# Recent Advances in Haploid Production in Higher Plants

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Studies on haploidy involve many important matters both in theory and application (cf. Katayama and Nei, 1964), and recently marked progress is being made in both areas. Haploids are spontaneously formed in ordinarily grown plants, and in progeny from interspecific or intergeneric crosses. They are also noticed in twins and triplets (cf. Kawakami, 1967). Artificial means of inducing haploids: pollination of X-ray irradiated pollen grains (Katayama, 1934); delayed pollination (Kihara, 1940); selection of specific strains or genotypes (Chase, 1949); and the use of an alien cytoplasm (Kihara and Tsunewaki, 1962) have been reported to be effective in certain materials. Moreover, the success of anther culture in *Datura* (Guha and Maheshwari, 1964) has inspired many investigators to study the experimental production of haploids. For the practical use of haploids in plant breeding it is essential to obtain a large number from a single treatment. Recent advances in the methods of haploid production toward this goal are reviewed here, and prospects for the future are discussed.

## 1. Success of anther culture

Although the possibility of using haploids for plant breeding was long ago pointed out, there has been no means of obtaining the large number of haploids necessary for practical breeding. In reviewing the studies on haploidy before 1964, Katayama and Nei (1964) conclusively suggested the idea of producing haploids by pollen culture. In the same year, success in the anther culture of *Datura* was preliminarily announced from the laboratory of Prof. P. Maheshwari of India, and a tangible report on this was made in 1966 (Guha and Maheswari, 1964, '66). Encouraged by their work, Tanaka *et al.* obtained a large number of tobacco haploids in the summer of 1967 at Hatano, Japan by culturing the anthers. Furthermore, they doubled the number of chromosomes in the haploids by colchicine treatment, and produced homozygous diploids. Results were reported at the semiannual meeting of the Japanese Society of Breeding in the autumn of the same year (Tanaka and Nakata, 1967, '68), and were published in two successive papers (Nakata and Tanaka, 1968; Tanaka and Nakata, 1969). After these achievements with tobacco, Niizeki and Oono (1968a, b) succeeded in the anther culture of the rice plant at Hiratsuka, Japan, and

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published in two papers. At almost the same time, the production of tobacco haploids by anther culture was successful in France (Bourgin and Nitsch, 1967; Nitsch *et al.*, 1968; Nitsch and Nitsch, 1969). Plant species in which haploids have been obtained so far by anther culture are listed in Table 1.

Table 1. Species in which haploid plants have been reported to be produced by the culture of anthers or pollen‡)

| Order | Family | Genus | Species | Reference‡‡) |
|---|---|---|---|---|
| DICOTS | | | | |
| Rhoeadales | Cruciferae | *Brassica* | *oleracea* | Kameya & Hinata 1970 |
| | | | *oleracea* × *alboglabra* | 〃 |
| | | *Arabidopsis* | *thaliana* | Gresshoff & Doy 1972b |
| Geraniales | Geraniaceae | *Pelargonium* | *hortorum* | Abo El-Nil & Hildebrandt 1971 |
| Tubiflorae | Solanaceae | *Atropa* | *belladonna* | Zenkteler 1971 |
| | | *Lycopericon* | *esculentum* | Sharp, Dougall & Paddock 1971, Gresshoff & Doy 1972a, Sharp *et al.* 1972, Debergh & Nitsch 1973 |
| | | | *pimpinellifolium* | Debergh & Nitsch 1973 |
| | | *Capsicum* | *annuum* | Wang, Sun, Wang & Chien 1973 |
| | | *Datura* | *innoxia* | Guha & Maheshwari 1964, 1966, 1967, Nitsch 1972, Nitsch & Norreel 1973 |
| | | | *metel* | Narayanaswamy & Chandy 1971, Nitsch 1972, Iyer & Raina 1972 |
| | | | *mateloides* | Nitsch 1972 |
| | | *Nicotiana* | *tabacum* | Bourgin & Nitsch 1967, Nakata & Tanaka 1968, Nitsch & Nitsch 1969, Sunderland & Wicks 1969, Tanaka & Nakata 1969, Burk 1970, Carlson 1970, Melchers & Labib 1970, Devreux *et al.* 1971, Nakata 1971, Sunderland 1971, Sunderland & Wicks 1971, Sharp, Dougall & Paddock 1971 |
| | | | *rustica* | Nitsch 1969, 1972 |
| | | | *otophora* | Collins *et al.* 1972, Collins & Sadasivaiah 1972 |
| | | | *glutinosa* (4n) | Nitsch 1969, 1972 |
| | | | *sylvestris* | Bourgin & Nitsch 1967, Nöth & Abel 1971, Nitsch 1972 |
| | | | *alata* | Nitsch 1969, 1972 |
| | | | *suaveolens* | Smith 1973 |
| | | | *glauca* × *langsdorffii* | 〃 |
| | | | *clevelandii** | Vysko & Novak 1974 |
| | | | *sanderae** | 〃 |
| | | *Solanum* | *verrucosum** | Irikura & Sakaguchi 1972 |
| | | | *nigrum** | Harn 1971, 1972 |
| | | | *dulcamara** | Zenkteller 1973 |

Table 1.— (continued)

| | | | | |
|---|---|---|---|---|
| MONOCOTS | | | | |
| Graminiales | Gramineae | *Oryza* | *sativa* | Niizeki & Oono 1968, Guha *et al.* 1970, Iyer & Raina 1972 |
| | | *Lolium* | *multiflorum* | Clapham 1971 |
| | | *Lolium* ×*Festuca* | *L. multiflorum* (4x) ×*F. arundinacea* (12x) | Nitzsch 1970 |
| | | *Hordeum* | *vulgare* | Clapham 1973 |
| | | *Triticum* | *aestivum* | Ouyang, Hu, Chuang & Tseng 1973, Wang, Chu, Sun, Wu, Yin & Hsü 1973 |
| | | *Triticale* | | Wang, Sun, Wang & Chien 1973 |
| | | *Aegilops* | *caudata*×*umbellulata* | Kimata & Sakamoto 1972 |
| | | *Setaria* | *italica* | Ban, Kokubu & Miyaji 1971 |
| Liliales | Liliaceae | *Lilium* | *longiflorum* | Sharp, Raskin & sommer 1971 |

+) From Smith (1974). The five species with asterisks are added to Smith's list.
++) References, except for those added five species and those referred to in the text, are not listed in the references of the present paper.

Two processes through which haploids are produced from pollen are known. In one, young pollen grains in the anthers, which are cultured under appropriate conditions, continue nuclear division after the first pollen mitosis and form multicellular embryoids which directly develop into haploid plantlets. This process is known in *Datura* (Guha and Maheshwari, 1967); *Nicotiana* (Nakata and Tanaka, 1968; Nitsch, 1969); and *Atropa* (Rashid and Street, 1973). In the other, immature pollen grains once dedifferentiate into haploid callus, from which haploid plantlets redifferentiate. This process is typically seen in *Oryza* (Niizeki and Oono, 1968a). In both cases, the nucleus must go on dividing, so that the pollen grain may develop into a haploid plant, instead of differentiating into generative and vegetative nuclei, and ceasing to divide. In *Nicotiana* and *Datura* the embryoid is formed chiefly by continuous division of the vegetative nucleus after the first pollen mitosis, and the generative nucleus degenerates or plays only a vestigial role (Sunderland and Wicks, 1971; Iyer and Raina, 1972).

The composition of the culture medium and the culture condtions are of great importance to the success of anther culture, but they are not so crucial as the selection of the most favorable stage in develoment when anthers are excised into the cultures. According to Nakata and Tanaka (1968), tobacco embryoids and haploid plantlets are directly formed from pollen grains at the one-nucleate stage just after the tetrad stage. Sunderland and Wicks (1969) believe that the first pollen grain mitosis is the critical point at which the transition to plantlet formation takes place in tobacco. In general, the crucial stage which determines whether the microspores continue to divide seems to occur immediately before, or at the time of, the first pollen mitosis. Many successful anther

cultures have been achieved by using anthers corresponding to the above pollen stages.

Some attempts to attain higher efficiency in haploid formation by anther culture have attracted our attention. These are: cold treatment (Wang *et al.*, 1974) to pollen at the first mitosis; adding iron to the medium as the ferric salt of ethylenediamine-di-O-hydroxyphenylacetic acid (FeEDDHA) (Chopra and Rashid, 1969); and the application of a chemical, para-fluorophenylalanine (PFP), to the medium to obstruct the growth of diploid callus (Niizeki and Kita, 1973).

The culture of isolated pollen from the anther is the final goal of anther culture. This methed could resolve the problems associated with the cultivation of intact anthers, which often results in the proliferation of diploid callus derived from the anther wall. Moreover it offers a more refined method for studying mutation and selection. Sharp *et al.* (1972) first obtained haploid clones originating from individual tomato pollen grains by using a nurse culture procedure in which a single pollen grain was cultured upon an intact anther. It is generally recognized that the coexistence of the anther and pollen grains is necessary for successful anther cultures. This implies that some specific substances are exuded from the anther wall which stimulate young pollen grains to further cell devision. Therefore, the identification of these substances, if they exist, is one of the foremost problems that be resolved for complete success in the cultivation of isolated single pollen grains.

Success in anther culture has given rise to many discussions on its application to haploid breeding methods and to some branches of biology, e. g. mutagenesis and genetics (Katayama and Tanaka, 1969; Sunderland, 1971; Melchers, 1972; Niizeki, 1972). This is because of some unique advantages of anther culture as a method of haploid formation, i. e. being able to produce many haploids at one time, or at least, of having the potentiality of becoming a general method applicable to every plant species.

## 2. Embryo culture in barley

For a long time breeders of barley had tried crosses between *Hordeum vulgare* and *H. bulbosum.* Their aim was to transfer desirable characters such as resistance to disease, winter hardiness and the cross pollinating habit from the wild species *H. bulbosum* to the cultivated barley *H. vulgare.* There are diploid (2n=14) and tetraloid (2n=28) cytotypes in *H. vulgare* and *H. bulbosum,* respectively. Although a triploid $F_1$ hybrid between *H. vulgare* (2x) and *H. bulbosum* (4x) was once reported by Kuckuck (1934), real success in obtaining viable $F_1$ hybrids from the crosses has been achieved only since the embryo culture technique has been used (Konzak *et al.*, 1951). Hybrid seeds from crosses between *H. vulgare* and *H. bulbosum* develop vigorously for about ten days after fertilization, but after that they start to shrivel and abort. However, if the hybrid embryos are removed from caryopses and are placed in cultivation

in ten to fourteen days after pollination, they continue to grow to viable seedlings. By this method of embryo culture, it has become possible to obtain viable plants from most of the cross combinations between the diploid and tetraploid forms of *H. vulgare* and *H. bulbosum* (Davies, 1958; Morrison *et al.*, 1959; Symko, 1969; Kasha and Kao, 1970; Lange, 1971a; Subrahmanyam and Kasha, 1973).

From six of the eight possible interspecific cross combinations between *H. vulgare* (2x, 4x) and *H. bulbosum* (2x, 4x), Lange (1971a) could obtain viable plants. Some of the crosses gave rise to haploids or dihaploids (derived from the autotetraploids) besides the normal interspecific hybrids. All the haploids and dihaploids resembled *H. vulgare*, regardless of the direction of the cross[1]. Symko (1969) obtained many seedlings from the cross, *H. bulbosum* (2x) ♀ × *H. vulgare* (2x) ♂, using embryo culture, and cytological examination revealed that all the plants had 2n=7 chromosomes (Table 2). Successsive counts at intervals of about one month revealed no change in chromosome number. The vegetative characters of most of these haploids at the tillering stage were intermediate between those of the two parental species, whereas all the haploids had the spike characters of *H. vulgare*. Kasha and Kao (1970) produced many haploids exclusively from the cross reciprocal to Symko's, i. e. *H. vulgare* (2x) ♀ × *H. bulbosum* (2x) ♂, using embryo culture (Table 2). They suggested that those haploids always had the *H. vulgare* genome, for all were vigorous *vulgare*-like plants. Kasha *et al.* (1970) also reported that nearly all the haploids or dihaploids produced from interspecific crosses between *H. vulgare* (2x, 4x) and *H. bulbosum* (2x, 4x) contained the gametic chromosome complement of *H. vulgare*, regardless of whether *H. vulgare* was used as the male or female parent.

Table 2. Results of reciprocal interspecific crosses between *Hordeum vulgare* (2n=14) and *H. bulbosum* (2n=14) using embryo culture techniques

| Cross | Female parent No. used | No. of spikes or florets pollinated | No. of seeds induced | No. of embryos cultured | No. of haploids |
|---|---|---|---|---|---|
| *H. bulbosum* ♀ × *H. vulgare* ♂ †) | 7 | 455 spikes‡) | 97 | 97 | 59 |
| *H. vulgare* ♀ × *H. bulbosum* ♂ ‡) | 19 | 1,238 florets | 638 | 209 | 23 |

†) From Symko (1969).
‡) From Kasha and Kao (1970).
‡‡) Each spike had 12 to 18 florets.

As the mechanism for producing the *vulgare*-like haploids or dihaploids from interspecific crosses between *H. vulgare* and *H. bulbosum*, Davies (1958) and

1) These results written in English are a part of Lange's extensive studies of interspecific crosses between the diploid and tetraploid cytotypes of *H. vulgare* and *H. bulbosum* presented in 1968 and 1969 in Dutch.

Symko (1969) considered the possibility of male parthenogenesis (merogony or androgensis), while Kasha and Kao (1970), Kao and Kasha (1970), Kasha *et al.* (1970) and Lange (1971a) all suggested selective chromosome elimination during haploid production, i. e. fertilized eggs, containing both male and female genomes, underwent selective and gradual elimination of their chromosomes into a haploid number during embryogenesis. It has been cytologically observed that embryos which were expected to give rise to 2n=7 haploids contained cells carrying variable numbers of chromosomes (from fourteen down to seven) in their early development. The frequency of haploid cells carrying seven chromosomes increased as the embryos developed until finally most of their cells possessed seven chromosomes (Lange, 1971b; Kasha *et al.*, 1972; Subrahmanyam and Kasha, 1973) (Table 3).

Table 3. Chromosome variations and abnormalities in embryos with 1 *vulgare* and 1 *bulbosum* genome

| Age in days | No. of embryos scored | No. of countable cells with chromosome numbers of +) | | | | | | | | Total No. of countable cells | % of cells with extra-chromatin material | Mean No. of cells per embryo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 7++) | 8 | 9 | 10 | 11 | 12 | 13 | 14 | | | |
| 3 | 6 | 3 (42.85) | | 1 | | 2 | | 1 | | 7 | 50.30 | 37 |
| 4 | 13 | | 3 | | 2 | 2 | 1 | 2 | 1 | 11 | 30.26 | 75 |
| 5 | 14 | 10 (37.03) | 6 | 4 | 4 | 1 | 1 | 1 | | 27 | 16.70 | 199 |
| 6 | 15 | 26 (52.00) | 14 | 5 | 3 | | | 1 | 1 | 50 | 9.81 | 370 |
| 7 | 17 | 68 (68.68) | 16 | 10 | 3 | 1 | | | 1 | 99 | 9.70 | 772 |
| 8 | 17 | 160 (90.90) | 11 | 2 | 2 | | 1 | | | 176 | 7.98 | 1178 |
| 9 | 10 | 177 (77.29) | 41 | 11 | | | | | | 229 | 4.54 | 2306 |
| 10 | 5 | 218 (90.45) | 13 | 7 | 2 | 1 | | | | 241 | 3.39 | 4710 |
| 11 | 4 | 431 (93.69) | 22 | 7 | | | | | | 460 | 2.36 | 7430 |

+) From Subrahmanyam and Kasha (1973).
++) Percentages given in parentheses.

The embryo culture of hybrid seeds from crosses between *H. vulgare* (2x) and *H. bulbosum* (4x) produced viable triploid hybrids with stable chromosome numbers (Lange, 1971a; Kasha and Sadasivaiah, 1971). This implies that chromosome stability in the embryo cells of the hybrids depends on their genomic constitutions. No matter what the basis for selective chromosome elimination, it is a general fact that embryos initially containing genomes in the ratio of 1 *vulgare* to 1 *bulbosum* gradually lose their *bulbosum* chromosomes during the early stage of development, while triploid embryos with 1 *vulgare* and 2 *bulbosum*

genomes are relatively stable in their chromosome numbers (Subrahmanyam and Kasha, 1973).

Although both Symko (1969) and Lange (1971b) indicated that chromosome elimination might also have occurred in the *H. vulgare* genome, various studies conducted by Kasha and others at Guelph, Canada have presented no conclusive evidence for the elimination of any *vulgare* chromosomes. That is, of the approximately 4,000 progeny from *H. vulgare* (2x) × *H. bulbosum* (2x), roughly 99% were haploids resembling *H. vulgare* and the other 1% was made up of diploid interspecific hybrids; no haploids of *H. bulbosum* being obtained even when *H. bulbosum* had been used as the female parent. No embryo cells were observed to contain less than the haploid number of chromosomes, only *vulgare* type chromosomes (though only three chromosomes, 5, 6, and 7 were distinguished) were exhibited in somatic cells of haploid and dihaploid seedlings. The marker genes on the *vulgare* chromosomes consistently expressed their effects in the haploids (cf. Subrahmanyam and Kasha, 1973). The chromosome number in the endosperm and plant tissue was less stable than that in the embryo, and also depended upon the genome constitution (Lange, 1971b; Subrahmanyam and Kasha, 1973).

Similar situations as in the relation between the balance of the parental genomes and chromosome elimination in barley hybrids have been known before, i. e. the restricted $F_2$ recombination in tobacco and cotton. In tobacco, recombinations of corolla characters, such as tube length and limb-width, in the $F_2$'s of *Nicotiana alata* × *N. langsdorffii* (Anderson, 1939) or *N. langsdorffii* × *N. sanderae* (Smith, 1950) were studied, and it was shown that the recombinations of these characters were not free, but were restricted to giving rise to offspring more or less like either of the parents or the $F_1$ hybrid. According to Stephens (1949), $F_1$ hybrids of *Gossypium hirsutum* (2n=26) and *G. barbadense* (2n=26) showed regular formation of 26 bivalents and were apparently fully fertile. However, free recombination did not occur between the parental characters, and the net effect of inbreeding was the establishment of types practically indistinguishable from the parent species without the establishment of intermediate types. In backcrosses of the $F_1$ hybrids a more rapid accumulation of the recurrent parent genotype occurred than was expected, as a result of random segregation and recombination.

The selective chromosome elimination in *Hordeum* is very similar to the phenomenon in which somatic cell hybrids between a human and a mouse progressively lose only their human chromosomes during cultivation (Weiss and Green, 1967; Matsuya *et al.*, 1968). The cause of selective chromosome elimination in both cases may be the same.

### 3. Use of alien cytoplasms

In 1958, Mazoti and Mühlenberg recognized the effect of an alien cytoplasm on parthenogenesis in maize (Chase, 1969). They examined the frequencies of haploids in paired stocks of the same genotype maize, which differed only in that one carried the maize cytoplasm and the other the teosinte cytoplasm. The result was that stock with the maize cytoplasm produced 1.69 haploids per 1,000 progeny and the other with the teosinte cytoplasm gave rise to 2.2 per 1,000. The authors concluded that the difference in cytoplasm had a significant effect on the frequency of parthenogenesis in maize. The teosinte cytoplasm, however, was no more effective in inducing parthenogenesis in maize than were other methods, such as delayed pollination and the selection of favorable genotypes.

Kihara (1951) substituted the cytoplasm of *Aegilops caudata* (genome formula CC, 2n=14) in common wheat (*T. aestivum*, AABBDD, 2n=42) by successive backcrossing in order to study the effects of the *Aegilops* cytoplasm on the manifestation of the common wheat genome. During this investigation, haploids were sometimes noticed among progenies of *T. aestivum erythrospermum* with the *Ae. caudata* cytoplasm. These amounted to nine (out of 287 examined) by the thirteenth generation of the backcross. Moreover, when the same alien cytoplasm was introduced into the *T. aestivum* strain Salmon (which is a 6x derivative of the 8x Triticale, formerly called Taylor's Triticale), the cytoplasm substitution line produced haploids at the very high rate of 52.9% (nine out of seventeen) in the second generation of the backcross (Kihara and Tsunewaki, 1962). Pure Salmon with the cytoplasm of common wheat produced no haploids. Salmon with the *Ae. caudata* cytoplasm has constantly given haploids at the rate of 20.3 to 38.0% in subsequent backcross generations (Tsunewaki *et al*. 1974) (Table 4). This line has given rise to many twins (most were the haplo-diplo type) at the rate of 6.5 to 15.2%, as well (Table 4). It was histologically confirmed that the occurrence of these haploids and twins could be attributed to parthenogenesis of the egg cells, and, in the case of twins, subsequent fertilization of the synergid (Tsunewaki *et al*., 1968).

As the nucleus of Salmon has been brought into various alien cytoplasms of related species, it has become clear that the cytoplasm causing parthenogenesis in Salmon is not confined to that of *Ae. caudata*. At present, six species in the genus *Aegilops* have been proven to have the cytoplasm that induces both haploids and twins in Salmon at a high rate (Table 4). Five of the species belong to the section Polyeides, in which the $C^u$ genome is in common. This indicates a close genomic relationship among donor species of the parthenogenesis-inducing cytoplasms to Salmon (Tsunewaki *et al*., 1974). To date, Salmon is the only known strain whose egg cells can parthenogenetically develop into embryos in alien cytoplasms, but *T. macha* seems to have the same characteristic because *T. mach* with an *Ae. colmnaris* cytoplasm gave rise to four haploids

out of twenty-four plants tested (Mr Mukai, personal communication).

Table 4. Haploid and twin inducing effects of six alien cytoplams to Salmon in artificially pollinated offsprings+)

| Cytoplasm donor | Backross generation | Year | ♂ parent | Twins % | Haploids % |
|---|---|---|---|---|---|
| *Ae. caudata* | $B_2$ | 1962 | Salmon | 8.4 | 27.9 |
| | $B_5$ | '65 | 〃 | 9.7 | 26.3 |
| | $B_6$ | '66 | 〃 | 13.0 | 38.0 |
| | $B_7$ | '67 | 〃 | 15.2 | 20.3 |
| | $B_8$ | '68 | 〃 | 6.5 | 25.2 |
| | $B_9$ | '69 | 〃 | 12.5 | — |
| | $B_{10}$ | '70 | 〃 | 14.3 | 36.7 |
| | $B_{11}$ | '71 | 〃 | 10.0 | 33.3 |
| | $B_{12}$ | '72 | 〃 | 6.7 | 37.5 |
| | $B_{13}$ | '73 | 〃 | 14.4 | 31.1 |
| *Ae. umbellulata* | $B_3$ | 1970 | Macha | 0.0 | 14.6 |
| | $B_4$ | '71 | Salmon | 0.0 | 0.0 |
| | 〃 | 〃 | CS++) × Salmon $F_1$ | 0.7 | 20.2 |
| | $B_5$ | '72 | Salmon | 5.6 | 0.0 |
| | $B_6$ | '73 | 〃 | 0.0 | 8.7 |
| *Ae. triuncialis* | $B_2$ | 1971 | Salmon | 0.0 | 0.0 |
| | $B_3$ | '72 | 〃 | 0.0 | 45.5 |
| | $B_4$ | '73 | 〃 | 7.4 | 24.6 |
| *Ae. colmnaris* | $B_1$ | 1973 | Salmon | 15.2 | 10.7 |
| *Ae. kotschyi* | $B_1$ | 1973 | Salmon | 4.0 | 12.5 |
| *Ae. variabilis* | $B_1$ | 1973 | Salmon | 6.9 | 37.0 |
| | 〃 | 〃 | Tve | 15.0 | 58.8 |
| | 〃 | 〃 | P168 | 8.3 | 90.9 |
| | 〃 | 〃 | JF | 26.7 | 63.3 |

+) From Tsunewaki *et al.* (1974)
++) CS, Tve, P168, JF are abbreviated names of varieties of *T. aestivum*.

## 4. Genic control of parthenogenesis

It is evident that the occurrence of haploids is controlled by the genetic constitution of the plant as well as by external conditions, for the spontaneous or induced frequency of haploid production largely differ from genotype to genotype in a species, as well as from species to species. For example, in maize, some stocks of certain genotypes produce greater numbers of haploids than do others when used as the male or female parent in crosses. Parthenogenetic development of the embryo in wheat is ordinarily a characteristic of a certain strain of *T. monococcum* (Katayama, 1933, '60). This haploid induction by alien cytoplasms is also restricted to a specific strain of *T. aestivum*. There has yet to be a report showing that the occurrence of haploids is controlled by a definite gene (or genes) or chromosome.

There is the phenomenon, however, that in a line of Salmon having *Ae. triuncialis* cytoplasm the parthenogenesis of egg cells seems to be controlled by a certain chromosome. Most cytoplasm substitution lines with the *Ae. triuncialis* cytoplasm show low crossed seed fertility (ca. 20%), even after five or six backcrosses. This low crossed seed fertility or high female sterility is intimately associated with the persistence of a chromosome derived from *Ae. triuncialis* (called i chrosome, hereafter), i. e. only egg cells containing the i chromosome can be normally fertilized and produce progeny, while egg cells without the i chromosome cannot be fertilized, or become lethal after fertilization (Endo and Tsunewaki, in press). This situation appears to be brought about by the i chromosome alone independent of the cytoplasm of *Ae. triuncialis*. Salmon with the *Ae. triuncialis* cytoplasm also contains one i chromosome in addition to the 42 chromosomes of Salmon, itself.

When the cytoplasm substitution lines of Salmon were backcrossed as female to normal Salmon, a large number of haploids, twins (all haplo-diplo type) and a triplet (haplo-diplo-diplo type) were obtained, as shown in Table 4. The chromosome numbers of these haploids and of haploid seedlings from twins and a triplet were examined in root tip cells. Thirty-four examined had twenty-one chromosomes, two had twenty, and the two remaining had twenty-two chromosomes. Hence, at least in Salmon, egg cells lacking the i chromosome seem to be able to develop parthenogenetically.

Suppose that the transmission rate of an i chromosome through the female gametes is about 25%, as in the ordinary univalents in the monosomics of wheat; then the expected number of $2n=22$ haploids containing the i chromosome will be eight or nine. This fact indicates that egg cells containing the i chromosome have relative difficulty in developing parthenogenetically, while $n=21$ egg cells free from the i chromosome were favorable for parthenogenesis. This phenomenon has two interpretations. One is that while the cytoplasm of *Ae. triuncialis*, itself, had a haploid inducing effect on Salmon, the i chromosome counteracts the effect of the cytoplasm in the gametes. The other is that the i chromosome inhibits fertilization of the i chromosome free egg cells ($n=21$) as stated above, which often results in parthenogenesis of the egg cells. Hence, the relative frequency of $2n=21$ haploids rises. Though no further detailed discussion on this subject is given here, it can be inferred that the i chromosome has some controlling function in the parthenogenesis in Salmon.

## 5. Concluding remarks and future prospects

Considering that gametes have totipotency, and that the haploids of higher plants are viable in spite of their half genic dose in comparison with diploids, haploid production in higher plants can be achieved by two approaches. One is to make the totipotency of gametes manifest without fertilization. As gametes are usually suppressed so as not to start cell division before fertilization, it

may be possible to remove this suppression or to activate parthenogenesis by some appropriate method. The other is to supply nutrients to haploid embryos until they are large enough to support themselves. Anther culture, for example, fulfills both of these requisites in terms of culturing anthers *in vitro*. In the embryo culture of barley, genomic unbalance causes haploid embryos as a result of chromosome elimination, and the embryo culture enables the haploid embryos to become seedlings. Compatibility of different genomes coexisting in a hybrid has been intensively discussed in terms of different compatibility in reciprocal crosses by Katayama (1933). Alien cytoplasms of *Aegilops* induce the parthenogenesis of egg cells through a disharmonious interaction with the nucleus of *T. aestivum* strain Salmon. The following fertilization of polar nuclei to develop endosperm is necessary if the parthenogenesis is to end in success. Disharmony between the nucleus and cytoplasm, or between genomes is ultimately attribtuable to genotypes, which suggests that a gene (or genes) is concerned in haploid induction. Among the various wheats only *T. monococcum* has the unique property for the parthenogenetic development of embryos (Katayama, 1933, '60). This may be associated with haploid inducing genes, or a special chromosome. The methods of haploid production reported above have the advantage of producing a large number of haploids. Anther culture is the most useful method for haploid production at present, thanks to its wide application to various plant species and its high efficiency in producing haploids.

As haploids have been obtained in large number and at a high rate, their usefulness has continued to increase. Above all, when they are combined with the recently developed method of culturing protoplasts, the value of haploids in plant breeding and genetics is conspicuously enhanced. Since successful isolation of protoplasts from the mesophyll tissues of tobacco was achieved with some enzymes (Takebe *et al.*, 1968), investigations using protoplasts have advanced rapidly. Protoplasts are able to regenerate cell walls and to undergo mitotic cell division (Nagata and Takebe, 1970). Takebe *et al.* (1971) and Nagata and Takebe (1971) succeeded in regenerating whole plants from protoplasts isolated from the mesophyll cells of tobacco. As with diploids, flowering haploid plants were later regenerated from leaf protoplasts of the haploid tobacco obtained from anther cultures (Ohyama and Nitsch, 1972; Bajaj, 1972). It has also become possible to fuse protoplasts from different species to produce hybrid somatic protoplasts or cells (Power *et al.*, 1970). Carlson *et al.* (1972) reported the only successful case of producing a mature interspecific hybrid plant by the fusion of leaf protoplasts from *Nicotiana glauca* (2n=24) and *N. langsdorffii* (2n=18), i. e. by parasexual hybridization between them. The parasexually produced mature hybrid (2n=42) produced fertile flowers and seeds, which germinated, and produced seedlings similar to a sexually produced amphiploid of the two species.

The combination of anther cultures with parasexual hybridization may be able to reproduce the life cycle of lower plants in higher forms. If this is realized, revolutionary changes will be brought about in the methodology of genetics and in the breeding of higher plants. It will be possible to apply the powerful techniques of microbial genetics to higher plants, with the efficient screening of useful mutants in a short time. In fact, this system has been almost established with the tobacco plant.

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