

Significance of Unreduced (2n) Gametes in Plant Breeding

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식물육종에 있어서 非環元 (2n) 配偶子の 중요성

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적 요

非環元 (2n) 配偶子は 생식세포 형성과정중 비 정상적인 세포분열의 결과로서 생식세포 염색체수 (n) 대신에 체세포 염색체수 (2n)를 가지는 생식세포 (화분 또는 난세포)를 말한다. 이러한 非環元 (2n) 配偶子들은 자연상태에서 거의 모든 식물종 들에서 낮은 빈도로 발생되며, 非環元 配偶子를 통한 유성 배수체 형성 (sexual polyploidization)은 자연상태에서 다배체종을 형성하기 위한 주된 통로의 역할을 하여 왔다. 식물에 있어서 非環元 配偶子들을 확인 할 수 있는 방법들은 : 1) 반수체 (n) 화분과 2n 화분에서 화분의 크기 또는 모양의 차이, 2) 후대의 배수성 분석 또는 감수분열시 이분체(dyads) 또는 사분체(tetrads)의 존재 여부, 3) 후대의 능력과 임성의 차이, 그리고 4) 동위효소 또는 DNA 표지인자의 이용 등이 있다. 非環元 (2n) 配偶子들은 양친으로부터 자손으로 상당한 비율의 비상가적(non-additive) 유전적 효과를 전달할 수 있고, 즉 異形接合性(heterozygosity)을 최대화 시킬 수 있는 독특한 방법을 부여 함으로써 새로운 품종을 합성하는데 좋은 수단이 될 수 있으며, 또한 배수성이 다른 중간 및 종내 교배시 임성을 회복시키는데 이용될 수 있다. 감자에 있어서 非環元 (2n) 화분의 형성 기작은 : 1) 평행 방추사 (parallel spindle) 또는 3각형 방추사 (tripolar spindle), 2) 미성숙 세포벽 분열 (premature cytokinesis), 그리고 3) 接合 突然變異體 (synaptic mutants) 등 3가지 방법이 밝혀졌다. 감자, 알팔파, 레드클로버 등과 같은 작물에서는 非環元 (2n) 配偶子들의 형성이 단일 열성 유전자에 의해 조절되는 것으로 밝혀졌으며, 밀에 있어서는 2개의 열성 유전자들에 의해서 조절되는 것이 유전적 분석을 통해서 밝혀졌다. 야생종으로부터 재배종으로, 특히 2배체로부터 4배체로 유전자원을 효과적으로 전달 할 수 있는 非環元 配偶子들을 이용함으로써 육종에 있어서 집단 유전적 변이성을 향상시킬 수 있을 것으로 사료된다.

I. INTRODUCTION

In plants, unreduced (2n) gametes are meiotic products (pollen or egg) having a sporophytic (somatic) chromosome number, resulting from abnormalities during either microsporogenesis or megasporogenesis.

They are known to occur naturally at a low frequency and play a major role in the evolution of polyploids in a number of plant species (Harlan and de Wet, 1975). Harlan and de Wet (1975) reviewed the occurrence of 2n gametes throughout the plant kingdom and concluded that sexual polyploidization has been the

major route to the formation of naturally occurring polyploids. Veilleux (1985) compiled reports of 2n gametes occurring in crop plants, involving either male or female gametogenesis.

Production of 2n gametes have been extensively studied in potato, *Solanum tuberosum* L. (Camadro and Peloquin, 1980; Mok and Peloquin, 1975; Watanabe and Peloquin, 1989), alfalfa, *Medicago sativa* L. (McCoy and Rowe, 1986; Veronesi et al., 1986; Vorsa and Bingham, 1979), red clover, *Trifolium pratense* L. (Broda and Smith, 1980; Parrott and Smith, 1984), pea, *Pisum sativum* L. (Myers et al., 1984), wheatgrass, *Agropyron cristatum* (L.) Gaertn. (Ray and Tokach, 1992), orchardgrass, *Dactylis glomerata* L. (De Haan et al., 1992; Maceira et al., 1992), ryegrass, *Lolium perenne* L. (Den Nijs and Stephenson, 1992), maize, *Zea mays* L. (Rhoades and Dempsey, 1966; Sarkar and Coe, 1971), and tetraploid wheat, *Triticum* spp. (Xu and Joppa, 1992; Xu and Dong, 1992).

Polyploidization can be achieved mainly in two ways: i) either by somatic doubling of the chromosomes (asexual polyploidization) or ii) function of 2n gametes produced by aberrations in the meiotic process (sexual polyploidization) (Camadro and Peloquin, 1980). Although both modes of polyploidization achieve a doubled chromosome number, advantages and disadvantages become evident when genetic variability, inbreeding, heterozygosity, and epistasis are considered (Camadro and Peloquin, 1980; Iwanaga and Peloquin, 1982). Harlan and de Wet (1975) suggested that almost all polyploid angiosperms primarily arose by way of 2n gametes through sexual reproduction rather than through spontaneous doubling of somatic chromosomes. de Wet (1980) proposed that tetraploidy can be achieved through two different processes: i) a 2n gamete is fertilized by a 1n gamete to produce a 3x individual which produces non-reduced 3n gametes that are

fertilized by 1n gamete and result in 4x individuals, or ii) a 2n gamete fuses with another 2n gamete which directly produces a 4x individual. The first polyploidization process is more common than the second and may occur in any species or ecotype. Unreduced gametes can also efficiently transfer germplasm from wild relatives to cultivated species, especially from diploids to tetraploids (Ehlenfeldt and Hanneman, 1984; Havey and Maxwell, 1988). Unreduced gametes are considered to be more desirable for maximizing heterozygosity (intralocus interactions) and epistasis (interlocus interactions) than doubled gametes of colchicine induced polyploids (Camadro and Peoloquin, 1980; McCoy and Rowe, 1986; Mok and Peloquin, 1975).

Heterosis in autotetraploids such as alfalfa, birdsfoot trefoil and potato appeared to be dependent on maximizing heterozygosity and, particularly, on the frequency of tri- and tetra-allelic loci (Bingham, 1980; Dunbier and Bingham, 1975). Dunbier and Bingham (1975) theorized that doubled-cross alfalfa populations derived from autotetraploid parents produced by chromosome doubling 2x haploids would outyield the single-cross populations because of a greater frequency of tetra-allelic loci.

The present review deals with the identification, genetic significance, mechanisms, genetic control and use of unreduced (2n) gametes in plant species.

II . IDENTIFICATION OF PLANTS PRODUCING 2N GAMETES

Unreduced gametes in plants can be identified for four possible ways as follow i) pollen size and/or shape differences between haploid (n) and diploid (2n) pollen, ii) ploidy analysis (chromosome number) of progeny or meiotic analysis (presence of dyads and/or triads at the microspore stage), iii) progeny performance and fertility and iv) dosage of isozyme or

RFLP loci. Pollen size and/or shape differences between haploid and diploid pollen were identified in red clover, pea, birdsfoot trefoil, alfalfa and potato, etc. Haploid pollen of pea and birdsfoot trefoil was tricolpate and globose-prolate in shape, while 2n

pollen was tetrahedral in shape (Fig. 1~3). In addition to the pollen shape, haploid and diploid pollen size differences also have been found in those species (Myers et al., 1984; Parrott and Smith, 1984; Rim and Beuselinck, 1991). Parrot and Smith (1984) also

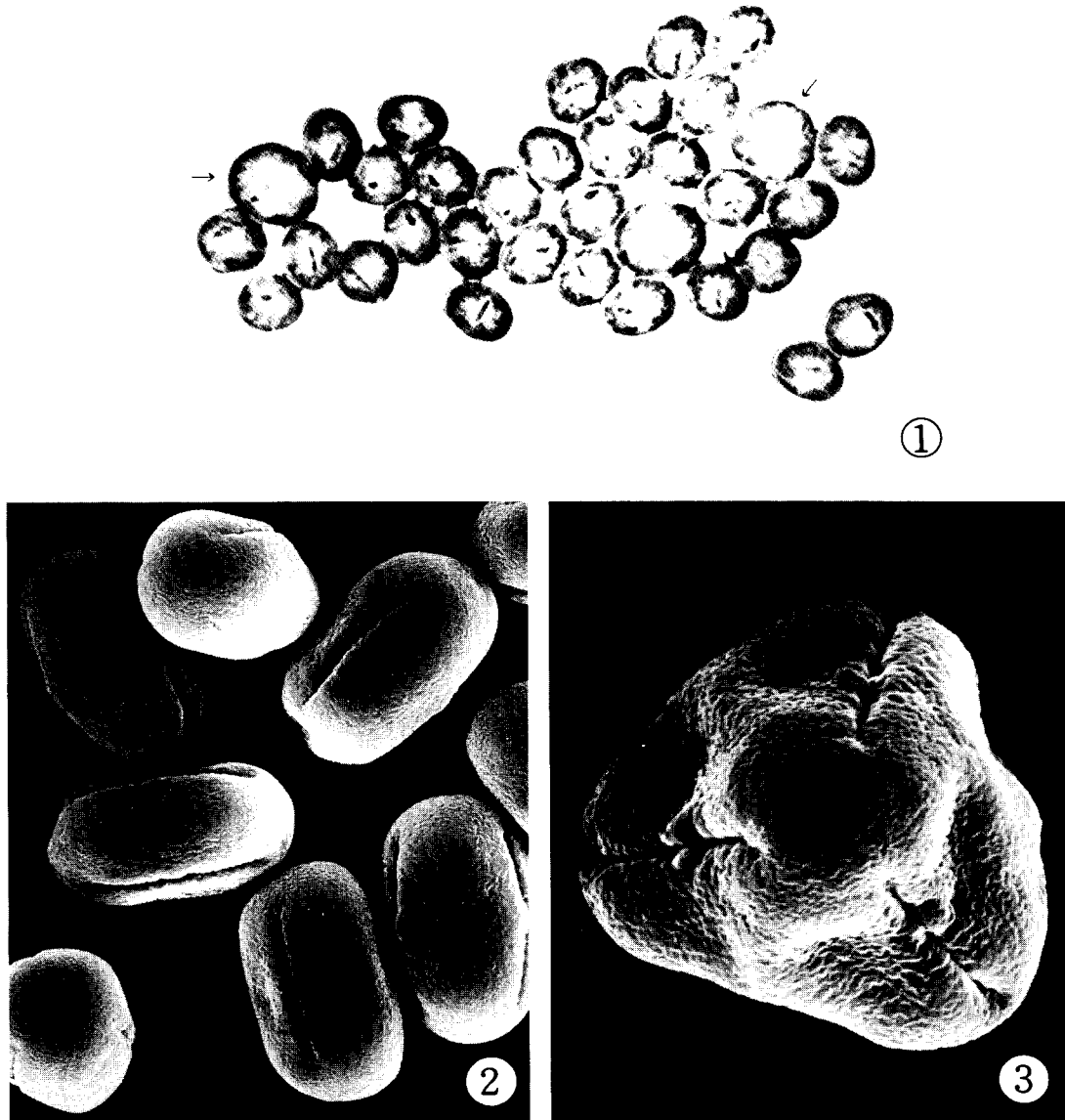


Fig. 1~3. Pollen morphology of birdsfoot trefoil (*L. tenuis* L.) : 1. Fresh pollen under light microscopy, 2n pollen identified by arrows among 1n pollen (x500), 2. Fresh *L. tenuis* 1n pollen under SEM (x4000), 3. Fresh *L. tenuis* 2n pollen under SEM (x4000).

reported distinctive morphologic differences between dry 1n and 2n red clover pollen: 1n pollen was described as being oblong and regular in shape, and shapes of 2n pollen were triangular, square, or irregular. A binomial distribution of pollen size has been found in alfalfa and potato, with the large grains containing 2n and the smaller grains 1n gametes (McCoy, 1982; McCoy and Rowe, 1986).

Progeny derived from $4x-2x$ or $2x-4x$ hybridizations with an unexpected chromosome number has usually been the first indication of functional 2n gametes (Veilleux, 1985). Tetraploid potato ($2n=4x=48$) was emasculated and crossed with diploid *S. phureja* L. ($2n=2x=24$) and tetraploid progeny were obtained due to 2n gametes from the male parent otherwise triploid (3x) progeny were expected (Mendiburu and Peloquin, 1977). In $2x-4x$ hybridization, diploid alfalfa, *M. falcata* ($2n=2x=16$) was crossed with tetraploid *M. sativa* ($2n=4x=32$). In F_1 , progeny were tetraploid instead of triploid. Identification of $4x-2x$ hybrids of alfalfa was simplified by utilizing male-sterile 4x female parents (Bingham, 1968; Cleveland and Stanford, 1959; Veronesi et al., 1986). The presence of dyads and/or triads at the microspore tetrad stage is generally good evidence for 2n gametes. However, in many cases, the frequency of 2n gametes was so low that meiotic analysis would have been difficult for examination of the underlying mechanisms.

Progeny performance and fertility can also be another indications to identify the 2n gametes. Tetraploid progeny obtained from $4x-2x$ or $2x-4x$ hybridization using 2n gametes was fertile and showed heterosis and yielded the parents (Mok and Peloquin, 1975a; Parrott and Smith, 1988).

Intensity of isozyme band can also recognized 2n gametes. In apple $2x-2x$ crosses, triploid progeny was obtained due to 2n gametes (Chyi and Weeden, 1984). They examined banding patterns of 6-Phospho-

gluconate Dehydrogenase and found asymmetric band intensity in triploid cultivars, explaining by the gene dosage effects.

III. GENETIC SIGNIFICANCE OF 2N GAMETES

Rosenberg (1927) first pointed out more clearly the phenomenon of meiotic nuclear restitution. Meiotic nuclear restitution may be defined as the formation of a single nucleus with unreduced chromosome number in place of two nuclei with reduced chromosome numbers, owing to a failure of either the first or second meiotic division (Ramanna, 1979). Depending on the meiotic divisions during which the nucleus restitutes, there can be two basic types of meiotic nuclear restitutions: i) the first division restitution (FDR) and ii) second division restitution (SDR) (Rosenberg, 1927). Cytologically, there are important differences between FDR and SDR. In FDR the nucleus does not undergo the normally expected disjunctional separation of homologous chromosomes at anaphase I; instead the entire diploid complement divides mitotically giving rise to a dyad with two unreduced microspores. Sister chromatids of each chromosome move to opposite poles. In SDR the nucleus restitutes after disjunctional separation of homologous chromosomes, i.e., after anaphase I. Sister chromatids do not separate to poles and the doubled chromosomes of a haploid set constitute the SDR gamete.

From the genetic point of view, FDR gametes may retain the parental genotypes to a great extent. In 2n gametes produced by FDR all heterozygous parental loci from the centromere to the first crossover and 50% from the first crossover to the second crossover on all chromosomes will be heterozygous. Approximately 70 to 80% of the heterozygosity (intralocus interaction) is transmitted from parent to offspring with FDR, and a

large fraction of the epistatic (interlocus interactions) are also maintained (Mendiburu and Peloquin, 1977; Mok and Peloquin, 1975a). However, in 2n gametes produced by SDR, all heterozygous parental loci from the centromere to the first crossover will be heterozygous in the 2n gametes. Only 40 to 45% of the heterozygosity is transferred from parent to offspring with SDR. First division restitution gametes are considered more desirable than SDR gametes in that they provide a unique method to maximize heterozygosity, i.e., to transfer a large proportion of the non-additive genetic effects (intra- and interlocus interactions) from parent to offspring (Mok and Peloquin, 1975a). Although gametes produced by SDR theoretically less heterozygosity than gametes by FDR, 2n gametes derived from SDR still permit efficient transfer of desirable traits and allelic diversity to higher ploidy levels.

IV. MECHANISMS AND GENETIC CONTROL OF 2N GAMETE FORMATION

The cytological mechanisms of 2n gamete formation have been identified in microsporogenesis (Iwanaga, 1984; Johnston et al., 1986; Mok and Peloquin, 1975a; Myers et al., 1984; Parrott and Smith, 1984; Ray and Tokach, 1992; Vorsa and Bingham, 1979) and in megasporogenesis (Iwanaga and Peloquin, 1982; Parrott and Hanneman, 1988; Pfeiffer and Bingham, 1983; Tavoletti et al., 1991). However, cytological mechanisms involved in 2n egg production have not been studied as extensively as the cytological mechanisms responsible for 2n pollen production, largely because of the time-consuming technical difficulties of precise embedding-sectioning procedures (Iwanaga and Peloquin, 1982).

Most polyploid gametes whose cytological mechanisms have been identified can be attributed to

some abnormality of meiosis. The various mechanisms by which polyploid gametes may arise through microsporogenesis have been reviewed by Veilleux (1985). He classified these mechanisms as three different categories as follow: i) premeiotic disturbances, ii) meiotic disturbances and iii) postmeiotic abnormal cytokinesis (Fig. 4). Among these three categories, unreduced gametes observed in most genera may be produced by abnormalities due to the category, meiotic disturbances. Although several different mechanisms of 2n gamete formation exist, the genetic consequences are equivalent to either FDR or SDR.

The three mechanisms of 2n pollen formation in potato have been discovered as follow: i) parallel spindles (ps) or tripolar spindles (ts), ii) premature cytokinesis (pc-1, pc-2) and iii) synaptic mutants (sy-2, sy-3, sy-4) (Iwanaga, 1984; Johnston et al., 1986; Mok and Peloquin, 1975b; Parrott and Hanneman, 1988; Ramanna, 1983). Unreduced pollen caused by ps or ts can be produced by disorientation of the meiotic spindles during metaphase II or anaphase II of microsporogenesis. The spindles may lie in one plane instead of two, in which case they are either parallel or tripolar. Parallel spindles lead to the formation of two restitution microspores, while tripolar spindles form one restitution microspore and two normally reduced microspores. It appeared that ps and/or ts are the most common mechanism(s) for 2n pollen formation in potato (Mok and Peloquin, 1975c), alfalfa (Vorsa and Bingham, 1979), and red clover (Parrott and Smith, 1984). In potato, Mok and Peloquin (1975c) observed that pc-1 and pc-2 cause the formation of SDR dyads as a consequence of a premature occurrence of cytokinesis after telophase I (for pc-1) and at prophase II (for pc-2) followed by the absence of the second meiotic division.

Synaptic mutants, variation in meiosis which affects chromosome pairing and/or chiasma formation,

have been identified in *S. phureja*, haploid of *S. tuberosum*, *S. phureja*-haploid *S. tuberosum* hybrids, haploid *S. tuberosum*-wild species hybrids, and *S. commersonii* Dun. (Iwanaga, 1984; Johnston et al., 1986; Parrott and Hanneman, 1988; Ramanna, 1983). Synaptic mutants are considered useful for potato

breeding when combined with ps, because haploids homozygous for synaptic mutants and ps produce fertile $2n$ pollen which transmit almost intact genotypes of the parents to the offsprings. Thus, the meiotic mutants provide a powerful breeding method for maximizing heterozygosity and epistasis.

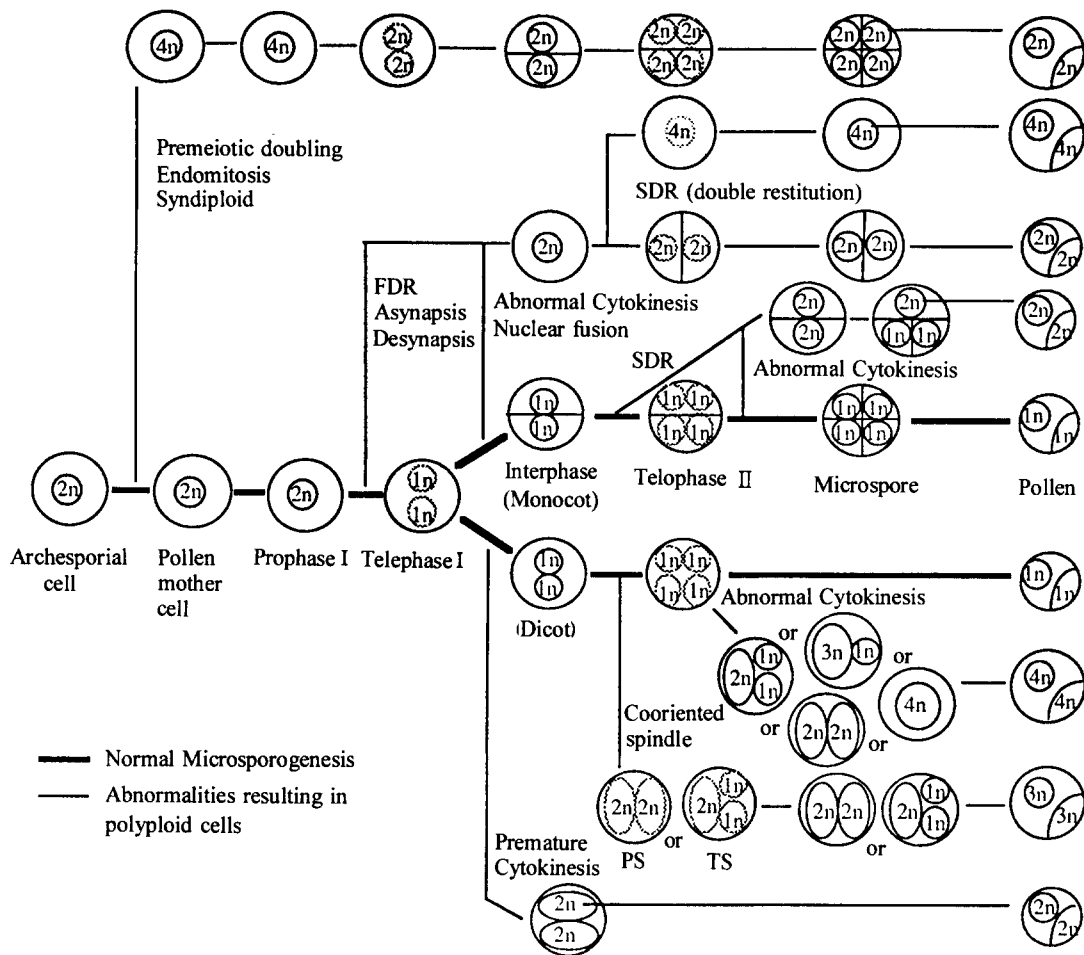


Fig. 4. Mechanisms of polyploid gamete formation during microsporogenesis.

The three mechanisms of $2n$ pollen formation observed in potato, alfalfa, red clover, and pea are controlled by a single recessive gene. Genetic control of $2n$ gamete formation by a single recessive gene also has been described in two other species; carrot, *Datura*

stramonium L. (Satina and Blakeslee, 1935) and maize (Rhoades and Dempsey, 1966). In carrot, Satina and Blakeslee (1935) first reported that the mechanism for $2n$ gamete formation was found to be the results of a recessive gene which altered microsporogenesis and

macrosporogenesis. Rhoades and Dempsey (1966) found a gene (el) in maize conditioning the formation of 2n eggs by SDR. However, Jacobsen (1980) found that inheritance of high seed set in meiotic doubling via $4x-2x$ crosses in potato was correlated with the quantitative inheritance of diplandroids (2n pollen) in dihaploid clones. He concluded that diplandroid formation, and their frequency, was controlled by major and minor genes. Romanna (1983) made crosses between two recessive homozygous (psps) parents in diploid potato to determine if meiotic nuclear restitution may be controlled by a single recessive gene, but he concluded that it was not plausible.

Recently, during the production of *Triticum-Aegilops* amphidiploids, Xu and Dong (1992) observed that some F_1 hybrids between *Triticum* spp. ($2n=4x=28$; AABB) and *Aegilops* spp. ($2n=2x=14$; DD) were self-fertile and seeds from fertile F_1 's were spontaneous amphidiploids. Detailed meiotic studies of the F_1 's between *T. persicum* Vav. and *A. squarrosa* L. showed that chromosome autoduplication of the F_1 hybrids was due to unreduced (2n) gametes and there were two ways by which unreduced gametes were formed; i) some pollen mother cells (PMCs) omitted the first meiotic division and only underwent the second meiotic division and ii) FDR with premature cytokinesis resulted in an asymmetrical dyad composed of one anucleate cell and one cell with a restitution nucleus. These mechanisms are different from ps and pc-1 and pc-2 described by Mok and Peloquin (1975c) in potato. Genetic analysis indicated that mechanisms in the *T. persicum* x *A. squarrosa* hybrids were controlled by two recessive genes located on the chromosome 2A and 6B (Xu and Joppa, 1992).

V. USE OF 2N GAMETES IN PLANT BREEDING

Since Mok and Peloquin (1975a) firstly

emphasized the breeding value of unreduced (2n) gametes in potato, uses of 2n gametes in breeding programs have been increased in monocots such as tetraploid wheat (Xu and Joppa, 1992; Xu and Dong, 1992), crested wheatgrass (Ray and Tokach, 1992), orchardgrass (De Haan et al., 1992; Maceira et al., 1992) and ryegrass (Den Nijs and Stephenson, 1988) as well as in dicots such as alfalfa (McCoy and Rowe, 1986; Veronesi et al., 1986; Vorsa and Bingham, 1979), red clover (Parrott and Smith, 1984), birdsfoot trefoil (Rim and Beuselinck, 1991). Unreduced (2n) gametes have received most attention in breeding of polyploid crops such as potato and alfalfa because performance of these species appears to be dependent on heterozygosity (Mendiburu and Peloquin, 1977; Veronesi et al., 1986). Consequently, the main target of the breeder in developing new cultivars is to maximize heterozygosity. 2n gametes can be an effective breeding tool in synthesizing new cultivars, providing a unique method to maximizing heterozygosity and can also be used to overcome infertility of interploidy crosses. The use of 2n gametes which can efficiently transfer germplasm from wild relatives to cultivated species, especially from diploid to tetraploid could make a contribution to the improvement of germplasm base in breeding programs.

The feasibility and advantages of improving interspecific germplasm transfer by sexual polyploidization with 2n gametes are evident in review of literatures. However, for efficient use of 2n gametes in breeding programs, it is necessary to: i) assess that the heterozygosity transmitted by 2n gametes shows heterosis, ii) screen a number of genotypes of diploid plant species to identify genotypes capable of producing high frequencies of 2n pollen, iii) increase the 2n pollen frequency through selection, as demonstrated by others (Parrott and Smith, 1984; Quinn and Smith, 1973) and iv) determine the inheritance of 2n pollen production in plant species.

VI. SUMMARY

Unreduced (2n) gametes are meiotic products (pollen or egg) having a sporophytic (somatic) chromosome number, resulting from abnormalities during either microsporogenesis or megasporogenesis. They occur naturally at a low frequency in many plant species. Unreduced (2n) gametes in plants can be identified for four possible ways as follow i) pollen size and/or shape differences between haploid (n) and diploid (2n) pollen, ii) ploidy analysis (chromosome number) of progeny or meiotic analysis (presence of dyads and/or triads at the microspore stage), iii) progeny performance and fertility and iv) dosage of isozyme and DNA markers. Unreduced (2n) gametes can be an effective breeding tool in synthesizing new cultivars, providing a unique method to maximizing heterozygosity, i.e., transferring a large proportion of the non-additive genetic effects (intra- and inter- locus interactions) from parent to offspring and can also be used to overcome infertility of interploidy crosses. Sexual polyploidization through 2n gametes has been a major route to the formation of naturally occurring polyploids. The three mechanisms of 2n pollen formation in potato have been discovered as follow: i) parallel spindles (ps) or tripolar spindles (ts), ii) premature cytokinesis (pc-1, pc-2) and iii) synaptic mutants (sy-2, sy-3, sy-4). Genetic analysis indicated that the mechanisms of 2n gamete formation were controlled by single recessive gene in potato, alfalfa, red clover, etc., and by two recessive genes in wheat. The use of 2n gametes which can efficiently transfer germplasm from wild relatives to cultivated species, especially from diploid to tetraploid could make a contribution to the improvement of germplasm base in breeding programs.

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