Where in the Genome is the Flax b1 Locus?

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Introduction

According to the Flax Council of Canada, flax (*Linum usitatissimum* L.) is one of the five major Canadian produced and exported crops. Canada is the number one producer of flax and according to statistics available from the Food and Agriculture Organization (FAO) Canadian farmers produced well over 700,000 tonnes as an annual average from the years 2000-2010.

For the past thousands of years flax has been grown for two separate utilities, its oil or its fibers. Fiber flax has long strong fibers within its stem that are increasingly important in the manufacturing of environmentally friendly products such as geotextiles, biofuels, biocomposites, and insulation. Oilseed flax, also known as linseed, has seeds that are high in fiber, contain 40-45% oil content, and 20-30% protein content. The oil is high in healthy polyunsaturated fatty acids, which are often also referred to as omega-3 fats. These qualities make flax oil and flax seed nutritionally beneficial to humans (Cunnane et al., 1993). The oil has also proven to be industrially useful in oil-based paints and stains, lubricants and as well as in the curing of linoleum flooring. As well, the meal left behind after the seed is crushed is a healthy feed for cattle due its high protein content.

Canada primarily produces Linseed type flax, the majority of which is for export to other countries. The export of Canadian linseed has been valued at between 150-180 million dollars (SaskFlax). The province of Saskatchewan contributes up to 70% of Canadian production and export, and 25-30% of the world's total production (FAOSTAT). Most flaxseed exported is crushed in order to extract the oil for use in the industrial purposes listed in the previous paragraph; the left over meal is used in high quality animal feed.

Until recently there have been two types of linseed flax, characterized by their seed coat color: brown and yellow. Brown seeds or, regular flax, contain greater than 50 percent linolenic (omega-3) fatty acid in their oil. Yellow seeds were previously required to identify Solin, a flax bred using mutation breeding from regular flax to contain less than five percent linolenic fatty acid, by the Canadian Grain Commissions standards (Mittapalli & Rowland, 2003). Solin was developed because it was more suitable for margarine and shortening than traditional flax oils (Saeidi & Rowland, 1997). However, there is no longer a market for solin flax and there is shifting interest into developing a yellow seeded high linolenic linseed flax variety because the food market prefers the yellow seed coat as well as a healthier oil profile.

Due to this new demand for high linolenic yellow linseed the Canadian Grain Commissions has removed the requirement for yellow seeds to be a phenotypic marker for the Solin oil profile. This requirement can be removed because the two traits are not linked and randomly assort when the genetics aren't fixed. However, the requirement for farmers to have uniform seed at grading has not been removed. This is causing grade reductions in farmers' seed when there are amounts of yellow seed "contaminants" in their brown seed. This grade reduction may be unnecessary

because it is likely that the brown and yellow seeds are not compositionally different from each other. Therefore, it is important for researchers to understand the genetic basis behind seed coat color development and its association with other important agronomic, quality, and morphological traits. This would allow for the mixing of seed colors by farmers without having grading penalties as well as the breeding of flax varieties with unique and useful combinations of quality traits and seed colors.

Materials and Methods

DNA was extracted using CTAB protocol from 94 recombinant inbred lines (RILs) of the B1VG population. This population was established by a cross between CDC Bethune and M96006. These lines were grown in the Phytotron growth chamber facilities. DNA of 8 individuals for each of the two seed color phenotypes were bulked into pools and bulked segregant analysis (BSA) was performed using 192 simple sequence repeat markers (SSRs). Markers showing indications of linkage were chosen to screen the entire population to get a measure of genetic distance.

Results and Discussion

Two populations were originally established to determine the location of the b1 locus in the flax genome. Preliminary observations of the populations showed indications of skewing that could be caused either by heterogeneity of the parental line, selection against lines with yellow seed coat or multiple genes being involved in seed coat color determination for one of the populations.

Table 1. Chi-squared test to determine if populations were segregating as expected.

Population	Observed Brown Seed	Observed Yellow Seed	Expected Yellow* Seed	Expected Yellow* Seed	Yate's# Corrected χ^2	χ^2 Critical Value
$b1^{vg}$	56	48	52.4	51.6	0.43	3.841
b1	80	24	52.4	51.6	28.74	3.841

From the above results (Table 1) it was shown that the B1VG population did fit the 1:1 ratio that is expected of a RIL population in the F7 generation but the B1 population did not. In order to determine the cause of this population skewing a two-gene model was fit.

Table 2. Chi-squared test to fit a two-gene model for the b1 RIL population.

Population	Observed Brown Seed	Observed Yellow Seed	Expected Brown Seed	Expected Yellow Seed	Yate's# Corrected χ^2	χ^2 Critical Value
b1	80	24	78	26	0.1154	3.841

Based on the results of this second chi-squared test (Table 2) there is evidence that this population is segregating for two genes that control seed coat color. Unfortunately it made this b1 population unusable for bulked segregant analysis. Therefore. BSA was only performed on the B1VG population.

Upon completion of the bulked segregant analysis markers on two separate linkage groups were showing evidence of linkage to seed coat color (Fig. 1). Because the chi-squared test for the B1VG population showed that only one gene was responsible then two of these markers must be

a result of a false positive which was relatively common in this bulked segregant analysis assay. These two chromosomes had very low marker coverage with only 3 polymorphic markers being present on chromosome 8 and two on chromosome 12. By future screening of the entire populations with these four linked markers genetic distance can be measured by determining recombination frequency. This will hopefully help to clarify the chromosome in question.

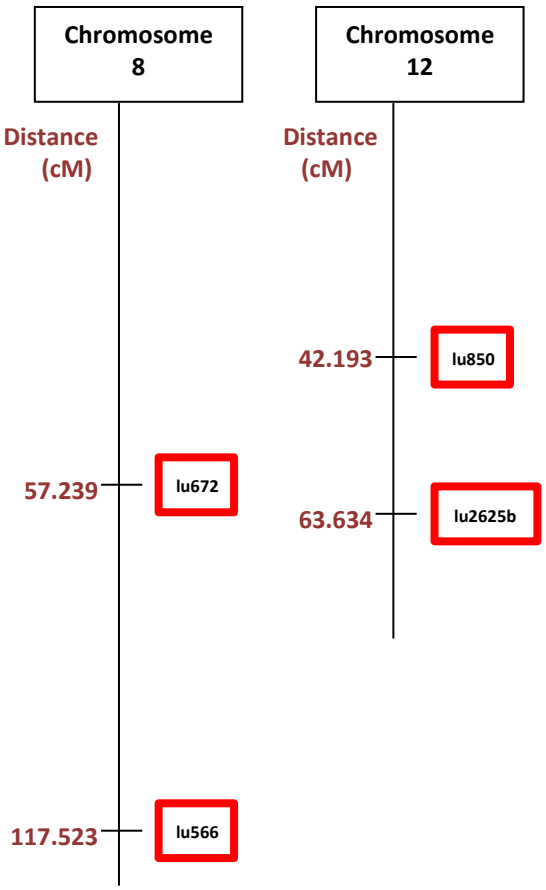

Figure 1. The markers showing evidence of linkage after bulked segregant analysis.

Conclusions

The evidence shows that the b1 population is likely segregating for two seed coat color genes and therefore is unusable for this study. But since the b1 and the b1vg populations are both segregating for the b1 locus the b1vg population can be used alone to determine the its location.

Upon completion of BSA two chromosomes were identified as potentially harboring this locus. Screening with markers from both of these chromosomes will be performed in order to identify the true location of the b1 seed coat color locus.

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