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Genetic analysis of preharvest sprouting tolerance in three wheat crosses

W. R. Lawson[AC], *I. D. Godwin*[AD], *M. Cooper*[A], *and P. S. Brennan*[B]

[A] Department of Agriculture, The University of Queensland, St Lucia, Qld 4072, Australia.
[B] Queensland Wheat Research Institute, Queensland Department of Primary Industries, PO Box 2282, Toowoomba, Qld 4350, Australia.
[C] Present address: Queensland Agricultural Biotechnology Centre, Queensland Department of Primary Industries, Level 4, Gehrmann Laboratories, The University of Queensland, St Lucia, Qld 4072, Australia.
[D] Corresponding author.

Abstract. Three recombinant inbred populations were assessed for tolerance to preharvest sprouting (PHS). Genetic analysis of the PHS scores, as assessed under artificial rain treatment, indicated that for 2 of the populations, tolerance to sprouting was simply inherited and was controlled by 2 independent genes, both of which are necessary for full tolerance. The data presented here show that in these 2 populations the trait is highly heritable under controlled environment situations. It was also demonstrated that the red seed colour gene, derived from Aus1490 and traditionally associated with tolerance, is not necessary for full tolerance to sprouting, although indirect selection for preharvest sprouting tolerance can be performed very effectively by selecting for red grain. The presence of white-seeded lines, recovered from this cross with a red-seeded donor of PHS tolerance, that are at least as tolerant as the most tolerant red-seeded individuals demonstrates that red-seeded donors of PHS tolerance should not be discarded for improvement of this trait.

Additional keywords: Triticum aestivum.

Introduction

The susceptibility of cereal crops in southern Queensland and northern New South Wales to germination in preharvest rains can dramatically reduce crop quality and yield. Preharvest sprouting (PHS) of the cereal grain leads to a weakening of dough strength owing to poor flour attributes, and as a consequence, there is a deterioration of end-product quality. Consumer demand for high quality products, especially in export markets, emphasises the problem posed by PHS sprouting. Traditionally, strong tolerance to sprouting has been closely associated with the presence of the red seed coat gene (Gfeller and Svejda 1960; Gale 1989). However, market trends show preferences for white-grained wheats, especially for bread and noodle products, and therefore, breeding of white-seeded PHS-tolerant cultivars has been an objective of wheat-breeding programs in northern NSW and southern Qld.

Numerous cultivars of both red- and white-grained wheat grown in Australia have been evaluated as potential sources of PHS tolerance in extensive studies by Mares (1987) and Henry and Brennan (1988), and breeding programs at the Queensland Wheat Research Institute, Toowoomba, are currently involved in transferring the sources of tolerance identified into adapted white-grained Australian varieties.

Screening for PHS tolerance is difficult owing to the low heritability of the trait and its tendency to be expressed as a quantitatively inherited character. Further, the PHS phenotype is complex in that the genes involved may be expressed in any one of 3 distinct tissues, the maternal plant, the endosperm, and the embryo, of which the latter 2 belong genetically to the next generation (Gale 1989). Thus, selection on phenotype alone in the F_2 is not possible.

The screening procedure currently in use at the Queensland Wheat Research Institute is designed to assess variation to sprouting in intact heads. Usually, heads of F_2 individuals contain seeds that are segregating. Therefore, in these individuals, all possible genotypes may be expressed. If seed in one head

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alone is segregating, then the mean estimate of PHS tolerance in that head is not an accurate assessment of the level of tolerance in that line or lines derived from that head. Recombinant inbred (RI) lines have been developed by selfing over a number of generations; therefore, seeds in one head have a high probability of being homozygous. Consequently, any estimate of PHS from one such head will be more indicative of the true genotype expected for that line and its progeny.

In this study, 3 wheat RI populations were characterised for their variation in tolerance to PHS under an artificial rain simulation treatment (McMaster and Derera 1975). Intact ears from the RI lines were numerically ranked for tolerance to sprouting. The heritability of the trait was determined for each population and a genetic analysis of the trait was conducted.

Materials and methods

Genetic material

Three RI populations of bread wheat, segregating for PHS tolerance, were obtained from the Queensland Wheat Research Institute (QWRI), Toowoomba. Each population was generated by a biparental cross between cultivars that contrasted for PHS tolerance. The susceptible cultivar Hartog was crossed with the PHS-tolerant lines Transvaal, Chile 59, and Aus1490. The resistant parents were chosen on the basis of their sprouting tolerance and low α-amylase production from studies conducted by Mares (1987) and Henry and Brennan (1988). Populations were inbred to F_7 by using single-seed descent, with a minimum of 50 lines produced for each population. The populations were designated P1 (Hartog × Transvaal), P2 (Hartog × Chile 59), and P3 (Hartog × Aus1490). Aus1490 has a single gene for red grain colour and the other 2 cultivars are white-grained (Mares and Ellison 1990).

Field design

The 3 populations were evaluated for their levels of sprouting tolerance in a field experiment at QWRI in June–December 1992. The parents and RI lines were evaluated in a randomised complete block design with 5 replicates for each entry, including the parents. Each plot consisted of 5 plants. The plots were arranged in an unbordered hill plot design. For each population, the 2 parents and 50 RI lines, chosen at random, were evaluated. Rainout shelters were erected prior to tillering to prevent any damage due to rainfall.

Evaluation for preharvest sprouting

At physiological maturity, indicated by the loss of green pigment from the spike, intact spikes were hand-harvested from the primary tillers. Loss of green colour normally occurs within 1 or 2 days of maximum grain dry weight (Hanft and Wych 1982). From each plot, 10 heads, uniform in size and maturity, were harvested by cutting the peduncle approximately 10 cm below the base of the spike. Spikes were then stored in dry, ambient conditions for 14 days before assessment by artificial rain simulation (McMaster and Derera 1975).

The 10 heads from each plot were arranged in trays by inserting the peduncles of each spike into holes drilled into steel trays. Prior to being placed into the rain simulator, each completed tray was sprayed to saturation point with Benlate (1 g/L; active ingredient benomyl, 500 mg/L) to prevent fungal contamination in the moist conditions. Artificial rain was provided by misting for 30 s every 60 min throughout the experiment (7 days). Temperature was maintained at 20±1°C.

Each spike was assessed for evidence of sprouting on Days 5 and 7 of the artificial rain treatment by using a scoring system based on that developed by McMaster and Derera (1975). A score of 1 indicated no visible sprouting over the spike, and a score of 10 indicated extensive sprouting where roots covered at least 75% of the spike and the coleoptiles were entering the first leaf stage. Any spikes exhibiting fungal contamination were discarded. Scored trays were returned to the growth chamber for a further 2 days after the Day 5 assessment. When a mean score of $\geq$5 was obtained for each sample of 10 heads on Day 5 of wetting, that line was considered to be non-tolerant and discarded. Such lines were assumed to have the maximum sprouting score of 10 at Day 7.

Data analysis

Analysis of variance was used to partition the phenotypic variation into components resulting from lines, experimental error, environmental variation, and sampling variance (variation within plots). The line mean heritability (h^2) under the artificial screen was estimated using the following equation:

$$h^2 = \sigma^2_L/[\sigma^2_L + (\sigma^2_\epsilon/n_r) + (\sigma^2_S/n_r n_s)]$$

where σ^2_L is the variance component for lines, σ^2_ϵ is the variance component for experimental error, σ^2_S is the sampling variance component, n_r is the number of replicates, and n_s is the number of samples or heads sampled per replicate.

Test of the genetic model

Where populations of individuals represent overlapping mixtures of different subpopulations, it is difficult to allocate individuals objectively to the correct subpopulations. This is the situation commonly encountered in analysis of genetic segregation ratios. The mixture method of clustering was developed by Basford and McLachlan (1985) and McLachlan and Basford (1988) to address the problem of objectively allocating individuals to underlying mixtures of subpopulations with normal distributions. As such, it is an appropriate method for objectively partitioning individuals in segregating generations into groups of individuals to enable a test of genetic models. The mixture method of clustering was applied to the data from this study. Genetic models, based on 1 or 2 genes, were tested for the 3 RI populations segregating for PHS tolerance, following the procedures used by Basnayake *et al.* (1995). For each population, the number of individuals in each group was identified, for both scoring days, by clustering the individuals on their PHS scores. The distribution of individuals among the groups was tested for goodness-of-fit to the expected segregation ratios of 1- and 2-gene models using the chi-square (χ^2) test. The lines were classified into 'tolerant', 'partially tolerant', and 'susceptible' groups. These terms indicated that there was 'little or no sprouting', 'an intermediate response' to sprouting, and 'a high degree of sprouting damage', respec-

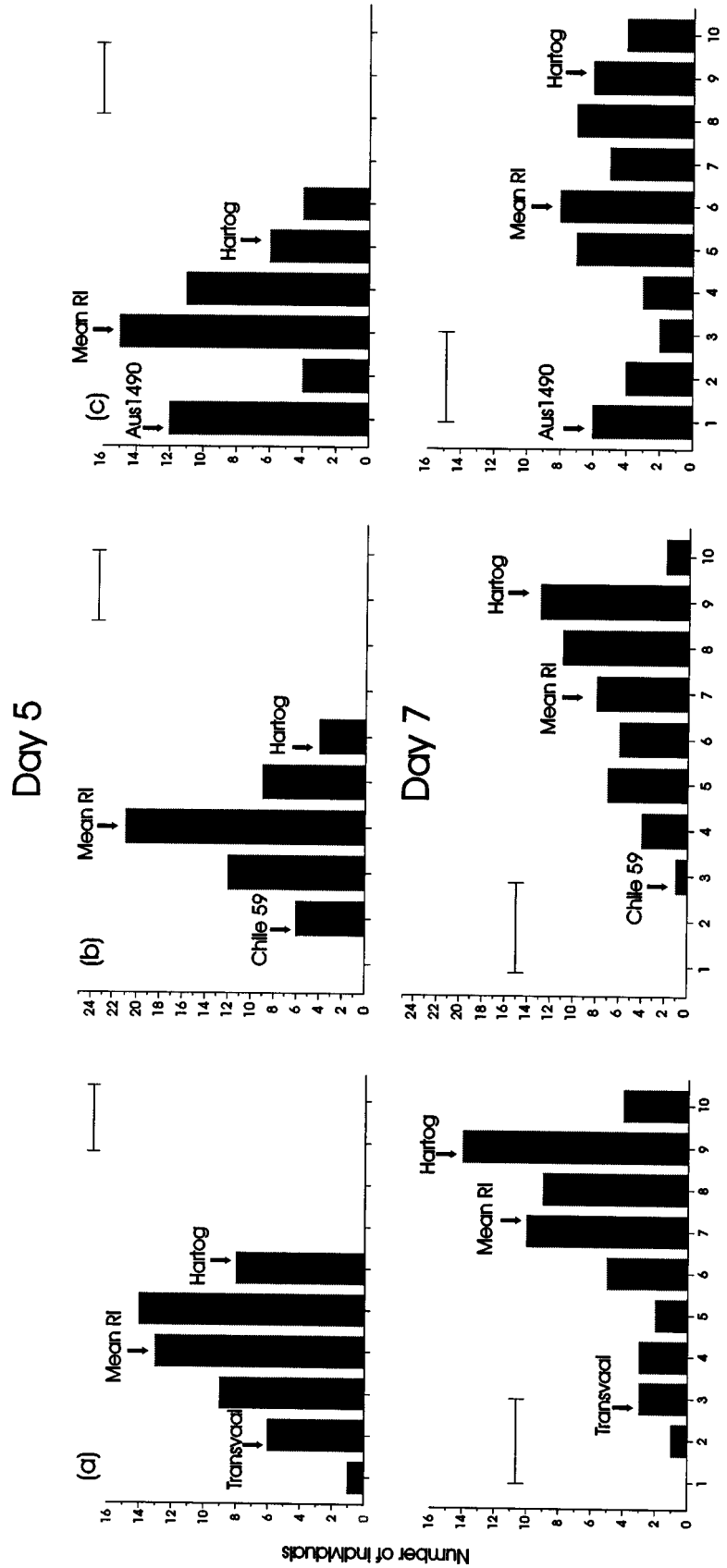

Fig. 1. Distribution of individuals in (*a*) Population 1 (Hartog × Transvaal), (*b*) Population 2 (Hartog × Chile 59), and (*c*) Population 3 (Hartog × Aus1490), according to their mean preharvest sprouting score. Horizontal bars represent l.s.d. at $P = 0{\cdot}05$.

tively. The scores differed depending on genotype and the day of scoring. The conversion of the numerical scores to the descriptive terms 'tolerant', 'partly tolerant', and 'susceptible' depended on both the cross under consideration and the day of testing, as explained fully under the *Genetic analysis* section of **Results**.

Seed colour test

To assess segregation for seed colour in the Aus1490 population, 3–5 seeds from each of the RI lines derived from the cross between Hartog and Aus1490, as well as the parents, were soaked in approximately 1 mL of 5% NaOH for 30–45 min. After the incubation period the parental seeds were used as references and the RI seeds were visually scored for 'red' or 'white' colour pericarp.

Results

Sprouting tolerance in parental lines and RI populations

All replicates of the parental lines were scored for their response to sprouting on Days 5 and 7 of wetting. Each resistant parent (Transvaal, Aus1490, Chile 59) was significantly ($P = 0{\cdot}05$) more resistant than the susceptible parent Hartog, although the resistant parents were not significantly different in their levels of resistance. Overall, Aus1490 showed the lowest level of sprouting on both scoring days (mean sprouting scores of 1 and 1·4, respectively), followed by Transvaal (2 and 3·04), then Chile 59 (2·4 and 3·7), then Hartog (6·04 and 9·38).

For each RI population tested, the lines showed a wide range of tolerance to sprouting (Fig. 1). The mean RI line score in each population was significantly different between Days 5 and 7, although there was no significant difference among the mean scores of the populations on Days 5 and 7. P1 and P3 showed a wider range of sprouting scores on both days when compared with P2 (Table 1), and both P1 and P3 had higher genotypic variance for both days than P2, although all 3 populations had similar sampling variance, except for P2 on Day 7, which was higher (Table 2). The estimates for line mean heritability were high for P1 and P3 but considerably lower for P2, reflecting the larger effects seen for environmental error and sampling variance in this cross and the lower genetic variance (Table 2). The estimates for error and sampling variance were quite large for each population relative to the genetic variation among the lines. In each case, the RI populations were ranked similarly for line mean heritability for both scoring days.

In all 3 populations there were RI lines with phenotypic values outside the range set by the parents; however, the sprouting scores for these individuals were not significantly different from the respective parents and were therefore not considered to be true transgressive segregants.

Table 1. Summary statistics of the sprouting responses of the recombinant inbred (RI) lines for three wheat populations (P1–3) measured under a controlled environment screen

Screening day	RI mean	Range	Phenotypic variance
	P1		
Day 5	4·51±0·18	5·24	1·65
Day 7	7·86±0·27	7·46	3·64
	P2		
Day 5	4·41±0·13	3·66	0·92
Day 7	7·72±0·24	5·50	2·77
	P3		
Day 5	3·64±0·22	5·72	2·44
Day 7	6·26±0·37	8·60	6·74

Table 2. Line (σ^2_L), error (σ^2_ϵ), and sampling (σ^2_S) variance components and heritability (h^2) estimates for sprouting tolerance in the three wheat populations (P1–3) screened under artificial rain simulation conditions at two times of scoring

Screening day	Variance components			Heritability
	σ^2_L	σ^2_S	σ^2_ϵ	(h^2)
		P1		
Day 5	1·28	1·23	1·19	0·83
Day 7	3·17	2·33	2·40	0·86
		P2		
Day 5	0·43	1·70	0·87	0·67
Day 7	1·24	7·10	2·25	0·46
		P3		
Day 5	2·27	1·08	1·16	0·89
Day 7	6·31	2·66	2·57	0·92

Table 3. Observed distributions, expected distributions for the 1- and 2-gene genetic models tested, and the χ^2 values for each recombinant inbred population for both days of scoring preharvest sprouting under artificial rain simulation conditions

Distributions with two values are tolerant : susceptible, those with three values are tolerant : partially tolerant : susceptible

Screening day	Observed distribution	Expected distribution	χ^2
	P1		
Day 5	7 : 42	1 : 3	2·45 n.s.
Day 7	8 : 23 : 18	1 : 2 : 1	4·26 n.s.
	P2		
Day 5	17 : 33	1 : 3	1·71 n.s.
Day 7	17 : 33	1 : 3	1·71 n.s.
	P3		
Day 5	15 : 35	1 : 3	0·43 n.s.
Day 7	11 : 23 : 16	1 : 2 : 1	1·32 n.s.

n.s., not significant ($P > 0{\cdot}05$).

Genetic analysis

One or 2 genetic models were proposed for each cross and then tested (Table 3). For the cross between Transvaal and Hartog (P1), the frequency of individuals on Day 5 was normally distributed, although slightly

skewed towards the susceptible parent, whereas on Day 7 the frequency distribution had spread and suggested a bimodal distribution (Fig. 1*a*).

Allocation of the RI lines in P1 into putative genotypic classes produced a partition on Day 5 that fitted a 1:3 (tolerant:susceptible) segregation ($\chi^2 = 2{\cdot}45$, $P > 0{\cdot}05$), and partitions on Day 7 of 1:2:1 (tolerant:partially tolerant:susceptible) ($\chi^2 = 4{\cdot}26$, $P > 0{\cdot}05$, Table 3). Mean sprouting scores of 1–2 were classified as tolerant, and mean scores of 3–6 as susceptible on Day 5, whereas on Day 7 mean sprouting scores of 2–5 were classed as tolerant, mean scores of 6–8 as partially tolerant, and scores from 9–10 as susceptible.

In the cross between Chile 59 and Hartog (P2), a nearly normal distribution was observed on Day 5 (Fig. 1*b*); the distribution was negatively skewed by Day 7. Allocation of the RI lines into putative genotypic classes produced a partition on both days that fitted a 1:3 (tolerant:susceptible) segregation ratio ($\chi^2 = 1{\cdot}71$, $P > 0{\cdot}05$). Mean sprouting scores of 2–3 were classified as tolerant, and mean scores of 4–6 as susceptible on Day 5; and on Day 7 mean sprouting scores of 3–6 were tolerant, and mean scores of 7–10 as susceptible.

The cross between the red-grained parent Aus1490 and Hartog (P3) produced a bimodal distribution on Day 5 and a trimodal response on Day 7 which skewed towards the susceptible end (Fig. 1*c*). When the lines were allocated into putative genotypic classes, they were partitioned into groups that fitted a 1:3 segregation (tolerant:susceptible) ($\chi^2 = 0{\cdot}43$, $P > 0{\cdot}05$) on day 5 (mean scores of 1–2 for tolerant and 3–6 for susceptible), and a 1:2:1 segregation (tolerant:partially tolerant:susceptible) ($\chi^2 = 1{\cdot}32$, $P > 0{\cdot}05$) on Day 7 (mean sprouting scores for the tolerant group were 1–3, for partially tolerant 4–7, and for susceptible 8–10).

Red v. white grain resistance

The lines derived from the cross using the red-grained cultivar Aus1490 (P3) exhibited the strongest and most durable tolerance. The seed colour of each RI line in this cross segregated 27:23 (red:white), which when tested for 1:1 segregation gave $\chi^2 = 0{\cdot}18$ ($P > 0{\cdot}05$), indicating that there was only one red colour gene segregating in this population.

The mean sprouting scores for the red and white genotypes were calculated for both scoring days. On Day 5 the mean score for the red genotypes was $3{\cdot}07$ and for the white genotypes was $4{\cdot}31$, and on Day 7 the mean sprouting score was $5{\cdot}23$ for the red genotypes and $7{\cdot}49$ for the white genotypes. For both scoring days the mean PHS scores were not significantly different between red-grained and white-grained groups. Most of the genotypes that fell within the tolerant group were red-grained (Fig. 2). Individual lines of both grain colours were represented in the tolerant (2 genes), partially tolerant (1 gene), and susceptible (no genes) groups (Fig. 2). On Day 5, 16 RI lines were grouped as tolerant and of these 16 lines, 13 (81%) were red. By Day 7, 13 RI lines, of which 12 (92%) were red, were tolerant. There was 1 white line which was not significantly different in its mean sprouting score from Aus1490 or the most resistant red-grain line. In addition, 17 RI lines were susceptible, of which 6 were red and 11 were white with one red line being as susceptible as the most susceptible white-grained lines.

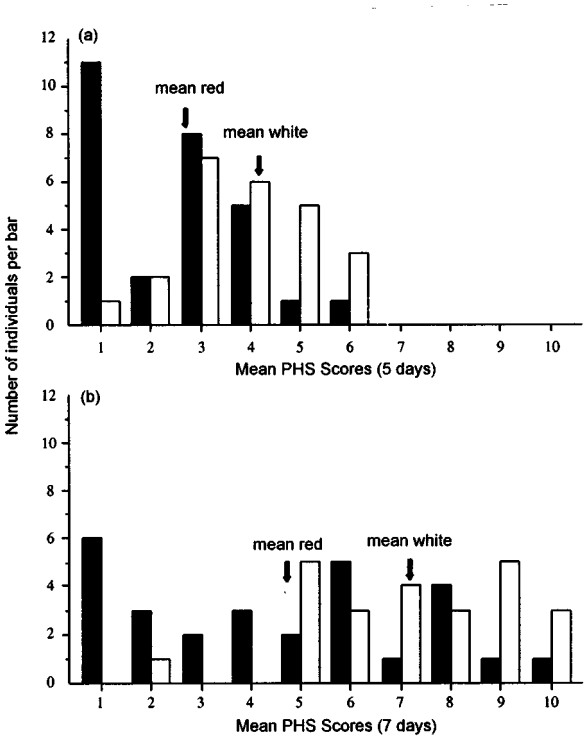

Fig. 2. Distribution of white (□) and red (■) grained individuals with their mean sprouting scores. Tolerant (2-gene) individuals have sprouting scores 1–3, partially tolerant (1-gene) individuals have sprouting scores 4–7, and susceptible (no gene) individuals have sprouting scores 8–10.

Discussion

The results suggest that tolerance to preharvest sprouting is under different genetic control in these 3 crosses. In 2 of the crosses (P1 and P3), sprouting is under simple genetic control, while in the third cross, P2, sprouting is under more complex genetic control. Analysis of variation within each RI population derived from the 3 biparental crosses indicated that

heritability estimates on a line mean basis were high for 2 populations (P1 and P3) and moderate for the other (P2). Therefore, characterisation of the variation for PHS on a line mean basis was possible with a high degree of confidence in 2 of the populations. The high heritability seen for 2 of the populations and the moderate estimate for the other suggested that the segregation patterns resulted from overlapping genotypic classes due to 2 genes. The mixture method of clustering (McLachlan and Basford 1988) provided the basis for an objective allocation of individuals into putative genetic classes. This method has previously been used for genetic analysis of physiological traits in sorghum (Basnayake et al. 1995)

The segregation of individuals within P1 (Hartog × Transvaal) and P3 (Hartog × Aus-1490) into the 3 classes, 1 : 2 : 1, indicated that tolerance to PHS was conferred by 2 independent genes that acted in an additive fashion. It is suggested that lines with 1 tolerant and 1 susceptible locus give a partially tolerant phenotype at Day 7, but are not distinguishable from the susceptibles at Day 5. This is strong evidence that both resistance genes are required for full tolerance. Genetic analysis of P2 (Hartog × Chile 59) indicated that in this cross variation for PHS tolerance was inherited as a trait where 2 genes were independently inherited but acted in a non-additive fashion and that the presence of either one or both of the genes resulted in tolerance.

Inheritance of PHS tolerance in P2 indicated the presence of different genes of a different genetic background to either P1 or P3. In this study no crosses were made between tolerant lines. Therefore, it is not possible to determine the homology between the tolerance genes identified in the 3 crosses. In addition, because gene action was only examined in RI populations, the tolerance genes were only observed in the homozygous state. Therefore, it is not possible to assess the degree of dominance expressed by the alleles at each of the hypothesised loci.

Traditionally, resistance has been closely associated with the presence of the red seed coat gene (Gfeller and Svejda 1960; Gale 1989). The presence of white-grained genotypes, recovered from this cross with a red-grained donor of PHS tolerance, at least as tolerant as most of the red-seeded individuals demonstrates that the presence of the red seed coat colour gene is not necessary for tolerance. It was noticed that of the resistant genotypes in P3, 12 were red and only 1 was white. The PHS score for both days for the white line was not significantly different from the resistant red parent, or from the most resistant red line. It was hypothesised that there were 2 genes for PHS in this cross, both of which were independently inherited and acted in an additive fashion. It is suggested that the red seed colour gene is tightly linked to one of these genes, hence the high percentage of red individuals in the tolerant class with one white line being a recombinant line. In support of the hypothesis, there were 2 red-grained individuals with scores of 9 or 10, indicating that they were equally as susceptible to sprouting as white-grained lines, whereas there was a white-grained genotype present within the resistant group that was not significantly different to the most resistant red genotypes. Therefore, it appears to indicate that in the red-grained genotypes the red gene is tightly linked to one of the resistance genes, accounting for the high percentage of reds in the resistant group, and that this linkage was broken by recombination in some cases resulting in a number of white-grained genotypes, one of which was in the tolerant class.

The presence of other mechanisms independent of the red gene that can impart resistance in white-grained genotypes was suggested by McCaig and DePauw (1992). Mares (1993) demonstrated that it was possible to separate dormancy from the red colour gene, though he found that the red gene was needed for full expression of dormancy. However, the presence of the red seed coat colour gene in susceptible lines indicated that the contribution of the red gene to PHS tolerance may be minimal, and tolerance was not associated with the gene for red grain colour but was due to other factors. In this study it has been shown that tolerance to sprouting in a white-grained genotype is possible and that the resistance shown was just as strong as in the red-grained genotypes.

This study of 3 wheat populations segregating for preharvest sprouting has examined 3 potentially useful sources of tolerance to sprouting (Transvaal, Chile 59, and Aus1490). The presence of white-grained genotypes that are at least as tolerant as most of the red-seeded individuals recovered from a cross between a red-grained donor (Aus1490) and a useful white cultivar (Hartog) demonstrates that strong tolerance to sprouting is achievable in a white-grained genotype. This study also demonstrates that a red-grained donor of PHS tolerance should not be discarded for improvement of this trait in breeding programs.

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