding program would be those which have long-time reproductive cycles. Certainly, the use of haploids in establishing true-breeding lines in such crops as perennial bulbs, biennial crops, shade trees and ornamental shrubs, and small and tree fruit crops will be of benefit to breeders of these species. The ability to produce fixed genotypes in one generation should be a great advantage in improvement of those crops. Once subsequent desirable genotypes are found they can be propagated readily by asexual methods. Another use of haploid sporophytes would be in the breeding and genetic studies of autoploid horticultural crops such as the strawberry and geranium. In these species it may be possible to conduct breeding at the tetraploid and diploid levels respectively followed by doubling to the octoploid and tetraploid level for further testing. In addition, genetic analysis could be much more readily accomplished at the lower ploidy levels.

It should be possible to obtain haploid plants on self-incompatible species and also male-sterile and cytoplasmic-male-sterile (CMS) genotypes. In the cole crops where bud-pollination may be troublesome or fail completely, the breeder could obtain superior self-incompatible genotypes using haploids. Tsikov et al. (94) found the yield of haploids from a CMS tobacco line to be much lower than the CMS fertile counterpart. The CMS haploids had a range of flower and stamen structures. Corollas varied from normal to split, stamens from normal to short filaments and anthers from normal to degenerative appearance, and failure to produce pollen. They concluded that the CMS derived haploid plants originated from vegetative cells of the immature microspore which accounted for the transfer of the cytoplasmic factor.

A specific instance where haploids may be particularly advantageous is in the breeding of the dioecious crop, asparagus. In asparagus, pistillate (XX) by staminate (XY) sib crosses are made to produce inbred populations. Uniform, all staminate populations with spears having a low fiber content are desired. Thevenin (93) used anther culture of staminate plants to produce haploids and doubled them to obtain homozygous "super males" (YY). These would produce all male populations when hybridized with pistillate (XX). Homozygous pistillate plants were obtained mainly by polyembryonic means, backcrossed to the staminate parent to produce an isogenic line and these (XY) lines were intercrossed so that uniform hybrids could be grown from seed.

Selection systems in cell culture

Plant breeders and geneticists could utilize haploid plants as a source material for producing haploid callus cells and/or for the isolation of haploid plant protoplasts (57). These 2 types of cells when cultured as single cells or very small clusters could subsequently be subjected to physiological and environmental factors after mutation treatment to selectively screen for superior haploid genotypes. The quantity of haploid plants needed for use in these selective screening systems would not have to be as large as for producing homozygous breeding lines, because variability would be introduced into the system by mutation, induced or natural. The general scheme in these selection systems is to parallel those employed by microbiologists for obtaining mutant genotypes. Thus, large quantities, 10^5 to 10^7, of isolated plant cells are readily cultured in synthetic media, exposed to a physical or chemical mutagenic agent, and plated on a differentiating media lacking a known growth requiring com-

Table 1. Horticultural crop species in which haploid plants have been obtained by anther culture methods.

Species	Medium	Meiotic Stage	Regeneration Medium	Reference
		Callus Pathway		
Asparagus officinalis	MS mod. NAA 0.1 x 10^{-6}M BAP 1.0 x 10^{-6}M	Uni	Omit NAA	Pelletier (77)
Brassica oleraceae	Nitsch mod. CM 10% or K 1.6 + 2,4-D 1.0	Uni	No Change or K 1.0 + NAA 1.0	Kameya & Hinata (46)
Capsicum annuum cv. Yeo Hsien (Small Red Pepper)	MS mod. K 1.0 RNA Nucleo. 40 2,4-D 1.0 or NAA 1.0	Uni	Omit auxin	Wang et al. (99)
cv. Grossum	LS + K 0.3 to 400 LS + IAA 0.5 to 3.0 LS + ino 100	Uni	LS + CH 400 IAA 0.5 inositol 100	George & Narayanaswamy (33)
Digitalis purpurea	Nitsch & Nitsch 2,4-D 5.0	Tetrad	Omit 2,4-D	Corduan & Spinx (23)
Fragaria x ananassa				
cvs. Tioga	LS + K 0.2 + IAA 2 + 2,4-D 0.4	1st mitosis	GDI	Rosati et al. (84)
Pocahontas	GD1		No change	
Gorella	GD1		No change	
Rabunda	GD1		No change	
Lilium longiflorum	MS (Sucrose 4%)	Uni	No change	Sharp et al. (86)
Lycopersicon esculentum	Gamborg PR4 mod. NAA 2.0 K 5.0 or	Tetrad	Shoot B + NAA 0.1 K 2.0	Gresshoff & Doy (35)
Races 25-27-28	B(mod.) + NAA 2 + K 1.0		Root B + NAA 8.0 K 0.1	
Rutgers	MS (low salt) IAA 4 + K 4 (Sucrose 3%)	Uni	No change	Padmanabhan et al. (74)
	GD + NAA 2.0 + K 5.0	Uni	Transplant with leaf callus	Padmanabhan et al. (74)
Pelargonium hortorum	White's medium NAA 2-2.5 + K 2.5 CM 15%	Tetrad	MS NAA 0.5 K 2.5	Abo El-Nil & Hildebrandt (1)
Ranunculus sceleratus L.	White's medium CM 10% 2,4-D 0.5, 1 or 2	Binuc.	No change	Konar & Nataraja (50)
Saintpaulia ionantha	Blaydes	1st mitosis	Root IBA 2.0	Hughes et al. (45)
Solanum nigrum	MS (Mod) K 2.2 2,4-D 2.2 NAA 1.9	Uni	Omit auxins	Harn (40)
		Embryoid Pathway		
Datura metel	Nitsch CM 15%	Uni		Narayanaswamy & Chandy (63)
Petunia hybrida	Nitsch BAP 2.0 NAA 0.5 PFP 15	Uni		
cv. Rose du Ciel	MS mod. BAP 0.2-1.0 NAA 0.5	Uni		Raquin & Pilet (83)
Solanum tuberosum	MS + NAA 0.01 or 1 or in combinations of K 0.1 – 10 or Cm 10-20% or CM with combination of auxin K & CM	Uni		Dunwell & Sunderland (28)
Nicotiana alata	Nitsch + IAA 0.1	Uni		Nitsch (68)
Nicotiana sanderae Hort.	Nitsch + IAA 0.1	Uni		Vyskot & Novak (96)

Growth regulator and supplement abbreviations: 2,4-D = 2,4-dichlorophenoxyacetic acid; BAP = 6-benzylaminopurine; CH = casein hydrolysate; CM = coconut milk; IBA = indolebutyric acid; IAA = indole-3-acetic acid; K = kinetin; NAA = naphthalene acetic acid; PFP = p-fluorophenylalanine. Growth regulator quantities listed are mg/liter. Medias: B = Blaydes (11); GD = Gresshoff & Doy (35); LS = Linsmaier & Skoog (53); MS = Murashige & Skoog (60).

pound. Or, by other means the cell population is exposed to nearly lethal levels of environmental stress factors such as salts, heavy metals, temperature extremes, air pollutants, herbicides, etc. In addition, the possibility exists to design selective methods for certain amino acids, sugar and flavor components, vitamins and other variables which contribute to the nutrient value of food crops.

The designing and utilizing of haploid single cell or protoplast systems for obtaining useful new genotypes of merit in horticultural breeding programs is, of course, only beginning. There are several biological criteria which must be met in the conduct of these experiments (Table 2) in order for the resultant plants to be useful to the plant breeder. It must be possible to readily isolate large numbers of cells either as callus or protoplasts directly from callus or leaf tissue, or other plant parts. The cells must be easily cultured in a defined media where they remain cytologically stable. They must respond to a physical or chemical mutagenic agent, and after being subjected to selection pressure, the conditions for regeneration of entire plants must be known. Also, the regenerated plants must exhibit the desired

Table 2. Criteria for haploid cell selection systems for plant breeding.

1. Isolate numerous cells	5. Appropriate selection regime
2. Culture cells in liquid media	6. Regenerate plants
3. Cells cytologically stable	7. Plant exhibits trait
4. Respond to mutagenic agent(s)	8. Inheritance of trait
9. Reisolated cells exhibit trait	

trait and its inheritance must be established. Lastly, reisolated cells should exhibit the trait as final confirmation.

The protocol for conducting these types of experiments and an indication of the possible results is now available from a few research experiments. The technique and procedure for liquid cell culture, isolating large numbers of single and small clusters of plant cells, plating them on defined media and regenerating callus was first successfully accomplished by Bergmann (6) with *Nicotiana* and *Phaseolus*. Nagata and Takebe (61) isolated tobacco protoplasts, plated them on defined media to produce callus colonies and regenerated whole plants. Similar systems resulting in regenerated plants have been reported for diploid petunia (10), haploid petunia (10), haploid tobacco (4), and diploid carrot (34). Protoplasts have been isolated from asparagus (54), and tomato (19), but the complete regeneration of shoots and roots has yet to be achieved. Variations in the plating technique for selection purposes have been employed since Bergmann (6) and Nagata and Takebe (16) dispensed the cells directly on the surface of the culture medium. Binding et al. (9) incorporated streptomycin in the media. Carlson (15) allowed the plated cells to grow for 14 days and overlaid them with media containing the selective agent, and in some cases, the cooled liquid selection media has been mixed with the isolated cells just prior to pouring the plates.

Binding et al. (9) derived streptomycin resistant lines in *Petunia* by selecting cells which were able to grow on a medium containing .05% of this antimetabolite. The resistant culture contained cells with 7, 14, and 28 chromosomes. Maliga et al. (56) performed similar studies with tobacco. Carlson's (15) work with methionine resistance in haploid tobacco protoplast cells is indicative of the potential the mutation – cellular selection systems employing analogs may play in improving crop species. He treated isolated protoplasts with 0.25% ethyl methane sulfonate (EMS) for 1 hr prior to plating them. After 2 weeks growth, the calluses were overlaid with a medium containing methionine sulfoximine (MSO). Three surviving calluses were diploidized and regenerated into whole plants. Two of the three had higher levels of methionine in the young, fully expanded leaves and they showed resistance to inoculation of *Pseudomonas tobaci*, which infects by release of a toxin that is an analog of methionine. The mutants did not express a level of resistance equal to that of Burley 21, a cultivar that carries genetic resistance.

As pointed out by Zenk (103), the work by Carlson (15) implied that the increased level of resistance to the wildfire toxin by the mutant lines may be due to the increased level of methionine. The levels of glycine, alanine and proline exhibited no significant variation. Earlier work by Braun (14) had shown that DL- or L- methionine would not reverse the pathological symptoms induced by the wildfire toxin or methionine sulfoximine. Zenk (103) also indicated that increased levels of methionine would not necessarily be responsible for the resistance, because the wildfire toxin inhibits activity of glutamine synthetase. Widholm (102) mentioned that methionine sulfoximine is an analog of glutamine rather than methionine. The results are indicative of new approaches to take in securing disease resistance in horticultural species.

Widholm (101) showed that 31 out of 32 carrot lines regenerated after cell selection for resistance to growth inhibition of 5-methyltryptophan maintained that resistance in cell cultures reisolated from carrot plants of each mutant type. Zenk (103) has reported preliminary observations on selection of resistance to 2,4-D. He employed haploid *Nicotiana sylvestris* cells which were suspension cultured and gradually increased the level of 2,4-D in succeeding passages over a period of a year and a half. This selection lead to a strain which resisted 10^{-3}M levels of 2,4-D. The wild type strain was susceptible to 2,4-D levels of 3×10^{-4}M, but resistance was exhibited by the selected strain. The increased resistance was thought to be due to an increased ability of the resistant strain to metabolize 2,4-D. The practical and fundamental applications of this type of research are obvious.

Genetic modifications

Haploid plants could well serve as a source of haploid protoplasts or callus cells which could be employed in various types of genetic modification experiments by plant breeders. To date, the general trend of these types of experiments has been to demonstrate that various kinds of transformations and transplantations of cellular organelles can be accomplished. For the most part, researchers have employed diploid plants or cells for these experiments. Haploid plants and cells would be useful in this type of research because all genes and genetic modifications which occur are immediately expressed. Hess (41, 42) showed transformation in *Petunia* by extracting DNA from an anthocyanin producing line and changing the color of his white-flowered type to anthocyanin producing. Leaf-shape was also modified and the changes were transmitted to succeeding generations (43). Recently, however, Hess' (41, 42) research has been challenged (8) on the basis of anatomical studies of the shoot apex.

Doy et al. (27) demonstrated bacterial virus transformation in haploid tomato cells which led to the coining of the term transgenosis to describe such events. Haploid tomato cells incapable of growth on galactose or lactose media as the carbon sugar source were infected with *E. coli* phage carrying the galactose or lactose operons. Only cells infected with the phages grew when plated on media containing galactose or lactose. Uptake experiments have also been demonstrated for viruses (3), nuclei (81) and chloroplasts (80). These types of genetic modification will merit use by plant breeders when the changes which are invoked can be directed in a known, useful manner.

Collateral studies

There are several supporting research areas relative to plant breeding where the use of haploid sporophytes as the plant material could be successfully utilized. For example, genetic analysis could be performed on haploid derived plant populations for determining inheritance patterns. For simply inherited traits, this procedure could eliminate the need for the F_2 generation. Karyological studies to determine the basic chromosome set could readily be done on haploid plants. In some cases they would directly represent the basic x chromosome complement. Even when derived from higher ploidy levels, the $2x$ or $3x$, material would be easier to study cytologically than the $2n$ sporophytic level for that particular species. Similarly, meiotic analysis on haploid plants could be used to complement karyological studies and to give a clear picture of chromosome homologies.

Use of haploid sporophytes for these studies would all have to be conducted based on the assumption that there is no preferential selection at either the gene or chromosome level for those genetic sets resulting from meiosis which constitute the derived haploid plant populations.

Other areas of research which merit attention are the use of haploid plants in studies of gene action, and physiological and biochemical activities. In many species the gametic chromosome set is totipotent with respect to complete plant development and thus offers the physiologist and biochemist genetic systems which are not hindered by dominance – recessive relationships.

Summary

Anther and pollen culture methods with some horticultural species have been successful in providing the plant breeder with haploid plants. Intensive research efforts are needed both at the basic level in understanding the mechanism of induction and growth of immature microspores into plantlets and to produce haploids on those economic crops where success has been elusive thus far. There are several areas where haploid plants have potential use by the plant breeder: these include the derivation of homozygous diploid breeding lines, cell selection systems, genetic modification procedures, and several areas collateral to plant breeding. Haploid plant breeding is not intended to be a "cure-all" for plant improvement but rather a technique and procedure which should complement long-standing, thoroughly studied breeding systems. The ingenuity and creativeness with which plant breeders employ haploids will result in their full potential. Or, as Chase (16) so aptly stated "the method will be most successful in practice if those who are masters of the haploid technique are also masters of plant breeding."

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