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Cytoplasmic male-sterile synthetics: a new approach to the exploitation of heterosis in rape

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Abstract In addition to their application in the production of F_1 hybrids in rape (*Brassica napus* L.), cytoplasmic male-sterility (CMS) systems may be used to produce synthetic varieties with much higher levels of heterozygosity than those expected in conventional rape synthetics. CMS synthetics are produced by compositing a CMS A-line with several male-fertile (MF) B-lines lacking nuclear alleles for fertility restoration, and increasing the resulting mixture by natural pollination. Over generations of increase, pollination of the CMS component by the MF component of the synthetic results in the progressive loss of A-line nuclear genes from the population. The initial proportions of CMS and MF plants are expected to be preserved over several generations of natural pollination if CMS and MF plants are equal in yield. Methods for estimating the heterozygosity level of CMS synthetics, taking into account the proportion of CMS plants, number of MF parents in Syn 0, and selfing rate (s) of MF plants, are presented. If completely inbred Syn 0 parents and s of 0 and 0.75 for CMS and MF plants respectively, are assumed, the heterozygosity level ($1-F$) of a synthetic derived from four inbred MF parents each comprising 6.25% of Syn 0 and one inbred CMS parent comprising 75% of Syn 0 is predicted to be 0.66 in Syn 5, compared to 0.30 in synthetics derived from four MF parents only. CMS synthetics offer a novel, low-cost approach to the exploitation of heterosis in rape and other species with mixed mating systems in which self-pollination predominates.

Key words Heterozygosity · Selfing rate · Mixed mating system · Synthetic varieties · Male sterility

Abbreviations *A-line* Cytoplasmic male-sterile line · *B-line* maintainer for A-line, with normal cytoplasm and no RF gene · *CMS* cytoplasmic male sterility · *H* heterozygosity level, or panmictic index ($1-F$) · *MF* male-fertile (or fertility) · *Rf* fertility restoration gene · *R-line* line containing Rf gene · *s* self-pollination rate

Introduction

Rape (*Brassica napus* L.) has a mixed mating system in which self-pollination predominates. Outcrossing is reported to range from 20% to 30% (Downey and Rakow 1987). Many authors have reported significant heterosis for grain yield in this species (Sernyk and Stefansson 1983; Grant and Beaversdorf 1985; Brandle and McVetty 1989), with the result that much effort has been invested in the development of F_1 hybrid cultivars. To date, most hybrid cultivars have been developed using cytoplasmically inherited male sterility (CMS) and nuclear fertility restoration (Rf) genes to control pollination. Several male-sterile cytoplasmic lines have been discovered and used in efforts to produce commercially acceptable hybrids. The area planted to such hybrids is increasing rapidly in the main areas of summer rapeseed production in Canada.

Although F_1 hybrids are higher yielding than pure-line rape cultivars, hybrid seed is costly and must be purchased annually. Relatively little effort has been devoted to the development of rape cultivar types that might exploit heterosis at reduced cost. The use of synthetic varieties in rape, first proposed by Schuster (1982), would permit rape cultivars to be multiplied for several generations before sale, thereby reducing costs to the grower. Léon (1991) reported a modest yield advantage of mixtures representing four-parent Syn 1 populations over the mean of the four parental lines. Because of the high selfing rate of rape, however, opportunities for the occurrence of highly heterozygous individuals due to

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outcrossing are limited in conventional synthetics. Measures to increase the outcrossing rate are required if heterosis is to be exploited in rape synthetics.

The deployment of CMS in synthetic varieties, first proposed by Atlin (1985) for potato varieties propagated by true seed, could permit the outcrossing rate of a rape synthetic to be increased dramatically. A synthetic variety exploiting CMS to increase outcrossing could be initiated by mixing seed of one CMS genotype (A-line) with seed of several non-restoring male-fertile homozygous lines (B-lines). The mean selfing rate of such a mixture would be the weighted average selfing rates (s) of its A-line and B-line components. This reduction in s from the 70% level expected in a synthetic produced only from B-lines is likely to have a substantial impact on the level of heterozygosity of the population in advanced synthetic generations. If it is assumed that rape plants with male-sterile cytoplasm do not yield less than male-fertile plants [an assumption supported by the observations of McVetty et al. (1989)], the reduced selfing rates of synthetic varieties initiated in this manner can be expected to remain constant over generations of increase.

The impact of including CMS parental inbred lines on heterozygosity in advanced generations of a synthetic variety is likely to be affected by the relative proportions of CMS and MF plants, and by the number, relatedness, and inbreeding coefficient of B-lines included in the Syn 0. This manuscript models the effect of selfing rate and parent number on the evolution of heterozygosity in CMS synthetics.

Theory

The selfing rate of CMS synthetics

A CMS synthetic may be produced by compositing seed of a CMS A-line with seed of several B-lines, and then allowing natural pollination to occur. The relative proportions of A-line and B-line in the Syn 0 generation will determine the mean selfing rate of the synthetic. Let P_A be the proportion of A-line seed in the Syn 0 composite, and let s_A be the A-line self-pollination rate. For the purposes of this discussion it will be assumed that the selfing rate of A-lines is 0, although A-lines are not usually completely sterile, particularly if high temperatures occur during flowering (Fan and Stefansson 1986; Burns et al. 1991). Also, let P_B be the proportion of B-line and s_B be the mean selfing rate of the cultivars making up the B-line component. The mean selfing rate (s) of the Syn 0 can therefore be predicted as:

$$s = (P_A \times s_A) + (P_B \times s_B) \quad [1]$$

which reduces to $P_B \times s_B$ under the assumption that $s_A = 0$. For example, if a synthetic is composited from 70% A-line with a self-pollination rate of 0 and 30% B-line (say, four B-lines each comprising 7.5% of the

mixture), and the B-lines have a mean selfing rate of 0.7, then the mean selfing rate of the Syn 0 generation is $0.3 \times 0.7 = 0.21$. The consequence of including A-line seed as 70% of the Syn 0 has therefore been to reduce the selfing rate from 0.70 (the rate expected for a synthetic originating only from B-lines) to 0.21; a reduction of this magnitude will substantially affect the inbreeding coefficient of the following synthetic generation and, perhaps, its yield and vigor.

It should be noted that a low selfing rate is achievable in CMS synthetics only if nuclear fertility restoration (Rf) alleles are not introduced in the MF component in Syn 0. If R-lines instead of B-lines are used as the MF component, offspring of CMS plants will be heterozygous for Rf alleles and would therefore be MF. Rf alleles are not required to achieve complete pollination in the progeny of CMS plants. We have observed that complete pollination of A-line plants in mixtures of CMS and MF plants can be effected when the MF proportion is as low as 20% (unpublished), making it unnecessary to restore fertility in order to obtain acceptable grain yields.

Inbreeding in CMS synthetics

Inbreeding in the male-fertile component

The evolution of inbreeding in a CMS synthetic may be modelled by considering that it consists of two distinct components differing in cytoplasmic genotype. If the selfing rate of the cytoplasmic male-sterile component is 0, then the male-fertile component can be considered a separate and closed population and its inbreeding coefficient (F) estimated in each synthetic generation according to the method of Busbice (1969). Briefly, in any synthetic generation, plants within the MF component of the synthetic arise from self-pollination with frequency s_B and from cross-pollination with frequency $1 - s_B$. For diploid plants arising from self-pollination, the inbreeding coefficient in generation t is:

$$F_t = (1 + F_{t-1})/2 \quad [2]$$

For diploid plants arising from cross pollination, F in generation t is:

$$F_t = r_{t-1} \quad [3]$$

where r_{t-1} is the mean coefficient of coancestry of plants in the previous generation. Because a rape synthetic is likely to be composited from unrelated inbred lines selected for high combining ability, the mean coefficient of coancestry among plants in the MF component is simply the probability that alleles taken at random from a pair of plants descend from the same Syn 0 parental inbred. Thus, r_{t-1} is $1/n$ for synthetic generations derived from n homozygous parental lines. The mean value of F for the MF component of a CMS synthetic in

generation t ($F_{B(t)}$) is thus:

$$\begin{aligned} F_{B(t)} &= s_B(1 + F_{B(t-1)})/2 + (1 - s_B)r_{t-1} \\ &= s_B(1 + F_{B(t-1)})/2 + (1 - s_B)/n \end{aligned} \quad [4]$$

It should be noted that synthetic varieties produced from composited seed of inbred lines differ from typical synthetics in self-incompatible forage species; in synthetics produced from inbred lines, matings among relatives (plants of the same inbred line) occur in the Syn 0 generation, whereas in synthetics produced in self-incompatible species, matings among relatives do not occur until Syn 1. For this reason, the inbreeding coefficient of the MF component of a CMS synthetic is greater than that of an equivalent forage synthetic in the same generation of increase.

Inbreeding in the CMS component

Each plant in the CMS component of the synthetic results from a mating between a MF plant and a CMS plant. The CMS component of the synthetic, therefore, is not closed; pollen fertilizing CMS plants must come from the MF component (unless the CMS component is not completely sterile). Each generation of increase of a CMS synthetic thus acts as a backcross, with the male-fertile component serving as the recurrent parent. Regardless of the initial A-line genotype, allele frequencies in the CMS component of the synthetic converge with those of the MF component; the frequency of A-line nuclear genes is reduced by 50% per generation.

Because F of a diploid individual equals the coefficient of coparentage (r) of its parents, the mean F of CMS plants (F_A) is therefore the probability that alleles drawn at random from MF and CMS parents are identical by descent. For this probability (which will be denoted r_{AB} in this discussion) to be greater than 0, the allele drawn at random from a CMS plant must have originated in the MF component. The probability that an allele drawn at random from a CMS plant in generation t originated in the MF component is determined by the number of generations of synthetic increase and can be expressed as $1 - (1/2)^t$, where t is the synthetic generation. Given that an allele in a CMS plant is derived from the MF component, the probability that it is identical by descent with an allele drawn at random from a MF plant is simply the probability that any two random alleles drawn from the MF component are identical by descent. This probability, assuming Syn 0 parents are unrelated, is equivalent to Busbice's (1969) r_1 and can be expressed as $(1/2n_B)(1 + F_0)$, where n_B is the number of MF parents included in Syn 0, and F_0 is the inbreeding coefficient of Syn 0 parents; this reduces to $1/n_B$ if Syn 0 parents are homozygous lines. Thus, for $t > 0$, the coefficient of coancestry of a plant in the MF component with a CMS plant in synthetic generation t

($r_{AB(t)}$), and F for offspring of matings between CMS and MF plants (F_A), is:

$$\begin{aligned} r_{AB(t)} &= [1 - (1/2)^t] \times 1/n_B \\ &= F_{A(t+1)} \end{aligned} \quad [5]$$

Inbreeding in the CMS synthetic as a whole

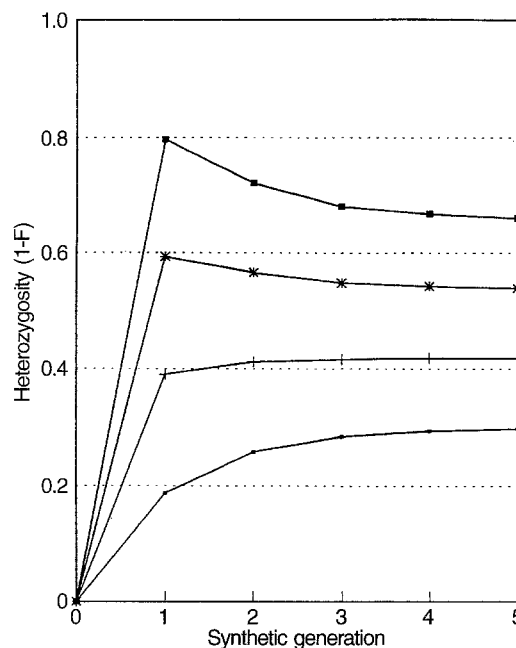
The mean inbreeding coefficient of plants in the population as a whole in any synthetic generation can now be estimated by calculating F separately for the CMS and MF components, and then taking a weighted average of these coefficients as follows:

$$F_t = (P_A F_{A(t)}) + (P_B F_{B(t)}) \quad [6]$$

Results and discussion

The effect of including a CMS component in synthetic varieties on heterozygosity ($1 - F$, or H) was modelled for synthetics derived from one homozygous CMS and four homozygous MF lines with proportions of CMS plants ranging from 0 to 75%, and a selfing rate of 0.75 for the MF component (Fig. 1). Including a CMS parent as 75% of Syn 0 resulted in a threefold increase in

Fig. 1 Effect of the proportion of CMS plants (■ = 0%; + = 25%; * = 50%; ■ = 75%) included in synthetic generation 0 on the level of heterozygosity of synthetic varieties established from four MF lines and one CMS line, assuming $F = 1$ for parental lines and selfing rate = 0.75 in MF plants



predicted H relative to a conventional synthetic in Syn 2. By Syn 5, the 75% CMS synthetic was predicted to have $H = 0.66$; H for the 100% MF synthetic was only 0.30. Increasing the CMS proportion of a synthetic above 75% is expected to increase H further but might have deleterious effects on seedset and yield of the CMS component; the author has observed that reducing the MF component of mixtures of A-line and R-line from 20% to 10% resulted in a significant yield reduction and an increase in the selfing rate of CMS plants (unpublished).

In addition to increasing heterozygosity by reducing selfing, the inclusion of a CMS component in a synthetic variety permits heterozygosity to be increased further by increasing the number of parents, an approach that normally has little effect in populations with high selfing rates. The effect of increasing the number of MF Syn 0 parents from two to four was compared for entirely MF synthetics versus synthetics that were 75% CMS (Fig. 2), assuming a selfing rate of 0.75 for the MF component. Increasing the parent number from two to four increases H in Syn 5 only from 0.20 to 0.30 in the MF synthetic, whereas the same increase in the number of MF parents in a 75% CMS synthetic increases H from 0.47 to 0.66.

The results presented above were derived under the assumption that the selfing rate of CMS individuals is 0. However, CMS plants usually produce some pollen, particularly when high temperatures occur during flowering (Burns et al. 1991; Fan and Stefansson 1986), with the result that self-pollination rates in some seed

production environments are likely to be high enough to significantly reduce heterozygosity in CMS synthetics from the levels predicted in this study. In cooler environments, however, self-pollination of CMS plants is low; the author has observed selfing rates of only 8% on A-line plants grown in mixtures containing 20% R-line and 80% A-line in Nova Scotia (unpublished), which has a cool Maritime climate with summer temperatures rarely exceeding 25 °C. In such environments, CMS synthetics may offer a novel, low-cost opportunity for the exploitation of heterosis in rape. CMS synthetics may also be superior to conventional varieties in other species with mixed mating systems, particularly under circumstances where self-pollination rates are high, inbreeding depression and heterosis are great, and complete pollination of male-sterile individuals can be effected by a small number of pollinator plants.

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Fig. 2 Effect of MF parent number on heterozygosity in synthetics containing 0 or 75% CMS plants (■ = 2 parents, 0% CMS; + = 4 parents, 0% CMS; * = 2 parents, 75% CMS; ■ = 4 parents, 75% CMS) assuming $F = 1$ for parental lines and selfing rate = 0.75 in MF plants

