Genetic diversity in canola for changing environments

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Abstract

The world is undergoing population growth and climate change at an unprecedented rate, and plant breeders must ensure that their breeding programs contain sufficient genetic diversity to respond to potential changes in the environment. Climate, plant diseases, and market demands for new quality traits, are included in this broad definition of environment. The Australian *Brassica napus* (canola) gene pool will be used to demonstrate how wide genetic diversity was exploited successfully to improve adaptation to a new and unique agricultural environment, while changing the crop from rapeseed to "double low" quality. The process began in 1970 with a broad range of parents from Europe, Canada and Asia, and two *B. juncea* accessions. During the next 30 years, 5 breeding cycles were completed in a closed population with an effective population size of approximately 11. The canola crop in 2000 was well adapted to the higher rainfall environments of southern Australia, and sufficiently resistant to the major disease (blackleg caused by *Leptosphaeria maculans*) that Australia became a significant exporter of canola. *L. maculans* has demonstrated a great capacity to adapt to polygenic resistance in released varieties, with an average change in resistance rating of -0.15 units per year after variety release on a scale of 0-9, where 0 is very susceptible and 9 is very resistant. From 1970 to 2000, breeders developed new varieties with a net annual rate of improvement in resistance of +0.13 units per year. However, the relatively low effective population size indicates a random loss of approximately 21% alleles due to genetic drift over 30 years, in addition to alleles lost through selection. Since 2000, the canola cropping region of Australia has suffered repeated droughts, and there is a demand for new canola types adapted to lower rainfall conditions, and with new quality traits. Techniques will be discussed that permit new genetic diversity to be introduced into elite locally-adapted breeding pools from interspecific crossing or foreign elite sources, without diminishing the performance of elite lines, so that canola breeders can continue to keep ahead of evolving pathogens, environments, and market demands. The process requires a decrease in cycle time, and improvement in heritability of traits through selection on near-homozygous lines.

Key words: *Brassica napus*, oilseed rape, rapeseed, climate change, population breeding

Introduction

Oilseed rape (*Brassica napus* L.) was introduced into Australia from Europe and Canada prior to 1970, but was extremely susceptible to blackleg. The oilseed rape industry almost disappeared until the 1990s, when new canola quality varieties, with moderate blackleg resistance, were released. From 1970 to 2000, Australian canola breeders successfully improved yield, adaptation, blackleg resistance and seed quality in Australian canola (Salisbury and Wratten 1999), such that the production of canola exceeded 1.6 million tonnes by 1998/99 (Colton and Potter 1999). Australian canola breeding underwent several cycles of recurrent selection in a closed population from 1970 to 2000.

Climate change will affect agricultural production in different ways in different regions, and crop breeders should be preparing for changing future environments by increasing genetic diversity in their breeding programs. However, the demand for adaptation to changing environments is not new; blackleg resistance will be used as an example of an environment that is constantly changing. Methods for introgressing new alleles into elite germplasm of canola breeding programs are needed, so that effective population size and genetic diversity can be increased. A small investment in genetic diversity now may pay large dividends later.

Materials and Methods

Pedigrees of Australian canola cultivars released between 1970 and 2004 were obtained from Salisbury and Wratten (1999) and IP Australia (http://www.ipaustralia.gov.au/pbr/). These were then plotted to demonstrate pedigree connections, based on year of release of each cultivar, to the ancestor varieties used in 1970 (Cowling, 2006). Pedigree trees were used to calculate the percent contribution of each ancestor variety in each released cultivar, and the frequency of occurrence in pedigrees of cultivars released from 1995 to 2002. The number of crossing cycles from 1970 to the year of release of each variety was calculated from the average of the male and female side of each pedigree. The inbreeding coefficient (F_X) of individual cultivars, and coefficient of ancestry or parentage (f) or pairs of cultivars, were calculated for representative cultivars from 1995 to 2002 following the methods outlined in Falconer and Mackay (1996). The common ancestor was assumed to be homozygous, so the contribution to F_X for each relationship path was calculated according to the following equation:

$$F_X = \Sigma\left(\frac{1}{2}\right)^n (1 + F_A)$$

where F_A is the inbreeding coefficient of the common ancestor, and $F_A = 1$ for a completely homozygous common ancestor.

The population coefficient of inbreeding over t cycles of recurrent selection was based on the formula for inbreeding coefficient over generations in a closed population (Falconer and Mackay, 1996):

$$F_t = 1 - (1 - \Delta F)^t$$

where ΔF is the rate of inbreeding, which in a random mating population is related to effective population size (N_e) as follows (Falconer and Mackay, 1996):

$$\Delta F = \frac{1}{2N_e}$$

F_t is equivalent to the cumulative loss of alleles through random genetic drift (Falconer and Mackay 1996).

Blackleg resistance of Australian canola cultivars is assessed by private and public breeders in a national field testing scheme that is co-ordinated through the Canola Association of Australia (CAA), and based on standard disease nursery protocols and quality assurance procedures. Breeders assess percentage stand decline due to blackleg disease on current and older cultivars, with standard control varieties nominated by the CAA, in replicated disease nursery trials at 5-10 locations across southern Australia each year. Predicted values of percentage stand decline for genotypes across years and sites are converted into a scale from 0 (very susceptible) to 9 (very resistant), and these cultivar blackleg resistance ratings are published annually by the CAA (http://www.canolaaustralia.com/information/pest_and_disease).

Annual blackleg resistance ratings, as published by the CAA, were used to assess to assess the average change in blackleg resistance rating on each canola variety over a minimum of 3 years with a minimum of 6 trial sites in each annual resistance rating. The average change in blackleg resistance rating per year was equal to the difference between the first and final resistance rating, divided by the number of years.

Results and Discussion

Genetic diversity in Australian canola

The ancestral population of Australian canola included European and Canadian types (the source of "double low" genes) and Asian lines of *B. napus* and *B. juncea* (L.) Czern. (the origin of polygenic blackleg resistance) (Cowling, 2006), but none of these were well adapted to the Australian environment. Recurrent selection was used to exploit this genetic variation and resulted in blackleg resistant and high quality varieties which form the basis of the current canola industry in Australia, and which are genetically distinct from Asian, European and Canadian types (Chen et al. 2007, these Proceedings). Recurrent selection is a powerful tool to exploit genetic variation in breeding programs for adaptation to new environments, and Australian canola breeding is a good example of this.

The average number of recurrent selection cycles in this closed population was approximately 5 cycles during 30 years 1970 – 2000), at an average cycle time of 6 years (Cowling 2006).

There was a high (>0.3) coefficient of parentage (f) between some pairs of Australian canola varieties in 2000 (Cowling 2006). This is higher than between pairs of North American two-row barley cultivars prior to 1990 (0.19) (Martin et al. 1991) and higher than the average coefficient of parentage in the North American soybean improvement program prior to 1980 (0.25) (St. Martin, 1982). Inbreeding is the result of low effective population size (N_e), and lack of immigration of new alleles. In Australian canola, $N_e \approx 11$, based on the number of ancestor parents left in pedigrees of canola varieties in 2000 (Cowling, 1996). This is similar to the estimate of N_e for the soybean improvement program (maturity groups 00 to IV) after 50 years of breeding in North America (St. Martin, 1982).

The relatively small N_e of the Australian canola breeding pool means that genetic drift is continually removing potentially valuable alleles. The coefficient of inbreeding in Australian canola ($F_t = 0.21$) (Cowling, 2006) may be interpreted as a 21% random loss of alleles after five cycles of recurrent selection, in addition to alleles lost through selection (Falconer and Mackay 1996). While genetic progress is possible in the short term, new genetic material must be brought in to ensure future genetic progress.

Changes in blackleg resistance over time

Based on the assumptions that the average final blackleg resistance rating of ancestor varieties in 1970 was 1.6, and the final blackleg resistance rating of cultivars released in 1996 was 5.6 in the year 2000 (the average final ratings of 1996 varieties Monty, Scoop, Grouse, Drum, Clancy and Pinnacle), then the net rate of improvement in blackleg resistance in the closed population was +0.8 resistance units per cycle, or +0.13 resistance units per year from 1970 to 2000 (Cowling, 2006). This includes any apparent fall in resistance of Australian cultivars in the years following release, since it is based on the final blackleg rating.

The average change in blackleg resistance ratings per year following release for 34 Australian canola varieties, which met the minimum requirements for inclusion, was -0.150 units per year, which was highly significantly different from zero (Cowling, 2006). Australian canola varieties slowly lose polygenic resistance in the years following release. The blackleg pathogen *Leptosphaeria maculans* can evolve in the field in response to changes in selection pressure brought about by wide scale cultivation of new varieties (Howlett 2004). While this potential for change is most commonly observed in response to the release of major resistance genes, the results presented here are unique in demonstrating possible changes in the pathogen population following release of polygenic resistant varieties.

Canola breeders have more than kept ahead of this erosion of polygenic resistance through genetic improvements in

resistance in the closed breeding population. The net resistance improvement (the sum of genetic improvement and genetic erosion of resistance) over 30 years is estimated to be +0.13 units per year. A model of net resistance improvement, and erosion after release, is presented in Fig. 1. Such a model is limited by the assumptions, but the implications are clear – based on the evidence, breeders will have to continually find new combinations of alleles, as well as new alleles, for polygenic resistance in order to keep ahead of adaptation in the blackleg fungus.

Indirect evidence suggests that transgressive segregation has occurred for blackleg resistance over and above the levels of resistance found in the ancestor varieties. This improvement of polygenic blackleg resistance over 30 years by Australian canola breeders is a major success story, and demonstrates the power of recurrent selection in relatively small populations.

Countering the effects of genetic bottlenecks

For most crops, one or more genetic "bottlenecks" have occurred when crop-types were selected from wild relatives due to the founder effect (Ladizinsky, 1985). Some crop species such as chickpea have experienced several bottlenecks during and after domestication, and consequently have very limited genetic diversity in the crop genepool (Abbo et al., 2003). The founder effect implies that wild relatives harbour valuable genetic variability with high breeding potential (Ladizinsky, 1985). The challenge is how to introgress these valuable minor alleles into elite gene pools in an economical and sustainable procedure in a commercial plant breeding program.

Experience shows that most plant breeders are reluctant to make wide crosses, as the performance of breeding populations is almost always negatively affected when exotic germplasm is introduced (Rasmusson and Phillips, 1997). A large number of backcrosses may be made to transfer major genes from wild relatives to elite gene pools in order to break linkage drag and restore performance of the elite cultivar. This obviously greatly reduces the probability of introgression of potentially valuable minor alleles from the non-adapted donor parent.

Response to selection (R) in any breeding program is related to index of selection (i), phenotypic variation (σ_p), heritability (h^2), and cycle time (t) as follows:

$$R = \frac{i\sigma_p h^2}{t}$$

A more rapid response to selection can arise from an increase in selection pressure, phenotypic variation, or heritability, or a decrease in cycle time.

Breeders will need to find new allelic diversity for polygenic blackleg resistance (heritable variance), and decrease cycle time, in order to maintain a net positive response to selection in polygenic blackleg resistance and "keep ahead" of the blackleg fungus. DH technology or single seed descent will help reduce the average cycle time below 6 years (the average from 1970 to 2000).

Breeders cannot be certain which immigrant varieties will be the source of valuable minor alleles, and cannot predict which interlocus epistatic interaction will result in the next major increase in adaptation, resistance or yield. A wide range of new germplasm should be used in crossing – quality and yield of *B. napus* is improving around the globe, and any exotic parent may provide valuable alleles for local breeding programs. However, canola germplasm from outside of Australia will most likely not be adapted to Australian conditions. Imported germplasm must be introgressed gradually without destroying local adaptation.

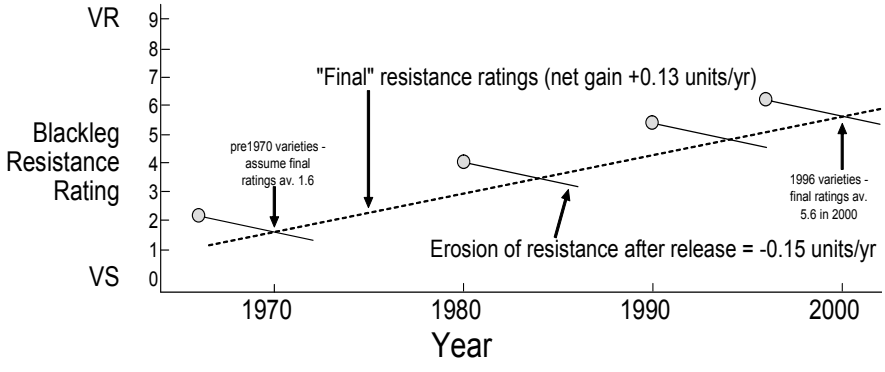

Figure 1. Model for net breeding gain in blackleg resistance (+0.13 resistance units per year) in Australian canola breeding programs from 1970 to 2000, with a simultaneous erosion of polygenic resistance of -0.15 resistance units per year. The "final" resistance rating occurs after an average of 4 years of testing, at which time the variety has lost 0.6 resistance units. 1996 varieties have an average final blackleg rating of 5.6, and 1970 varieties are assumed to have a final blackleg resistance rating of 1.6. Blackleg resistance varies from 0 (very susceptible, VS) to 9 (very resistant, VR). Modified from data presented in Cowling (2006).

New methods of introgression of new alleles should be investigated, such as the RIPE system in barley (Kannenberg and Falk, 1995). The RIPE system introduces new alleles in small increments, while preserving local adaptation in the elite population. This allows a balance to be maintained between introgression of new genetic diversity and improving local

adaptation. The RIPE system limits the number of backcrossing steps, before selection occurs on selfed BC_2 progeny. The probability of selecting a valuable minor allele is reasonable if the number of BC_2 progeny is large enough. The RIPE system also ensures there is a reasonable chance of transferring the new improvements to the elite population, by fixing and selecting BC_2 progeny before the next round of crossing to the elite population.

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