Towards Positional Cloning and Functional Analysis of Glossy Mutants of Barley

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Barley (*Hordeum vulgare* ssp. *vulgare*) is an economically important cereal crop that is among the best adapted grains and grows in diverse climatic conditions (Ullrich, 2010). It is the fourth most produced cereal crop in the world with end uses in animal feed (75%), malt production (20%) and human food (5%) (Blake, Bowman, & Abdel - Haleem, 2010). It is a member of the tribe *Triticeae* along with other important crops like wheat and rye.

Breeding for high quality and high yielding barley varieties is mostly based on crosses from elite varieties, which leads to severe reduction of genetic diversity. Novel favorable alleles for diverse traits in particular resistances against abiotic and biotic stresses can be introduced by screening the primary gene pool for improved traits. Wild barley (*Hordeum vulgare* ssp. *spontaneum*) is the progenitor of cultivated barley, and can produce fully fertile hybrids with their domesticated counterparts (Zohary, 1969). A comprehensive collection of 318 wild barley accessions from diverse geographical regions in the Fertile Crescent comprises the Wild Barley Diversity Collection by Dr. Brian Steffenson. The WBDC has been screened quite extensively for novel resistances against rust, mildew and spot blotch diseases (Steffenson et al., 2007, Ames et al. 2015, Roy et al., 2010). To work towards introducing wild barley alleles into adapted breeding material, Nice et al. (Unpublished) have generated a Wild barley Introgression Panel (WBIP) for which 25 lines, representing 90% of the WBDC allelic diversity have been used to generate BC2 backcross populations with Rasmusson of ~30 lines each with a total of 796 individuals. The WBIP and the WBDC are an ideal resource to screen for traits in their original genetic environment and in the adapted background of the 6-row elite variety Rasmusson.

Epicuticular waxes are an important component of elite cultivars of most crops. The waxes present in the cuticle provide a waterproof layer to the plants (Post-Beitenmiller, 1996), reduce leaf and surface temperature (Blum, 1975), reduce water loss due to transpiration, thereby increasing transpiration efficiency (Febrero et al., 1998), increase host plant resistance to pathogens (Janick, 1998), etc. However, almost all of the wild barley in the Fertile Crescent are glossy (wax-less) in nature (Steffenson 2016, Personal Communications). This begs a very important evolutionary question: why does wild barley not possess any waxes that have been deemed to be so important for water use efficiency, even when they are present in dry and arid climate? Is the presence of wax in barley a consequence of evolution or domestication? To date it remains unclear what caused the loss of the glossy phenotype or the emergence of waxiness. It also is not clear whether glossy or waxy phenotype constitutes the "ancestral" trait for *Hordeum.*

Two *glossy* phenotypes, *glossy sheath* and *glossy spike* were found to be segregating in the WBIP, which have been mapped to a ~7 cM region on chromosome 3HL, and another to a ~4 cM region on chromosome 1HS. The QTLs on chromosome 3HL and 1HS are coincident with classical barley mutants *glossy sheath 2 (gsh2)* and *Glossy Spike (Cer-yy),* respectively. The *gsh2* mutants are characterized by an allelic series of recessive mutations exhibiting a lack of epicuticular waxes in the stem, sheath and spike, while the *Cer-yy* mutants are characterized by an allelic series of dominant mutations showing a lack of wax in the spike. Our main objectives are to: 1) fine map and clone genes responsible for these phenotypes, 2) perform a thorough SEM, and chemical analysis and establish differences in wax accumulation and composition between mutants and wild-type, 3) learn the physiological implications of the presence/ absences of waxes in barley via induced drought and senescence studies; and 4) characterize the wax related traits of the WBDC and understand the genetic basis of wax emergence in cultivated barley.

For the first objective, we have developed fine-mapping populations for both the glossy sheath and the glossy spike phenotype. A near-isogenic line (NIL) with a *gsh2* mutant allele in the genetic background of cv. Bowman was used to create and phenotype F_2 populations (N= ~3200) by crossing with four elite barley cultivars, cvs. Bowman, Morex, Harrington, and Steptoe. About 400 progeny from the Morex F2 which exhibited the glossy phenotype for the recessive mutation were screened with 35 polymorphic SNPs with the MassARRAY system and KASP assays narrowing the confidence interval to <1 cM, corroborating mapping results of the AB-NAM population. A chain of BAC sequences, which harbor about 10 high confidence genes, physically linked flanking markers. Likewise, a NIL of a *Cer-yy* dominant glossy mutant allele was used to create F2 populations with cvs. Bowman and Morex. Initial screening with flanking markers has identified several dozen recombinants, which are now being further characterized. We have confirmed the glossy (loss of wax) state of these mutants histologically via scanning electron microscopy (SEM) showing a dramatic reduction of wax compared to wild type. Chemical composition analysis will be performed via GC-MS to characterize the constituent compounds that make epicuticular waxes in barley mutants and wild type along with comparative analysis of waxes present in barley with those present in other members of the *Triticeae* family, *Brachypodium distachyon* and *Triticum aestivum*. Drought response of the NIL pairs carrying mutant alleles for *gsh2* and *Cer-yy* in the Bowman genetic background and Bowman will be discussed to understand the implications of presence or absence of waxes in barley. Another experiment exploring the differential rates of senescence of the glossy mutants when compared to wild type will be conducted. The WBDC is currently being grown, and will be characterized for wax related traits and their associations with different important physiological functions to further explore their importance to barley.