QTLS FOR WESTERN GALL RUST (*ENDOCRONARTIUM HARKNESSII*) RESISTANCE IN LODGEPOLE PINE (*P. CONTORTA SPP. LATIFOLIA*)

Changxi Li [1] & Francis C. Yeh [2]
Department of Renewable Resources, University of Alberta, Edmonton, Alberta, T6G 2H1, Canada
[1] Current address: Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, T6G 2P5, Canada.
[2] Corresponding author: Telephone (780)-492-9053; Fax (780)-492-0097; email francis.yeh@ualberta.ca

Received June 12, 2002; accepted July 27, 2002

ABSTRACT

We mapped three quantitative trait loci (QTL) that increased resistance to western gall rust (WGR) fungus (*Endocronartium harknessii* (J. P. Moore) Y. Hiratsuka) in lodgepole pine (*Pinus contorta* spp. *latifolia*). Each of two sets of 45 open-pollinated seedlings of a parent tree was inoculated with a geographic sources of WGR fungal isolates sampled from lodgepole pine or jack pine (*Pinus banksiana* Lamb.) hosts. Using single-tree maps derived from corresponding haploid megagametophytes recovered from inoculated seedlings, QTLs for resistance to WGR fungus were identified through a full scan on a genomic map with 148 framework and 77 accessory Randomly Amplified Polymorphic DNA (RAPD) markers. Three putative QTLs for resistance to WGR fungus were mapped to linkage group 6. Two explained 42.9 % and 27.1 % of the total phenotypic variance in resistance to the WGR fungal isolate of lodgepole pine host. The third QTL accounted for 22.6 % of the total phenotypic variance in resistance to the WGR fungal isolate of jack pine host. These results suggest that resistance to the WGR fungus in lodgepole pine involved a small number of genes of large effect.

Key words: major gene resistance, Western gall rust, lodgepole pine.

INTRODUCTION

Endocronartium harknessii (J. P. Moore) Y. Hiratsuka, the fungus that causes western gal rust (WGR) in hard pines, is an endocyclic rust possessing only one spore state and has no alternative hosts (HIRATSUKA 1991). It infects more than 20 native and exotic hard pines (TUSKAN & WALLA 1989). In Canada, the major hosts of WGR fungus is lodgepole pine (*P. contorta* var. *latifolia*) and jack pine (*P. banksiana* Lamb.). Damage by this disease is especially severe in immature and managed stands, resulting in great economic loss in terms of growth vigor and wood value (PETERSON 1971; HIRATSUKA & POWELL 1976; BELLA & NAVRATIL 1988; BURNES *et al.* 1988). A study of Randomly Amplified Polymorphic DNA (RAPD) variation across Canada found great differentiation between the WGR isolates of lodgepole and jack pine hosts (LI *et. al.* 2001). The frequencies at 15 of 18 polymorphic RAPD markers were significantly heterogeneous, and of these 15, ten were host specific. RAPD patterns were uniform in isolates from lodgepole pine. However, isolates of jack pine differed significantly in the frequency of four RAPD markers among locations, with an east-west trend of decreasing RAPD similarity. This large differentiation between the WGR isolates of lodgepole pine and jack pine hosts might suggest that selective pressure for host specificity in sampled populations was strong.

Lodgepole pine has been shown to exhibit great variation in resistance to the WGR fungus in natural infections (MARTINSSON 1980; YANCHUK *et al.* 1988; WU *et al.* 1997) and in inoculation experiments (VAN DER KAMP 1988; KOJWANG & VAN DER KAMP 1991; BLENIS *et al.* 1993; YANG *et al.* 1997). However, the host response to infection is complex. In a test with natural inoculums on a heavily infected site, the number of galls per tree at 13 years after planting varied in a continuum (VAN DER KAMP & TAIT 1990). On the other hand, non-infection and complete galls as well as partial galls were commonly observed among young seedlings in greenhouse inoculation studies (KOJWANG & VAN DER KAMP 1991; BLENIS *et al.* 1993; YANG *et al.* 1997). These results indicate that the expression of resistance in host might be influenced by the age of test, source of inoculums, inoculation method and scoring criteria. They could also suggest that single gene major resistance and polygenic resistance were both important in the lodgepole pine-western gall rust pathosystem (KOJWANG & VAN DER KAMP 1991).

The advent of molecular markers, and in particular RAPD, has greatly facilitated the construction of

saturated genomic maps suitable for detecting individual quantitative trait loci (QTL) in forest trees. Each random primer typically produces several polymorphic RAPD markers and since many random primers are available, a large number of RAPD markers are available for analysis. RAPD markers are particularly suited to constructing genomic maps in conifers. The genetic makeup of a haploid megagametophyte represents the maternal contribution to the corresponding embryo as both megagametophyte and the egg cell are derived from the same product of maternal meiosis (FOSTER & GIFFORD 1974). Thus, the use of megagametophytes avoids the problem of dominance and recombination between RAPD markers in megagametophytes of single trees directly reflects their linkage relationships. Single-tree mapping with RAPD markers is the most common approach for constructing saturated genomic maps in conifers (TULSIERAM *et al.* 1992; NELSON *et al.* 1993; BINELLI & BUCCI 1994; NELSON *et al.* 1994; KAYA & NEALE 1995; WILCOX 1995; YAZDANI *et al.* 1995; ECHT & NELSON 1997; KRUTOVSKII *et al.*1998), including lodgepole pine (LI & YEH 2001).

Segregating RAPD markers among megagametophytes of single trees also present the unique opportunity to identify QTLs. By recovering megagametophytes from germinating seedlings for DNA extraction, it is possible to score segregating RAPD markers in megagametophytes and measure the phenotypic traits in seedlings derived from the corresponding embryos (O'MALLEY *et al.* 1994). This approach to identify QTLs has been used by DEVEY *et al.* (1995) for white pine blister rust (*Cronartium ribicola* Fisch) resistance in sugar pine (*P. lambertiana* Dougl.) and by WILCOX *et al.* (1996) for fusiform rust (*Cronartium quercuum* (Berk)) resistance in loblolly pine (*P. taeda* L.). The basis of their strategy was that when large numbers of RAPDs were studied in megagametophytes, some would co-segregate with the disease phenotypes in corresponding seedlings if that trait is controlled by resistance genes inherited from the seed tree and the challenging inoculum is avirulent to the genes.

The results of RAPD-based QTL mapping in tree-pathogen systems are not consistent with the control of multiple genes each of small effect. On the contrary, it is commonly observed that one or few genes of large effect control disease resistance, as reported for white pine blister rust in sugar pine (DEVEY *et al.* 1995), black leaf spot (*Gnomonia ulmea*) in Chinese elm (*Ulmus parvifolia*) (BENET *et al.* 1995), fusiform rust in loblolly pine (WILCOX *et al.* 1996), and pine needle gall midge (*Thecodiplosis japonensis*) in Japanese black pine (*Pinus thunbergii*) (KONDO *et al.* 2000). In lodgepole pine, however, the molecular genetic basis of resistance to WGR largely remains unclear and the knowledge regarding the interaction between the host and the isolates is also very limited.

We report the mapping of QTLs controlling resistance to two geographic sources of WGR fungus in lodgepole pine using single-tree RAPD genomic maps derived from megagametophytes and measuring the WGR scores in seedlings derived from the corresponding embryos.

MATERIALS AND METHODS

Mapping population

Open-pollinated seeds of a mother tree were collected near Wolfcreek, Alberta, at 1036 m elevation, latitude 54°36' N and longitude 119° 03' W. The tree was 74 years of age at the time of seed collection in 1976. It was chosen for study because it was free of WGR fungus infection in the natural stand and its open-pollinated half-sib progenies have exhibited large phenotypic variance in resistance to WGR fungus both in long-term field tests and in greenhouse inoculation studies (unpublished data). Variation in resistance to WGR fungus among progenies within family might indicate the segregation of QTL for resistance in this family.

Plant material

Seeds were germinated in the greenhouse at the University of Alberta. Plastic trays (Ventblock ® 45, Beaver plastic Ltd.) each containing 45 cavities (350 cm^3/cavity) were used to cultivate the seedlings. We filled each cavity with peat moss that was adjusted to pH 5.5 by the addition of 355 g of dolomite lime to each 113-L bale of peat moss.

Two seeds were sown into each cavity and deionized water was applied daily to maintain soil moisture during the first two weeks. After two weeks, each cavity was randomly thinned to have one germinating seedling. Megagametophytes were separated from the extending cotyledon needles of the germinating seedlings prior to natural abscission. Each megagametophyte averaged 2.0 mg in weight and was stored individually in a 1.5 ml micro-centrifuge tube at –20°C until required for DNA extraction for use in genomic map construction. The corresponding seedlings were cultivated in a greenhouse under day/night regimes of 16/8 hours, supplemented by high pressure sodium vapour light (with PPFD intensity of 300–340 $\mu mol \cdot m^{-2} \cdot s^{-1}$) when natural daylight was shorter than 16 hours. Watering and fertilizing (1:1 of N–P–K 20–20–20) took place at one and two week intervals,

respectively. Temperatures were set at 25/20 °Celsius for day/night during the study.

Phenotypic data

Two WGR spore sources that were different in their RAPD profiles (LI *et. al* 2001) were used as the inoculums. One spore source was sampled from single gall isolate on a lodgepole pine near St. Albert (53° 12' N; 113° 40' W). The second spore source was sampled from single gall isolate on a jack pine in Dragline Lake, Manitoba (51°35' N; 100°40' W). Only the inner layer of aeciospores was collected. WGR fungal isolate of each gall was removed by brushing the gall and sieving the isolate through a thin nylon cloth. Cleaned aeciospores were kept separate by gall, air-dried for 24 h and placed into 30-mL vials with silica gel crystals for storage at –20 °C. The spore viability was tested on 0.2 % agar on a microscope slide at 25 °C before inoculation. Germination rates of spores were above 85 %. Seven weeks after germination, one half of the seedlings (45) were inoculated with the spore source of lodgepole pine host. The other half (45) were inoculated with the spore source of jack pine host. The inoculation protocol adhered to the torn needle technique developed by MYRHOLM & HIRATSUKA (1993) because it minimized the possibility of "escaping" infection and allowed for the production of galls on young seedlings. After inoculation, the seedlings were lightly misted with distilled water and covered with a plastic sheet to maintain high humidity for spore germination and infection. They were kept at 15 °C without artificial lighting for 48 hours. Then, the plastic sheet was removed and seedlings returned to growth conditions under day/night regimes of 16/8 hours supplemented by high pressure sodium vapour light (with PPFD intensity of 300–340 $\mu mol.m^{-2}.s^{-1}$) when natural daylight was shorter than 16 hours.

Six months after inoculation, WGR disease infections were evaluated on the 0 to 5 scoring criteria developed at the Northern Forestry Center, Canada (KLEIN *et al.* 1991). A rating of 0 typified the lack of symptoms; 1 was for visible discoloration or a definite indication of infection such as acute bending of stem; 2 was for a define canker but no swelling; 3 was for some swelling with rough bark and open necrotic canker; 4 was for partial gall often with rough bark and necrotic canker; and 5 corresponded to a complete globose gall.

Framework linkage map construction

Extraction of total DNA, PCR amplification of DNA and RAPD analysis followed the protocols of LI & YEH (2001). Primer screening is essential to obtain reproducible RAPD profiles. The random primers used in this study were 10-base, oligonucleotide primers obtained from University of British Columbia and from Operon Technologies. After an initial screening of 840 random primers against eight megagametophyte DNA samples, we selected 110 primers that were segregating and produced sharp and reproducible RAPD markers over several independent runs.

Framework linkage maps with RAPD marker order at a LOD interval support of at least 3.0 were constructed using the DNAs of 90 saved megagametophytes of the germinating seedlings. Details of map construction have been presented elsewhere (LI & YEH 2001). Briefly, 328 RAPD markers with fragment sizes that ranged between 260 and 3080 base pairs were found segregating at the 110 RAPD primers. Of these 328 RAPD markers, 148 were mapped to 16 framework linkage maps and 77 were mapped as accessory markers onto the framework linkage maps. The 16 framework linkage maps covered a distance of 2,287 cM.

QTL mapping

The disease scores of the seedlings corresponding to the megagametophytes used in framework linkage map construction were transformed by log (1+score) to improve the normality of the raw data. We carried out the QTL analysis for each spore source separately by interval mapping using MAPQTL 4.0 (VAN OOIJEN & MALIEPAARD 1996) under the backcross model. Interval mapping expands single-locus association to a pair of adjacent markers. This provides a precise location for the QTL but requires that accurate marker distances are known. The LOD threshold values were determined using a permutation test implemented in MAPQTL 4.0. The permutation test was based on the method outlined by CHURCHILL and DOERGE (1994). For each permutation, the log (1+score) transformed disease scores were randomly shuffled. The QTL mapping analysis was then performed using the randomly shuffled data and the corresponding maximum LOD score was stored. The entire procedure was repeated 1,000 times. The LOD threshold was calculated by choosing 100 $(1-\alpha)$ percentile (α is the type I error) of the LOD score distribution. Type I error of 0.05 and 0.01 were used to calculating the suggestive and significant LOD thresholds, respectively. For each LOD peak, we used a 1.5 LOD support interval to determine to the left and right boundaries.

Subsequent to interval mapping, we used the Multiple-QTL Model (MQM) procedure in MAPQTL 4.0 to fine map multiple QTLs within a linkage group that were detected by the interval mapping. This

procedure requires accurate knowledge of markers already known to be linked to the QTL because the markers close to a detected QTL were used as cofactors to absorb the effects of nearby QTLs. Thus, RAPD markers that were nearest to the peaks were defined as the cofactors in our data. This increases the power for mapping other segregating QTLs and enables the separation and mapping of linked QTLs (VAN OOIJEN & MALIEPAARD 1996).

RESULTS

Host resistance to WGR infection differed for the two geographic sources of WGR fungus. The rust disease scores of seedlings inoculated with isolates sampled from lodgepole and jack pine hosts both ranged from 0 to 5, with averages of 1.80±0.54 and 3.70±0.26, respectively. The permutation procedure generated the suggestive LOD thresholds of 2.40 and 2.10 for QTL mapping to the lodgepole and jack pine isolates, respectively. The corresponding significant LOD thresholds were 3.70 and 3.10. Based on these LOD thresholds, we mapped three putative QTLs (QTL1, QTL2, and QTL3) on to linkage group 6 (Table 1), each reached the significant LOD threshold with an overall false positive rate of less than 0.01. QTL1 and QTL2 increased resistance to WGR fungal isolate of lodgepole pine host while QTL3 increased resistance to WGR fungal isolate of jack pine host.

QTL1 was between RAPD markers U443_1920 and U690-1495, with an interval length of 10.1 cM. The most likely location of QTL1 was 5 cM from RAPD marker U443_1920. This QTL explained 42.9 % of the total phenotypic variance in WGR resistance and lowered the rust score of seedlings by 1.2.

QTL2 was between RAPD markers U680_2255 and U428-1260, with an interval length of 27.7 cM. It accounted for 27.1 % of the phenotypic variance in WGR resistance and lowered the rust score of seedlings by 0.9. In all likelihood, QTL2 was 15 cM from RAPD marker U680_2255.

QTL3 was between RAPD markers U443_1920 and U690-1495, with an interval length of 10.1 cM. Its most probable position was 2 cM from RAPD marker U443_1920. QTL3 explained 22.6 % of the total phenotypic variance in WGR resistance and lowered the rust score of seedlings by 0.6.

The relative map positions of the three putative QTLs on framework linkage group 6 and their 1.5 LOD support intervals were depicted in Figure 1. QTL1 and QTL2 are almost 50 cM apart. QTL3 has a support interval that overlapped with that of QTL1. It is located left of QTL1, at a distance of about 3.0 cM.

Table 1. Putative QTLs for resistance to western gall rust in lodgepole pine family using MAPQTL (4). RAPD names include primer identities (U: University of British Columbia, Canada), and fragment size in bp with (-) representing coupling phase and (_) representing repulsion phase. Locations and LOD peaks of QTL are in Kosambi centimorgan (cM) away from their left-most RAPD markers.

Trait / QTL[a]	Linkage group	Flanking markers	Interval length (cM)	LOD position (cM)[b]	Peak[c]	Support interval (cM)[d]	% variation explained[e]	Genetic effect[f]
RWGRL								
QTL 1	6	U443_1920 U690-1495	10.1	5.0	8.9**	20.1	42.9	-1.2
QTL 2	6	U680_2255 U428-1260	27.7	15.0	5.5**	37.7	27.1	-0.9
RWGRJ								
QTL 3	6	U443_1920 U690-1495	10.1	2.0	3.9**	33.8	22.6	-0.6

[a] RWGRL stands for resistance to western gall rust from lodgepole pine source.
RWGRJ stands for resistance to western gall rust from jack pine source.
[b] Distance from the leftmost marker on the interval, indicating the most likely QTL position corresponding to the LOD peak.
[c] Peak value of maximum LR test statistic observed for QTL, with "**" indicating significant level of 0.01 or a overall false positive rate less than 0.01.
[d] QTL support intervals were determined at LOD of 1.5.
[e] Percentage of phenotypic variance in WGR score as explained by the QTL.
[f] QTL effect as measured by the additive effect of the QTL allele.

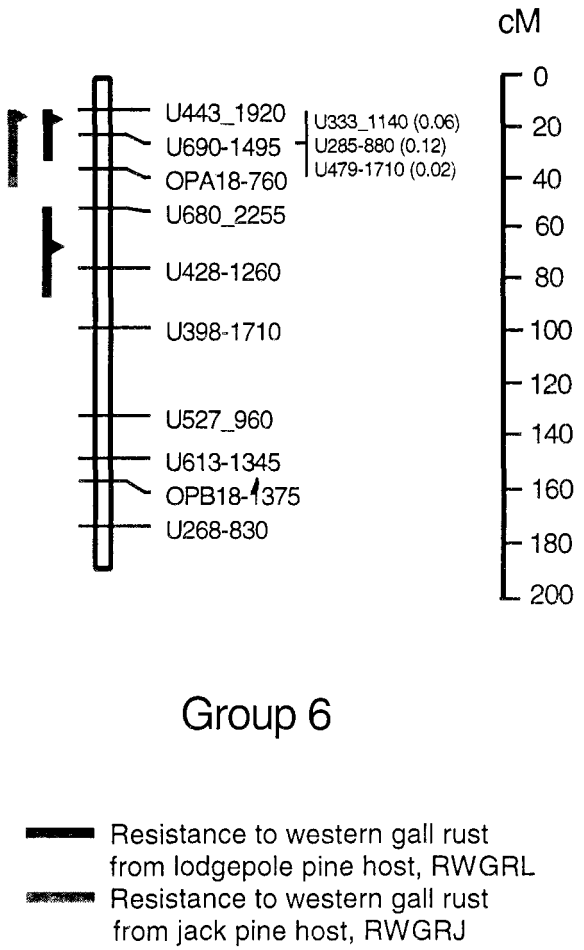

Figure 1. Putative QTLs for resistance to western gall rust in a lodgepole pine family. The arrow keys and bars on left of the framework linkage map 6 indicated the most likely positions of the QTL and their corresponding support intervals at LOD of 1.5. RAPD names include primer identities (U: University of British Columbia, Canada; OP: Operon Technologies, Alameda, California) and fragment size in bp with (-) representing coupling phase and (_) representing repulsion phase. RAPD markers were placed on the map with likelihood order support ≥3.0. To the right of the nearest framework markers are the accessory RAPD markers, with recombination fractions shown inside the parentheses. The relative genetic map distance was scaled on the right in Kosambi centimorgan (cM).

DISCUSSION

There have been criticisms of the use of RAPDs because they are sensitive to experimental conditions (SMITH *et al.* 1994; HARRIS 1995). We have, however, found that using a consistent quantity of DNA per amplification and high quality *Taq* Polymerase, and carrying out an extensive primer screening, we could generate reliable and reproducible RAPD profiles in lodgepole pine (FAZEKAS & YEH 2001; LI & YEH 2001).

RAPD markers are well suited for genomic mapping of QTLs in lodgepole pine in that they are highly polymorphic and a small amount of DNA template (as low as 4 ng) is required for each amplification. Each megagametophyte harvested from a germinating seedling averaged 2.0 mg in weight and yielded between 1–2 µg of template DNA. Thus, at least 250 RAPD reactions were possible and they generated on average 250 sharp and reproducible RAPD markers that were segregating in the population. Consequently, a large number of RAPD markers were available from each megagametophyte for mapping. Our genomic map has 148 framework and 77 accessory RAPD markers evenly spaced along the genomes. With this level of map density, we mapped three QTLs that increased host resistance to WGR (Table 1), a fungal disease that causes great economic loss in growth vigor and wood value of lodgepole and jack pine in Canada. QTL1 explained 42.9 % and QTL2 explained 27.1 % of the total phenotypic variance in WGR resistance to the fungal isolates of lodgepole pine host and lowered the rust score of seedlings by 1.2 and 0.9, respectively. QTL3 explained 22.6 % of the total phenotypic variance in WGR resistance to the fungal isolate of jack pine host and lowered the rust score of seedlings by 0.6. Our finding demonstrates that host resistance to WGR infection could be under oligogenic control in lodgepole pine. This is congruent with earlier report that major gene resistance could play a part in the lodgepole pine-western gall rust pathosystem (KOJWANG & VAN DER KAMP 1991). One or few QTLs of large effect controlling disease resistance have also been reported in sugar pine (DEVEY *et al.* 1995), Chinese elm (BENET *et al.* 1995), hybrid *Populus* (NEWCOMBE & BRADSHAW 1996), loblolly pine (WILCOX *et al.* 1996) and Japanese black pine (KONDO *et al.* 2000) as well as in plant species (YOUNG *et al.* 1993; CHEN *et al.* 1994). However, the small sample size of 45 used in this study might overestimate the QTL effects (BEAVIS 1994). On the other hand, there are perhaps other QTLs controlling WGR resistance in this family. However, linked RAPD markers might not be segregating. In addition, the power of QTL detection would necessarily be low for small sample sizes. In all probability, therefore, we could not detect QTLs of small effect with the sample size of 45 in this study.

To gain insight into the virulence variation between geographically separate spore sources of WGR, we challenged the seedlings with two genetically distinct isolates sampled from lodgepole and jack pine hosts. The average disease scores were 1.80±0.56 and 3.70±0.26 for isolates of lodgepole and jack pine hosts, respectively, suggesting that the isolate of jack pine

host was more virulent than the isolate of lodgepole pine host. This differential response of lodgepole pine seedlings to the two geographic sources of WGR was also revealed in the QTL results. We identified two QTLs (QTL1 and QTL2) that together accounted for 70 % of the phenotypic variation in resistance to the WGR isolate of lodgepole pine host. In contrast, there was one QTL (QTL3) that only explained 22.6 % of the total phenotypic variance in WGR resistance to the isolates of jack pine host. Evidence of fungal specificity when mapping QTL was also reported in loblolly pine (WILCOX *et al.* 1996). This apparent interaction between lodgepole pine and WGR fungus suggests that the variability of virulence should be considered when mapping QTLs for use in marker-assisted selection (MAS) to improve WGR resistance in lodgepole pine.

The successful application of MAS depends on the ability to identify markers closely linked to the genes underlying the QTL. This has been reported on RAPD markers for disease traits in forest trees. DEVEY *et al.* (1995) mapped a marker within 1.0 cM and six markers within 5 cM of the resistance gene to white pine blister rust in sugar pine. In Chinese elm, BENET *et al.* (1995) mapped a marker at a distance of 4.3 cM from the black leaf spot resistance gene. WILCOX *et al.* (1996) mapped a marker within 2 cM of the fusiform rust resistance gene in loblolly pine. In Japanese black pine, KONDO *et al.* (2000) mapped a marker at a distance of 5.1 cM from the pine needle gall midge in Japanese black pine. We report here a region on the framework linkage map 6 in lodgepole pine with five markers linked to WGR resistance genes (Figure 1). One of these, U443_1920, is within 2 cM and 5 cM of the gene or genes that confer resistance to WGR fungal isolates of jack and lodgepole pine hosts, respectively. QTL3 has a support interval at 1.5 LOD that overlapped with that of QTL1 (Figure 1). It is about 3.0 cM left of QTL1. Since the size of our mapping population is only 45 and there were large differences in the disease scores for the two rust sources, it is conceivable that QTL1 and QTL3 is the same QTL. This is because the power to detect a QTL and to estimate its effect depends on the size of the mapping population and the precise measure of the target trait. That one gene or genes in lodgepole pine could increase the resistance to WGR isolates sampled from both lodgepole and jack pine hosts is not unexpected. Lodgepole pine and jack pine are closely related and probably evolved from a common progenitor (CRITCHFIELD 1985; YE *et al.* 2002).

The co-segregation of RAPD markers and quantitative trait loci in our single-tree mapping population is the basis for identifying QTLs that confer resistance to WGR in lodgepole pine. This was pursued within an open-pollinated family where the maternal contribution to the segregating RAPD markers in the offspring was accessible in the saved megagametophytes. Co-segregation between RAPD markers from the seed tree and WGR fungal resistance in the offspring is expressed when the fertilizing pollen-cloud is a random sample of a population in linkage equilibrium, denoting that free recombination of genes is expected regardless of their linkage relationships. With life-history attributes that favors outcrossing, large effective population size and extensive gene flow (YANG & YEH 1995), it is reasonable to believe that the fertilizing pollen-cloud is a random sample of a population in linkage equilibrium. Consequently, deviation from free recombination of genes originates from the seed tree. This strategy to mapping QTLs avoids the generation of mapping population with special cross and is particularly fitting for Alberta because full-sib progenies from control crosses are not available.

Tree improvement of Alberta lodgepole pine is at an early stage and could benefit from MAS because of the long time required to complete a cycle of breeding and selection. The three QTLs mapped in this study should be useful for MAS to increase WGR resistance in lodgepole pine. Experiments are currently undertaken to verify the three QTLs for WGR resistance in independent populations. As well, we are mapping QTLs that confer resistance to WGR in other open-pollinated half-sib families. This is because the occurrence of detected associations could vary between families given that different parents could be heterozygotic in different loci, both pertaining to the RAPD markers and QTLs. Moreover, the linkage phase between RAPD markers and QTL could vary between parents because of different cis-trans configurations.

This is the first study to map WGR resistance QTL in lodgepole pine. Our results indicate that the percentage of the total variation explained by the three QTLs is very high (Table 1). This suggests that effective selection for WRG resistance in lodgepole pine is feasible with a few markers tightly linked to the QTLs. Nevertheless, our study is only the first step in MAS because QTL analysis needs to be validated in independent population and refined to determine the precise locations and effects of the individual QTLs. Finally, the QTLs we identified may also be of use to map the genomic regions that control WGR resistance in related pines. Saturating the QTL regions for disease resistance using additional genetic markers has been successful in pines (HARKINS *et al.* 1998; HAYASHI *et al.* 2001). The addition of reference markers and transforming into sequence characterized amplified region (SCAR) markers of RAPDs tightly linked to the WGR QTLs detected in this study (U443_1920; U690-1495; U680_2255; U428-1260) will facilitate the

identification of causative genes for WGR resistance in lodgepole pine and enable the comparative mapping of QTLs that confer resistance to rust disease among the hard pines.

ACKNOWLEDGEMENTS

This research represents a portion of a dissertation submitted by Changxi Li to the University of Alberta in partial fulfillment of the requirements for the Doctor of Philosophy degree. We thank Dr. Narinder Dhir for providing the seeds, Dr. Yasu Hiratsuka for advice on the inoculation, Dr. Ivan Scotti and an anonymous reviewer for suggestions, and the Natural Sciences and Engineering Research Council of Canada for grants A2282 to Francis Yeh and STR0167526 to Francis Yeh and Yasu Hiratsuka.

REFERENCES

BEAVIS, W. D. 1994: The power and deceit of QTL experiments: lessons from comparative QTL studies. *Proceedings of the Corn and Sorghum Industry Research Conference*. American Seed Trade Association, Washington, D. C. pp. 250–266.

BELLA, I. E. & NAVRATIL, S. 1988: Western gall rust dynamics and impact in young lodgepole pine stands in west-central Alberta. *Can J For Res* **18**:1437–1442.

BENET, H., GURIES, R. O., BOURY, S. & SMALLEY, E. B. 1995: Identification of RAPD markers linked to a black leaf spot resistance gene in Chinese elm. *Theor. Appl. Genet.* **90**: 1068–1073.

BINELLI, G. & BUCCI, G. 1994: A genetic linkage map of *Picea abies* Karst., based on RAPD markers, as a tool in population genetics. *Theor. Appl. Genet.* **88**:238–288.

BLENIS, P. V., PINNELL, H. D. & JOHN, S. E. T. 1993: Stand, family, and rust–resource effects on four attributes of lodgepole pine resistance to western gall rust. *Can. J. For. Res.* **23**:144–150.

BURNES, T. A., BLANCHETTE, R. A., WANG, C.-G. & FRENCH, D. W. 1988: Screening jack pine seedlings for resistance to *Endocronartium harknessii*. *Plant Dis.* **72**:614 –616.

CHEN, F. Q., PREHN, D., HAYES, P. M., MULROONEY, D., COREY, A. & VIVAR, H. 1994: Mapping genes for resistance to barley stripe rust (*Puccinia striformis* f. sp. *Hordei*) *Theor. Appl. Genet.* **88**:216–219.

CHURCHILL, G. A. & DOERGE, R. W. 1994: Empirical threshold values for quantitative trait mapping. *Genetics* **138**:963–971.

CRITCHFIELD, W. B. 1985: The late Quaternary history of lodgepole pine and jack pine. *Can. J. For. Res.* **15**:749 –772.

DEVEY, M. E., DELFINO-MIX, A., KINLOCH, B. B. JR., NEALE, D. B. 1995: Random amplified polymorphic DNA markers tightly linked to a gene for resistance to white pine blister rust in sugar pine. *Proc. Natl. Acad. Sci.* **92**:2066–2070.

ECHT, C. S. & NELSON, C. D. 1997: Linkage mapping and genome length in eastern white pine (*Pinus strobus* L.). *Theor. Appl. Genet.* **94**:1031–1037.

FAZEKAS, A. & YEH, F. C. 2001: Random amplified polymorphic DNA diversity of marginal and central populations in *Pinus contorta* subsp. *latifolia*. *Genome* **44**:13 –22.

FOSTER, A. S. & GIFFORD, E. M. 1974: Comparative morphology of vascular plants. 2nd edn. W. H. Freeman and Company, San Francisco, Calif. pp:382–404.

HARKINS, D. M., JOHNSON, G. N., SKAGGS, P. A., MIX, A. D., DUPPER, G. E., DEVEY, M. E., KINLOCH, JR. B B. & NEALE, D. B. 1998: Saturation mapping of a major gene for resistance to white pine blister rust in sugar pine. *Theor. Appl. Genet.* **97**:1355–1360.

HARRIS, S. A. 1995: Systematics and randomly amplified polymorphic DNA in the genus *Leucaena* (Leguminosae, Mimosoideae). *Pl. Syst. Evol.* **197**:195–208.

HAYASHI, E., KONDO, T., TERADA, K., KURAMOTO, N., GOTO, Y., OKAMURA, M. & KAWASAKI, H. 2001. Linkage map of Japanese black pine based on AFLP and RAPD markers including markers linked to resistance against the pine needle gall midge. *Theor. Appl. Genet.* **102**: 871–875.

HIRATSUKA, Y. 1991: Nuclear cycle, taxonomy, and nomenclature of western gall rust. *In*: Rusts of pine. (eds. Y. Hiratsuka, J. K. Samoil, P. V. Blenis, P. E. Crane, and B. L. Laishley). Proc 3rd IUFRO Rusts of Pine Working Party Conference, Sept.18–22, 1989, Banff, Alberta. Information Report NOR–X–317. Forestry Canada, Northwest Region, North Forest Center, Edmonton, Alberta, pp: 92–101.

HIRATSUKA, Y. & POWELL, J. M. 1976: Pine stem rusts of Canada. Forest Technical Report 4 Environment Canada, Canada Forest Service, North Forest Center, Edmonton, Alberta.

KAYA, Z. & NEALE, D. B. 1995: Utility of random amplified polymorphic DNA (RAPD) markers for linkage mapping in Turkish red pine (*Pinus brutia* Ten.). *Silvae Genet.* **44**: 110–116.

KLEIN, J. I., HIRATSUKA, Y., VESCIO S. & MARUYAMA, P. J. 1991: Disease resistance evaluation of jack pine for western gall rust. *In*: Rusts of pine. (eds. Y. Hiratsuka, J. K. Samoil, P. V. Blenis, P. E. Crane, and B. L. Laishley). Proc 3rd IUFRO Rusts of Pine Working Party Conference, Sept.18–22, 1989, Banff, Alberta. Information Report NOR–X–317. Forestry Canada, Northwest Region, North Forest Center, Edmonton, Alberta, pp: 268–273.

KOJWANG, H. O. & VAN DER KAMP. B. J. 1991: Infection of lodgepole pine clones by single-gall isolates of western gall rust. *In*: Rusts of pine. (eds. Y. Hiratsuka, J. K. Samoil, P. V. Blenis, P. E. Crane, and B. L. Laishley). Proc 3rd IUFRO Rusts of Pine Working Party Conference, Sept. 18–22, 1989, Banff, Alberta. Information Report NOR–X–317. Forestry Canada, Northwest Region, North Forest Center, Edmonton, Alberta, pp: 194–199.

KONDO, T., TERADA, K., HAYASHI, E., KURAMOTO, N., OKAMURA, M. & KAWASAKI, H. 2000: RAPD makers linked to a gene for resistance to pine needle gall midge in Japanese black pine (*Pinus thunbergii*). *Theor. Appl. Genet.* **100**:391–395.

KRUTOVSKII, K. V., VOLLMER, S. S., SORENSEN, F. C., ADAMS, W. T., KNAPP, S. J. & STRAUSS, S. H. 1998: RAPD genome map of Douglas-Fir. *J. Hered.* **89** (3):197–205.

LI, C. & YEH, F. C. 2001: Construction of a framework map in *Pinus contorta* spp. *latofolia* using Random Amplified

Polymorphic DNA markers. *Genome* **44(2)**:147–153.

LI, C., YEH, F. C, & HIRATSUKA, Y. 2001: RAPD variability among geographic isolates of western gall rust fungus, *Endocronartium Harknessii* (J. P. Moore) Y. Hiratsuka in Canada. *Can. J. For. Res.* **31**:1304–1311.

MARTINSSON, O. 1980: Stem rusts in lodgepole pine provenances. *Silvae Genet.* **29**:23–26.

MYRHOLM, C. L. & HIRATSUKA, Y. 1993: A new method for inoculating jack pine seedlings with the western gall rust fungus *Endocronartium harknessii. Can. J. Plant Pathol.* **15**:29–33.

NELSON, C. D., NANCE, W. L. & DOUDRICK R. L. 1993: A partial genetic linkage map of slash pine (*Pinus elliottii* Engelm. var. *elliottii*) based on random amplified polymorphic DNAs. *Theor. Appl. Genet.* **87**: 145–151.

NELSON, C. D., KUBISIAK, T. L., STINE, M. & NANCE, W. L. 1994: A genetic linkage map of longleaf pine (*Pinus palustris* Mill.) based on random amplified polymorphic DNAs. *J. Hered. 85*: 433–439.

NEWCOMBE, G. & BRADSHAW, H. D. JR. 1996: Quantitative trait loci conferring resistance in hybrid poplar to *Septoria populicola*, the cause of leaf spot. *Can. J. For. Res.* **26**:1943–1950.

O'MALLEY, D. M., CRANE, B., MCKEAND, S. E., LIU, B. H. & SEDEROFF, R. R. 1994: Genomic mapping of quantitative traits in loblolly pine. *In*: Proc TAPPI Biological Sciences Symposium. Minneapolis, Minnesota, October 3–6. pp: 173–177.

PETERSON, R. S. 1971: Wave years of infection by western gall rust on pines. *Plant Dis. Rep.* **55**:163–167.

SMITH, J. J., SCOTT-CRAIG, J. S., LEADBETTER, J. R., BUSH, G. L., ROBERTS, D. L. & FULBRIGHT, D. W. 1994: Characterization of random amplified polymorphic DNA (RAPD) products from *Xanthomonas campestris* and some comments on the use of RAPD products in phylogenetic analysis. *Mol. Phyl. Evol.* **3**: 135–145.

TULSIERAM, L. K., GLAUBITZ, J. C., KISS, G. & CARLSON, J. E. 1992: Single tree genetic linkage mapping in conifers using haploid DNA from megagametophytes. *Bio/Technology*, **10**: 686–690.

TUSKAN, G. A. & WALLA, J. A. 1989: Isozyme characterization of *Peridermium harknessii* and *Cronartium quercuum* f. sp. *banksianae* with starch gel electrophoresis. *Phytopathology* **79**:444–448.

VAN DER KAMP, B. J. 1988: Susceptibility of lodgepole pine provenances to geographically separate western gall rust spore sources. *Can. J. For. Res.* **18**:1203–1205.

VAN DER KAMP, B. J. & TAIT, D. E. N. 1990: Variation in disease severity in the lodgepole pine-western gall rust pathosystem. *Phytopathology* **80**:1269–1277.

VAN OOIJEN, J. W. & MALIEPAARD, C. 1996: MAPQTL (tm) version 4.0: Software for the calculation of QTLS positions on the genetic maps. CPRO-DLO, Wageningen, The Netherlands.

WILCOX, P. L. 1995: Genetic dissection of fusiform rust resistance in loblolly pine. Ph.D. Dissertation. University of North Carolina State University, Raleigh. 125 pp.

WILCOX, P. L., AMERSON, H. V., KUHLMAN, E. G., LIU, B.-H., O'MALLEY, D. M. & SEDEROFF, R. R. 1996: Detection of a major gene for resistance to fusiform rust disease in loblolly pine by genomic mapping. *Proc. Natl. Acad. Sci.* **93**:3859–3864.

WU, H. X., YING, C. C. & MUIR, J. A. 1997: Effect of geographic variation and jack pine introgression on disease and insect resistance in lodgepole pine. *Can. J. For. Res.* **26**: 711–726.

YANCHUK, A. D., YEH, F. C. & DANCIK, B. P. 1988: Variation of stem rust resistance in a lodgepole pine provenance-family plantation. *For. Sci.* **34**:1067–1075.

YANG, R.-C. & YEH, F. C. 1995: Patterns of gene flow and geographic structure in *Pinus contorta* Dougl. *For. Genet.* **2**:65–75.

YANG, R.-C., DHIR, N. K., YEH, F. C. & HIRATSUKA, Y. 1997: Geographic variation in susceptibility of Alberta lodgepole pine to western gall rust. *Can. J. For. Res.* **27**: 1398–1405.

YAZDANI, R., YEH, F. C. & RIMSHA, J. 1995: Genomic mapping of *Pinus sylvestris* (L.) using random amplified polymorphic DNA markers. *For. Genet.* **2**(2): 109–116.

YE, T. Z., YANG, R.-C. & YEH, F. C. 2002: Population structure of a lodgepole pine (*Pinus contorta*) and jack pine (*P. banksiana*) complex as revealed by Random Amplified Polymorphic DNA. *Genome* **45**:530–540.

YOUNG, N. D., DANESH, D., MENANCIO-HAUTEA, D. & KUMAR, L. 1993: Mapping oligogenic resistance to powdery mildew in mungbean with RFLPs. *Theor. Appl. Genet.* **87**: 243–249.