Mapping the Genes Controlling Inulin Content in Wheat

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Abbreviations

1-FFT—2,1-fructan:2,1-fructan 1-fructosyltransferase
1-SST—sucrose:sucrose 1-fructosyltransferase
AMMI1—Additive Main Effects and Multiplicative Interaction
ANOVA—analysis of variance
CIM—composite interval mapping
G x E—genotype by environment
HPLC— high-performance liquid chromatography
IPC1—interaction principal component
LOD—logarithm of odds
QTL—quantitative trait loci

ABSTRACT

As a widely consumed staple food, wheat (*Triticum aestivum*) is a good vehicle for inulin, a complex carbohydrate that improves gastrointestinal microfauna populations, thereby increasing the body's ability to take up micronutrients and improving the immune system. This set of studies was conducted to determine the amount of variation for inulin content in wheat, the trait's heritability and the level of environmental effects on this trait, and to find quantitative trait loci for inulin content. Amongst 87 varieties, inulin content ranged from 0.4 to 14.6 milligram inulin per gram dry weight with a median value of 7mg/g dry weight, demonstrating that there is statistically significant genetic variation for inulin content. When 20 varieties were grown in 6 different locations, inulin content was found to have a low heritability (H^2=0.20), and GxE effects were a significant factor in predicting inulin content. From a population consisting of 101 doubled-haploid lines created from a cross between AC Reed and Grandin, composite interval mapping detected major QTLs on chromosomes 2BL-2 and 5BS, which explained 20.15% and 15.28% of the variation for inulin content, respectively. The results of these studies indicate that, although its heritability is relatively low, there is sufficient genetic variation for improving inulin content via biofortification in a wheat breeding program and that marker-assisted selection would be useful.

INTRODUCTION

Inulin is a complex carbohydrate found in a variety of fruits and vegetables. Fructans or fructooligosaccharides are included in the classification of inulin. It has several beneficial effects including stimulating the growth of beneficial bacteria in the gut. This allows them to outcompete harmful bacteria, improve the immune system, and aid in the uptake of certain

micronutrients (Niness, 1999). As a widely consumed staple food, wheat is a good vehicle for inulin. In Third World countries where many suffer from micronutrient malnutrition (Welch and Graham, 2004), wheat with increased inulin content would be especially helpful for alleviating health problems.

Inulin

Inulin is a complex carbohydrate in a chain formation consisting of two to sixty fructose molecules with a glucose molecule at each end. The fructose molecules are held together by β(2-1) linkages. The glucose molecules are attached by α(1-2) linkages (Fig. 1) (DeLeenher and Hoebregs, 1996; IUB-IUPAC, 1982; VanHaastrecht, 1995). Inulin is found as a storage carbohydrate in over 36,000 plants including crops such as onion, banana, garlic, chicory root, Jerusalem artichoke, and wheat. There is a wide range of inulin content in different food crops (Table 1). In wheat, low molecular weight inulin is found in the inner pericarp, testa, and endosperm; high molecular weight inulin is found in the outer pericarp, and negligible inulin is found in the embryo (Schnyder et al., 1993). Because inulin is found in the endosperm, it is present in white flour, which does not contain bran, and thus, will be present in white bread and other foods made with white flour. When inulin extracted from chicory was added to bread as a dietary fiber, quantifications of bread quality as well as taste testers found the product to be acceptable (Wang et al., 2002). This suggests that wheat with higher inulin content can be used in baked products without decreasing their quality or palatability.

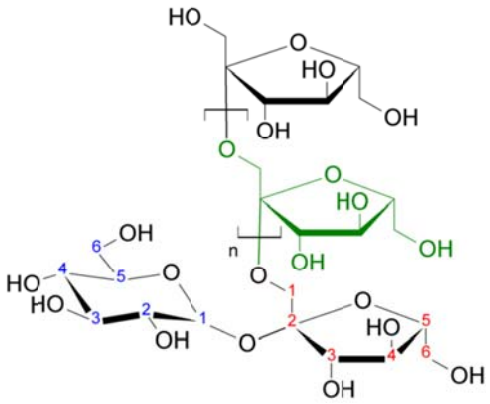

Fig. 1. Structural formula of inulin showing glucose molecule attached to fructose by β(2-1) linkage (image from Wikipedia, http://en.wikipedia.org/wiki/File:Inulin_strukturformel.png, accessed May 2011).

Table 1. Inulin content as percent of fresh weight in commonly consumed foods (VanLoo et al., 1995).

Food source	Inulin content (%)
Chicory	15-20
Jerusalem artichoke	14-19
Garlic	9-16
Leek	3-10
Artichoke	3-10
Onion	2-6
Rye	0.5-1
Barley	0.5-1.5
Banana	0.3-0.7

The biochemical pathway of fructan synthesis is a topic that is still actively researched. The theoretical pathways can be seen in Fig. 2. All involve using an enzyme to catalyze reactions of sucrose to make increasingly long chains of a glucose attached to many fructose molecules (SRI International, 2010). The inulin biosynthesis pathway that has been experimentally proven to exist is shown in more detail in Fig. 3. In this pathway, inulin formation is initiated by the enzyme sucrose:sucrose 1-fructosyltransferase (also known as 1-SST or by its Enzyme Commission designation EC 2.4.1.99). This enzyme acts to catalyze the reaction of two sucrose molecules to form β-D-glucose and 1F-beta-D-fructosylsucrose (shown in the diagram as GF_2 for glucose-fructose-fructose). 1F-B-difructosylsucrose is essentially the smallest molecule that can be classified as inulin. Inulin formation is continued with the aid of

another enzyme, EC 2.4.1.100, which is commonly called 2,1-fructan:2,1-fructan 1-fructosyltransferase or 1-FFT. As seen in Fig. 3, this enzyme works by adding another fructose molecule, derived from sucrose to the growing inulin molecule (Koops and Jonker, 1996). The activities of these enzymes take place in the vacuole (Wagner et al., 1983; Darwen and John, 1989). Additionally, the enzymes are not interchangeable as they have specific and separate functions. 1-FFT cannot catalyze the initial step of inulin formation, while 1-SST is not able to accelerate the formation of polymers that already have five or more fructose molecules in their chain. The two enzymes' only shared function is the catalysis of the formation of molecules with three or four fructose molecules, though these processes are more efficiently achieved by 1-FFT (Koops and Jonker, 1996).

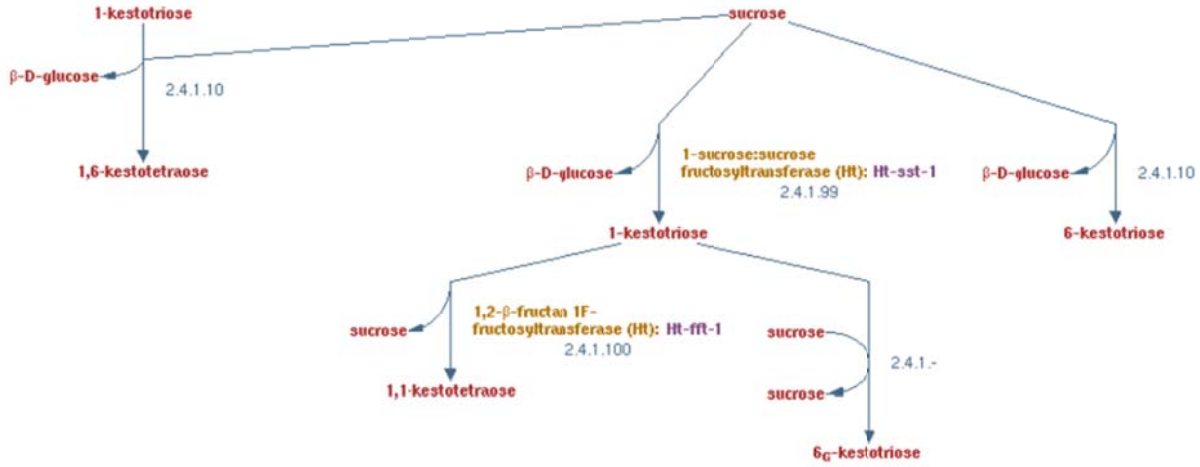

Fig. 2. Theoretical pathway for the biosynthesis of fructans. (Image from http://biocyc.org/META/new-image?type=PATHWAY&object=PWY-822&detail-level=2&ENZORG=TAX-4233, accessed May 2011).

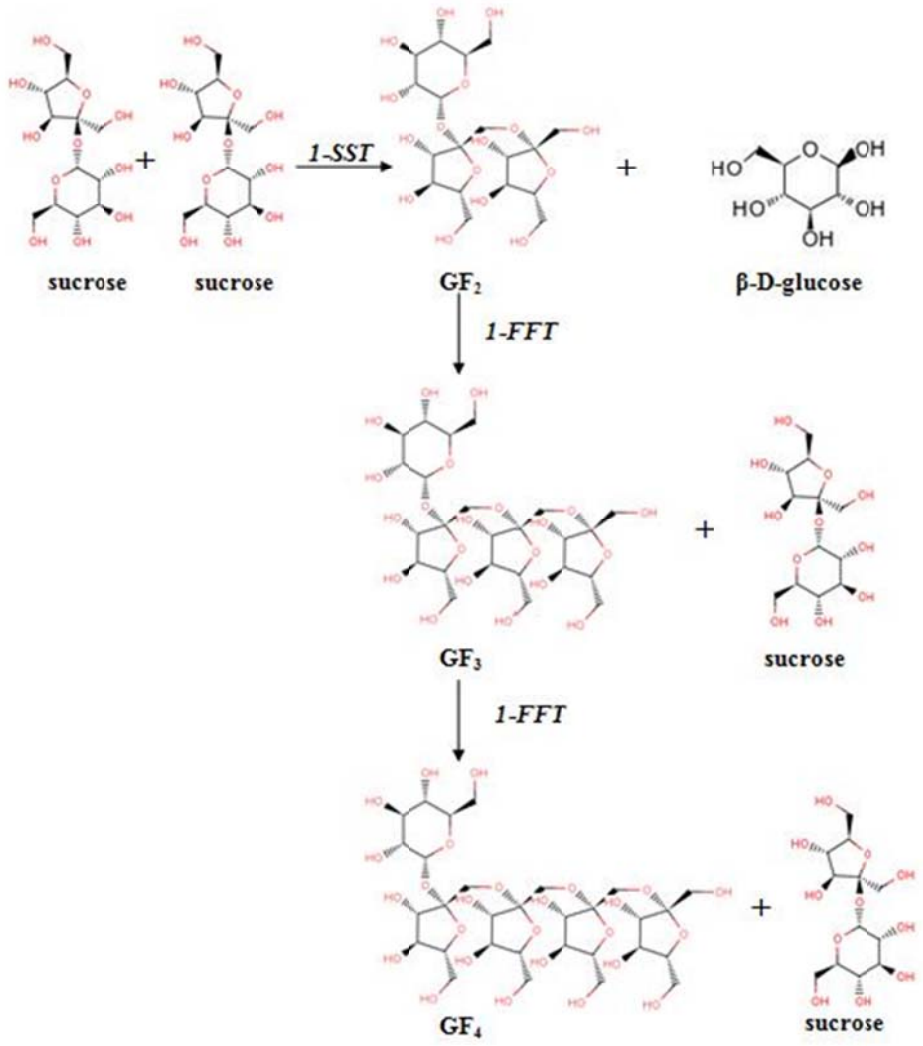

Fig. 3. The pathway for inulin synthesis. The enzyme 1-FFT repeatedly adds fructoses to the chain to make a longer inulin molecule.

Health Benefits of Inulin

Inulin cannot be broken down like other carbohydrates by human digestive enzymes in the upper gastrointestinal tract because of the β(2-1) linkages (Roberfroid and Delzenne, 1998) so it continues through the digestive track to the colon. This results in inulin's reduced caloric value and its function as a dietary fiber. In the colon, fermentation of inulin by bacteria that live there stimulates the growth of *Bifidobacteria* spp., which are known to be beneficial. Because they are being nourished, these beneficial bacteria can better compete with harmful bacteria, which can improve the overall human digestion and health. *Bifidobacteria* are also known to

benefit the immune system and to improve the uptake of ions and the synthesis of B vitamins (Niness, 1999). The improved uptake of minerals is a result of the fermentation of inulin by the gastrointestinal bacteria, which creates an acidic environment that is more favorable for ion uptake (Delzenne et al., 1995; Ohta et al., 1995). Calcium absorption may also be improved by mechanisms including enhanced pools of soluble and ionized calcium, increase in the colon's absorptive surface, and direct interaction of inulin with the intestinal tissue (Raschka and Daniel, 2005).

Studies have shown that, in rats, a diet high in inulin can increase the absorption of Ca^{2+}, Mg^{2+}, iron ions, and Zn^{2+} in the colon (Scholz-Ahrens et al., 2001; Delzenne et al., 1995; Ohta et al., 1995). The increased absorption of Ca^{2+} and Mg^{2+} increased bone mass and biomechanical properties of the bones, suggesting that inulin could indirectly help in fighting diseases like osteoporosis (Lobo et al., 2006). At this time, only a limited number of studies have been reported on inulin as it affects human micronutrient uptake (Scholz-Ahrens et al., 2001). Only calcium uptake has been proven to be significantly improved by high amounts of inulin in the diet (Roberfroid and Delzenne, 1998; Courdray et al., 1997). Longer term studies in humans will be necessary to determine inulin's effects on micronutrient uptake, bone health, and skeletal development (Scholz-Ahrens et al., 2001). Another study, in rats treated with azoxymethane to induce colon carcinogenesis, showed that they developed fewer tumors in their colons when inulin was added to their diet. This study implies that the low digestibility of inulin could help in preventing colorectal cancer (Jacobsen et al., 2006). Though sensitive people could experience some intestinal discomfort, higher inulin intake has not been demonstrated to have a toxic effect (Coussement, 1999). When participants consumed 22 to 34 grams of inulin per day as part of an energy intake study, they reported only mild to moderate amounts of discomfort in the form of

flatulence and bloating. Additionally, they reported that these symptoms improved within two to four weeks of beginning the added-inulin diet (Kruse et al., 1999).

Results of Huynh et al. study and related works

Currently, there is very little published information on inulin content in wheat. One paper has been published in Australia by Huynh et al. (2008a). Analyzing the fructan levels of doubled haploid lines created from a cross between a high-fructan variety and a low-fructan variety, this group found quantitative trait loci (QTLs) on chromosomes 2B, 3B, 5A, 6D, and 7A. Additionally, they reported that the QTLs on chromosomes 6D and 7A had large, favorable effects, explaining 17% and 27 % of variation in inulin content, respectively. These two QTLs were also seen in another cross. Although QTLs for increased fructan in vegetative tissue have been found in barley (Hayes et al., 1993), onion (McCallum et al., 2006), wheat stems (Ruuska et al., 2006), and perennial ryegrass (Turner et al., 2006), fructan content in vegetative tissues is not likely to be applicable to the fructan levels of the grains (Schnyder et al., 1993; Nardi et al., 2003).

Objectives

The overall goal of this project was to determine the feasibility of breeding wheat for increased inulin content. The diversity study aimed to determine whether there is enough genetic variation for inulin content amongst different varieties and experimental lines of wheat. The objectives of the genotype-by-environment (GxE) interaction experiments were to determine the heritability for inulin content and the level of environmental effects. The goal of the QTL study was to determine the number and location of QTLs for inulin content in a spring wheat population.

MATERIALS AND METHODS

Plant Materials

1. Genetic Diversity for Inulin Content

The wheat varieties and experimental lines used in this study were advanced lines in the Cornell Small Grains Breeding and Genetics Research Project and were grown in Ithaca, NY during the field season of 2004 (Appendix A).

2. GxE Interaction Effects on Inulin Content

The wheat varieties in this study (Table 7) were chosen because they represented a range of inulin concentration . In 2008, these lines were grown at six locations: the Helfer, Caldwell, and Snyder fields at the Cornell University Agricultural Experiment Station, in Ithaca, NY; in Akron, CO, and in an irrigated field and a dry field in Fort Collins, CO.

3. Inulin Content QTL Mapping Study

The wheat population used in this study consisted of 101 doubled-haploid lines developed by Agriculture Canada at Winnipeg, Province of Manitoba, Canada. It was derived from a cross between two varieties, AC Reed (soft white spring) and Grandin (hard red spring) (Breseghello et al., 2005). These lines were grown on the Snyder field of the Cornell University Agricultural Experiment Station in Ithaca, NY during the spring of 2009 and were hand harvested during that summer. Additionally, samples of the parent varieties grown in 2006, 2008, and 2009 were included.

Inulin extraction and analysis

Measuring the inulin content of the wheat lines was completed using a simplified enzymatic hydrolysis and high-performance liquid chromatography (HPLC) method as described by Quemer et al. (1994) with some modifications.

For each sample, approximately 10 grams of seed were ground in a household coffee grinder to a fine powder. The grinder was cleaned between samples to prevent cross contamination. Then, between 0.2 and 0.25 grams of the ground powder of each sample were weighed out and put into a 15 mL centrifuge tubes, which were then filled with boiling deionized water. For the standards, between 0.025 and 0.05 grams of pure inulin from dahlia tubers (Sigma I-3754) were weighed and placed in 15 mL centrifuge tubes, which then were filled with boiling deionized water. Before adding water, the tubes were weighed with sample in them with their caps on. They were weighed again after the water was added. This allowed for an indirect measurement of how much water was added to each sample tube. All tubes were vortexed and then shaken for an hour. Next, the tubes were centrifuged at 4000 rpm for 10 minutes.

Two aliquots of the extract were taken from each sample. An aliquot of 1000 μL was put into a 1.5 mL Eppendorf tube with 30 μL of inulinase solution (Sigma I-2017) to hydrolyze inulin into fructose and glucose. These samples were then incubated in a water bath at 60°C for an hour and centrifuged for 10 minutes at 13,000 rpm. From this mixture, 50 μL was put in a Dionex autosampler tube that contained 100 μL of 1.0 mM rhamnose, 850 μL of deionized water, and 8 μL of chloroform. Another aliquot of 100 μL was placed directly into a Dionex autosampler tube containing 100 μL of 1.0 mM rhamnose, 8 μL of chloroform, and 800 μL of deionized water. The Dionex autosampler tubes were vortexed before being placed into an AS-50 Autosampler from which 10 μL were injected directly into the Dionex eluent stream. The Dionex system was equipped with a GS50 Gradient Pump, ED 50 Electrochemical Detector with Integrated Amperometric Detector, and a CarboPac PA1 guard column (4x50 mm) coupled to a CarboPac PA1 analytical column (4x250 mm). Table 2 describes the linear gradient used for the Dionex system.

Table 2. The linear gradient of NaOH and deionized water (diw) eluents for the Dionex system.

Time (minutes)	% diw	% 300 mM NaOH
0.00	96.0	4.0
15.00	96.0	4.0
29.00	40.0	60.0
36.50	0.0	100.0
40.50	0.0	100.0
40.51	96.0	4.0
43.00	96.0	4.0

Data analysis

Inulin concentration was calculated by the following equations

$$I = k(G_i + F_i)$$

$$G_{inulin} = G_{untreated} - G_{treated}\text{; where } k = \frac{180 + 162(n-1)}{180n} \text{ and } n = \frac{F_{inulin}}{G_{inulin}} + 1$$

$$F_{inulin} = F_{untreated} - F_{treated}$$

where I=inulin; G_i=G_{inulin}=glucose from inulin; $G_{untreated}$=glucose from untreated sample; $G_{treated}$=glucose from inulinase-treated sample; F_i=F_{inulin}=fructose from inulin; $F_{untreated}$=fructose from untreated sample; $F_{treated}$=fructose from inulinase-treated sample;180=molecular weight of glucose or fructose; 162=molecular weight of the anhydrous form of these molecules; and n= the sum of fructose and glucose molecules per inulin molecule (Quemer et al., 1994). $G_{untreated}$, $G_{treated}$, $F_{untreated}$, $F_{treated}$ were measured by HPLC as mentioned above.

In the GxE interaction study, average inulin content was calculated by averaging the concentrations for the two technical replicates and then averaging that value for each of the two field replicates. Finally, that value was averaged for all three locations in each state. The data were analyzed using analysis of variance (ANOVA) in JMP with several different models. The best fit model used the factors Location, Source, LocRep nested within Location, and Source by Location where Location was the field where the wheat was grown. Source was the variety of wheat, and LocRep was the replicate in the field location. The factor Source was considered a fixed effect, and the factors Location and LocRep[Location] were random effects. Rep which

was the technical replicate was eliminated from this model because it did not improve the fit of the model.

QTL Analysis

The marker and mapping data used for the QTL analysis were obtained from an earlier study. The genetic map was constructed with 340 molecular markers including 222 AFLP, 42 RFLP, 75 SSR and one STS (Breseghello et al., 2005). The QTL analysis was completed in QTL Cartographer (Wang et al., 2011) using composite interval mapping (CIM) with 1000 permutations and an α-level of 0.05. From this permutation test, a threshold of logarithm of odds (LOD)=3.99 was determined to be appropriate for deciding which QTLs were significant.

RESULTS AND DISCUSSION

Genetic Diversity for Inulin Content

Inulin content was assessed for 87 varieties. The mean percent inulin was used to determine the range of inulin values (Fig. 4). The values for inulin content ranged from 0.4 to 14.6 milligrams inulin per gram dry weight with a mean of 7.3 mg/g and a median of 7.0 mg/g. This equates to about a 3.6 fold range in inulin content. Significant variation for inulin content amongst different varieties of wheat, barley, rice, and rye has been reported (Table 3). The results from the present study show lower inulin content than the values reported previously. However, the calculated range is comparable to those that have been published.

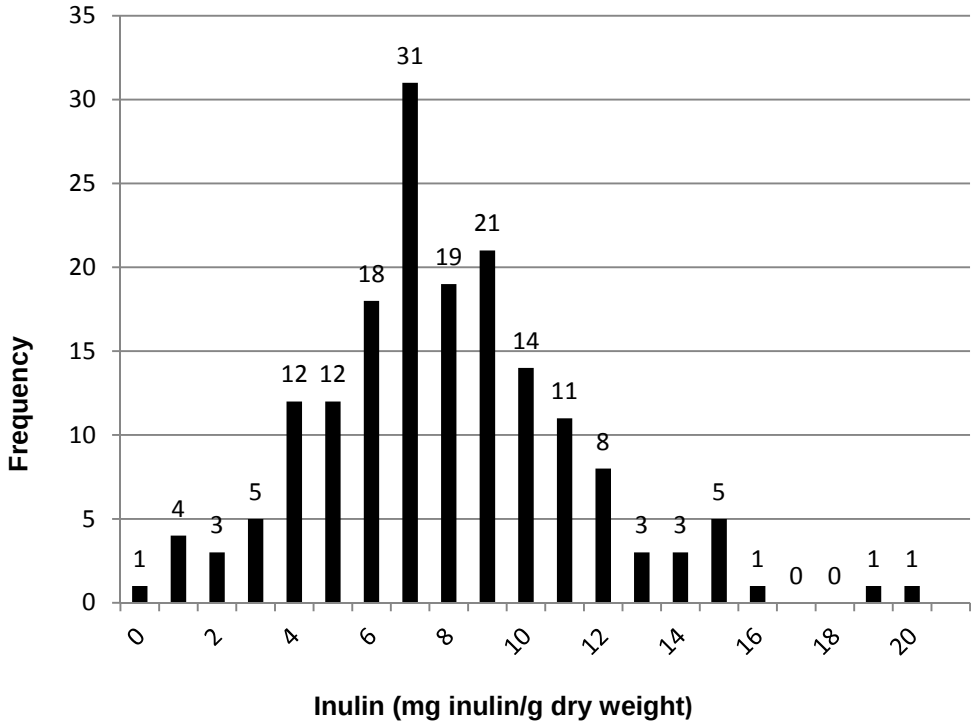

Fig. 4. Range of inulin content in varieties tested.

Table 3. Range in inulin concentration (milligrams inulin per gram dry weight) for barley, rice, rye, and wheat

Food crop	Range	Factor of difference	Source
barley	5-15	3.00x	Cerning-Beroard and Guilbot, 1975
rice (brown)	1.7-8.4	4.94x	Genc et al., 2005
rice (milled)	0.2-2.9	14.5x	Genc et al., 2005
rye (flour)	0.5-10	2.00x	Asami et al., 1989
wheat	55-85	1.54x	Genc et al., 2005
wheat (green house)	7-16	2.29x	Huynh et al., 2008b
wheat (field)	15-23	1.53x	Huynh et al., 2008b
wheat (flour)	10-40	4x	Cerning-Beroard and Guilbot, 1975; Nilsson and Dahlquist, 1986

The data were analyzed in the JMP computer software program (JMP 8.0 2008, SAS Institute Inc.) using analysis of variance and a Tukey multiple-comparison test on the replicated checks to determine whether varieties had significantly different mean inulin contents. Amongst varieties used as replicated checks, AC Reed, Caledonia, and KanQueen were significantly different from Opata (Table 4). Variety was determined to be a significant indicator of inulin content ($p=0.0075$) (Table 5).

Table 4. Tukey test performed on replicated checks. Varieties not having the same letter are significantly different (p<0.05). Mean inulin reported in milligrams inulin/g dry weight.

Variety		Mean
AC Reed	A	10.4
Caleconia	A	10.2
Kan Queen	A	9.8
M6/Synthetic	A B	9.8
Cayuga	A B	9.7
Foster	A B	9.4
Grandin	A B	7.8
Jennah Ketifu	A B	7.8
Charmi	A B	7.4
Opata	B	6.0

Table 5. ANOVA table for one-way analysis of percent inulin by variety.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Variety	9	0.0000569	6.326e-6	3.67	0.0075*
Error	20	0.0000345	1.726e-6		
C. Total	29	0.0000914			

GxE Interaction Effects on Inulin Content

The analysis of variance model explained 94.31% of the variation in inulin content (R^2=0.9431) indicating that all of the factors except replicate contributed to the variation in inulin content (Table 6). The heritability of inulin content was estimated to be 0.20 by the equation for broad sense heritability, $H^2 = \frac{\text{var}(g)}{\text{var}(p)}$. Inulin content of the lines evaluated in this experiment was somewhat predictable as their rank was similar in both Colorado and NY. However, three varieties, AR 910-0-1, M00-3701, and T143, showed a strong location interaction with relatively higher inulin concentration when grown in New York (Fig. 5, Table 7). The interaction of these varieties with location most likely accounts for the significant GxE interaction effects. The Additive Main Effects and Multiplicative Interaction (AMMI1) biplot (Fig. 6) illustrates the structure of the data. The abscissa represents differences in main effects, namely genotype means (over environments) and environment means (over genotype), and the ordinate shows the first interaction principal component (IPC1) scores or the interaction between

the two factors. For instance, varieties Jagger (Jagg) and NuFrontier (NuFr) differed mostly by main effects, varieties VA97W-375WS (VA97) and M00-3701 (M003) differed mostly by interaction effects, locations Akron (Akro) and Snyder (Sny) differed in both respects, and varieties Jagger (Jagg) and Ripper (Ripp) were similar in both respects. Figures 5 and 6 illustrate the variability in inulin content due to variety and environment. With low heritability and significant GxE interaction, it will be necessary to evaluate inulin content over multiple environments to accurately assess genotypic values.

Table 6. Data for the effect of each factor in the ANOVA model.

Source	DF	Sum of Squares	F Ratio	Prob > F
Location	5	0.000709	174.555	<0.0001*
Source	19	0.000391	25.302	<0.0001*
LocRep[Location]	6	0.00000931	1.910	0.0851
Source*Location	95	0.000414	5.370	<0.0001*

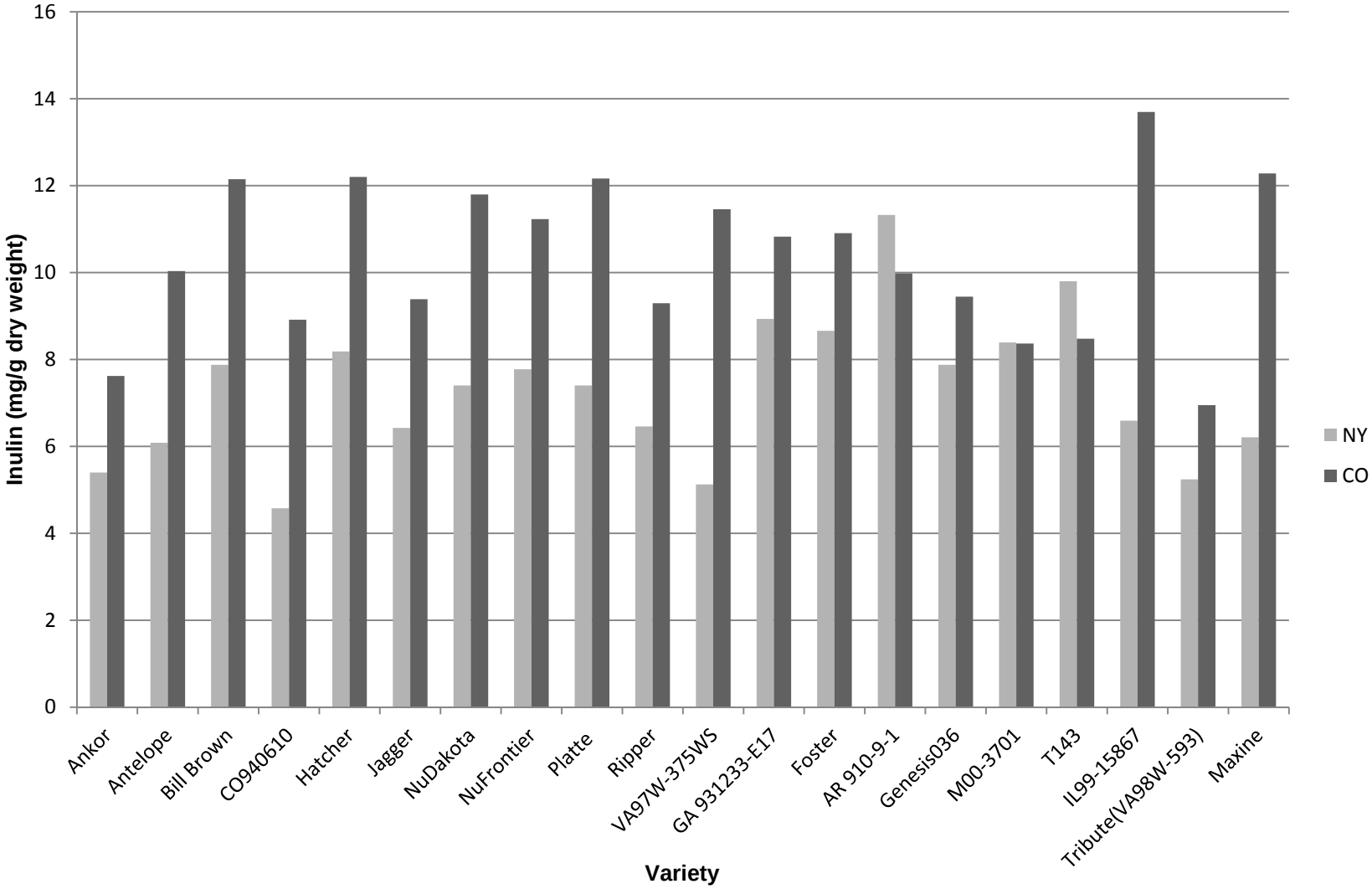

Fig. 5. Inulin content for each of the twenty varieties tested in the GxE study.

Table 7. Average inulin content for each variety in each field location in New York and each field location in Colorado. Units are milligrams inulin/gram dry weight.

Variety Name	New York			Colorado		
	Helfer	Caldwell	Snyder	Akron	Ft.Collins (Dry)	Ft.Collins (Wet)
Ankor	5.950	5.050	5.200	8.212	7.274	7.372
Antelope	7.725	6.200	4.325	10.301	10.914	8.883
Bill Brown	8.300	8.300	7.025	12.569	11.935	11.938
CO940610	5.150	4.925	3.650	9.188	9.260	8.295
Hatcher	8.125	8.075	8.350	14.175	11.520	10.902
Jagger	7.650	6.675	4.950	9.737	9.213	9.208
NuDakota	9.025	6.750	6.425	12.651	12.192	10.545
NuFrontier	8.750	8.475	6.100	12.242	11.174	10.273
Platte	8.600	7.925	5.675	12.157	11.925	12.402
Ripper	7.400	6.175	5.800	10.444	8.908	8.527
VA97W-375WS	6.075	5.950	3.350	12.754	11.663	9.953
McIntosh (GA 931233-E17)	11.475	7.275	8.050	12.433	8.817	11.229
Foster	9.300	8.800	7.875	10.848	10.542	11.327
AR 910-9-1	13.325	9.650	11.000	11.889	8.624	9.432
Genesis036	10.275	7.225	6.125	10.668	8.564	9.104
M00-3701	10.925	7.300	6.950	10.495	7.852	6.749
T143	11.500	8.975	8.925	8.554	8.923	7.956
IL99-15867	7.325	6.625	5.825	14.599	13.610	12.871
Tribute(VA98W-593)	5.550	5.650	4.525	7.668	7.065	6.109
Maxine	7.150	6.400	5.075	12.862	10.415	13.562

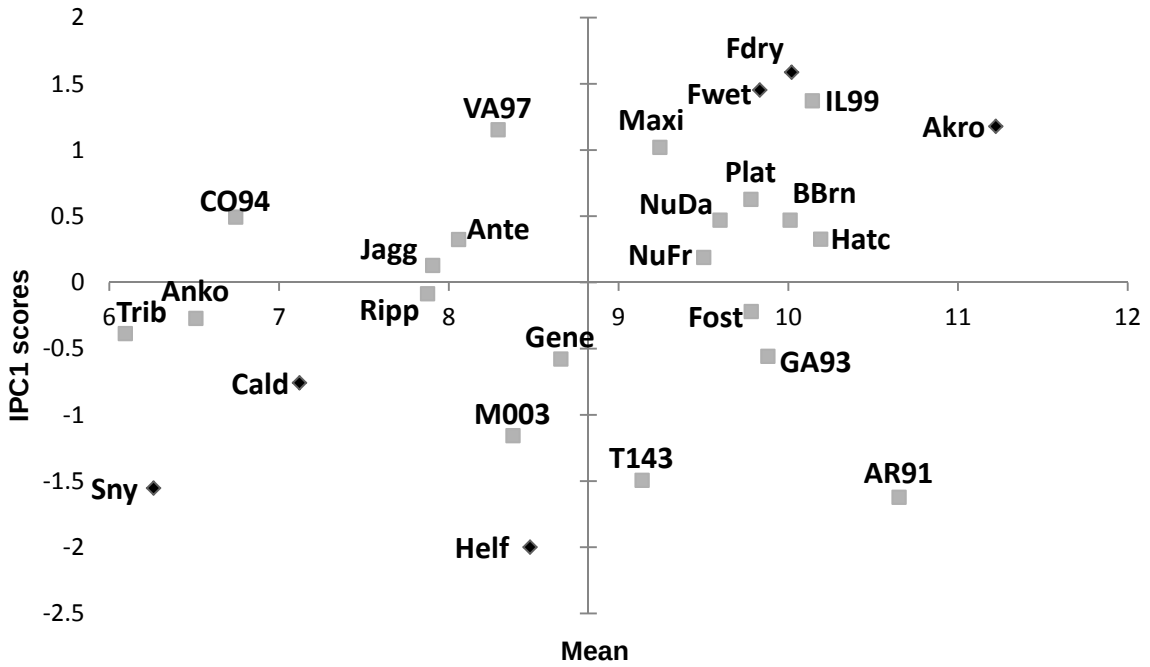

Fig. 6. The AMMI1 biplot for the GxE interaction effects experiment. Variety and location names are represented by abbreviations that consist of their first four letters or numbers. The abscissa shows main effects, namely genotype means (over environments) and environment means (over genotypes), and the ordinate shows the IPC1 scores. The vertical center line indicates the grand mean (an inulin content of 8.82204) and the horizontal center line indicates an IPC1 score of zero. Means are in units of % inulin and IPC1 the square root of this. This graph accounts for 93.85% of the treatment sum of squares. The root mean square residual for the AMMI1 model is 0.62328.

Inulin Content QTL Mapping Study

At the $p<0.01$ level, significant QTLs were detected on linkage groups 2BL-2 (long arm of chromosome 2 from B genome) and 5BS (short arm of chromosome 5 from B genome). For both QTLs, higher inulin alleles were from Grandin, and together were responsible for an 18% increase in inulin as compared to the alleles from Reed (Table 8). The QTL on chromosome 2BL-2 alone explained 20.15% of variation in inulin content while the QTL on chromosome 5BS alone explained 15.28% (Table 8). Figure 7 shows the map locations of the QTLs. Huynh et al. reported a QTL for higher inulin content on chromosome 2B as well. However, the location of

this QTL cannot be compared to the location of the one on chromosome 2B in the present study because the two studies did not have any markers in common.

Table 8. Significant QTLs for inulin content as determined by CIM.

Chromosome	Markers	R^2	Additive	LOD
2BL-2	beyond gcag9 toward end	0.20148	from Grandin, 0.1042	5.45877
5BS	between gwm499 and bcd1823	0.152838	from Grandin, 0.07634	4.350705

Fig. 7. Genetic linkage groups on which QTLs for inulin content were found. These linkage groups are named by homeologous group, genome, and chromosome arm. Regions filled in grey indicate loci of QTLs detected by CIM.

CONCLUSION

In order to improve a trait in a breeding program, we need to know whether there is genetic variation for the trait, how the environment affects the trait and if there are genotype-by-environment interaction effects. The number of genes that control the trait of interest and their location in the genome are important for using marker-assisted selection in a breeding program. The results of the diversity study showed that there is significant genetic variation for inulin content amongst different wheat varieties and experimental lines with a range of inulin concentrations from 0.4 to 14.6 milligram inulin per gram dry weight. In the GxE study, heritability was found to be relatively low with a value of 0.20. Most of the varieties had higher inulin content when grown in Colorado. However, three varieties (AR910-9-1, T143, and M00-3701) showed a strong interaction with location with relatively higher inulin content in NY. In

the QTL mapping study, two major QTLs for inulin content were identified on chromosomes 2BL-2 and 5BS. Based on these experiments on inulin content in wheat, the most efficient breeding strategy would be to use marker assisted selection to backcross the two QTLs into an elite variety.

Potential for breeding wheat varieties with sufficiently higher inulin content

Though other food crops have higher levels of inulin, wheat is so widely consumed that it could increase the inulin levels in diets worldwide. In human studies, doses of inulin as small as 4 grams per day increased the amount of *Bifidobacteria* in the colon (Buddington et al., 1996; Williams et al., 1994). This suggests that, considering wheat to have an average of 0.0073 grams of inulin per gram of dry weight (from the diversity study), daily consumption of wheat would need to be about 550 grams of wheat (pre-cooking weight) per day in order to achieve biologically significant inulin intake if it were the only source of inulin. Only three nations, Tunisia, the United Arab Emirates, and Azerbaijan, currently consume enough wheat daily per capita (552.6, 559.3, and 602.5 grams respectively) to obtain a useful amount of inulin from wheat assuming average inulin content (Food and Agriculture Organization of the United Nations, 2011). Thus, in a developing nation such as India, where daily wheat consumption is 164.94 g/day (Food and Agriculture Organization of the United Nations, 2011), wheat inulin content would need to reach 2.42% to provide people with enough inulin for health improvements. Because some varieties have inulin content as high as 1.46%, it would likely be possible to breed wheat with increased inulin to reach a content of 2.42%. In addition, a broader survey of different wheat varieties would likely identify genotypes with higher levels than those found in this study.

Future work

Future research will be necessary to achieve the goal of creating a wheat variety with substantially higher inulin. Additional QTL mapping studies using populations from parents with a larger difference in inulin content will be necessary to map more QTLs. Also, it will be necessary to perform the experiments in more environments as inulin shows considerable variation across environments. Future efforts could use fine mapping to more precisely identify the chromosome regions and genes affecting inulin content and to develop markers more closely linked to the genes. Furthermore, once remaining questions regarding inulin's effects are addressed, this work can be utilized to maximize benefits in human nutrition and health.

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Appendix A. Mean inulin content (milligrams inulin/grams dry weight) and percentage relative standard deviation, which tells precision and repeatability of the measurement, for each variety tested in the diversity study.

Variety Name	Mean	Relative St. Dev.	Variety Name	Mean	Relative St. Dv.
Rural New Yorker	7.04	8.0%	VA00W-526	3.60	0.5%
Pride of Genesee	5.64	15.8%	MSU Line E1007	4.40	18.6%
American Banner	11.42	0.7%	P961341A3-1-2	6.17	14.1%
Goldcoin	9.68	3.4%	X00*1118	6.12	3.6%
Grandprize	7.14	19.4%	P91202RB1-3-3-4-5	1.99	4.5%
Honor	8.74	1.9%	IL99-15867	0.70	13.0%
Forward	6.69	8.2%	Caldwell	3.19	0.8%
Valprize	7.90	10.4%	KY93C-0378-5-2	3.63	0.7%
Yorkwin	6.33	1.8%	MO980829	7.60	12.7%
Nured	6.19	1.4%	VA97W-375WS	0.41	141.4%
Cornell 595	9.40	5.7%	X00-1079	4.83	11.9%
Genesee	10.29	2.2%	B980582	9.24	9.1%
Avon	8.55	0.7%	McCormick(VA98W-591)	6.52	23.3%
Yorkstar	9.38	8.1%	Honey	3.24	26.2%
Arrow	8.72	13.4%	Maxine	1.88	59.3%
Ticonderoga	11.09	4.5%	Patton	8.06	0.6%
Purcell	6.98	4.7%	Pioneer Var25R23	4.28	5.8%
Houser	7.74	8.2%	Red Caledonia	7.33	19.8%
Geneva	6.79	21.6%	TW044-094	10.74	6.0%
NYBatavia	8.08	11.5%	Roane	8.07	20.1%
Cayuga	5.98	9.6%	Sisson(Va96W-250	8.44	23.4%
D8006	5.71	20.0%	Genesis R036	14.60	8.3%
Pioneer Var25W33	6.03	4.3%	Pioneer Var25R54	11.12	32.9%
NY87048W-7388	4.82	7.9%	TW005-008	8.31	58.2%
NY89088-9118	5.68	5.2%	Seafire	9.93	9.7%
CaledoniaResel-A	8.02	2.19%	NY93255-7340	7.96	8.0%
Harus	5.88	4.5%	Royale	5.37	21.2%
NY89063-9126	3.57	12.1%	SREXP43	6.07	20.5%
T143	18.77	3.80%	Blizzard	5.13	7.9%
G39186	11.45	6.67%	Icebreaker	2.33	117.3%
Foster	13.40	7.20%	TW044-065	7.70	23.4%
AR 910-9-1	12.75	3.97%	SR12	6.71	10.1%
T141	11.39	9.14%	Tribute(VA98W-593)	1.41	
GA 931233-E17	14.25	1.43%	Agripro Douglas	4.79	9.3%
M00-3701	12.80	13.39%	Red Caledonia	5.73	35.4%
B980696	10.41	2.44%	Genesis 9953	8.76	27.9%
AR 93027-3-2	9.43	7.44%	Pioneer Var2510	4.37	43.2%
KY93C-1238-17-1	4.96	47.52%	Genesis R045	6.75	12.2%
MD 11-52	6.90	36.9%	Pioneer Var25R35	2.75	3.1%
Danny exp.	6.40	27.5%	Cardinal	6.58	2.7%
Jolly exp.	6.66	2.5%	Warwick	6.97	10.3%
M99*3098	8.53	9.5%	Pioneer Var25R37	6.66	2.5%
MV 5-46	10.73	6.5%	SW43	11.18	37.2%
IL97-3632	9.69	4.2%			