

Emergence and Establishment Problems of Hulless Barley – A Possible Solution.

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Introduction

Plant emergence and establishment is a critical issue in modern farming systems, particularly with the adoption of minimum tillage practices. Poor plant emergence and establishment can affect early plant vigour, weed competition, water use efficiency and, ultimately, grain yield. Poor establishment can be a problem in covered barley but is a frequent and more serious problem in hulless barley. For the successful development of a hulless barley industry, this issue needs to be thoroughly investigated and solved.

Initially, we considered emergence and establishment problems to be associated with the embryo being damaged during harvesting, resulting in poor seed viability and germination. Despite modification to harvest and seed cleaning procedures, we continue to be frustrated by yield trials with variable and inconsistent plant emergence and establishment.

In 1998 yield trials, the actual number of emerged plants ranged from 75 plants/m² to 95 plants/m² for hulless genotypes and from 120 to 140 plants/m² for covered genotypes. Variations in plant establishment and seeding rate make interpretation of grain yields comparisons between hulless and covered genotypes very difficult. To compensate, the number of seeds sown/m² in yield trials in 1999 were increased from 150 to 180 seeds/m².

Preliminary investigations into the morphology of the coleoptile revealed that hulless barleys may produce a shorter coleoptile than covered barleys. In addition, hulless barleys appear to be more susceptible to growing an 'abnormal' coleoptile particularly when the embryo is damaged.

It is likely that a large proportion of variation for coleoptile length is under simple genetic control. Selection for improved coleoptile length in hulless barley, therefore, may improve establishment.

The aim of this study was: a) to determine the relative differences in coleoptile length between hulless and covered barley genotypes, b) to determine the effect of grain damage on coleoptile length in hulless barley and, c) to identify chromosomal regions conferring coleoptile length in barley which could, in turn, be used to select for coleoptile length in hulless barley.

Materials and Methods

Germination

120 grains of each barley variety and line were counted and placed in petri dishes lined with 2 pieces of Whatman No. 1 90 mm filter papers. 4 ml of R.O. water was added and petri dishes were sealed with parafilm to prevent moisture loss. Samples were incubated at 20C for 5 days (EBC, 1998).

Measurement of coleoptile length

After 5 days germination, 100 grains with healthy root and acrospire growth were selected and placed ventral side down on moistened Grade R6 45N 320 mm x 460 mm filter papers (25 seeds per filter paper). The filter papers were rolled being careful not to disturb the orientation of the grains. They were wrapped in aluminum foil and placed upright in a container with approximately 2 cm of R.O. water in the bottom and incubated at 20C for a further 7 days. After 7 days the length of the coleoptile for each grain was measured in millimetres and recorded. Coleoptile length means and standard deviations were calculated for each variety and line using Microsoft Excel 6.0.

Comparison of coleoptile lengths in hulless and covered barleys

Twenty-three covered and hulless barley genotypes were screened for coleoptile length. These included 7 hulless cultivars from Australia (Namoi and Morrell), Canada (Candle and Richard), Europe (Nudinka), Mexico (CIMMYT_42002) and USA (Merlin); 10 hulless breeding lines developed at the University of Adelaide; 2 Australian malting cultivars Franklin and Schooner; and 4 Australian feed cultivars Barque, Chebec, Galleon and Skiff. Grain samples were taken from SABIP Stage 3, 2 and 1 Trials grown at Charlick Research Station, Strathalbyn; Clinton (Yorke Peninsula); and Weetulta (Yorke Peninsula) in 1997.

Effect of visual grain damage on coleoptile length in hulless barley

The cultivars and lines assessed included 2 hulless Australian cultivars Namoi and Morrell, and 2 hulless breeding lines developed at the University of Adelaide, 89/126/E*22 and 90/78*10. Grain samples were taken from SABIP Stage 3 Hulless Trials grown at Clinton (Yorke Peninsula) in 1997. Grains were sorted and classified into the following categories: 'Husk Intact'; 'Visually Whole Embryo'; 'Slightly Damaged Embryo' (estimated <10% damage); 'Moderately Damaged Embryo' (estimated 25 – 50% damage); 'Badly Damaged Embryo' (estimated >50% damage); and 'Broken Grain – Embryo Intact'. Up to 50 grains per genotype were classified and coleoptile lengths measured for each category.

Mapping of coleoptile length in barley

The parents of three NBMMP mapping populations, Chebec x Harrington, Galleon x Haruna nijo and Alexis x Sloop, were assessed for variation in coleoptile length. From this the Chebec x Harrington and Galleon x Haruna nijo populations were identified as suitable for mapping.

Eighty-six doubled haploid lines from the Chebec x Harrington mapping population and sixty-nine doubled haploid lines from the Galleon x Haruna nijo mapping population were assessed for coleoptile length. Grain samples were taken from a field trial grown at Charlick Research Station in 1997 and 1998 respectively.

A total of 247 and 278 RFLP marker loci in the Chebec x Harrington and Galleon x Haruna nijo mapping populations (Langridge *et al.*, 1995) respectively, were used for simple and interval regression analysis, the latter by the method of Haley and Knott (1992). A minimum LOD threshold of 3.0 was used. All mapping marker analyses were performed with the computer program 'QGENE' (Nelson, 1997).

Results and Discussion

Comparison of coleoptile lengths in hulless and covered barleys

Covered barleys had coleoptiles that were between 13 – 59 mm longer than the hulless barleys tested (Figure 1). The genetic variation for coleoptile length was less between covered cultivars (64 – 78 mm) than the hulless commercial cultivars (14 – 48 mm). The semi-dwarf covered cultivars Skiff and Franklin produced the short mean coleoptile (Figure 1). With the exception of Merlin, a semi-dwarf, ‘waxy’ variety developed in USA, the hulless breeding lines had coleoptiles that were longer than commercial hulless cultivars. Merlin’s longer coleoptile may be explained by the better overall grain quality. Merlin grain samples appeared to have less embryo damage than other cultivars. The embryo on Merlin grain seems to be smaller and less protruding and as a result may have been less susceptible to damage during harvesting. In addition, Merlin had a greater percentage of grains that had their palea and lemma still intact (21% compared to zero for Namoi). The presence of the husk would also offer the embryo protection during harvesting. Similar coleoptile lengths to Merlin were observed for Skiff derived hulless lines (89/03*48, 89/126/A*09, 89/126/E*22 and 89/126/J*11) but were still 14 – 19 mm shorter than Skiff. Galleon derived hulless lines (89/20*78 and 89/20*70) produced coleoptiles that were longer than all commercial hulless cultivars. The Galleon derived lines were, however, 21 and 23 mm shorter respectively, than Galleon. The Galleon derived hulless line 89/20*58 produced a coleoptile that was 37 mm shorter than Galleon. In field trials this line has had serious plant emergence and establishment problems and these may be related to its short plant height, considering it is the shortest of the three Galleon derived lines assessed. Hulless lines 89/15*44, 90/77*41 and 90/78*10 all produced the longest coleoptiles but are still 15 – 22 mm shorter than Galleon. In field trials, these three lines have exceptional plant emergence and establishment scores and this may be correlated to their medium – tall plant heights. The standard errors were larger for all hulless barley genotypes tested. This trend may be explained by the enormous variability in grain quality i.e. presence of intact husk, embryo damage and broken grains.

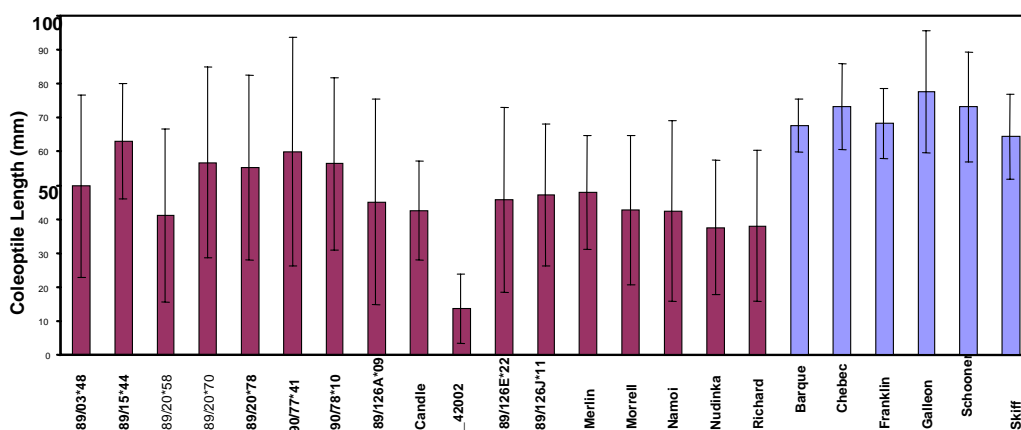


Figure 1. Summary of coleoptile lengths for hulless and covered barley genotypes grown at Charlick Research Station in 1998

Effect of visual grain damage on coleoptile length in hulless barley

A superior hulless barley coleoptile was produced for all four hulless barley genotypes when the palea and lemma remained intact (Figure 2). The husk, therefore, is likely to be important in the development of a healthy coleoptile resulting in sound plant emergence and

establishment. It is likely that the husk is acting as a protective barrier against embryo damage. Coleoptile lengths were shorter for ‘Visually Whole Embryo’ than ‘Husk Intact’ which suggests that it will be difficult to avoid any embryo damage despite our modifications to harvest and seed cleaning procedures. Despite the ‘Broken Grain – Embryo Intact’ classification producing the shortest mean coleoptile length, all coleoptiles were healthy. Under field conditions these broken grains should emerge and establish healthy plants. However, depth of sowing would need to be carefully monitored. Short, ‘weak’ coleoptiles were observed for all four hulless barley genotypes for ‘Slightly Damaged Embryo’ and ‘Moderately Damaged Embryo’ classifications. Namoi was the only genotype that we observed germination and coleoptile growth for the ‘Badly Damaged Embryo’ classification. As expected seed viability for all genotypes for these three ‘Damage Embryo’ classifications was very low (Figure 3). The embryo damage appeared to contribute to an increased frequency of ‘abnormal’ coleoptiles. Under field conditions it is highly unlikely that these ‘abnormal’ coleoptile would emerge.

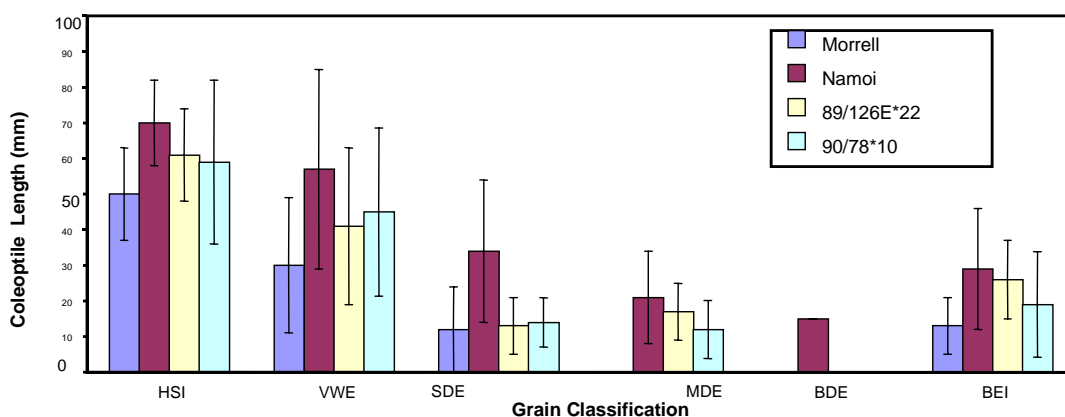


Figure 2. Effect of visual grain damage on coleoptile length in hulless barley genotypes.

Mapping of coleoptile length in barley

Table 1 is a summary of the mean coleoptile lengths for the parents of the Chebec x Harrington, Galleon x Haruna nijo and Alexis x Sloop mapping populations. From the analyses of variance there were significant ($P < 0.05$) differences between Chebec and Harrington, and Galleon and Haruna nijo at both Brinkworth and Tuckey. There was no significant difference for coleoptile length identified between Alexis and Sloop at either Brinkworth or Tuckey. The overall mean coleoptile length was 64 mm for Chebec x Harrington population compared to 73 mm for Galleon x Haruna nijo population. However, large standard errors were observed for individual lines in the latter population. It is difficult to explain these large standard errors but it may be related to the extent of grain and embryo damage arising from mechanical harvesting. Visually, the lines could be separated into two distinct groups a) Galleon types – large grains, thick husk, very little damage to the grain or embryo and generally developed a very robust coleoptile, and b) Haruna nijo types – smaller grains, thin husk, evidence of harvest damage (‘skinning’ and embryo damage) and developed a more ‘fragile’ coleoptile.

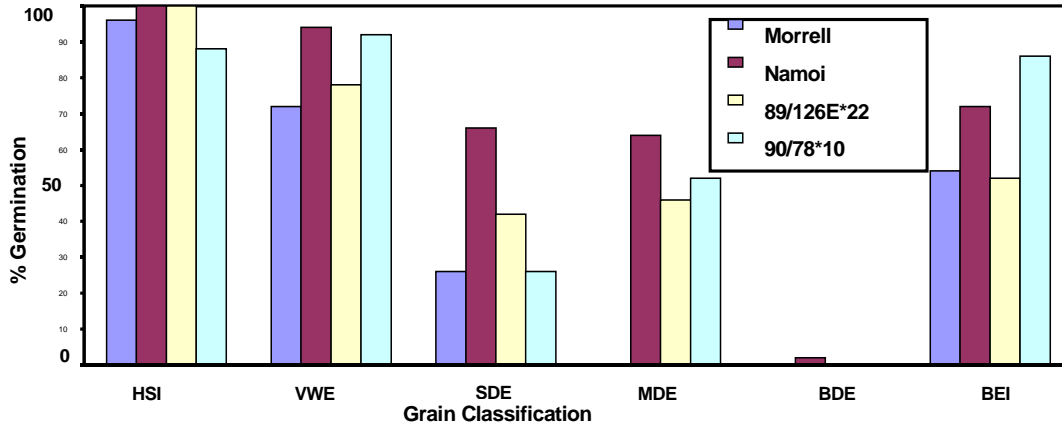


Figure 3. Effect of visual grain damage on the seed viability for four hulless barley genotypes

Table 1. Summary of the coleoptile length data for parents of the three NBMMP mapping populations. Data expressed as a mean of two sites (Brinkworth and Tuckey) from 1997.

Cultivar	Mean Coleoptile Length (mm)
Chebec	78.6c*
Harrington	55.9a
Galleon	94.5e
Haruna nijo	84.0d
Alexis	70.0b
Sloop	67.0b

*Same letter denotes values are not significantly different (significant at $P < 0.05$ level).

Simple and interval regression analysis (Figure 4) revealed a significant association ($LOD = 3.39$) between coleoptile length and RFLP markers on a region of chromosome 7H in the Chebec x Harrington mapping population. Chebec marker alleles at this locus conferred long coleoptile length. Bulked segregant analysis in hulless x covered breeding populations revealed a significant association between RFLP markers in the identical region of chromosome 7H (Box, unpublished data). Simple and interval regression analysis revealed a significant association ($LOD = 3.10$) between coleoptile length and RFLP markers on a region of chromosome 5H in the Galleon x Haruna nijo population. Galleon marker alleles at this locus conferred long coleoptile length. The region conferring long coleoptile length in this population was found to map very closely to the Xylanase structural gene found to be strongly associated with cereal cyst nematode resistance derived from Galleon (Barr *et al.*, 1987).

There appears to be a single gene difference for coleoptile length between Chebec and Harrington and a different single gene between Galleon and Haruna nijo. It is difficult to determine if the chromosome 7H region derived from Chebec contributes to above average coleoptile length or that a Harrington allele at this locus contributes a below average coleoptile length. Galleon and its derivatives are important parents, contributing cereal cyst nematode resistance, high yield and wide adaptation, in the SA hulless barley improvement program. Selection for cereal cyst nematode resistance in hulless crosses involving Galleon and its derivatives could result in improved coleoptile length. This selection for cereal cyst nematode resistance and, inadvertently improved coleoptile length can be observed in the Galleon derived hulless lines (89/20*58, 89/20*70 and 89/20*78) (Figure 1). Both 89/20*70 and 89/20*78 are resistant to cereal cyst nematode and have up to 7 mm longer coleoptiles

than the hulless commercial cultivars, whereas 89/20*58 is susceptible to cereal cyst nematode and produces a coleoptile length up to 7 mm shorter than Merlin but 3 mm longer than Richard and Nudinka. Further studies are required to assess the relative effect of the alleles on Chebec chromosome 7H, Harrington chromosome 7H, Galleon chromosome 5H and Haruna nijo chromosome 5H in alternative genetic backgrounds.

Conclusion

Covered barley genotypes had coleoptiles that were between 13 – 59 mm longer than hulless barley genotypes. The standard errors were larger for all hulless barley genotypes tested. This trend may be explained by the enormous variability in grain quality i.e. presence of intact husk, embryo damage and broken grains.

A superior hulless barley coleoptile was produced for all four hulless barley genotypes when the palea and lemma remained intact after harvesting and threshing. The husk, therefore, is likely to be important in the development of a healthy coleoptile resulting in sound plant emergence and establishment. In addition, hulless barleys are more susceptible to growing an ‘abnormal’ coleoptile particularly when the embryo is damaged.

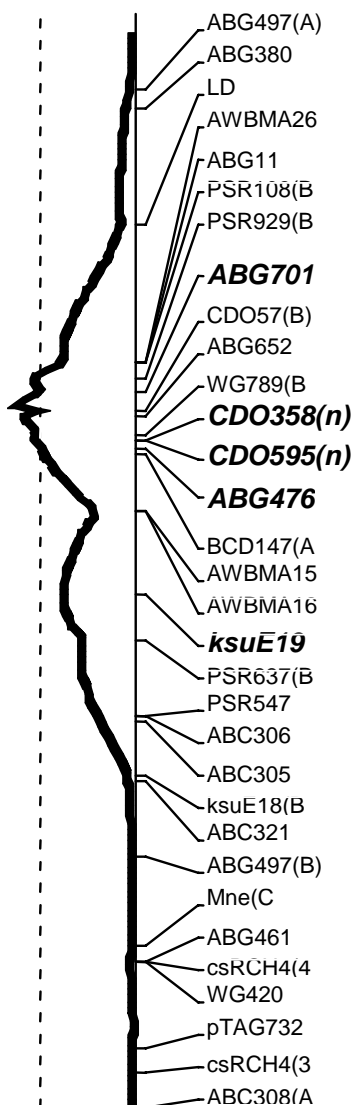
Chromosome regions involved in the control of coleoptile length were identified on chromosome 7H in the Chebec x Harrington population and on chromosome 5H in the Galleon x Haruna nijo population. A gene for hulless caryopsis has been located in the same region on chromosome 7H found to be associated with coleoptile length. A gene for cereal cyst nematode resistance derived from Galleon has been mapped in close proximity to the region identified in this study conferring coleoptile length on chromosome 5H.

It is possible that alleles for long coleoptile length from Chebec and alleles for hulless caryopsis from alternative hulless parents would be in repulsion and therefore very difficult to select in combination. In contrast, selection for cereal cyst nematode resistance in hulless crosses involving Galleon may contribute to improved coleoptile length and improved establishment. Results presented here also emphasize the importance of care in harvesting and handling of hulless grain.

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Chromosome 7H
LOD = 3.39



Chromosome 5H
LOD = 3.10

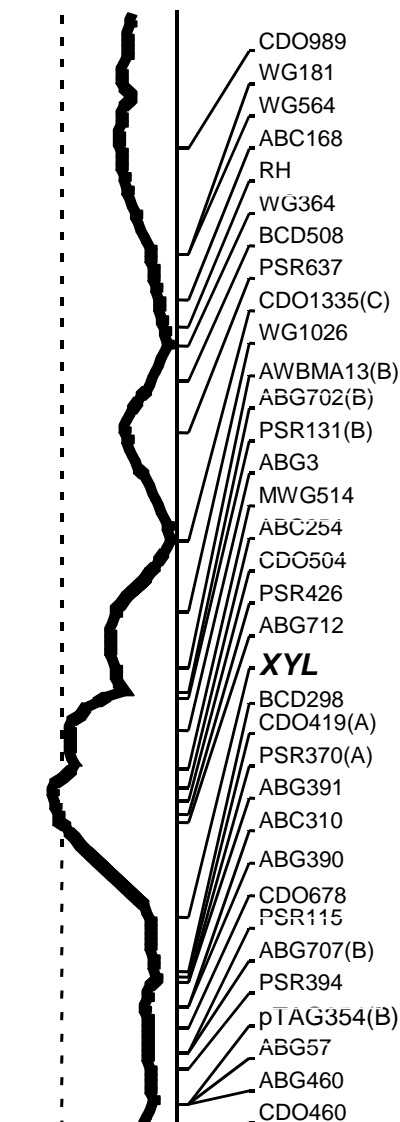


Figure 4. Partial interval analysis maps for coleoptile length for Chebec x Harrington and Galleon x Haruna niyo mapping populations

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