Inheritance of erucic acid content in European and North American populations of wild mustard *Sinapis arvensis*

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ABSTRACT

The presence of wild mustard *Sinapis arvensis* as a weed in *Brassica* crops has an impact on the commercial crop=s seed oil and meal quality. Wild mustard in North America has historically been relatively low in erucic acid content. Collections of wild mustard from Europe (EU) and western Canada (NA) were analyzed for fatty acid composition. The average erucic acid content in the EU accessions was 28.1% (SD 3.49%), while the NA accessions had an average of 9.9% (SD 7.40%). Single seed analysis of the selfed plants identified significant heterogeneity within both populations for the expression of erucic acid, with EU consistently higher in erucic acid than NA. Reciprocal crosses were made between the EU and NA single plants and the F_1 hybrid seed was analyzed. Reciprocal differences were small and the results supported the additive genetic control of the trait, consistent with the model of erucic acid inheritance reported in other studies of *Brassica* and related species. The results also support the presence of distinct alleles in the NA and EU populations. The NA accessions are either homozygous for an allele which produces 0-5% erucic acid designated *e e*, heterozygous *E1 e* with the *E1* allele producing 10% erucic acid or homozygous *E1 E1* resulting in 20% erucic acid. The EU accessions are homozygous for the allele *E2* which produces between 10-15% erucic acid with the majority of the population homozygous *E2 E2* with 25-30% erucic acid. The F_2 populations were analyzed and, in two of the three sets of crosses, supported the model of a single gene locus with multiple alleles.

Key Words: wild mustard - erucic acid - genetic analysis

INTRODUCTION

Wild mustard *Sinapis arvensis* L., also known *Brassica kaber* (DC.) Wheeler, is a common weed in the canola production areas of western Canada. The difficulty in separation of the seed of wild mustard from the crop (*B. napus* L. and *B. rapa* L.) has led to establishment of low tolerances for wild mustard plants in the canola production field. Admixture of wild mustard with commercial canola or rapeseed results in decreased oil and meal quality due to the lower oil content and higher levels of glucosinolates and erucic acid in the contaminating weed (Daun, DeClercq, and Mazur 1983). In commercial canola seed, a tolerance of 5% admixture has been determined to be adequate to ensure that the low levels of erucic acid required for canola oil are not compromised (Canadian Grain Commission 2002). This tolerance reflects the unusually low level of erucic acid found in *S. arvensis* seeds grown in North America compared to populations in other parts of the world, including Europe (Daun, DeClercq, and Mazur 1983). Understanding the inheritance of erucic acid in *S. arvensis* and the genetic composition of North American and European germplasm would provide an assessment of the potential for higher levels of erucic acid to develop in western Canadian wild mustard populations.

MATERIALS AND METHODS

The source of the European seed stocks and the collection of wild mustard samples from western Canada (North America) is described by Daun et al. 2003. Seeds from the original seed stocks of both the North American (NA) and European (EU) wild mustard accessions were germinated in petri dishes with a solution of 0.01% gibberellic acid. Seedlings were planted into Metromix-filled Jiffy pots and, after approximately three weeks, were transplanted into 6" pots.

Reciprocal crosses were made between the EU and NA accessions. Flowering racemes that were not used in the crosses were sprayed with a 3% NaCl solution to overcome self

incompatibility and covered with a bag to ensure self-pollination. Seed from eleven unique crosses and their reciprocals, as well as the selfed seed from all parents, was harvested and the fatty acid analysis conducted according to Hougen and Bodo (1973).

The crosses between the European accession EU5 and three North American accessions NA4, NA2 and NA10 were chosen to represent high X low, high X intermediate and high X high combinations for the F_2 generation study. F_1 plants were sprayed with 3% NaCl solution every two days on newly opened flowers and covered with a bag to prevent cross-pollination. The plants were gently shaken every day within the bag to enhance pollen movement. Bud pollination was also undertaken after two weeks of salt treatment on those plants which did not have adequate pod development. The seed was harvested at maturity and 20 or 40 single seeds were analyzed for fatty acid composition from plants that produced 50 seeds or more.

Chi-square analysis was used to test the genetic model, with n=2 degrees of freedom and P> 0.05 used for the acceptance of the hypothesis.

RESULTS

The average erucic acid content in the EU accessions was 28.1% (SD 3.49%), while the NA accessions had an average of 9.9% (SD 7.40%). Single seed analysis of the selfed plants identified significant heterogeneity within both populations for the expression of erucic acid, with EU consistently higher in erucic acid than NA. EU5 had the highest erucic acid levels of the European accessions (30.7% range 23.8-35.6). The North American accessions had a range in erucic acid levels from 0 to 20%: NA 4 0.0%, NA2 6.4% (range 2.0-11.7) and NA10 19.8% (range 10.8 B29.9).

The fatty acid analysis of the bulk F_1 seed and the single seed analysis of the F_2 populations (20 or 40 seed samples) were used to test the proposed model of erucic acid inheritance with multiple alleles *e, E1* and *E2* at a single gene locus. In this model, the EU accessions are homozygous for the allele *E2* which contributes 15% erucic acid. The allele *E1* contributes 10 % erucic acid. The NA accessions are homozygous for the null allele e (0% erucic) or the *E1* allele (20% erucic) or are heterozygous *e E1*, (10% erucic) as *S. arvensis* populations are heterogeneous due to outcrossing.

High X Low Crosses. The proposed genotypes of the two parents EU5 and NA4 are *E2 E2* and *e e*. The erucic acid level in the F_1 hybrid (*E2 e*) was 16.4% (17.4% reciprocal), supporting the expression of 15% erucic acid with the single dose of the *E2* allele. The segregation of four F_2 and three reciprocal F_2 families was tested for the fit to a single gene segregation ratio 1:2:1, assigning the single seeds with 0- 4.8% as the *e e* genotypes, the single seeds with the range of 14.8- 20.8 as the *E2 e* and the single seeds with the range 28.7 -34.3% as the *E2 E2* genotypes. The hypothesis was accepted for the four F_2 families and the three reciprocals.

High X High Crosses. The proposed genotypes of the two parents EU5 and NA10 are *E2 E2* and *E1 E1*. The erucic acid level of the F_1 *E2 E1* was 20.4% (reciprocal 25.6%) supporting the contribution of the *E2* allele of 15% and the *E1* allele of 10%. Four F_2 families and four reciprocals were tested and the hypothesis was accepted in three of the four populations and reciprocals.

High X Intermediate Crosses. The NA2 accession had a range of erucic acid 2.0 -11.7%, with an average level of 5.4%. We propose that this reflects the heterogeneity in the NA2 population with the expression of the genotypes *e e* (0-5%) and *e E1* (10-15%). In the F_1 of the crosses to EU5 (*E2 E2*), the level of erucic acid 20.9% (reciprocal 21.9%) is consistent with the model of a mixture of genotypes *e E2* (15%) and *E1 E2* (20-25%). However, in the F_2 single seed analysis (20 seeds), there was no recovery of the *e e* genotype, as the lowest erucic acid value was 7.1% and there were very few single seeds with 10-20% erucic acid (genotype *e E2*), as would be expected from the selfing of the F_1 genotype *e E2*. None of the seven F_2 families= segregation ratios fit the expected segregation for the F_1 genotype *E1 E2* as there were seeds in the low erucic acid class ranging from 7.1- 10.3% in each of the families. This level of erucic acid does

not fit the expected expression of the homozygous *E1 E1* genotype (20%). The proposed model did not provide an adequate genetic explanation for this cross. Additional crosses and seed analyses should be conducted.

DISCUSSION

The proposed single gene model with multiple alleles for erucic acid in the diploid *S. arvensis* is consistent with the classic studies in the tetraploid *B. napus*, describing a two gene model with multiple alleles acting in an additive fashion (Harvey and Downey, 1964; Stefansson and Hougen 1964. Chen et al. 1995 proposed a monogenic additive model for the inheritance of erucic acid in *S. arvensis* : 1 *EE* 18-22% erucic acid:2 *Ee* (9-14% erucic acid): 1 *ee* (<1% erucic acid). This study has identified distinct alleles in the North American and European populations, proposed as *e*, *E1* and *E2*. A modified monogenic model is proposed to accommodate the multiple alleles. The introduction of the *E2* allele from European wild mustard into North American populations would result in higher levels of erucic acid and a reduction of seed oil quality in a commercial crop with wild mustard contamination.

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