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Identification of Microsatellite Markers Linked to Photoperiod Insensitive Gene *Ppd-D1a* in Wheat

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ABSTRACT To facilitate breeding of lines with either the *Ppd-D1a* or *ppd-d1a*, we screened 342 F_2 progenies from a cross between Laura (photoperiod insensitive, *Ppd-D1a*) spring wheat and SWP5304 (photoperiod sensitive, *ppd-d1a*) for their time to heading under 10 hour day length, and with a set of 37 microsatellite primers previously mapped to chromosome 2D. Bulk segregant analysis was used to identify four linked microsatellite loci. The *Ppd-D1a* locus was flanked by *Xgwm484* with 13.7 cM distance and *Xgwm455* with 27 cM. These markers may be useful in selection of the desired photoperiod sensitivity in segregating populations grown in Northern latitude.

Keywords : wheat, photoperiod, SSR, Ppd gene, MAS

Wheat is adapted to a variety of environmental conditions and is grown world-wide partially due to a range of photoperiod response. In general photoperiod sensitive genotypes requiring long days to flower may be advantageous in northern regions, while insensitive types are necessary in more southerly wheat growing areas. Winter and spring wheats growing in more northern latitudes tend to be photoperiod sensitive, while insensitive genotypes tend to grow in southern latitudes (Scarth and Law, 1985). The dominant alleles *Ppd-A1a*, *Ppd-B1a* and *Ppd-D1a* on chromosomse 2A, 2B and 2D respectively confer insensitivity to photoperiod, while the recessive counterparts confer sensitivity (Whitechurch and Slafer, 2002). The photoperiod insensitive genes allow wheat to flower under short day conditions and generally shorten the days to heading. Also, the photoperiod genes have been reported to have a variety of pleiotropic effects e.g. on low-temperature tolerance (Mahfoozi *et al.*, 2001), plant structure (i.e. height, tillering, spikelet number, rate of spikelet initiation and spikelet fertility) (Worland *et al.*, 1998; Miralles and Richards, 2000), and vernalization (Brooking and Jamieson, 2002). Many of these traits as well as others have a direct influence on yield (Borner *et al.*, 1993). Unfortunately for the breeder, the effect on yield is not simple and is highly dependent on interactions between genotypes and environment (Worland *et al.*, 1998). Another confounding factor is that different alleles have different pleiotropic effects (Worland *et al.*, 1998; Whitechurch and Slafer, 2002). Thus, breeders need to select genotypes based on given environments and on the decision to breed widely or locally adapted varieties.

Phenotypic selection of appropriate photoperiod genotypes is difficult due to genetic and environmental interactions. Under 12-hour day length, the separation of sensitive and insensitive lines is marked. However, under normal field growing conditions in northern latitudes the difference in heading date between photosensitive and insensitive is variable depending on date of planting and other genetic and environmental factors, and is compressed to just a few days difference at best. Thus, selection in the field among segregating individuals is difficult. Photoperiod response is a good candidate for marker assisted selection (MAS) if linked molecular markers are identified. The object of this study was to identify markers linked to photoperiod gene, *Ppd-D1a*.

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MATERIALS AND METHODS

Plant Materials

A total of 348 F_2 wheat seeds from a cross between a photo-sensitive variety (SWP5304) as a female parent and a photo-insensitive variety (Laura) as a male parent segregating for *Ppd-D1a* and *ppd-d1a* were provided by the Montana State University, Department of Plant Sciences and Plant Pathology in Bozeman, Montana. Photoperiod sensitivity was determined by growing individual F_2 plants growing in containers with 10 hour days at 22℃ and 18℃ nights. Spike emergence was scored daily starting at 50 days after planting.

Bulk segregant analysis (BSA)

Molecular markers that had been previously mapped on chromosome 2D were screened using bulked segregant analysis of F_2 population. Three photosensitive bulks and three photo-insensitive bulks were created by combining equal amounts of DNA from 6 photosensitive F_2 plants and from 6 photo-insensitive F_2 plants, respectively. Thus, a total of 36 DNA samples were included in the six bulks.

Microsatellite analysis

The wheat microsatellite markers including 17 *Xgwm* primers (Roder *et al.*, 1998), 10 *Xgdm* primers (Pestova *et al.*, 2000), and 7 *barc* primers (USDA-ARS and U.S. Wheat Barley Scab Initiative) were screened. The PCR condition was followed as described by Roder *et al.*, (1998). Once a polymorphism was identified between photosensitive and photoinsensitive bulks, the polymorphic microsatellite marker was applied to the complete F_2 population.

Statistical analysis

Recombinational frequencies and linkage relationships were determined for 4 microsatellite markers and heading date. The linkage maps were constructed using G-Mendel software (Liu and Knapp, 1990) at LOD>3.0 with the Kosambi function. We created a similar map using MapMaker (Lander *et al.*, 1990).

RESULTS AND DISCUSSION

Spike emergence phenotyping

The heading dates among F_2 progeny varied from 53 to 177 days (Fig. 1). At this time sixty-one individuals had still not flowered, and the experiment was terminated. It was difficult to group individuals into the homozygous dominant insensitives class and the heterozygous class, because the heading dates of insensitive plants ranged between 53 days and 92 days without break. The sensitive types all had heading dates of greater than 120 days.

Microsatellite markers linked to *Ppd-D1a*

Of thity-four microsatellite markers screened, four were polymorphic between the photoperiod insensitive and sensi-

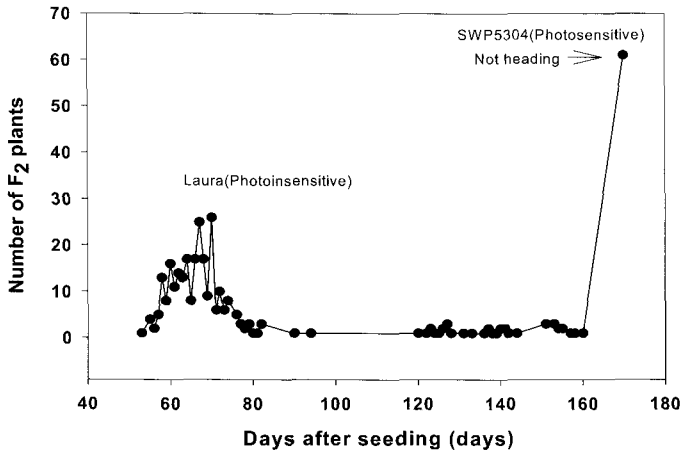
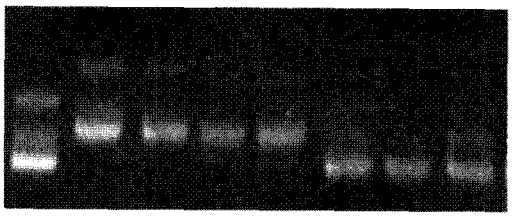


Fig. 1. Frequency distribution of heading days after seeding in F_2 plants from a cross between photo-sensitive (SWP5304) and photo-insensitive (Laura) wheats.

tive bulks. Markers *Xgwm484* (Fig. 2), *Xgwm455* (Fig. 3), and *Xgdm5* (Fig. 4) were codominant. Marker *Xgwm296* (Fig. 5) was a dominant marker with a band found in the sensitive bulks. No significant segregation distortion was

I. Bulked segregant assay



II. F_2 segregant assay

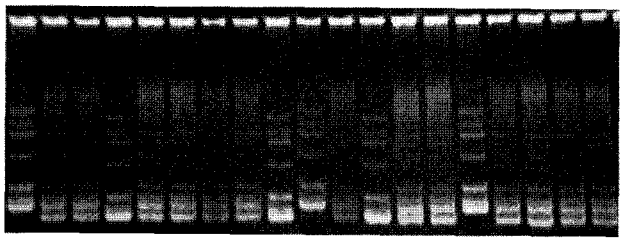
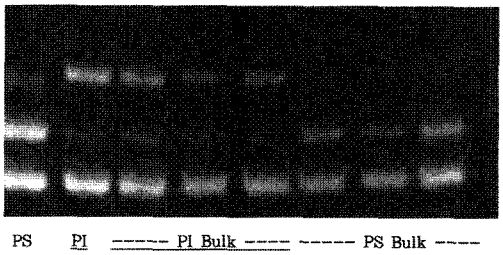


Fig. 2. DNA bands amplified from the photo-sensitive and photo-insensitive parents, three photo-insensitive bulks, three photo-sensitive bulks and F_2 progenies using the microsatellite marker *Xgwm484* : PS=Photo-sensitive, PI=Photo-insensitive, H=heterozygous F_2 plants.

I. Bulked segregant assay



II. F_2 segregant assay

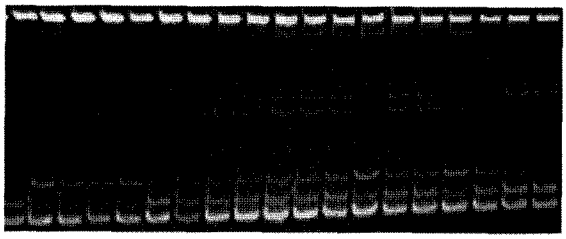
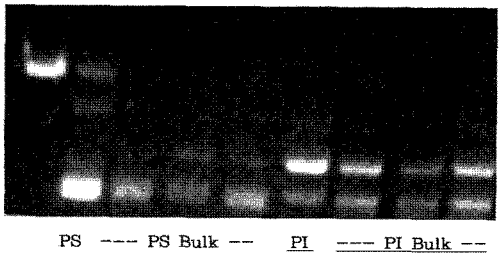


Fig. 3. DNA bands amplified from the photo-sensitive and photo-insensitive parents, three photo-insensitive bulks, three photo-sensitive bulks and F_2 progenies using the microsatellite marker *Xgwm455* : PS=Photo-sensitive, PI=Photo-insensitive, H=heterozygous F_2 plants.

I. Bulked segregant assay



II. F_2 segregant assay

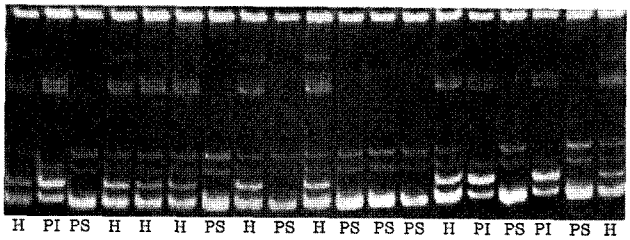
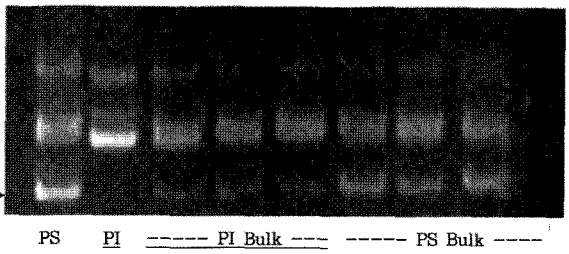


Fig. 4. DNA bands amplified from the photo-sensitive and photo-insensitive parents, three photo-insensitive bulks, three photo-sensitive bulks and F_2 progenies using the microsatellite marker *Xgdm5* : PS=Photo-sensitive, PI= Photo-insensitive, H=heterozygous F_2 plants.

I. Bulked segregant assay



II. F_2 segregant assay

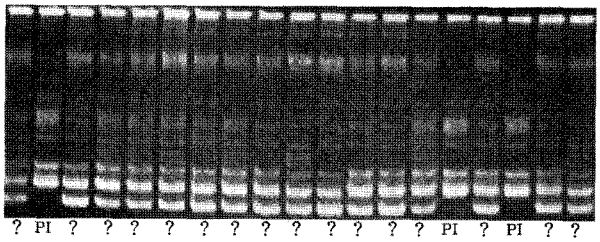


Fig. 5. DNA bands amplified from the photo-sensitive and, photo-insensitive parents, three photo-insensitive bulks, three photo-sensitive bulks, and F_2 progenies using the microsatellite marker *Xgwm296* : PI=Photo-insensitive, ?=Photo-sensitive or heterozygous F_2 plants.

Table 1. Segregation analyses for the *Ppd-D1a* gene and linked microsatellite markers in F_2 population from a cross between photo-sensitive (female) and photo-insensitive (male) wheats.

Gene or marker	No. of F_2 plants	No. of Observed†			Expected ratio	χ^2	P‡
		PP	Pp	pp			
Ppd-D1a	348		256	92	3 : 1	0.383	0.5<P<0.9
Xgwm 484	347	81	175	91	1 : 2 : 1	0.602	0.5<P<0.9
Xgwm 455	348	74	181	93	1 : 2 : 1	2.638	0.1<P<0.5
Xgdm 5	342	79	171	92	1 : 2 : 1	0.988	0.5<P<0.9
Xgwm 296	345	77	268		1 : 3	1.323	0.1<P<0.5

†Phenotype or genotype : PP=homozygous photoinsensitive. Pp=heterozygou, pp=homozygous photosensitive.
‡P>0.05=fit to the expected segregation ratio of the F_2 population.

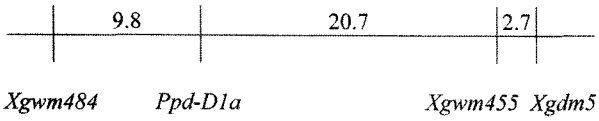


Fig. 6. Linkage map for *Ppd-D1a* on the basis of wheat microsatellite markers on 2D

observed for any of the loci when screened using the entire F_2 population (Table 1). All 5 loci were linked, with heading date flanked by *Xgwm484* and *Xgwm455* as shown on linkage map 2D (Fig. 6). The recombination frequency (RF) between *Xgwm484* and heading date was 13.6% with a Kosambi map distance of 13.9 cM. The RF between heading date and *Xgwm455* was 24.6% with a map distance of 27 cM. *Xgwm455*, *Xgdm5* and *Xgwm296* were tightly linked with map distances between markers of 2.7 cM and 1.9 cM, respectively. These markers have been mapped to similar positions on the 2D chromosomes (Roder *et al.*, 1998, Pestsova *et al.*, 2000).

Molecular markers could be used to classify individuals into three classes. Individuals with homozygous bands derived from the photo-insensitive parent, SWP5304, for *Xgwm484* and *Xgwm455* alleles were classified into homozygous dominant, individuals with heterozygous bands were classified into heterozygous, and the others with homozygous bands derived from the sensitive parent, Laura, were classified into homozygous recessive (Fig. 2 & 3). Recombinant individuals were not included in this classification. The mean heading dates for the F_2 individuals classified as homozygous for photo-insensitive alleles, heterozygous at both loci, and homozygous for photo-sensitive alleles were 61.7, 81.8, and 162.2, respectively. The heading date means for the three classes were significantly different at the P< 0001. Although rare misclassification may have occurred due to double crossovers, the differences between the means were so great that it would be immaterial. The fact that significantly more days to heading were observed in the heterozygous class than the homozygous dominant class may indicate that *Ppd-D1a* does not have complete dominance.

An ideal result would have been to identify microsatellite markers more closely linked to the *Ppd-D1a* locus. However, since two of the markers are flanking, they can be used in MAS. If the selection accuracy of using either *xgwm484* or *xgwm455* is 86.4% and 75.4% respectively, then the selection accuracy using both markers would be 96.7% (Liu *et al.*, 2002). Thus, use of the identified microsatellite markers could be helpful in selection for photoperiod response lines in segregating populations grown under long day conditions.

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