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Single cross alfalfa (*Medicago sativa* L.) hybrids produced via 2n gametes and somatic chromosome doubling: experimental and theoretical comparisons

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Summary. The potential breeding value of 2n gametes from diploid alfalfa (2n = 2x = 16) was tested by comparing single cross alfalfa hybrids produced via 2n = 2x gametes from diploids versus n = 2x gametes from somatic-chromosome-doubled, tetraploid counterparts. Three diploid clones, designated 2x-(*rprp*), homozygous for the gene *rp* (conditions 2n gamete formation by a first division restitution mechanism) were colchicine-doubled to produce their tetraploid counterparts, designated 4x-(SCD). These six clones were crossed as males to the same cytoplasmic male sterile clone. Yield comparisons of progeny from the six clones demonstrated a significant yield increase of the hybrid progeny from 2n = 2x gametes from the diploids over the hybrid progeny from n = 2x gametes from the chromosome doubled tetraploid counterparts. The yield gain ranged from a 12% increase to a 32% increase. Theoretical comparisons indicated the 2n = 2x gametes from diploids would have 12.5 to 50% more heterozygous loci, on average, than the n = 2x gametes derived from somatic doubling. These results confirm the importance of heterozygosity on alfalfa yield, and the results demonstrate that 2n gametes formed by first division restitution offer a unique method for producing highly heterotic alfalfa hybrids.

Key words: 2n Gametes – Alfalfa – Lucerne – *Medicago sativa* L. – Colchicine doubling – First division restitution

Introduction

Heterosis in alfalfa (2n = 4x = 32) and other autopolyploids such as potato is dependent on maximum heterozygosity (Bingham 1980). Quantitative genetic theory and experimental data have demonstrated the importance of tri- and tetra-allelic loci in increasing yield (Demarly 1963; Dunbier and Bingham 1975) as well as showing that inbreeding depression is a function of the loss of first order interactions from tri- and tetra-allelic loci (Busbice and Wilsie 1966). Isozyme analysis has confirmed the presence of multiple alleles at a locus (Quiros 1982); however, in relation to maximum heterozygosity tetra-allelic loci cannot be distinguished from four different blocks of linked genes referred to as linkats (Demarly 1979).

In alfalfa, methodology for the efficient and highly reproducible production of haploids (2n = 2x = 16) (Bingham 1971) has been instrumental in the development of populations with defined genetic structures. Chromosome doubling of cultivated diploids (2n = 2x = 16) results in tetraploids with a maximum of two alleles at a locus. Therefore, the genotypic structure of single- and double-cross populations derived from chromosome doubled diploids can be predicted. In an elegant study, Dunbier and Bingham (1975) demonstrated theoretically, that the double cross population would have a greater frequency of tetra-allelic loci, and experimentally, that the double cross population outyielded the single cross population indicating a positive correlation between performance and heterozygosity. Given the irrefutable evidence for the importance of maximizing heterozygosity Bingham (1980) outlined modifications of current methods of alfalfa variety development that would maximize heterozygosity.

In addition, two unique methods for maximizing heterozygosity are potentially available: 1) somatic hybridization of unrelated diploid hybrid cells via protoplast fusion and 2) union of male and female 2n gametes from unrelated diploid hybrids. Somatic hybrids between *M. sativa* and *M. falcata* L. (the same biological species) have been reported (Teoule 1983). However, two major limitations exist in attempts to apply protoplast fusion to variety development: 1) Regeneration from protoplasts is limited to only a few genotypes (McCoy and Walker 1984) and 2) The highly heterozygous tetraploid would have to be vegetatively propagated on a commercial scale.

A recessive gene controlling male 2n gamete formation by a mechanism genetically equivalent to first division restitution (FDR) has been described in alfalfa (McCoy 1982; Vorza and Bingham 1979). The importance of 2n gamete formation by a FDR mechanism is that 100% of the heterozygosity from the centromere to the first crossover, and 50% distal to the crossover or between the first and second crossover is transferred from the sporophyte to the gamete (Peloquin 1981). In alfalfa, there is frequently only one chiasma per bivalent with the result that the genetic constitution of the sporophyte is transferred to the gametes relatively intact (Bingham 1980). An FDR mechanism for female 2n gamete formation has not been identified although plants with a high frequency of 2n eggs formed by second division restitution have been described (Pfeiffer and Bingham 1983). In addition, a pleiotropic effect of the *jp* gene (conditions the failure of the post-meiotic-cytokinesis during microsporogenesis) is to produce an elevated frequency of 2n eggs (McCoy and Smith 1983).

The breeding value of FDR 2n gametes in producing highly heterotic tetraploids is well documented for potato (Mok and Peloquin 1975; Mendiburu and Peloquin 1977; Peloquin 1981). In alfalfa, a tetraploid clone resulting from the union of 2n male and female gametes outyielded the best somaclonal variant and a chromosomal doubled clone from the same genetic background (Pfeiffer and Bingham 1984).

This report examines the theoretical and experimental value of alfalfa 2n gametes (2n=2x=16) from diploids versus normal n gametes (n=2x=16) from their chromosome-doubled tetraploid counterparts. Forage yield of progeny derived from 2n gametes from diploids versus n gametes from tetraploids using the same female parent were determined.

Materials and methods

A number of diploid alfalfa plants homozygous for the gene *rp* (McCoy 1982), that conditions 2n pollen formation by a mechanism genetically equivalent to first division restitution, were identified in the progeny produced by crossing unrelated heterozygous (*Rprp*) plants. These *rprp* plants were grown in the field at Reno, Nevada and three unrelated plants were visually selected on the basis of vigor and fertility under open pollination. The three clones were designated 2x-A(*rprp*), 2x-B(*rprp*) and 2x-C(*rprp*). All three clones were chromosomally doubled with colchicine to produce their respective tetraploid counterparts designated 4x-A(SCD), 4x-B(SCD) and 4x-C(SCD).

The seven populations used in this study (Fig. 1) were produced by crossing the three diploid *rprp* clones, the three tetraploid counterparts and a normal tetraploid clone with the same cytoplasmic male sterile clone, designated 4x(cms)-1 (clone 6–4 ms obtained from Dr. E. T. Bingham, University of Wisconsin-Madison). Plants from each cross were established in conetainers in the greenhouse in March 1981 and then transplanted to the field in June 1981. Plants were space planted in rows 90 cm apart with 30 cm between plants. A completely randomized design with three replications and 12 plants per replication was used. Fresh weight yields were collected in the fall of 1981, four times in 1982 and in the spring of 1983.

Results

Theoretical considerations

In the 4x-(SCD) clones every locus which is heterozygous must be a duplex genotype (*BBbb*) since it was produced via somatic doubling of the chromosome number. The gametic output of the duplex genotype assuming random chromosome inheritance will be genotypes *BB, Bb,* and *bb* at frequencies 1/6, 4/6, and 1/6, respectively. Thus 1/3 of the n gametes (n=2x=16) will be homozygous or inbred and 2/3 will be heterozygous. This result generalizes for all heterozygous loci.

The 2x-(*rprp*) clones have the same genes and gene frequencies as their 4x-(SCD) counterparts but their 2n gametes (2n=2x=16) will on average have a very different genetic structure. Consider that after the equivalent of FDR in the 2x-(*rprp*) clones a chromo-

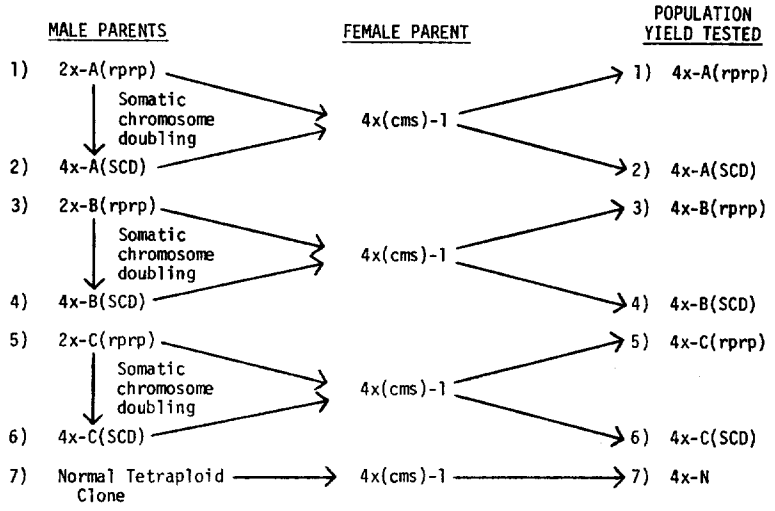

Fig. 1. Production of the seven populations that were yield tested. The female parent was the same cytoplasmic male sterile clone in all crosses

some arm attached to any given centromere will consist of only two regions: that which was transferred from another centromere and that which was not. On that region of a chromosome not transferred by crossover all heterozygous loci will be heterozygous in the gamete. The region which was transferred by crossover will also be heterozygous if it is transferred to the gamete with its symmetrical crossover counterpart attached to the other centromere which occurs by chance 1/2 of the time. The rest of the time the crossed over region will be in a gamete with a corresponding but uncrossed region of the same arm and for the crossed over region all loci will be homozygous in the 2n gametes. If we designate the proportion of genes on a chromosome arm that are transferred to another centromere due to a cross-over as c and assume genes are dispersed equally over the chromosome arms, then (c/2) is the proportion of heterozygous loci in the sporophyte which are homozygous in the gamete. The level of retained heterozygosity in the gametes is 1-(c/2). The value of c is a function of crossover rate and to compare the diploids and their 4x-(SCD) counterparts we assume ploidy level does not affect frequency of crossovers.

The value of c is 0.0 when the crossover is in the other arm of a given chromosome and the assumption was made that on average no more than 50% of a chromosome arm is expected to be transferred in a cross-over. Thus the range of values of c are 0.0 to 0.5 and the ratio of the levels of heterozygosity of the 2x-(*rprp*) to its 4x-(SCD) counterpart is [1−(c/2)]/(2/3). The percent increase in heterozygosity of 2n=2x pollen from the 2x-(*rprp*) plants versus n=2x pollen from the 4x-(SCD) plants is in the range of 50 to 12.5% corresponding to values of c from 0.0 to 0.5. When the 2x-(*rprp*) clone and its 4x-(SCD) counterpart are crossed to the same male sterile the progenies are expected to differ on average only with respect to the percentage of heterozygous loci. Within the context of previously mentioned assumptions and the additional assumption that value of c is nearly constant for the species, then the differences between progenies of the 2x-(*rprp*) clones and the 4x-(SCD) counterparts are a unique measure of the potential value of 2n gametes in maximizing heterozygosity.

Table 1. The analysis of variance for yield of families produced via 2n=2x gametes from diploids versus normal gametes from the colchicine doubled tetraploid counterparts

Sources	df	Mean squares	F
Parental genotype (A)	2	3,729	3.6 ns
2n=2x gametes from diploids versus n=2x gametes from tetraploid counterparts (B)	1	29,564	28.7**
A×B	2	2,194	2.1 ns
Error 2	4	1,031	
Harvest (C)	5	572,913	378.0**
C×A	10	514	< 1.0 ns
C×B	5	5,534	3.6 ns
C×B×A	10	453	< 1.0 ns
Error	68	1,515	

** Indicates F is significant at the $\alpha = 0.01$ level

Table 2. Mean fresh weight for the seven populations evaluated and percent yield gain of the families produced via 2n gametes from diploids versus normal gametes from the colchicine doubled tetraploid counterparts

Population yield tested	Mean fresh weight (g)	gain[a]
4x-A (rprp)	196.4	19%
4x-A (SCD)	165.1	
4x-B (rprp)	213.5	32%
4x-B (SCD)	162.0	
4x-C (rprp)	176.7	12%
4x-C (SCD)	157.3	
4x-N	195.8	

[a] Percent yield gain of the family produced by 2n gametes from a diploid versus normal gametes from the tetraploid counterpart (e.g. 2x-A yielded 19% more than 4x-A)

Experimental results

A split plot in time analysis indicated highly significant yield differences between the progeny produced via 2n=2x gametes from diploids versus the progeny produced from n=2x gametes from the chromosomally doubled tetraploid counterparts (Table 1). In addition a highly significant yield difference with harvest dates was observed. The percent yield gain was from 12% for the progeny produced from crossing 2x-C (*rprp*) versus the progeny from crossing 4x-C (SCD) with the same male sterile, to 32% yield gain for the progeny from 2x-B (*rprp*) versus the progeny from 4x-B (SCD) (Table 2). The absence of any significant interactions (Table 1) indicated a significant difference in yield which can be associated with the retention of heterozygosity in 2n gametes formed via a FDR mechanism.

Discussion

The theoretical comparison of FDR 2n gametes from diploids versus normal n gametes from chromosomally doubled tetraploid counterparts demonstrated that 2n

gametes from diploids would have 12.5 to 50% greater heterozygosity than the n gametes from tetraploids. Experimentally, a significant yield gain of 12 to 32% was achieved with progeny from FDR 2n gametes versus progeny from n gametes from tetraploids. These results demonstrate that FDR 2n gametes provide a unique pathway to maximizing heterozygosity in alfalfa, and they provide additional confirmation for the importance of increasing heterozygosity in order to improve alfalfa vigor.

Previous studies demonstrated theoretically and experimentally that maximum heterozygosity is important for improved yield (Demarly 1963; Dunbier and Bingham 1975; Bingham 1980). Pfeiffer and Bingham (1984) found that the best tetraploid clone produced from 2n gametes from both diploid parents out-yielded the best somaclonal variant from the same genetic background and the somatically doubled tetraploid clone. This report is the first documentation that 2n gametes from diploids produce superior alfalfa yield on a family basis, even though the 2n gamete provides only half of the genetic contribution.

Numerous reports have shown that potato yields can be significantly increased when FDR 2n gametes are used in 4x-2x crosses (Mok and Peloquin 1975; Mendiburu and Peloquin 1977; Peloquin 1981). This report demonstrates an increase in alfalfa yield on a family basis when the identical genes are present initially, but the only difference is how they segregate in the population of gametes.

Given that 2n gametes do offer significant advantages their direct usage in a seed-reproduced autopolyploid such as alfalfa presents numerous technological barriers. One breeding scheme involving the union of 2n eggs and 2n pollen from diverse backgrounds has been proposed (McCoy and Walker 1984). In addition there is potential for using 2n pollen in a hybrid breeding program using tetraploid, cytoplasmic male steriles in 4x-2x crosses. This would allow the breeder to capitalize on advantages of disomic inheritance over tetrasomic inheritance in the development of diploid populations to be used as males. The results presented here indicate that elite selected diploids concurrently selected for 2n pollen production could be used in 4x-2x crosses to transfer the selected genotype relatively intact. In a sense the unit of selection, the sporophyte, could be considered a living gamete because of the near identity between somatic and gametic cells.

The results of this small study indicate a definite effect of genome heterozygosity on alfalfa performance. An important aspect of this report is that utilization of genotypes of defined genotypic structure can result in elucidating the relative importance of specific genetic events on alfalfa performance. This study is an initial step in the development of future research using meiotic mutants and quantitative genetics to further decipher the importance of genome organization on alfalfa improvement.

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