Effect of Earliness per se gene on selected kernel physical traits in barley

Ahmed Ibrahim[1, 2], **Matthew T. Harrison**[1], Peter Johnson[1], Holger Meinke[1] and Meixue X. Zhou[1]

[1] Tasmanian Institute of Agriculture (TIA), 165 Westbury Rd Prospect, TAS 7320, Ibrahim.Ahmed@utas.edu.au
[2] Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria

Abstract
Kernel physical traits in barley are important in determining the suitability of a variety for either malting or feed. A pair of near isogenic lines (NILs) differing in the earliness per se gene (Eps5HL) on chromosome 5HL were evaluated for these traits. NILs had a significantly higher percentage of big kernels ($P < 0.05$). Significant differences ($P < 0.05$) in 1000-kernel weight were found between the two NIL lines and between the parents. For kernel hardness, Franklin had a higher particle size index (PSI) (56%) than TX9425 (51.3%) but no difference was found between the NIL lines. We therefore concluded that the Eps5HL locus of the NILs showed significant effects on kernel size and 1000-kernel weight, but not on kernel hardness.

Keywords
Kernel size, 1000-kernel-weight, kernel hardness, particle-size-index.

Introduction
Physical grain characteristics such as size, weight and hardness, are important quality traits that are required for determining the flour quality in cereals (Munck 1995). Hardness in barley is the kernel resistance to breakage or endosperm texture, which is due to the interplay of some complex factors (Fox et al. 2007a). Of these factors, chemical (starch, protein) and structural (endosperm texture) modifications are the major components that affect the quality of the grains in barley (Camm and Rossnagel 2017). Malting barley varieties have generally softer endosperm than feed barley varieties (Fox et al. 2007b; Psota et al. 2007). Higher starch content is negatively correlated to kernel hardness while increased protein and β-glucan are reported to positively influence the grain hardness and consequently the malting quality in barley (Henry and Cowe 1990). Kernel size and weight are other quality components that have significant influence on malting quality (Agu et al. 2007). Bigger kernel size is directly related to the percentage of starch and energy in the grain (Bleidere 2008) and reduced protein proportion (Fox et al. 2006). Because of their importance, variations in kernel hardness, size and weight in barley are usually the breeder's prime interest for quality improvement. Fortunately for breeders, hardness (Fox et al. 2007a), size (Fregeau-Reid et al. 1995) and weight (Ferrio et al. 2006) are under genetic control, although environment can have a significant impact. Quantitative trait loci (QTL) regulating grain hardness was reported on chromosome 5H (Beecher et al. 2002) and later by Fox et al. (2007a), and also for grain size on chromosome 5H (Walker et al. 2013). QTL for grain weight was found on chromosome 3H (Chen et al. 2012; Wang et al. 2014) and 5H (Walker et al. 2013). Some developmental genes such as Vrn-H1 and earliness per se genes are also located in similar positions and have been implicated with grain size (Coventry et al. 2003; Walker et al. 2013). A better understanding of the relationships between earliness per se genes and grain physical characteristics such as hardness, size and weight is necessary (Bleidere 2008; Coventry et al. 2003). In view of this, here we investigate the influence of a QTL (on chromosome 5H consisting of an earliness per se gene) on these quality traits using a pair of near isogenic lines.

Methods
This experiment was conducted in Tasmanian Institute of Agriculture (TIA) at Mt Pleasant Laboratories, Launceston (Latitude: -41.4702 Longitude: 147.1392). A pair of near isogenic line (NILs) consisting of earliness and late alleles (Eps5HL-e and Eps5HL-l) derived from Franklin and TX9425 were used. The NILs were sown in randomised complete block design with three replications. Local agronomic practices were followed. Kernels were harvested from the three middle rows of all the plots for each genotype, air dried, threshed and cleaned. 1000 kernels were randomly counted and weighed to measure the 1000-kernel weight. The procedure was repeated 3 times for each sample. Determination of kernel size index (KSI) was conducted by shaking 100 grams of the kernels of each of the genotypes in a PFEUFFER SORTIMAT K3 set for one minute. Samples passed through an instrument consisting of four different sieve sizes (<2.2, 2.2, 2.5 and >2.8 mm). The kernel samples retained on each of the sieves were weighed. The kernel size index

(KSI) was calculated: KSI (%) on sieve = weight (g) on the sieve/ total weight (g) of the sample × 100 (Hossain et al. 2010).
In order to determine the grain hardness, 10 g of the sample with the size of >2.8 mm for each genotype were assessed for particle size index (PSI). The sample from each genotype was milled using 0.18KW RockLabs ring miller New Zealand (1985), ring size (O ring chrone 3) (Figure 1A) and set for 3 minutes. The same machine that was used for milling was modified for the shaking by using 125 mm sieve of ABICHEM Test Sieve BS 410 (Figure 1B) set for 5 minutes and following the most suitable criteria described by Orth (1977). The whole procedure was repeated three times for each genotype. The (PSI) was calculated as: PSI [%] = (weight of sample throughs [g] / total sample weight [g]) × 100 (Psota et al. 2007).

Figure 1. (A) The RockLabs ring miller (0.18KW) New Zealand with CH3 ring for milling and (B) The RockLabs ring miller (0.18KW) New Zealand with 125mm sieve of ABICHEM Test Sieve BS 410 for the shaking.

Results and Discussion

Evaluation of kernel size is normally done on 4 segments: <2.2, 2.2 - 2.5 mm, and >2.8 mm in Australia (Fox et al. 2006). The NIL lines had a higher percentage (90%) of kernels over the size of 2.8 mm (Figure 2A and B) compared with the parents (75%), TX9425 and Franklin. Kernel size in cereals, especially barley, could be influenced by the environment, for example water use, drought and heat stress (Fettell et al. 2001) at flowering or kernel filling stage. However, the trait is largely controlled by genetic factors (Fox et al. 2006) with the dominant grade of 2.8 mm kernels having higher heritability estimate in barley (Fox et al. 2006) and thus could be easily selected for improvement. Furthermore, this grade of kernels has the highest diastatic power compared with other grades (Agu et al. 2007). Therefore, to increase the overall kernel size, breeding for increased kernel size of >2.8mm will offer a valuable strategy in Australia. There has been no report on the effect of Eps gene on grain size except that by Coventry et al. (2003). Our results also showed that the Eps gene in this NIL had no significant effect on grain size (Figure 2).

Kernel weight, being the first improved agronomic trait by the ancient farmers, is strongly under genetic control (Ferrio et al. 2006). High heritability and genetic advance has been observed for this trait (Yadav et al. 2015) indicating that direct selection can be effective. The late maturity variety Franklin had higher 1000-kernel weight than TX9425 (Figure 2C). Similarly, 1000-kernel weight of the NIL line carrying the late allele (Eps5HL-l) was higher than the early counterpart with (Eps5HL-e) allele. Of all the developmental stages in barley, the post-anthesis stage is the largest determinant of grain weight (Al-Ajlouni et al. 2016). Developmental stages including those post-anthesis are regulated by the Eps genes (Hill and Li 2016; Ibrahim et al. 2016). It is therefore possible that the differences obtained for 1000-kernel weight could be attributed to the influence of the Eps5HL locus in the NIL lines.

Particle size index (PSI) is an efficient method of identifying the QTL regions for hardness (Fox et al. 2007a). A kernel sample is considered hard when lower percentages of flour pass through the sieve. Therefore a sample with low PSI value is related to hard kernels (Salmanowicz et al. 2012). Variations in PSI have been reported in both wheat (Orth 1977) and barley. Of the 40 genotypes evaluated, Fox et al. (2007a) recorded the highest PSI value of 29.9% for Kaputar. Genetic studies of kernel hardness showed that the trait is strongly under genetic control (Fox et al. 2007b). The heritability estimate for this trait ranges from moderate to high (Fox et al. 2009) and therefore can be easily inherited in general (Yamazaki and Donelson 1983). In our experiment, a higher PSI value was observed for Franklin than the TX9425. Eps genes are reported have significant effects on grain protein (Herndl et al. 2008) which may be inversely related to starch content and consequently PSI values. However, Yamazaki and Donelson (1983) obtained no relationship between PSI value and protein content in wheat. Our results showed that the NIL lines had

similar PSI values (53%) indicating that the Eps5HL locus had no effect on kernel hardness, even though PSI and Eps5HL gene reside on chromosome 5H.

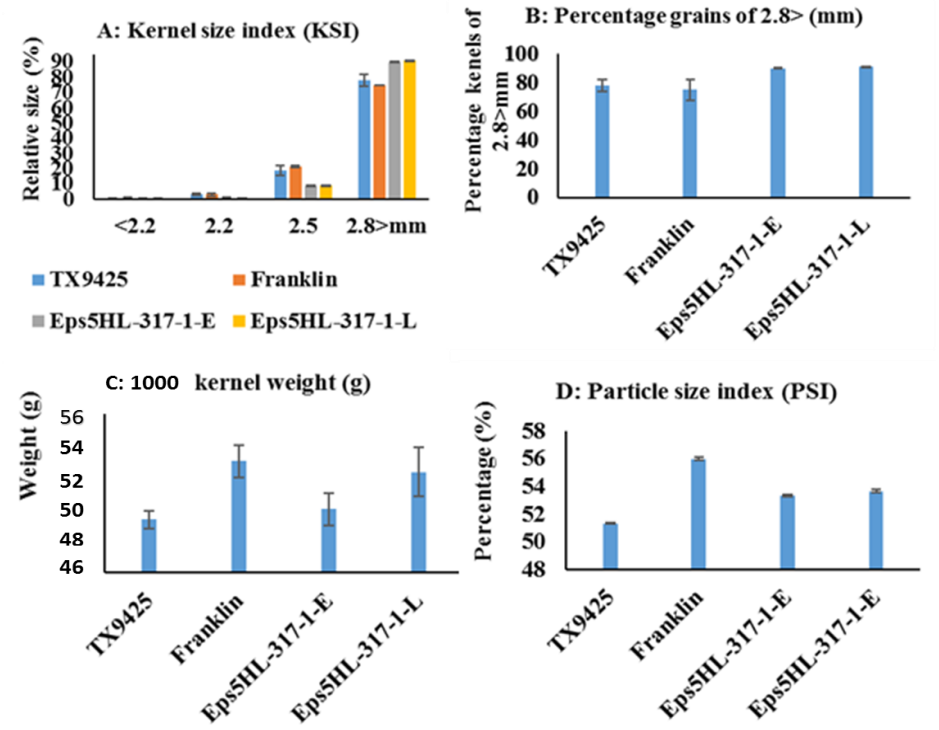

Figure 2. (A) The kernel size index (KSI), (B) Percentage kernels of >2.8mm, (C) 1000-kernel weight and (D) particle size index (PSI) of near isogenic lines carrying Eps5HL locus and the parents, TX9425 and Franklin.

Conclusion

Kernel physical traits in barley are important in determining the suitability of a variety for both malting and feed. The near isogenic lines (NILs) used for this study had ninety percent of their kernel ranges >2.8 mm while only 75% of the kernels from the parents were >2.8 mm. A significant difference in 1000-kernel weight was found between the two NIL lines and between the parents. This difference could be due to the effect of the Eps5HL locus. Franklin had higher particle size index (PSI) (56%) for kernel hardness than TX9425 (51.3%). Both NIL lines (early allele (Eps5HL-e1 and late Eps5HL-l1) had a close PSI value of 53.3 and 53.6%, respectively. It is concluded that the early allele of the Eps5HL locus had negative effects on 1000-kernel weight but not on kernel hardness.

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