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Quantitative trait loci and molecular markers associated with wheat allelopathy

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Abstract Wheat (*Triticum aestivum* L.) has been examined for allelopathic potential against annual ryegrass (*Lolium rigidum*). The bioassay technique, 'equal-compartment-agar-method', was employed to evaluate seedling allelopathy in a doubled-haploid (DH) population derived from cv Sunco (weakly allelopathic) and cv Tasman (strongly allelopathic). A significant difference in allelopathic activity was found among the DH lines, which inhibited the root length of ryegrass across a range from 23.7 to 88.3%. The phenotypic data showed that wheat allelopathic activity was distributed normally within this DH population and a substantial transgressive segregation for seedling allelopathic activity was also found. Analysis of restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) and microsatellite (SSRs) markers identified two major QTLs on chromosome 2B associated with wheat allelopathy. The linkage analysis of genetic markers and the QTLs may improve genetic gains for the allelopathic activity through marker-assisted selection in wheat breeding. The development of wheat allelopathic cultivars could reduce the over-reliance of weed control on synthetic herbicides.

Keywords *Triticum aestivum* · Allelopathy · QTL · Genetic marker · Weed suppression

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Introduction

Farmers have become increasingly reliant on synthetic herbicides for weed control in various conservation farming systems. However, the extensive use of herbicides has resulted in the rapid development of herbicide resistance in weeds. Globally, at least 260 weed biotypes, 156 weeds species, including 94 dicots and 62 monocots, have now been reported to have acquired resistance to important herbicides (Heap 2002). For example, annual ryegrass (*Lolium rigidum* Gaud.), an important weed in Australia, has evolved resistance to six major herbicide classes (Preston et al. 1999). The ineffectiveness of herbicides on resistant weed species, and environmental imperatives, have prompted the search for non-herbicidal innovations to manage weed populations (Wu et al. 1999).

To-date, biological methods are the least exploited area in weed management. The potential for using allelopathy in weed management has been well documented (Rice 1995). The application of crop allelopathy in weed suppression involves two crop growth stages, i.e. the vegetative stage and the post-harvest stage. At the vegetative growth stage, crop-seedling allelopathy could be exploited to suppress weeds. At the post-harvest stage, crop-residue allelopathy could be used for weed suppression, especially during the establishment period of the following crop. Wu et al. (1998) found that wheat residue allelopathy differed significantly on the suppression of annual ryegrass among accessions. Further study showed that root exudates of wheat seedlings inhibited root growth of annual ryegrass over a range from 10% to 91% in a worldwide collection of 453 wheat accessions, and the allelopathic activity of wheat seedlings on ryegrass was not correlated with wheat competitive parameters, such as the plant height and root length of wheat seedlings (Wu et al. 2000a).

The development of allelopathic crop cultivars for weed suppression has been increasingly recognised as one of the possible components in integrated weed management (Wu et al. 1999; Olofsdotter et al. 2002). The use of crop cultivars with elevated allelopathic activity could reduce the need for commercial herbicides to early season application, with late-season weed control provided by the heightened advantages of crop competitiveness.

However, the development of allelopathic crop cultivars has been greatly hindered by the limited knowledge about the genetic control of crop allelopathy. Recent research has generally shown that allelopathic activity is quantitatively inherited in rice and wheat (Dilday et al. 1998; Wu et al. 2000a). Wu et al. (2000a) found that wheat-seedling allelopathy on *L. rigidum* was normally distributed in the collection of 453 wheat accessions, indicating that this weed-suppressing ability is a quantitative trait. The genetic control of allelopathic activity was further studied using near-isogenic wheat lines derived from Hartog (weakly allelopathic) × Janz (strongly allelopathic). The allelopathic activity of BC_2-Hartog lines (backcrossed to Hartog) was weak, similar to that of Hartog. Janz lines had strong allelopathic activity, similar to that of Janz. These results suggested that there is a strong genetic basis in conferring allelopathic activity.

Recently, molecular techniques have been employed to investigate the genetic markers associated with rice allelopathic activity (Ebana et al. 2001; Jensen et al. 2001). Jensen et al. (2001) investigated the quantitative trait loci (QTLs) of rice-allelopathy in a population of 142 recombinant inbred lines derived from a cross between IAC 165 (*japonica* upland variety) and CO 39 (*indica* irrigated variety). IAC 165 was strongly allelopathic against barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.], whereas CO 39 was weakly allelopathic. Of 140 genetic markers examined, four QTLs were identified on three chromosomes, which collectively explained 35% of the total phenotypic variation of the allelopathic activity in the population (Jensen et al. 2001). In a similar study with RFLP analysis, Ebana et al. (2001) identified seven QTLs associated with rice allelopathy on six chromosomes, explaining the phenotypic variation in the range from 9.4% to 16.1%.

No QTL studies of wheat allelopathy have been reported. The aim of this study was to identify genetic markers (QTLs) conferring wheat allelopathic activity using a doubled-haploid population of wheat derived from Sunco×Tasman.

Materials and methods

Plant material

The doubled-haploid (DH) wheat population of Sunco×Tasman, consisting of 271 DH lines, was produced using the wheat × maize-derived DH production technique from F_1 plants by Kammholz et al. (2001), and kindly supplied by Mr. John Sheppard of Leslie Research Centre, Queensland Department of Plant Industries, Australia.

Map construction

A subset of 189 DH lines and the two parents were subject to genetic-marker analysis. The construction of a genetic map of this population has been explained in detail (Chalmers et al. 2001), which consisted of 363 genetic markers covering all 21 wheat chromosomes.

Evaluation of wheat allelopathic activity

The 271 DH lines and the two parental lines were phenotyped for allelopathic potential on the growth of annual ryegrass by the Equal-Compartment-Agar-Method described previously (Wu et al. 2000b). Briefly, 12 germinated wheat seeds (surface-sterilized) of each accession were uniformly selected and aseptically sown on an agar surface in three rows on one-half of a glass beaker pre-filled with 30 ml of 0.3% water-agar (no nutrients). The beaker was covered with a piece of parafilm and placed in a controlled growth cabinet with a daily light/dark cycle of 13 h/11 h and a temperature cycle at 25°C/13°C. After growing the wheat seedlings for 7 days, 12 germinated seeds of ryegrass were sown on the other half of the agar surface in three rows. A piece of pre-autoclaved white paperboard was inserted across the centre and down the middle of the beaker with the lower edge of the paperboard kept 1 cm above the agar surface. The entire beaker was thereby divided into two equal compartments, each occupied separately by wheat and ryegrass seedlings. The beaker was again covered with parafilm and placed back in the growth cabinet for 10-more days. Ryegrass was grown alone as a control. After 10 days of co-growth of ryegrass with wheat, the longest root lengths of the ryegrass seedlings were measured. A randomized complete block design with three replicates was used. The mean value of the three replicates was taken to conduct QTL analysis.

Statistical analysis

Experimental data were subjected to the analysis of variance using Genstat 5 Release 3.2 (1995) and the treatment means were tested separately for (l.s.d.) at P=0.05 or 0.01 where appropriate. Percentage of the inhibition on root length of ryegrass was calculated as (control−raw data)/control*100. Transgressive segregation among DH lines, Sunco and Tasman, was tested by the l.s.d. test at P=0.05. Where the mean trait value for a line exceeded that of the nearest parent, it was considered a transgressive segregant.

Mapmanager (QTXb15, Manly et al. 2001) and Windows version QTL Cartographer (Basten et al. 1994, 2002) were used for QTL analysis with a significance threshold of LOD=3. With composite interval mapping, Model 6 (Basten et al. 2002) was used for background marker selection with a 2-cM walking speed and 10-cM window size.

Results

The parental lines, Sunco and Tasman, differed significantly (P<0.05) in their allelopathic activities against annual ryegrass, giving a root length of ryegrass of 18.4 mm and 6.9 mm, respectively. This result revealed that Tasman was more allelopathic than Sunco. Phenotyping results showed that there was a high variation among the 271 DH lines in their allelopathic activities, inhibiting the root length of ryegrass across a range from 23.7 to 88.3%. There was substantial transgressive

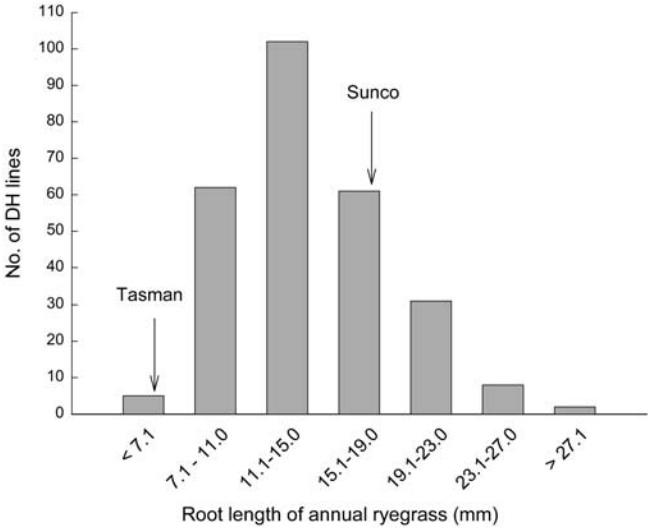

Fig. 1 Histogram of distribution for allelopathic activity in the 271 doubled-haploid lines derived from Sunco×Tasman. Parental means for Sunco and Tasman are indicated

segregation for seedling allelopathic activity against ryegrass within the Sunco×Tasman DH population. A normal distribution of wheat allelopathic activity was found in this DH population (Fig. 1, Skewness=0.609, Kurtosis=0.593), suggesting that more than one gene is likely to be affecting genetic expression of the allelopathic trait. The DH population resulted in a mean value of ryegrass root length of 14.3 mm, which is not significantly different to the mid-parent value of 12.7 mm. Of the 271 DH lines screened, five lines were strongly allelopathic, giving a root length of ryegrass of less than 7.0 mm, in comparison to the nil-wheat control of 37.3 mm. Ten DH lines were weakly allelopathic, giving a root length of ryegrass of more than 23.0 mm.

Mapmanager was used initially to locate QTLs. The results revealed that the significant interval covers nearly all regions of chromosome 2B and most markers on this chromosome met the LOD=3 criterion (Fig. 2). No markers from other chromosomes were found significant at the LOD=3 level.

Regression based on simple interval mapping revealed that the marker P32/M48-316 on chromosome 2B accounted for 29% of the total variance with LOD 14.7. Analysis using the composite interval mapping-approach indicated that at least two QTLs were identified on chromosome 2B, which had opposite genetic effects (Fig. 3). The first QTL at the location around the fifth marker position from the left, i.e. the marker P32/M48-316 position (Chalmers et al. 2001), was donated by the parent Tasman, and had a LOD score of 4.4; while the second QTL at the location around the eighth marker position, i.e. the marker P32/M48-93 position (Chalmers et al. 2001), came from the parent Sunco with a LOD value of 3.6.

Discussion

Crops have been made resistant to insects, pathogens and herbicides with transgenes, but biotechnology has not produced crops that control weeds with allelochemicals. Duke et al. (2001) proposed two strategies for producing allelopathic crops by biotechnology: (1) enhancement of existing allelopathic potential, and (2) insertion of genes to produce allelochemicals that are not found in the crop. So far, however, attempts have not yet been made to develop allelopathic cultivars due to the little understanding of the mechanism and genetic control of crop allelopathy.

The present research has identified two major QTLs on chromosome 2B associated with wheat-seedling allelop-

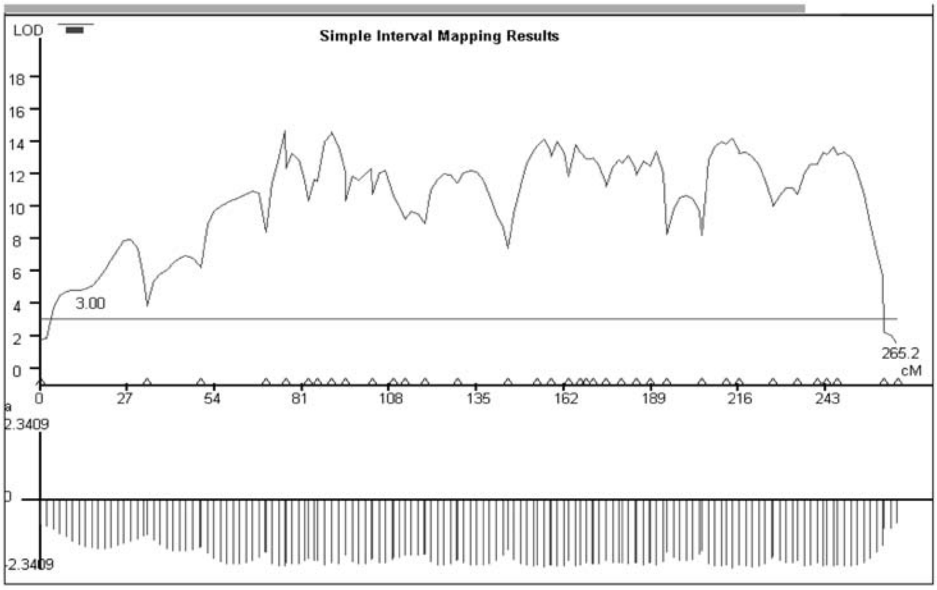

Fig. 2 Simple interval mapping on chromosome 2B

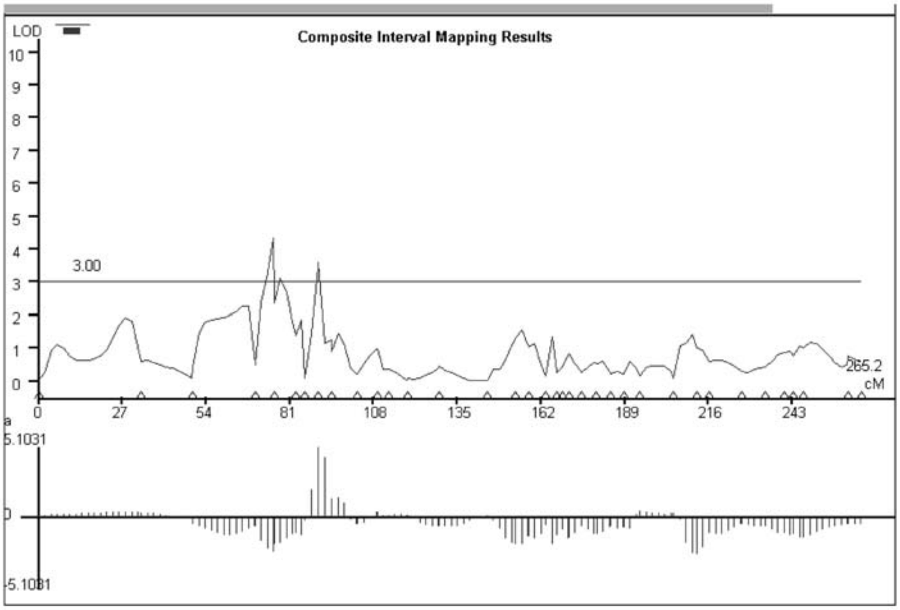

Fig. 3 Composite interval mapping on chromosome 2B

athy. Composite interval mapping indicated that there was a QTL cluster on the 2B chromosome, and this cluster completely masked the genetic effect of the second QTL inherited from the parent Sunco. The genetic effects of the two QTLs detected by composite interval mapping were *in trans* (Darvasi and Pisanté-Shalom 2002). A number of sections of chromosome 2B had notable genetic effects on the allelopathic activity and were inherited from the parent Tasman even though the LOD values of these sections were below 3.0 (Fig. 3). The *cis* effects of these chromosome-2B sections exaggerated the genetic effects from Tasman, thereby masking the effects of the second QTL from Sunco (Fig. 2). The use of composite interval mapping unravelled the masks implied on the Sunco-related QTL and gave more-detailed and accurate information of QTL effects and positions on chromosome 2B. These results demonstrate the weakness of simple interval mapping in dealing with more than one QTL on a chromosome (Zeng 1993) and the power of composite interval mapping in dissecting multiple QTLs on one chromosome (Zeng 1993, 1994).

The two QTLs on chromosome 2B had relatively high significance; it is therefore necessary to further validate these two QTLs over a range of genetic backgrounds. Once validated, the robust genetic markers will then be able to be implemented in wheat breeding-programs to select allelopathic wheat cultivars via marker-assisted selection.

It is worth noting that a complicated genetic control was revealed. No QTLs detected were able to account for the relatively big phenotypic differences in allelopathic activity between Sunco and Tasman. The two QTLs detected on 2B were inherited from different parents and had similar genetic scopes. There were a number of chromosome regions which had LOD values above 1.5 and their genetic effects were inherited from the Tasman parent. The genetic effects associated with these chromosome regions may explain that Tasman has a much higher allelopathic activity than Sunco. Since the genetic population used in this study is relatively small and can not reliably detect small effect QTLs (Beavis 1994, 1998; Melchinger 1998), these putative QTLs with small effects were not reported in this study.

It is unrealistic to expect that the use of allelopathic cultivars will completely kill weeds in the field. Wu et al. (2002) found that allelochemicals exuded by the living roots of young wheat seedlings were not present in concentrations high enough to kill weed plants; however, wheat root exudates significantly suppressed the root growth of annual ryegrass up to 91% in controlled experiments. The use of allelopathic cultivars could therefore have long-term effects on weed ecology. It could gradually reduce the number of weed seeds in the seed bank and weed population. Duke et al. (2001) claimed that even if allelopathic traits could only marginally reduce herbicide use or tillage, the monetary savings to farmers over time would be significant, and the reduced environmental impact would be highly desirable.

This present study on the genetic markers associated with allelopathic activity was performed using the existing doubled-haploid mapping population Sunco× Tasman, initially developed for studying wheat quality traits, such as flour colour and texture (Kammholz 2001). Although cv Tasman was strongly allelopathic, cv Sunco belonged to the intermediate allelopathic groups (Wu et al. 2000a). To verify these initial results on the genetic control of wheat allelopathy, it is necessary to construct new mapping populations derived from two extreme allelopathic groups, i.e. the strongly allelopathic and weakly allelopathic groups reported previously (Wu et al. 2000a). Wheat accessions, such as Tasman, Khapli, Wattines, AUS# 12627, Triller, SST 6, AUS# 18060, Tunis 2, AUS# 18056 and Meering, were strongly allelopathic, while the accessions Canada 3740, AUS# 12788, Sunstate, RAC 710, Excalibur, Afghanistan 19, L 1512-2721, HY-65, Canada 51 and PF 8716 were weakly allelopathic. Wu et al. (2002) further reported that there were significant differences in the chemical profiles between these two allelopathic groups, with strongly allelopathic accessions producing significantly higher amounts of allelochemicals in the shoots and roots of wheat seedlings, and also exuding larger quantities of allelochemicals into the growth medium. These wheat accessions could be good genetic materials for future studies on the genetics of wheat allelopathy and the identification of genetic markers governing the production and exudation of responsible allelochemicals.

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