BREEDING STRATEGIES FOR THE DEVELOPMENT OF HYBRID CANOLA

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INTRODUCTION

During the past decade, the development of Brassica napus hybrids has attracted more interest and research activity than any other area of canola breeding. The reasons for this are obvious: the proprietary nature of hybrids makes them commercially attractive, while the existence of substantial heterosis, and genetic mechanisms for pollination control make hybrid development scientifically and technically feasible. Although the development of commercial canola hybrids has proven a more difficult task than was envisioned a decade ago, steady progress has been made, and the first cycle of hybrid products are beginning to emerge from several breeding programs. The objective of this paper is to provide an overview of the current "state of the art" of hybrid canola development, with special emphasis on hybrid breeding strategies, experiences to date with pollination control systems, and commercial hybrid seed production. The economic considerations vis-a-vis hybrid development, while obviously critical, will not be dealt with in detail here.

PREREQUISITIES FOR SUCCESSFUL HYBRID DEVELOPMENT

The scientific and technical feasibility of hybrid canola is dependant upon three factors: (1) the existence of substantial, high-parent heterosis in elite germplasm, (2) the existence of genetic pollination control mechanisms which can limit self-pollination to negligible levels, and (3) efficient pollen transfer from male to female parents on a field scale.

Heterosis:

Significant high-parent heterosis (HPH) has been reported by several researchers in both spring- and winter-type B. napus (Shiga, 1976; Bartkowiak-Broda, 1983; Grant and Beverdorf, 1985; Lefort-Buson et al., 1987). In most of these studies, heterosis was greatest in crosses involving parents of different geographic origins (i.e. Asian x European). Since most of these studies were based on limited data (i.e. a single season at one or two locations), HPH estimates are probably inflated; nonetheless, they provide sufficient evidence of the existence of HPH to justify the development of commercial hybrids.

Pollination Control Mechanisms:

Two types of pollination control mechanisms (PCMs) have been utilized in canola - cytoplasmic-genetic male sterility (CMS), and sporophytic self-incompatibility (SI).

All CMS systems have two genetic components: a male sterile cytoplasm, and (a) nuclear fertility restoration factor(s). Three CMS systems have been investigated experimentally as PCMs for B. napus: the nap, pol, and ogu. These systems has been reviewed by Rousselle et al. (1984) and Thompson and Hughes (1986). Of the three, the ogu and pol systems are currently being used for commercial hybrid development.

Sporophytic SI has been introgressed into B. napus from the progenitor species, B. campestris and B. oleracea, both of which are self-incompatible. S-alleles exist as an allelic series at a single locus (the s-locus) in both component genomes. S-alleles from both genomes are,

with few exceptions, dominant to self-compatiblity (SC) when transferred to B. napus; the F_1 of a cross between SI and SC lines is thus usually SI. In crosses between SI lines, the s-allele carried by one will usually be dominant to that carried by the other. A dominance heirarchy exists among s-alleles in both genomes; the nature of this heirarchy, and the behaviour of individual alleles has been much better characterized in B. oleracea than in B. campestris, since SI is used as a PCM in vegetable Brassica species (see Thompson, 1983).

Pollen Transfer in the Field:

Brassica napus is a mostly self-pollinated species, but exhibits up to 40% outcrossing in some environments. While wind can be an effective pollen vector, the sticky, entomophilous pollen is best adapted to transfer by insects, particularly bees (Free, 1968). Temperature and humidity also influence pollen movement, as they affect anther dehiscence, pollen viability, and bee activity.

HYBRID BREEDING STRATEGIES

In canola breeding programs focusing on open-pollinated cultivar development, selection is based on per se performance only. Hybrid breeding strategies combine selection for per se and cross performance, so are both more complex and more resource-intensive than traditional cultivar development programs.

The abundant literature on hybrid breeding in corn provides canola breeders with an inventory of possible strategies. In this section, frequent reference will be made to such strategies, and their potential use in hybrid canola development. Generally speaking, all hybrid breeding strategies include three phases: (1) improvement and sampling of source material, (2) inbred development and testing, and (3) hybrid synthesis and evaluation.

Improvement and Sampling of Source Material:

The source material for inbred extraction may vary from narrow crosses to broad-based populations. Where a recurrent selection scheme of some sort is applied to source material in conjunction with a hybrid breeding program, broad-based populations will be used. Various intrapopulation improvement methods can be used to improve the general level of performance of a population over time for important traits. Where narrow crosses are used as source material, recurrent selection of the form used in populations cannot be applied. However, the use of narrow crosses has an advantage in that it allows sampling of a broad range of backgrounds, with rapid exploitation of newly-available commercial material. When individual populations or crosses are used as source material in canola, one very important strategic component of a hybrid breeding program will be lacking, however: the potential for developing and exploiting heterotic patterns.

In corn, the historical development of source material has placed heavy emphasis on heterotic patterns. The concept of heterotic patterns is based on the observation that there is often less heterosis, on average, in crosses between material drawn from the same genepool, or geographic locale, than there is between materials which have evolved in isolation from one another. The positive correlation between geographic and genetic distance has also been observed in crosses involving Asian and European winter-type B. napus (Shiga, 1976; Lefort-Buson et al., 1987), and those involving European and Canadian spring canola cultivars (Grant and Beversdorf, 1985).

The relationship between geographic and genetic distance is a very crude one, however, and is often violated in canola. A better approach is to synthesize two heterotic pools from elite starting material, using

cross-performance information obtained from diallel crossing. Synthesis of populations on this basis is described by Hallauer and Miranda, Fo. (1981). The genetic distance between such heterotic blocks can be increased over time by reciprocal recurrent selection (RRS).

Inbred Development and Testing:

The most common method of producing inbreds from any form of source material is through selfing. In B. napus, the possibility of using haploidy, based on microspore culture, also exists. Promising inbreds are identified on the basis of both per se and cross performance. How, and in what generation performance data is obtained depends on the nature of the source material, and the types of progeny families being evaluated.

In canola, a large proportion of material can be eliminated during the first two generations of selfing on the basis of visual selection, quality (oil, protein, and/or glucosinolate content), and reaction to blackleg (Phoma lingam). By the S_3, or F_5, per se and/or testcross yield performance assessment will have begun. If hand-crossing is necessary to evaluate cross performance, selection on the basis of per se performance will probably be carried out first, followed by testcrossing. If male sterile or SI inbreds are available for use as testers, then testcrossing can begin simultaneous with per se testing.

The evaluation of finished inbreds in appropriate mating designs allows separation of general (GCA) and specific combining ability (SCA). Inbreds with high GCA are particularly valuable in the first few cycles of a hybrid breeding program, or where there is no provision for development of heterotic blocks.

In programs utilizing RRS to develop heterotic blocks, full-sib or half-sib selection can be used to identify inbreds. Full-sib RRS identifies good specific combinations (full-sibs) whose inbred components can also be evaluated for per se performance. Half-sib selection identifies inbreds with high combining ability, using either an inbred from the opposite population, or the population itself, as a tester.

Hybrid Synthesis and Evaluation:

Inbreds with adequate per se performance and high combining ability can be used to synthesize hybrid combinations for yield evaluation. With CMS systems, normal, fertile inbreds can be crossed to elite restorer inbreds to produce single-cross hybrids for multilocation testing. When the best combinations are identified, the fertile inbreds can be converted to "A lines" by backcrossing in the male sterile cytoplasm. The best A lines can then be used as testers for one or two cycles, to identify promising restorer inbreds.

Where SI is used, true single-cross hybrids cannot be commercialized, so modified single-cross, three-way or double-cross products must be used (see Thompson, 1983). In this case, an isoline cross or a single-cross involving at least one SI inbred can be used to identify new, specific hybrid combinations for testing.

When RRS is used in a hybrid breeding program, a considerable gain in efficiency can be realized by converting at least one of the populations to a pollination control mechanism. In the case of CMS, a restorer population can be developed, while with SI, one or more s-alleles can be introgressed into one or both populations.

COMMERCIAL HYBRID SEED PRODUCTION

Production of seed of inbred components and of commercial canola hybrids is considerably more complex than is production of seed of open-pollinated (OP) cultivars. In addition to the standards of purity required in all cultivars, attaining consistently high hybridity in hybrid seedlots is also essential, to meet legal requirements where they

exist, and to ensure performance. Hybridity can be defined simply as the percentage of seed in a seedlot of a hybrid cultivar which is true hybrid seed, as opposed to selfed seed of the female parent. Hybridity is a concern because the genetic PCMs used in canola are all influenced to some degree by environmental factors. Under stress conditions, the effectiveness of a PCM may be reduced, resulting in selfed seed production on the female (seed) parent in hybrid seed production fields.

The specific approaches to hybrid seed production which are being used with CMS and SI are outlined below. Specific problems associated with field hybrid seed production in each system are also mentioned.

CMS-based Hybrid Seed Production:

CMS-based hybrids would normally be single-cross hybrids, produced by crossing a restorer inbred (pollen parent) with an A-line (seed parent). Restorer (R) inbreds carry the male sterile cytoplasm, but are also fixed for a nuclear restorer factors which, ideally, will completely restore fertility. Because they are completely self-fertile, R inbreds can be increased in the same way as OP cultivars. Maintaining purity is essential, however, since contamination of an R line results in segregation for fertility/sterility, and can lead to incomplete restoration in the hybrid product.

Seed of an A-line is increased by crossing onto it with pollen from its isoplasmic maintainer (B) line (i.e. line of similar nuclear genotype, but carrying a male fertile cytoplasm). A-line increases must thus be planted in strips, so that the male fertile B-line can be removed from the stand after flowering.

The F_1 seed is also produced in strip plantings, with the A-line and R-line parents being planted in alternating strips. The R-line strips must be cut out of the production field after flowering in order to prevent contamination of the hybrid seedlot at harvest. Since hybrid seed is only harvested from the A-line strips, the ratio of land occupied by A-line/R-line must be maximized in order to minimize seed production costs. For adequate pollen movement between R-line and A-line strips to ensure optimum F_1 seed yields, hybrid seed production fields should be stocked with honeybee hives.

SI-based Hybrid Seed Production:

In order to produce selfed seed of SI inbreds, the SI must be suppressed using CO_2 or saline (NaCl) solution. Use of CO_2 is obviously limited to enclosures such as greenhouses or plastic tunnels, where high enough CO_2 concentrations can be achieved for effective selfing (ca. 5%). NaCl solutions can be applied in the field, and while selfed seed yields per plant are low, this should be less costly than using CO_2.

All SI-based hybrids involve two generations of crossing, the first to produce hybrid components, the second to produce the hybrid itself. This approach minimizes the amount of costly SI inbred seed required per unit of hybrid seed produced, and also ensures increased cross-compatibility in the final hybrid product. SI-based hybrids may be modified single-crosses, three-way crosses, or double-crosses, and may involve all SI inbreds, or a combination of SI and SI inbreds.

The first generation, or component production is similar for all types of SI-based hybrids. If SI and SC inbreds are used in component synthesis, they must be planted in alternating strips, with component seed being harvested from the SI inbred only. Where both inbreds are SI, component seed can be harvested from both inbreds in a mixed stand.

In the second generation, hybrid seed for MSC or TWC hybrids using a second SC inbred must be produced in strip plantings, with hybrid seed being harvested from the component only. In the case of DC hybrids, however, because both components are SI, hybrid seed can be produced on a mixed planting of the two components, with the entire stand being

harvested. The latter approach should reduce production costs vs. strip plantings.

With SI-based hybrids, as with CMS-based products, honeybees should be stocked in seed production fields to ensure adequate pollen movement between strips.

EXPERIENCE TO DATE IN APPLYING HYBRID BREEDING STRATEGIES IN CANOLA

Several public and private sector breeding programs in N. America and Europe are actively pursuing hybrid development in both spring- and winter-type Brassica napus. This section provides an update on current activities, with particular emphasis on our own experiences at Pioneer/A.C.T. over the past five years.

Hybrid breeding activity in spring canola is both more widespread and more advanced than in winter canola. In Canada, three private companies (Garst/I.C.I., King Agro, and Pioneer/A.C.T.) have large-scale, spring canola hybrid programs. In addition, the Canadian government, through Agriculture Canada, Saskatoon, is involved in commercial hybrid cultivar development. In Europe, there is a large hybrid breeding effort at the French government research centre, INRA, at Rennes. The latter program collaborates with the Ringot (Serasem/UNCAC) commercial breeding program. Other smaller efforts exist in several European centres. In winter canola, the two programs with substantial hybrid breeding efforts are the INRA program at Rennes, France, and the Pioneer/A.C.T. program, based in Canada and France.

In terms of the types of hybrids under development at different centres, the pol and ogu CMS systems and sporophytic SI are all being used in commercial hybrid development. As we have few details on the specific breeding strategies being employed at various centres, we will briefly review some of our own.

At Pioneer/A.C.T., we have tested both the pol and the nap CMS systems and SI. The nap system proved unsuitable for commercial applications due to the environment-sensitive nature of the cytoplasm, and the genetic complexity of maintenance of sterility. We had similar experiences with the pol system. Our experiences to date with SI have been "cautiously positive". While expression of individual s-alleles is influenced by both genetic background and environment, we have identified a few alleles with consistently good expression, and some backgrounds which give better then average expression of most alleles. Because of the large number of alleles available from both B. campestris and B. oleracea, we expect to find other alleles as good, or better, than those we are currently using.

Our initial approach to hybrid development was probably similar to that of most of our competitors: we installed PCMs in cultivar derivatives, and tried to reproduce on a large-scale the results we had obtained from limited testing of hand-crossed hybrid combinations. We failed to obtain the expected hybrid performance, because of both problems with the CMS systems which reduced the hybridity of field-produced seedlots, and because the crosses themselves had only marginal heterosis when evaluated more extensively.

With SI, we have taken a more rigorous approach to inbred development. Inbreds must demonstrate above average per se performance before their combining abilities are evaluated. Using this approach, one to several new inbreds with high GCA can be identified annually for conversion to SI. During conversion to SI, expression of incompatibility is evaluated during backcrossing in all inbred backgrounds. Alleles and backgrounds with poor SI are eliminated from the program.

In the Pioneer/A.C.T. programs, inbreds for conversion to SI are identified among pedigree-derived and doubled haploid lines, and in full-sib components from RRS population improvement schemes. Details on the

results of three cycles of RRS in our spring canola program are provided in the article, "Intra- and Interpopulation Improvement in Spring Brassica napus", by Patel et al., presented elsewhere in the proceedings of this congress.

Evaluation of inbred performance continues during backcrossing, especially with winter-type material. Since backcrossing s-alleles into finished inbreds delays hybrid commercialization, we also produce SI inbreds and isolines directly from source material segregating for SI. In the long term, all of our inbreds will come from this source.

While breeding strategies themselves must remain central to any hybrid development program, molecular biology promises to provide a number of ways of accelerating the efficiency of hybrid development. As single genes which confer SI or male sterility are cloned or synthesized artificially, the possibility of converting male fertile or SC inbreds to sterility or incompatibility through transformation will be opened up. A system of this sort has already been developed by P.G.S, of Gent, Belgium, and is being tested experimentally (Mariani et al., 1990). The greatest long-term contribution of molecular biology to hybrid breeding will probably be in the area of RFLP development, which will provide canola breeders with both an analytical and predictive tool capable of accelerating the rate, and improving the efficiency of hybrid breeding.

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