

Interspecific Hybridisation and Polyploidisation as Tools in Ornamental Plant Breeding

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Abstract

Interspecific hybridisation and polyploidy are recognized as the most important sources of evolution and domestication of flowering plants. In ornamental plant breeding these phenomena go hand in hand and can be observed in the breeding history of many ornamental crops (*Rosa*, *Chrysanthemum*, *Gladiolus*, *Alstroemeria*, *Lilium*, orchids etc). With lily as model crop techniques developed for overcoming pre- and post-fertilisation barriers are reviewed. For overcoming F₁-sterility mitotic and meiotic polyploidisation are applied and can result in fertile allopolyploids. The mechanism of viable pollen production of mitotic and meiotic polyploidisation is quite different. Mitotic polyploidisation possess one homologous chromosome set. They undergo normal meiotic division like diploid cells. However, meiotic polyploidisation often show irregular chromosome division resulting in two of unreduced chromosome number instead of reduced chromosome number in tetrads. In contrast to mitotic doubling homoeologous recombination can occur. There are two significant mechanisms, FDR and SDR, for the formation of 2n-gametes. The FDR-gamete increase heterozygosity while SDR-gamete increase homozygosity. Genomic in situ hybridisation (GISH) has been used to discriminate parental chromosomes and to detect homoeologous recombination. Mitotic polyploidisation showed no homoeologous recombinations between the parental genomes whereas in meiotic polyploids it can be detected frequently. The use of 2n-gametes is therefore the most promising way for the introgression of desirable characters in the breeding with interspecific hybrids. Although spontaneous occurring in the domestication of many ornamental crops the systematically detected unreduced gametes proved to be highly efficient tool for introgression of characters.

INTRODUCTION

The role of interspecific hybridisation is the most important source for variation in ornamental breeding. Interspecific hybrids have the potential to capture hybrid vigour as well as combine traits that do not occur within a single species (Volker and Orme, 1988). Because breeder rather than geneticist always want to add a new type of characteristics to the current cultivars, interspecific hybridisation is indispensable to combine diverse gene pools. Thus interspecific or intergeneric hybrids have the enormous potential to extend not only their qualitative but also quantitative traits such as the type of flower, plant phenotypes, other single dominant traits from parent species with an environmental adaptation or improve the flower vase life. While natural hybrids can exist between species whose flowering times overlap, pre- and post- fertilisation barriers hinder the frequency of these hybrids. In lily interspecific hybridisation has greatly contributed to the breeding for the commercial Longiflorum-Asiatic hybrids, which are becoming more important on the market. The point of current breeding of important horticultural hybrids has two distinct points. One is interspecific hybrid and the other is polyploids origin. In fact, more than 60-70 % are polyploid origin in angiosperm (Soltis and Soltis, 1993). However, the systematic use of this genetic phenomenon is more important to commercial breeding. As in natural condition the polyploid gametes have originated spontaneously from interspecific hybrids. In those early days the knowledge regarding on the polyploidisation was very

poor, the horticultural breeders were successful in creating considerable genetic variation. These efforts were subsequently responsible for selecting thousands of horticultural varieties in some of the crops. However, the genetic consequence of the interspecific hybridisation has not been dealt in finding the efficient way for the introgression. Therefore, it is important to elucidate the cytogenetic composition of many of these crops in order to practice a more systematic breeding. The number of produced interspecific hybrids and their backcross progenies solely depended on the seed set after normal sexual hybridisation until plant tissue culture techniques has not been settled. However, the prevalence of pre- and post-fertilisation barriers to crossing species, as well as the high degree of sterility of the F₁-hybrids, hinder introgression between two species and, when successful, it was a highly laborious and frustrating process. Nevertheless, the introduction of ovule culture and embryo-rescue methods has greatly facilitated wide hybridisation followed by backcrossing in a wide variety of plant species (Hadley and Openshaw, 1980; Van Tuyl et al., 1990; Van Tuyl and De Jeu, 1997; Buitendijk et al., 1995; De Jeu and Jacobsen, 1995). Although these techniques opened the possibilities for the production of interspecific hybrids and backcross progenies on a large scale, the next important steps of selecting genotypes from the backcross derivatives, i.e., selection methods, were required. In this context, the recently developed molecular cytogenetic techniques are potentially of great value for improving the efficiency of introgression. The development of polyploid forms of breeding in ornamental plants has also been important. In *Cyclamen*, experiments to develop triploids between the diploid and tetraploid have been attempted (Takamura and Miyajima, 1999). In *Delphinium*, new cultivars, which resulted from hybridisation among plants with different ploidy levels, have appeared in recent years.

A combination of in situ hybridisation and molecular markers can be not only useful for monitoring the hybrids and back cross progenies for introgression but also for elucidating the modes of origin of 2n-gametes, the extent of genetic recombination and the phylogeny of the species and hybrid cultivars. In the paper the role of polyploidisation in interspecific hybridisation of ornamentals with *Lilium* as model crop is reviewed.

INTERSPECIFIC HYBRIDISATION

Techniques for Overcoming Pre-Fertilisation Barriers

In order to introduce valuable economic traits such as disease resistance, flower shape and colour, from wild species into the cultivar assortment, it is essential to overcome interspecific crossing barriers. Several techniques hitherto, as the cut style method, the grafted-style method and the in vitro isolated ovule pollination technique, have been developed to overcome pre-fertilisation barriers (Van Tuyl et al., 1991). To overcome pre-fertilisation barrier, the environmental conditions such as high temperature, hormone treatment (Ascher, 1973), specific protein and exudates (Martin, 1970) and lipids (Wolter-Arts et al., 1998) can be applied.

Techniques for Overcoming Post-Fertilisation Barriers

Post-fertilisation barriers can be circumvented by in vitro rescue methods as embryo, ovary-slice and ovule culture in case of lily and other important ornamentals. Interspecific hybridisation is highly relied on the genotype combination among species. Fertilisation can be classified into embryo and endosperm formation. Failure of the fertilisation in interspecific hybridisation is assumed mainly due to genetic discrepancy and chromosome degeneration during cell division of the zygote. In interspecific hybridisation of *Lilium*, the embryo and endosperm formation and development can be categorized in different groups. Because of the development of embryo is highly affected by the development of endosperm. If there is no endosperm formation, normally the embryo cannot survive anymore. In this case, very early embryo rescue such as ovary slice culture may helpful. There are several obligations in interspecific hybridisation, which are important to know why interspecific hybridisation cannot be successful. Firstly, fertilisation has to be successful both embryo and endosperm. Secondly, embryo development has to be checked.

Thirdly, endosperm development has to be monitored. Based on the results of certain cross combination, suitable rescue method can be applied (Van Tuyl et al., 2002). In this case, the time of embryo rescue looks important but it is not always effective for the embryo rescue. For unknown reasons embryos degenerate in a very early stage even several days after fertilisation although it is not clear whether or not the fertilisation has been occurred normally. In the case of early embryo degeneration the ovary slice culture method is preferred. Many cases showed that endosperm grows longer than the embryo. When the embryos are degenerated in a middle stage of the development, ovule culture can be applied. In all crosses made in *Delphinium* by Honda & Tsutsui (1997), pollen germination was normal on the stigma, and numerous pollen tubes reached the upper part of the ovary. The observations may confirm that although fertilisation itself might occur normally, a post-fertilisation barrier arose. Hybrid embryos from these crosses probably were aborted due to the lack of endosperm development. Interspecific hybrids in ornamental crops have been reported in *Lilium* (Van Tuyl et al., 1996), *Lupinus* (Gupta et al., 1996), *Sandersonia* (Morgan et al., 2001), *Alstroemeria* (De Jeu and Jacobsen, 1995), *Tulipa* (Van Creij et al., 1997) and many others crops (Ewald, 1996; Sharma et al., 1996).

Integrated Techniques for Overcoming Pre- and Post-fertilisation Barriers

Integrated techniques have been considered to handle both pre- and post-fertilisation barriers. Unlike the other techniques, which retain the zone of inhibition (stigma and style) and manipulate pollen germination and pollen tube growth to overcome pre-fertilisation barriers, in vitro pollination brings pollen grains in direct contact with the ovules, and is, therefore, considered more effective (Willemsse et al., 1995).

In *Lilium* various combinations of in vitro pollination (cut-style and grafted-style method) and embryo rescue (ovary, ovule and embryo culture, placental pollination), were applied in order to control the whole fertilisation process (Van Tuyl et al., 1991; Janson, 1993; Chi, 2000). This resulted into a range of new interspecific hybrids (Van Creij et al., 1993; Van Tuyl et al., 1996). Using these techniques wide interspecific lily crosses with species and cultivars from the different sections of the genus *Lilium* (*L. longiflorum*, *L. henryi*, *L. canadense*, *L. concolor*, *L. dauricum*, *L. candidum*, *L. rubellum*, *L. martagon*, Asiatic and Oriental hybrids) could be made. Hybrids originating from inter-sectional crosses (e.g. *L. longiflorum* x *L. concolor*, *L. longiflorum* x *L. dauricum*, *L. longiflorum* x *L. henryi*, *L. longiflorum* x *L. martagon*, *L. longiflorum* x *L. candidum*, *L. longiflorum* x Asiatic hybrids (LA), *L. longiflorum* x Oriental hybrids (LO), *L. longiflorum* x *L. rubellum*, *L. longiflorum* x *L. canadense*, Oriental x *L. auratum* x *L. henryi*, *L. longiflorum* x (*L. longiflorum* x *L. rubellum*) Oriental x Asiatic hybrids (OA) and *L. henryi* x *L. candidum*) have been produced. Especially the Oriental x Asiatic hybrids are a break-through in lily breeding and a promise for the future. Similar results were obtained from interspecific crosses in *Tulipa* and intergeneric crosses between *Nerine* and *Amaryllis* (Van Tuyl et al., 1990, 1991; Van Creij et al., 1999). Until now in vitro fertilisation for bulbous crops has not been achieved using isolated sperms and eggs.

POLYPLOIDISATION

Polyploidisation Techniques for Overcoming F₁-Sterility and Introgression

The introgression of summer-hardiness traits into *Impatiens hawkeri* germplasm has been hampered by sterility in the interspecific hybrids (Arisumi, 1974, 1975) and in their offspring (Pasutti and Weigle, 1980). Amphidiploidy has been used to create male and female fertility (Arisumi, 1975; Pasutti et al., 1977), but this has made introgression more difficult because segregation in progenies is hindered (Arisumi, 1975). Naturally occurring pollen fertility has been found sporadically among interspecific hybrids without the use of amphidiploidy (Weigle & Pasutti, 1976; Pasutti, 1977; Pasutti & Weigle, 1980). Methods for estimating pollen fertility include counting unstained, normal-looking pollen grains (Arisumi, 1975) or grains stained with acetocarmine (Arisumi, 1974; Merlin & Grant, 1986). Hicks et al. (1987) showed that an *Impatiens platypetala* x *hawkeri* F₁ hy-

brid produced pollen that could be germinated quickly in a petri dish on the surface of 2 % agar, and that there was a close relationship between pollen germination in vitro and seed set on a seed parent tester in vivo. Acetocarmine or Aceto orcein and FDA (Fluoro diacetate) have been used for the staining of fertile pollen in many crops. Especially pollen from interspecific or intergeneric hybrids can be checked for their fertility. Under the stereo microscope, pollen from *Liliaceae* can be observed easily for their size as n- and/or 2n-pollen. Because pollen of distantly related interspecific or intergeneric hybrids can not be germinated in a normal condition, however, a low percentage pollen are able to germinate. In this case we have a clue that these viable pollen is not reduced but exists of only unreduced pollen. Most of unreduced gametes are produced by FDR with maximum heterozygosity. Theoretical point of view of FDR, there are some extent of homoeologous recombination between parental genomes. Lim et al. (2001, 2003) showed using GISH that lily chromosome segments from wild species are transferred into domesticated hybrids by different mechanisms of 2n-gametes production.

Mitotic Polyploidisation

In almost all cases, the F_1 hybrids of distantly related species are highly sterile. In most cases sterility is resulted from the disturbed chromosome division during meiosis which leads to the formation of spores with unbalanced chromosome constitution leading to sterility. The most widely used method of restoring fertility in interspecific hybrids is by doubling the chromosome number of the F_1 in order to produce an amphidiploid. Because of homologous chromosomes pair with each other, meiosis will be normal. The pollen can be viable with balanced euploid number. One great drawback of this approach is, however, that because of the preferential pairing of chromosomes between the constituent genomes of the hybrid, the possibility for homoeologous chromosome pairing and crossing-over is dramatically reduced. Since homoeologous recombination is a crucial prerequisite for introgression of specific desirable characters into a cultivar, chromosome doubling of the F_1 hybrid is not a desirable method. However, whole chromosomes can be added to the backcross progenies as has been demonstrated in the case of diploid ($2n=2x=24$) hybrid of *Lilium longiflorum* \times *L. rubellum* and their BC_1 progeny using GISH (Lim et al., 2000). However, in this intersectional hybrid there is evidence for homoeologous chromosome pairing and crossing-over but such recombinant products cannot be transmitted into next generation due to sterility. Although there is a drawback being reduced homoeologous recombination, there are many chromosomes from donor into recipient plant. In this case whole chromosomes are introgressed rather than chromosome segments. As will be described below for a different hybrid, through the use of 2n-gametes homoeologous recombinant products can be recovered in the backcross progeny. In Table 1 of ten genera examples are listed in which restoring of fertility was accomplished by chromosome doubling.

Meiotic Polyploidisation (Occurrence of 2n-Gametes)

Occasionally, plant species and interspecific hybrids produce 2n-gametes instead of the normally expected haploid, or n-gametes. When they occur in interspecific hybrids in some extent of viability, 2n-gametes can be used for the production of sexual progeny either through crossing or selfing. Progenies in these cases consist of polyploids and offer an alternative to somatic chromosome doubling. If 2n-gametes from only one parent was crossed to diploid counterpart parent, 3x progeny will be produced in general. Indeed, meiotic doubling has been practised successfully in some of the crops such as *Alstroemeria* (Ramanna, 1992; Buitendijk et al., 1997; Kamstra et al., 1999), and *Lilium* (Van Tuyl, 1989; Lim et al., 2000, 2001, 2003).

Unlike mitotic doubling, sexual polyploidisation saves time that would have required for colchicine treatment. But the most important advantage of meiotic doubling is that homoeologous recombination occurs during meiosis in the diploid interspecific hybrid and many alien chromosome segments can be transmitted. Because, unlike in a somatically doubled allotetraploid, the homoeologous chromosomes are forced to pair

during meiosis diploid interspecific hybrid. This forms the basis for the occurrence of intergenomic recombination in the $2n$ -gametes. Such homoeologous recombination due to crossing-over has been clearly demonstrated in the case of *Alstroemeria* (Kamstra et al., 1999), *Lilium* (Karlova et al., 1999; Lim et al., 2001) and *Gasteria lutzii* × *Aloe aristata* (Takahashi et al., 1997) (Table 2).

There are two main mechanisms known so far viz. FDR and SDR (Veilleux, 1985). Recently a third mechanism 'IMR' was reported by Lim et al. (2001) in lily. Because homoeologous chromosome pairing can be highly variable in interspecific hybrids, ranging from the formation of only univalents, i.e., without any crossing-over, to complete pairing as bivalents (high degree of crossing-over) in some of the spore mother cell, several mechanisms can be considered. The most common mechanism of $2n$ -gamete formation is FDR. There are two types of FDR gametes, so called, FDR with homoeologous recombination and FDR without homoeologous recombination. It is interesting that FDR with homoeologous recombination is more often than FDR without homoeologous recombination analysed in lily BC_1 plants.

In view of the occurrence of different types of meiotic restitution mechanisms in distant hybrids, it is possible to predict the potential outcome of using $2n$ -gametes in breeding provided we have knowledge of the modes of their origin. From the literature on the occurrence of $2n$ -gametes in plants it is clear that they occur in almost all plants (Harlan and de Wet, 1975) but their frequencies vary greatly. There are claims that different mechanisms of nuclear restitution are determined by single genes in crops like potato (Mok and Peloquin, 1975) and many other crops (review, Britagnolle and Thompson, 1995; Veilleux, 1985). Table 2 summarize the occurrence of $2n$ -gametes in a number of ornamental crops. Although the occurrence of unreduced gametes in generally is rare it appeared from our own research that induction or increase of unreduced gametes is possible by i.e. temperature shock treatment during flower development.

Although it can be laborious it is possible to select genotypes that produce reasonably high frequencies of $2n$ -gametes so that they can be used in breeding. When sterile hybrids produce even low frequencies of $2n$ -gametes it is possible to obtain sexual polyploids easily simply because they are the only viable gametes. On the other hand, when both n - and $2n$ -gametes are produced in a fertile diploid plant, the progeny consists of a mixture of diploids and polyploids (Van Tuyl et al., 1989). The number of diploids is normally higher than that of triploids because of counterpart gamete is also haploid so that embryo-endosperm ratio will be stable. The selection of polyploids will be laborious in this case. However, in a considerable number of plant species a phenomenon called 'triploid block' operates and in these the selection of polyploids can be highly efficient. As regards the second question on how to proceed with triploids and allotetraploids derived from uni- and bi-lateral sexual polyploidisation, there is considerable literature on using both triploids and allotetraploids but certain aspects have been ignored in the past. Generally triploids are regarded as sterile and of little interest in breeding. However in vegetative propagated ornamentals like *Alstroemeria* and lily the triploid ploidy level is preferred over diploids and tetraploids. A cursory survey indicates that triploids have been used successfully as parents in planned crossings (reviews, Brandham, 1982) as well as during spontaneous polyploidisation, e.g., *Narcissus*, extensively (Throckmorton, 1980). An interesting feature of triploids is that it is possible to generate progenies with near diploid (circa diploid) or tetraploid chromosome constitution by making $3x-2x$ (or reciprocal) and $3x-4x$ (or reciprocal) respectively. The progeny with a near diploid constitution are potentially useful for breeding at the diploid level as has been demonstrated in the case of analytical breeding in potato and other polysomic polyploids. Like in other plant species, near diploid progenies have been obtained by crossing a triploid *Lilium* hybrid ($2n=3x=36$) with diploid ($2n=2x=24$) genotypes (Lim et al., 2003). The different ploidy levels observed between $3x-2x$ and $3x-4x$ crosses are possibly caused by preferential fertilisation or survival resulting in a different ratio of chromosome numbers between the embryo and endosperm. Though *Lilium* has tetrasporic, eight nucleate type of embryo sac formation (Fritillaria type), the observed difference between the progeny types in $3x-2x$ and $3x-4x$

crosses is comparable to that of observed in monosporic eight nucleate type (Polygonum type) that predominate in most genera of Angiosperm. An important feature of the genome constitution of the progenies was that the homoeologous recombinant chromosomes were transmitted intact from BC₁ to BC₂ progenies in variable numbers. In addition, there was evidence for the occurrence of new homoeologous recombinations in the triploid BC₁ (Lim et al., 2003).

Genetic and Epigenetic Interactions in Allopolyploid Plants

One putative hybrid (2n=24) from *Delphinium hybridum* 'Galahad' (2n=32) × *D. grandiflorum* (2n=16) showed that some of phenotypic characters were similar to *D. hybridum* 'Galahad', others are look like *D. grandiflorum*. The leaf shape of this hybrid was intermediate between those of the parents. The percentage of viable pollen of parents was about 76 %, whereas that of the *D. hybridum* 'Galahad' × *D. grandiflorum* hybrid was only 8.5 %. The hybrid failed to yield viable seeds either by self-pollination and back crossing (Honda et al., 2003).

Whereas wild allopolyploid plants are well adapted for a long time, man-made allopolyploids are typically unstable. Some progenies of 3x × 2x cross of interspecific lily hybrids died in vitro stage, while others grew very vividly. This instability refers to the appearance of unexpected changes in phenotype, such as homeotic transformation and lethality, and in genome structure, such as chromosomal rearrangements and changes in the number and distribution of repeated DNA sequences within heterochromatin. However, the molecular mechanisms underlying these adaptive steps remain obscure (Comai, 2000). Interspecific hybrids between distant relatives are usually sterile. Allopolyploids represent a special type of hybrid that contain two parental genomes undergo little intergenomic recombination and thus maintain their integrity through sexual generations. Domestication of closely related interspecific hybrid might be more reliable. Because the homoeologous chromosomes between parental species or subspecies are able to pair at higher level so that many breakpoints of homoeologous recombination are possible just nearly the same act as homologous intra-specific recombination. That's how natural allopolyploids have been adapted more stably rather than man-made allopolyploids. In an ancient era some species in the same genus were more homologous in their genomes, that may affect more chance being natural allopolyploids. Therefore, karyotypic stability is achieved at the expense of the evolutionary flexibility provided by unhindered recombination between the parental genomes. One factor that favours allopolyploids may be heterosis generated by the combination of homoeologous genes (Allard et al., 1993; Jiang et al., 1998).

Although the prominent role of polyploids in plant breeding has long been recognized, underlying mechanisms of gene expression, causing the novel variation are still poorly understood. New research approaches are being used to study these mechanisms and the results should provide a more complete understanding of polyploidy (Osborn et al., 2003).

CONCLUSIONS

In ornamental plant breeding interspecific hybridisation and polyploidisation are phenomena which go hand in hand and can be observed in the breeding history of many ornamental crops. Pre- and post-fertilisation barriers can be overcome by the application of a range of techniques, varying from pollination techniques, ovule and embryo rescue to polyploidisation. The doubling of chromosomes and the use of 2n-gametes overcomes F₁-sterility and enhance introgression of characters. Search and induction of unreduced gametes in interspecific hybridisation programmes is an underestimated field of research. The application of molecular genomic and cytogenetic techniques like FISH, GISH and MAS can be of great help for fastening interspecific hybridisation programmes.

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Tables

Table 1. Examples of mitotic doubling to restore F1-fertility.

Crop	Combination	Reference
<i>Lilium</i>	<i>L. longiflorum</i> × <i>L. henryi</i>	Asano (1982)
<i>Lilium</i>	<i>L. candidum</i> × <i>L. henryi</i> ; <i>L. longiflorum</i> × Asiatic, Oriental × Asiatic; <i>L. longiflorum</i> × Oriental; <i>L. longiflorum</i> × <i>L. rubellum</i> and others	Van Tuyl et al. (1989, 2000, 2002)
<i>Hemerocallis</i>	<i>H.</i> cultivars	Arisumi (1964)
<i>Nerine</i>	<i>N. bowdenii</i> × <i>Amaryllis belladonna</i>	Meijer et al. (1998)
<i>Alstroemeria</i>	<i>A. aurea</i> × <i>A. caryophyllaea</i>	Lu & Bridgen (1997)
<i>Cyclamen</i>	<i>C. persicum</i> × <i>C. purpurescens</i>	Ihizaka & Uematsu (1995)
<i>Delphinium</i>	<i>D. zalil</i> × <i>D. nudicale</i>	Legro (1964)
<i>Dendranthema</i>	<i>D. grandiflorum</i>	Endo et al. (1997)
<i>Iris</i>	<i>I. laevigata</i> × <i>I. ensata</i> <i>I. hollandica</i> × <i>I. tingitana</i>	Yabuya (1985) Van Eijk & Eikelboom (1990)
<i>Impatiens</i>	<i>I. platypetala</i> × <i>I. mooreana</i>	Arisumi (1978)
<i>Tagetes</i>	<i>T. erecta</i> × <i>T. patula</i>	Bolz (1961)
<i>Nicotiana</i>	<i>N. trigonophylla</i> × <i>N. tabacum</i>	Chung et al. (1996)

Table 2. Occurrence of 2n-gametes in ornamental plants.

Crop	Hybrid	Reference
<i>Alstroemeria</i>	Interspecific hybrids	Kamstra et al. (1999)
<i>Antoxanthum</i>	Species	Bretagnolle (2001)
<i>Lilium</i>	Interspecific hybrids	Van Tuyl (1989), Lim et al. (2001)
<i>Narcissus</i>	Cultivated <i>Narcissus</i>	Brandham (1986)
<i>Gladiolus</i>	Cultivated <i>Gladiolus</i>	Ohri and Khoshoo (1983)
<i>Gentiana</i>	Species	Yuan et al. (1998)
<i>Ranunculus</i>	Interspecific hybrids	Okada H. (1984)
<i>Rosa</i>	Interspecific hybrids	Crespel et al. (2002)
<i>Orchidaceae</i>	Species, interspecific hybrids	Storey (1956), Teo (1984), Lee (1987)
<i>Hemerocallis</i>	Cultivated <i>Hemerocallis</i>	Tomkins et al. (2001)
<i>Hypericum</i>	Species	Matzk et al. (2003)
<i>Tulip</i>	Species	Kroon and Jongerius (1986)
<i>Primula</i>	Interspecific hybrids	Kato and Mii (2000), Kato et al. (2001)