AFLP markers linked to seed quality traits in *Brassica juncea*

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ABSTRACT

Canola quality *Brassica juncea* has been under development at the Saskatoon Research Centre for several years. Breeding efforts have focused on the development of germplasm with improved seed quality characteristics including superior fatty acid profiles, low glucosinolates and high oil content. Future breeding efforts in this crop would be greatly facilitated through the development of molecular markers for genes controlling seed quality traits. A mapping population was developed from the cross between a mustard quality *B. juncea* line and a canola quality *B. juncea* line. Seventy five doubled haploids from this cross were grown in the field in Saskatchewan over two years and assessed for seed quality characteristics. A bulked segregant analysis was performed on DNA from bulked DH samples. The bulks were selected based on the field data. A bulked segregant analysis using AFLP was performed in an attempt to discover linked markers for glucosinolate content (butenyl glucosinolates, allyl glucosinolates), fatty acids (erucic acid, oleic acid), oil content and thousand kernel weight. Several potential AFLP markers linked to the genes controlling these traits were identified through the bulked segregant analysis and these markers will be mapped and used to develop PCR based markers for use in the plant breeding program.

Key words: *Brassica juncea*, seed quality, AFLP, bulked segregant analysis

INTRODUCTION

Brassica juncea has traditionally been grown as a condiment mustard crop and has been shown to have good adaptation to the hotter drier areas of western Canada. *Brassica juncea* was originally chosen as a species for conversion to canola quality because it exhibits superior drought and heat tolerance, disease resistance and pod shatter resistance compared to *Brassica napus* (Woods *et al*.,1991, Burton *et al*., 1999). Mustard *B. juncea* was found to be higher yielding than either *B. napus* or *B. rapa* under droughty conditions (Woods *et al*., 1991). Initial results with the canola quality AAFC line J90-4316 indicated the canola quality germplasm has heat tolerance similar to *B. juncea* mustard (Angadi *et al*., 2000).

AFLP analysis has been used to develop linkage maps in several species including *B. juncea* (Negi *et al*., 2000 and Lionneton *et al*., 2002). Molecular markers have been identified for seed coat colour (Negi et al. 2000) and for seed oil content and fatty acid composition in mustard quality *B. juncea* (Lionneton *et al*., 2002). Our goal was to develop an AFLP map from a cross of mustard quality *B. juncea* and canola quality *B. juncea* that would allow us to identify markers for seed oil and meal quality traits. Markers identified through the process would then be converted to PCR based markers to allow for high throughput uses in our germplasm improvement program.

MATERIALS AND METHODS

A mapping population was developed from the cross of *B. juncea* J90-2733 x *B. juncea* J90-4317. The parental lines were selected on the basis of their divergent seed quality characteristics; *B. juncea* J90-2733 contains very high oil, high glucosinolates and high erucic acid, *B. juncea* J90-4317 contains low oil, low glucosinolates and low erucic acid. A mapping population of 75 doubled haploid lines was developed and evaluated for two years in a replicated field nursery. Bulks were made of DNA from selected DH's for the various traits. DH's were only used in the bulks if they were consistent in all replicates, in both years of the field evaluation. Seed quality characteristics, including glucosinolate content has been determined. A genetic linkage map was created and several AFLP markers have been identified for the various traits.

RESULTS

The DH population was used to first construct a genetic map using AFLP markers. We have placed approximately 300 markers into linkage groups (data not shown). Field evaluation data was collected over two field seasons. Several AFLP markers were identified in this mapping population for seed quality characteristics (Table 1). There was a large range of variation for the seed quality traits under investigation across the DH population. Oil content ranged from 38-49.1% (on a dry seed basis), allyl glucosinolates ranged from 1.2-74.6 µmoles/g of oil free meal, butenyl glucosinolates ranged from 0.8-91.6 µmoles/g of oil free meal, erucic acid level ranged from 0.9-43.0 percent. A lower level of variation was observed in the DH population for thousand kernel weight (TKW) which ranged from 2.5-4.1g.

Several clusters of markers were found for traits such as allyl and butenyl glucosinolates and erucic acid and oil content. Markers linked to allyl and butenyl glucosinolates were identified on linkage group 2. Additional linked markers for either allyl or butenyl glucosinolates were found on linkage groups 8 and 11. In this mapping population, markers linked to oil content were found on linkage group 3 and 13 and these markers also appeared to be linked to erucic acid content. Examination of the seed quality data of the DH's used for the low erucic acid bulk vs the high erucic acid bulk indicated that the average oil content of the lines used in the low erucic acid bulk was 40.2% whereas the average oil content of the DH lines used for the high erucic acid bulk was 46.1%. Markers were also identified that were linked to thousand kernel weight (TKW) on linkage group 8 and 15.

Table 1. Identification of linked markers for seed quality characteristics in the *B. juncea* J90-4317xJ90-2733 DH mapping population

Linkage group[a]	Markers identified	Linked trait
LG2	3	allyl glucosinolates
	3	butenyl glucosinolates
LG3	3	erucic acid/oil
LG8	4	TKW
	2	allyl glucosinolates
LG11	1	butenyl glucosinolates
LG13	3	erucic acid/oil
LG15	1	TKW
LG17	2	erucic acid

a- linkage group number represents our linkage group number as assigned during AFLP based map construction- does not relate to published linkage groups at this point for *B. juncea*

DISCUSSION

Analysis of our mapping population has identified several potentially useful AFLP markers that can now be converted to PCR based markers for confirmation of their utility and use by the breeding programs. We have identified what appears to be a major region located on our linkage group 2, containing genes involved in allyl and butenyl glucosinolate content in *B. juncea*. We also observed an association between erucic acid level and oil content. Lionneton *et al*., 2002 did not find an association between erucic acid content and oil content in *B. juncea* however the explanation may be that the germplasm used in their study was of the condiment mustard type and the parental lines used had oil contents between 32-36%. The range of oil content in our mapping population was between 38-49% oil and therefore we may have identified different genes then those identified in Lionneton *et al*., 2002.

All the required genetic elements (high oil content, zero erucic acid, increased oleic acid content/decreased linolenic acid content, very low glucosinolate content with no allyl glucosinolate present) to make *B. juncea* a canola crop competitive with *B. napus* in terms of seed quality are available in various lines. The challenge is to integrate all these elements together in one line of *B. juncea*. The development of DNA markers for these seed quality traits

will greatly assist in this work. There is excellent potential to convert the markers identified in this study into PCR based markers to allow for high throughput use in our germplasm improvement program.

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