Effect of the *Gpc-B*1 Allele On Grain Protein Concentration in Hard Red Winter Wheat (*Triticum aestivum* L.) in the Pacific Northwest of the US.

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Abstract:

Grain protein concentration (GPC) influences end-use quality of products made from wheat (*Triticum aestivum* L.); therefore, this trait is commonly a high priority for breeders. However, most variation in GPC is due to variability in the environment, instead of genetics effects. In addition, GPC and grain yield are negatively correlated. Due to the negative correlation between yield and GPC, and the high variation caused by the environment, enhancing genetic expression of this trait will be important to improving grain protein concentration in hard wheat varieties. Previous studies have shown varying results in spring wheat in regards to increasing GPC, with some studies increasing GPC by up to 1.5%. The objective of this study was to evaluate the *Gpc-B1* allele in hard winter wheat cultivars. Near-isogenic lines (NIL) with and without the *Gpc-B1* allele were created in four populations: Farnum by Lassik, WA8061 (an advanced breeding line) by Lassik, Farnum by Hollis, and Farnum by Eddy. Presence of this allele was validated using the diagnostic marker *Xuhw89*. Field trials were planted in Pullman and Lind, Washington under a randomized complete block design which included NIL and check cultivars with and without the *Gpc-B1* allele. These two locations differ by annual rainfall averages and yield potential. Results indicate that the inclusion of the *Gpc-B1* allele for high GPC in hard red winter wheat does increase GPC, although genetic background does play a role. A negative correlation with grain yield and GPC was found, showing inclusion of the *Gpc-B1* allele significantly decreased grain yield potential. Interestingly, the Farnum by Hollis NIL with the *Gpc-B1* allele showed not only high GPC, but also higher grain yield potential than the NIL without this allele. These data demonstrate that inclusion of the *Gpc-B1* allele increases GPC in the tested hard red winter lines, and that the well-known negative correlation between GPC and grain yield can be selected against.

Introduction:

Grain protein concentration (GPC) is an important trait for wheat breeders because of the impact it has on the end-use quality of products produced from wheat (*Triticum aestivum* L.). High GPC has been associated in an increase of quality of bread and pasta products (Distelfeld *et al*., 2006). In addition, wheat is a main source of protein for humans and livestock. From 1998 – 2007, the FAO estimated an average of 10% GPC, which accumulates to 60 million Mg of protein annually for humans and livestock.

Although protein concentration's importance, efforts towards breeding specifically for high GPC are slow. The cause of this slower progress is due to environmental effects, complex inheritance, and the negative correlation between protein concentration and grain yield (Carter et al., 2012). Therefore, in the past selection for higher yielding cultivars has probably countered increases in GPC. The increase in GPC in modern cultivars will require high nitrogen (N) inputs, in order to maintain high grain yield (Brevis and Dubcovsky, 2010). Another complexity with increasing GPC, is the limit of genetic variation for this trait. Recently, introgressions from related wheat varieties have added genetic diversity for GPC. Wild emmer wheat, *Triticum turgidum* spp. *dicoccoides* (DIC) has contributed uniquely to increase GPC; often being recognized as having high GPC, often higher than many commercial varieties (Brevis and Dubcovsky, 2010). One gene identified from this wheat relative, termed *Gpc-B1*, has been shown to increase GPC in spring wheat up to 1.5% (Brevis and Dubcovsky, 2010; Carter et al., 2012). Although important for the spring wheat crop, where the minimum protein content is 14%, hard winter wheat varieties could also benefit from incorporation of this allele, especially if it is associated with improved end-use quality.

The objective of this research was to test the *Gpc-B1* allele in hard red winter wheat near-isogenic lines in Washington to determine the effect of this allele on grain protein concentration.

Materials and Methods:

Plant material:

Plant material was developed by crossing Farnum with Lassik, Hollis, and Eddy. WA8061, a sister line to Farnum, was also crossed with Lassik to create a breeding population. Farnum and WA8061 both contain the *Gpc-B1* allele. Near-isogenic lines (NIL) were developed by identifying F_4 derived breeding lines which were heterozygous for the *Gpc-B1* allele. Selected lines from each cross were allowed to self-pollinate, then markers were again used to identify lines homozygous with and without the *Gpc-B1* allele, thereby creating NIL within each genetic background. Farnum (containing *Gpc-B1*) and Bauermeister (without *Gpc-B1*) were used as check cultivars.

Field design:

Near isogenic lines were planted in two different locations in the state of Washington. The two locations – Lind and Pullman – were selected because of the differing average annual rainfall (Lind=10.00 inches, Pullman=21.00 inches). Lines were planted in a randomized complete block design in 2012 (Pullman) and 2013 (Lind and Pullman). The design had three replicates and included a positive and negative control.

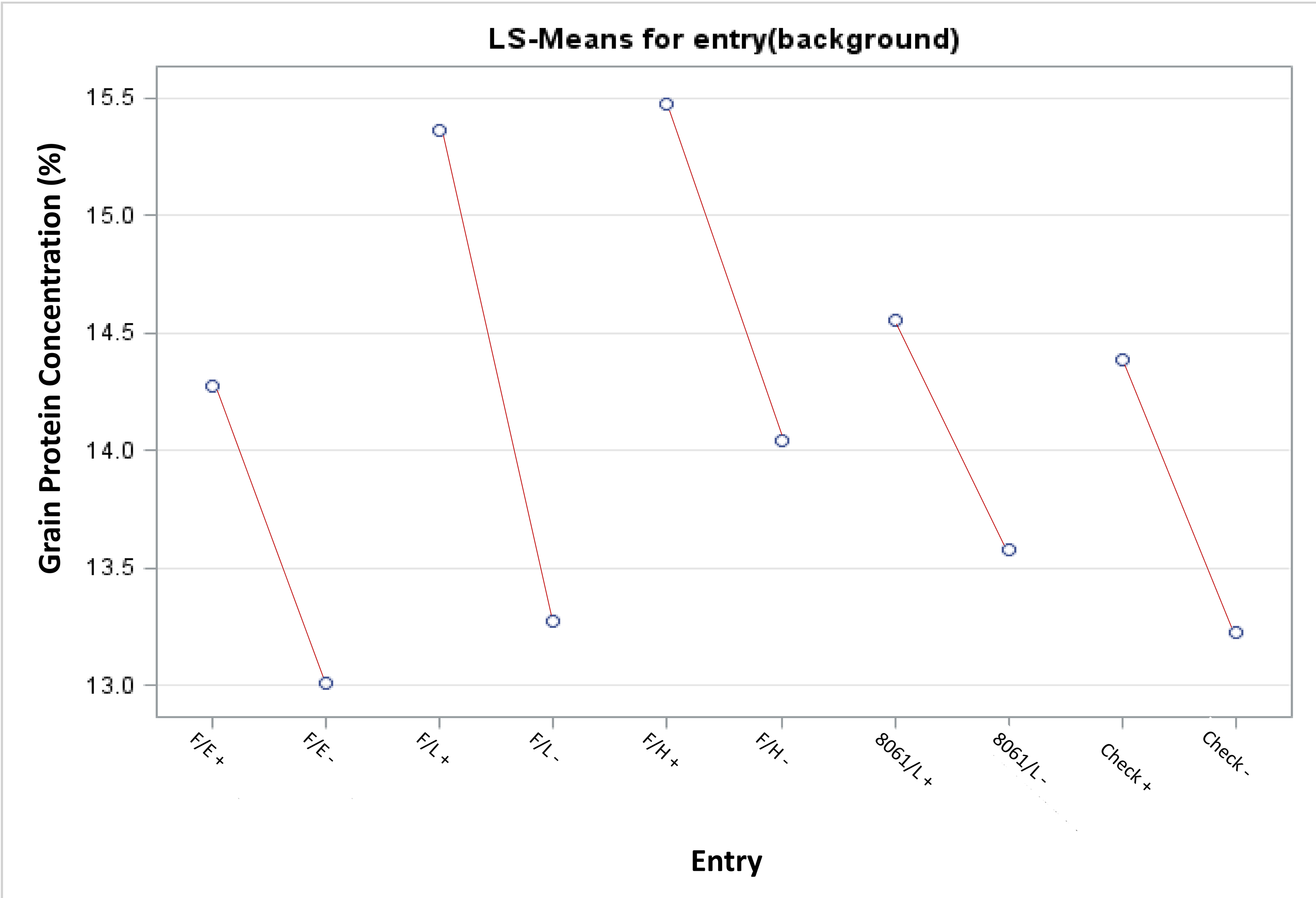
DNA marker analysis:

The near isogenic lines were analyzed with the sequence-tag site (STS) *uhw89* genetic marker. The codominant STS marker spans the complete DIC *Gpc-B1* locus (Distelfeld *et al*., 2006). This marker was used to identify lines with and without the *Gpc-B1* allele.

Table 1: Analysis of variance of grain protein content for four different near-isogenic lines derived from different hard red winter wheat backgrounds planted at three locations in the Pacific Northwest.

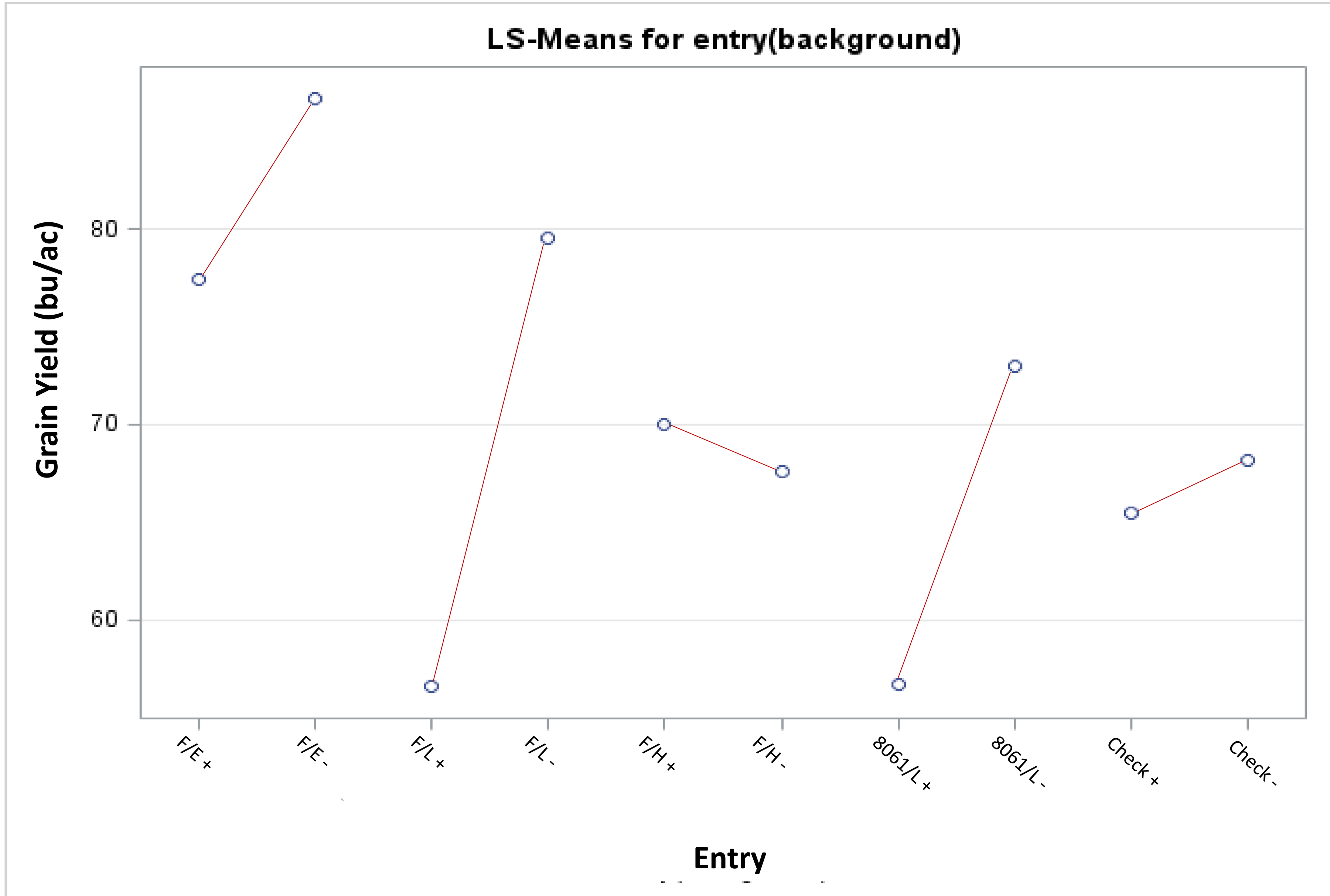
Source	*DF*	*Type I SS*	*Mean Square*	*F Value*	*Pr> F*
Location	2	47.1527	23.5763	15.18	<.0001
Bloc(location)	6	12.2473	2.0412	1.31	0.2619
Marker	1	43.2640	43.2640	27.86	<.0001
Background	4	14.0362	3.5091	2.26	0.071
*Marker*Background*	4	3.2638	0.8159	0.53	0.7174

Figure 1: Graph depicting the LS-means for grain protein concentration of near-isogenic lines with the *Gpc-B1* allele and those without across four different genetic backgrounds.



Note: F/E= Farnum/Eddy; F/L = Farnum/Lassik; F/H = Farnum/Hollis; 8061/L = WA8061/Lassik; "+" = contains *Gpc-B1* allele, "-" = does not contain *Gpc-B1* allele.

Figure 2: Graph representing the LS-means for grain yield potential (bu/ac) between four sets of near-isogenic lines with and with the *Gpc-B1* allele. Lines with the *Gpc-B1* allele showed higher grain protein content, but yield relationship varied between backgrounds.



Note: F/E= Farnum/Eddy; F/L = Farnum/Lassik; F/H = Farnum/Hollis; 8061/L = WA8061/Lassik; "+" = contains *Gpc-B1* allele, "-" = does not contain *Gpc-B1* allele.

Results:

- Bloc and the Marker*Background interaction were not significantly different (Table 1).
- Location demonstrated significant differences in GPC (Table 1). The Lind location demonstrated 1% higher GPC than did the Pullman locations. The Location*Background and Location*Marker interaction was not significantly different (data not shown), indicating that presence of the allele and NIL had similar results across locations.
- Results from data analysis shows that protein concentration was significantly different (P<0.0001) by an average of 1.4% between cultivars with the *Gpc-B1* allele and cultivars without (Table 1).
- Collectively, genetic background had no significant effect on the *Gpc-B1* allele (Table 1).
- Individually, the *Gpc-B1* allele had varying effects on protein content across cultivars of different genetic backgrounds (Figure 1):
  - Eddy with the *Gpc-B1* allele increase protein by 1.3%
  - Farnum by Lassik background with the *Gpc-B1* allele increase protein by 2.1%
  - Hollis background with the *Gpc-B1* allele increase protein by 1.4%
  - WA8061 by Lassik background showed no significant differences.
- Analysis showed that the *Gpc-B1* allele had no significant effect on heading date or test weight.
- Analysis showed that the *Gpc-B1* allele has a significant effect on grain yield potential.

Discussion:

- Contrary to the results in spring wheat (Brevis and Dubcovsky, 2010; Carter et al., 2012), the *Gpc-B1* allele did not decrease test weight in winter wheat.
- When crossed with Farnum, NIL with Eddy, Lassik, and Hollis backgrounds demonstrated a significant difference in protein concentration between the lines with and without the *Gpc-B1* allele. This trend was similar among both years and locations of field data (Pullman 2012, Pullman 2013, and Lind 2013).
- In the Hollis background, the *Gpc-B1* allele clearly showed an increase in GPC, regardless of yield potential.
- For the overall data set, there was a negative correlation between yield and protein (Figure 2); with an r^2 value of -0.56. However, the analysis of yield among backgrounds did not follow the same trend. Looking at backgrounds, cultivars with an Eddy or Lassik pedigree had significant differences between lines with and without the *Gpc-B1* allele – lines without the *Gpc-B1* allele had higher yields. Yet, NIL with a Hollis background demonstrated an opposite effect. Lines with the *Gpc-B1* allele (in the Hollis background) averaged higher yields than lines without the *Gpc-B1* allele (a difference of 2 bu/ac).
- Although the negative correlation between GPC and grain yield is well known, the fact that NIL with the *GPC-B1* allele in the Farnum by Hollis background showed both higher GPC and higher grain yield is noteworthy. This demonstrates that the negative correlation can be broken and breeding can make improvements on grain yield and GPC simultaneously.

Conclusions:

- Knowing the effect of the *Gpc-B1* allele across backgrounds will be useful for plant breeders when selecting for higher grain protein content, and identifying parents in crossing.
- In winter wheat, inclusion of the *Gpc-B1* allele increased GPC, but in some backgrounds also demonstrated a negative correlation with grain yield. Further investigation is ongoing to look at the relationship between the *Gpc-B1* allele, GPC, and grain yield.

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References:

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