Relationship between efficiency of nitrogen use and industrial quality in Argentine varieties of Bread Wheat (*Triticum Aestivum* L.) with different gluten composition

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ABSTRACT

The efficient use of nitrogen in crops can minimize environmental risks and maximize returns to farmers. This can be achieved by adjusting the fertilization management and/or using the genetic variability. In addition to its effect on yield, N has a significant effect on the quality of wheat grains. The latter is also influenced by the gluten protein composition and the environment. The aim of this work was to study the relationship between N recovery efficiency and its partitioning between grain and stover with industrial quality attributes in Argentine wheat bread varieties of different cycle and with different allelic composition of gliadins and glutenins. In the current work, field trials of 27 Argentine bread wheat varieties of different cycle were carried out during 2011/12 season, in Azul, Province of Buenos Aires, Argentina. The fertilizer treatments were: control treatment (T) and fertilized treatment (F) with split application of urea at variable rates to reach 210 kg n/ha. The following characteristics were determined: grain yield and its components, parameters of N use efficiency, alveographic parameters, sedimentation volume, protein content, wet gluten content and N stover content. The protein pattern of each variety was identified by polyacrylamide gels. The genotype was the main source of variation for total recovery efficiency, grain recovery efficiency and stover recovery efficiency. The N harvest index decreased from 0.81 in control to 0.73 in fertilized treatments on average, and variety*treatment interaction was significant. The protein content in grains and the dough extensibility were highly dependent on the level of n while the baking strength it was of the genotype. The ability to partition n to grain explained the relationship between the extensibility and the n recovery efficiency. The assessment of high molecular weight glutenin alleles was not strictly coincided with quality group of the variety showing the influence of other protein fractions and their interaction with the environment. The expression of the genetic potential attributed to the allelic composition of gluten may be regulated in part by n use efficiency attributes.

Keywords: nitrogen recovery, end use quality, glutenins, gliadins.
INTRODUCTION

Nitrogen (N) is one of the main inputs in cereal cultivation and has become an important agricultural pollution factor through leaching and fertilizer runoff. Therefore, a more efficient use of N in production represents a current challenge for agronomic research, and especially for plant breeding programs, with the aim of minimizing environmental risks and maximizing producers’ incomes (Muurinen et al., 2006).

The response to fertilization in different production situations is markedly affected by genetic differences in regards to potential yield and N absorption and retranslocation to harvestable organs (Van Sanford and MacKown, 1987). The duration of the cycle of the variety is another important variation factor (Rostami and O Brien, 1996). The genotype and N level affect the nutrient use efficiency in terms of yield and captured N (Barracough et al., 2010), as well as the grain recovery efficiency of the applied fertilizer (Guarda et al., 2004).

There are two ways of increasing the N use efficiency (EUN) in wheat crops, adjusting the management of fertilization or achieving better varieties. The efficiency of fertilization can be increased with managements and crop rotations that impact on the N levels in the soil, as well as fertilizer applications adjusted to the crop’s demand, taking into account the achievable yield and the climate. The other way to improve EUN is to develop varieties able of recovering more N from soils and fertilizers and to use it to generate more grain (Barracough et al., 2010). It should be noted that the captured N not translocated to grains returns to the system by means of mineralization, and becomes available (to a greater or lesser extent) for the next crop. Although the latter does not generate a direct productive result, it contributes to improve sustainability.

In addition to its effect on yield, the N captured and translocated to grains exerts an important effect on the amount of protein in grains and other quality parameters. The protein content in grain, strongly associated with the gluten content, is partially determinant of industrial quality (Labuschagne et al., 2006; Pinilla Quezada et al., 2008). The type of proteins of gluten (gliadins and glutenins) are characteristic of each variety and provide specific properties related to their end use.

The effect of N on the rheological parameters of the dough, such as those obtained using the Chopin Alveograph: baking capacity (W), tenacity (P) and dough extensibility (L) (Alzueta et al., 2008; Fuertes-Mendizábal et al., 2010, 2012). The dough extensibility (L) is the alveographic parameter most influenced by the availability of N (Park et al., 2006), and thus stable genotypes in N recovery efficiency in grain and agronomic efficiency show less variation in the values of this parameter even between years with different rainfall distribution (Lerner et al., 2013). However, little is known about the relationship between the industrial quality of the flours and the determinants of grain N recovery: capture and partition.

Materials and Methods

Field tests

The tests were conducted during the 2011/12 campaign in the experimental field of the Faculty of Agronomy, National University of the Center of the province of Buenos Aires, Azul, Buenos Aires (36° 49’ 53” South latitude, 59° 53’ 23” West longitude). We included twenty-seven Argentine long-cycle (LC) bread wheat (Triticum aestivum L.): ACA 201 and 304, BIOINTA 2005, Buck Baqueano, Malevo, 55 CL2, SY 100 and SY 200, Klein Carpincho, Gladiador and Yarará, Relmó INIA Torcaza; and short-cycle (SC): ACA 231 and 304, BIOINTA 2005, Buck Baqueano, Malevo, 55 CL2, SY 100 and SY 200, Klein Carpincho, Gladiador and Yarará, Relmó INIA Torcaza; and short-cycle (SC): ACA 801, BIOINTA 1003, Buck AGP Fast, Meteoro and SY 300, Don Mario Atlax and Arex, Klein León, Nutria, Proteo and Rayo, Santa Rosa BAT 102, 111 and 112 (experiments), Sursem SRM 2331. Sowing dates were July 5 and August 8, 2011, and the target densities were 400 and 450 plants/m² for the first and second group, respectively.

Genes in the chromosomes in groups 1 through 6 control the coding of gluten proteins. The glu-A1 loci, glu-B1 and glu-D1 loci encode high molecular weight glutenin (HMWG) subunits. Payne et al. (1987) observed that HMWG subunits were directly associated with gluten strength and, by analyzing numerous varieties of bread wheat, established an index called Scoreglu-1, where a quality value was assigned to each variety (from 3 to 10) depending on the subunit composition of these proteins. For the construction of this classification, the sedimentation test (SDSS) (Axford, 1979) was used to estimate gluten strength, which is very important to determine the final use of flours (Peña, 2002; Cornish et al., 2006).

Low molecular weight glutenins (LMWG) are controlled by genes from the loci glu-A3, glu-B3 and glu-D3 located on the short arms of chromosomes 1AS, 1BS and 1DS, near the genes encoding certain gliadins (γ- and ω-gliadins) and therefore they are partially jointly inherited (Metakovský et al., 1990). In a first glance, there are two broad groups of gliadins, those resembling the Chinese Spring (CSS) variety and those similar to the Cheyenne variety (CNN). The latter provides better rheological properties to the flours.

On the other hand, the substitution of the short arm of the chromosome 1B of wheat by the short arm of the chromosome 1R of rye (1B/1R translocation) has a negative impact on wheat bread quality due to the loss of a group of LMWG and gliadins (ω-gliadins), which is replaced by secalines (Martin and Carrillo, 1999). This last factor has been corrected in the aforementioned Score. Another factor that may affect the relationship between different protein groups is the overexpression of the Bx7 subunit (HMWG), which only associates to subunit 8 (Marchyllo et al., 1992) and improves quality.

The objective of the present work was to study the relationship between the efficiency of N capture and its partition between grain and stover with attributes of industrial quality in Argentine varieties of wheat bread of different cycles and with different allelic composition of gliadins and glutenins.
We worked with two levels of nitrogen (N): control (T), which corresponded to emergency base fertilization with a dose of urea equivalent to 50 kg/ha of diammonium phosphate (9 kg N/ha), and fertilized (F), with split application of urea, 40% at emergence and 60% at the end of tillering, at variable doses according to soil analysis at planting in order to achieve a total of 210 kg/ha of N (210 kg N/ha - N Soil) that correspond to the requirements of N for an objective yield of 7 t/ha. Triple superphosphate was applied at pre-sowing to ensure phosphorus non-deficiency. Pests, diseases and weed were controlled when appropriate.

Rainfall and temperature

Figure 1 shows the the average monthly rainfall and temperatures for the campaign (Regional Center for Agrometeorology, Centro Regional de Agrometeorología, FA-UNCPBA, 2011). During the crop cycle the cumulative precipitations were 229.3 mm for long cycles and 174.6 mm for short cycles. It should be noted that in November a total of 79.9 mm was concentrated.

Measurements and calculations in the crop

At harvest and on a 1m² plot we determined total dry matter (TDM), grain yield (GY), dry matter of stubble (DMS), grain weight (P1000). We calculated number of grains (NG), harvest index (HI) and number of spikes (Nsp). We determined percentage of proteins in grain (%Pro) (NIR) and percentage of N in stover (% NS) (Micro-Kjeldahl) and we calculated total absorbed N (NabsT), N absorbed in grain (NabsG), N absorbed in stover (NabsS) and N harvest index (ICN= NabsG/NabsT). We calculated the Total N Recovery Efficiency (TRE), in grain (REG) and in stover (RES) from the calculated percentage of N in grain (N= Protein / 5.75) (% NG) and the percentage of N in stover, according to the following formulas derived from Guarda et al. (2004):

- Total N recovery efficiency (%): \( \frac{\text{N (kg/ha)} - \text{N grain+stover treat. T (kg/ha)}}{\text{N applied (kg/ha)}} \times 100 \)

- Recovery efficiency of N in grain (%) = \( \frac{\text{N grain treat. N (kg/ha)} - \text{N grain treat. T (kg/ha)}}{\text{N applied (kg/ha)}} \times 100 \)

- Recovery efficiency of N in stover (%) = \( \frac{\text{N stover treat. N (kg/ha)} - \text{N stover treat. T (kg/ha)}}{\text{N applied (kg/ha)}} \times 100 \)

Quality assessment

Industrial quality rheological parameters were obtained from the flour obtained from grinding the harvest grains: baking capacity (W), tenacity (P), extensibility (L) and P/L ratio using a Chopin Alveograph (AACC International, 1999). We performed the sodium dodecyl sulphate sedimentation (SDSS) sedimentation test (Dick and Quick, 1983) on wholegrain flour. Varieties were classified according to their quality group (INASE, 2014).
Identification of the protein pattern

Electrophoresis was performed on polyacrylamide gels (SDS-PAGE, one-dimensional, T= 13.5%), with sequential extraction of gliadins and glutenins according to Gupta and Mc Ritchie (1991). The quality of the different variables was measured according to Score glu-1 (Payne, 1987), which takes into account the HMWG composition and the 1B/1R transposition correction. In turn, the patterns of LMWG were numbered by electrophoretic mobility similarity, the type of gliadins was determined as well as the presence of overexpression of the Bx7 subunit and 1B/1R translocation.

Experimental design and statistical analysis

The experimental design was in three complete random blocks with split plot. Macro-plots of 13.3 m² were used, randomizing the varieties in the main plots and the fertilization treatments in the secondary plots. The results were analyzed by means of Analysis of Variance (ANOVA) and comparison of means by Duncan’s test (α≤0.05) and Principal Component Analysis (PCA) using the Infostat statistical package (Di Rienzo et al., 2014). For each attribute studied by ANOVA, the percentage of sum of squares of each source of variation with respect to the sum of squares of the model (% SC) was calculated, in order to estimate the contribution of each factor on the variability of these attributes.

RESULTS AND DISCUSSION

Yield and components

The variables TDM, GY, DMS, HI and Nsp were significantly differed between varieties and treatments (Table 1). Lerner et al. (2013) reported similar effects of the variety and nitrogen fertilization treatment on GY, although their interaction was significant. On the other hand, the interaction variety*N was significant for the variables %NG,%NS, NabsT, NabsG and NabsS, although the value %SC value was low in all cases (Table 2).

This indicates that the genotypic differences in N capture and its partition to grain depended on the availability of the nutrient for the crop. In contrast, Le Gouis et al. (2000) found that the interaction variety*N was not significant for %NG in experiments conducted in France, although it was significant for yield and its components. The main effect of the fertilization treatment on NabsG was due to responses in both GY and %NG. Similarly, the average response in NabsS corresponded to variations in DMS and %NS, determining higher NabsT values (Table 3).

Significant differences were observed between cycles for TDM, GY, DMS, HI, Nsp, %NG, %NS, NabsT, NabsG and NabsS (Tables 1 and 2). The effect of this factor in favor of long-cycle varieties on NabsG was due to the responses of GY and %NG (Table 3). The interaction cycle*N was significant for TDM, DMS, %NG, NabsT and NabsS (Tables 1 and 2). Also, we noted a higher response to fertilization in %NG for short-cycle varieties, whereas for NabsS a higher response to the addition of N was found in the long-cycle varieties, probably due to the longer stage of biomass accumulation (Table 3).

Nitrogen use efficiency

Significant differences were found between varieties in terms of total N recovery efficiency (TRE), in grain (REG) and in stover (RES)(Table 4 and Figure 2). Genotype proved to be a significant source of variation for the recovery efficiencies, as reported by Guarda et al. (2004). On the other hand, Figure 2 shows that the behavior of the different varieties in REG did not strictly coincide with what was observed in RES and therefore in TRE. This can be partially explained by the genotypic variability found in the N harvest index (NHI) (Table 2 and Figure 3), which determines different patterns of N partitioning within the plant, similar to the report of Barraclough et al. (2010).

Long-cycle varieties presented significantly higher TRE (LC= 43.30 a; SC= 33.55 b) and RES (LC= 19.33 a; SC= 13.04 b) values when compared with short cycle varieties, although there were no differences in REG (LC= 23.97 a, SC= 20.51 a). Therefore, the differences found between cycles in N capture were reflected in changes in the ability to allocate N in stubble rather than in grains. This may be due in part to the different doses applied to the different cycles, which are involved in the calculation of RES, due to the variation in the availability of N in the soil at the time of sowing.

We observed significant differences between varieties and between treatments in NHI, but not between cycles (Table 2 and Figure 3). Fertilized treatments presented, on average, lower values of this variable than the controls (T= 0.81 a, N= 0.73 b). This shows a limitation in the capacity of retranslocation of N to harvestable destinations with high doses of fertilizer, which is in agreement with Le Gouis et al. (2000). The interactions cycle*N and variety*N were significant, although with reduced %SC. However, it is worth mentioning some varieties that did not respond to general- ity, such as BIO1003, AGPFAST, Meteoro, León, Proteo, Torcasa, BAT111 and BAT112, which were stable in ICN between fertilization treatments.

Industrial quality

The percentage of protein in grains (% Pro), which is a parameter of commercial quality partially associated to industrial use, presented significant effects of the cycle, the variety and the level of N. There were significant interactions cycle*N and variety*N. The most important sources of variation were fertilization and genotype, accounting for 55.8% and 26.7% of variability, respectively, while %SC and cycle interactions were low (Table 5).

The baking capacity (W), the main attribute of industrial quality, presented significant differences between cycles, varieties and N levels, and the interaction variety*N was significant. But in this case, the genotype, clearly associated with the protein composition of gluten, was the source of preponderant variation followed by fertilization. As for the deter-
Table 1. Percentage of sum (TDM), grain yield of squares (%SC) and p-value of the sources of variation (SV) considered for total dry matter (GY), dry matter of stover (DMS), harvest index (HI) Harvest (IC) and number of spikes per m2 (Nsp).

*p-value<0.05; **p-value<0.01; ***p-value<0.0001.

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Table 2. Percentage of sum of squares (%SC) and p-value of the sources of variation (SV) considered for percentage of nitrogen in grain (%NG), percentage of nitrogen in stubble (%NS), total absorbed nitrogen (NabsT), nitrogen absorbed in grain (NabsG), nitrogen absorbed in stover (NabsS) and nitrogen harvest index (NHI).

*p-value<0.05; **p-value<0.01; ***p-value<0.0001. The variables expressed as percentage were transformed (√) for analysis.

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Table 3. Means of total dry matter (TDM, kg/ha), grain yield (GY, kg/ha), dry matter of stover (DMS, kg/ha), harvest index (HI), number of spikes per m2 (Nsp), percentage of nitrogen in grain (%NG), percentage of nitrogen in stover (%NS), total absorbed nitrogen (NabsT), nitrogen absorbed in grain (NabsG), and nitrogen absorbed in stover (NabsS) for the combinations of long cycles (LC) and short cycles (SC) and control (T) and fertilized (N) treatments. Means with a common letter are not significantly different (Duncan, α= 0.05). The variables expressed as percentage were transformed (√) for analysis.

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Table 4. Percentage of sum of squares (%SC) and p-value of sources of variation (SV) considered for the total recovery efficiency (TRE), in grain (REG) and stover (RES).

*p-value<0.05; **p-value<0.01; ***p-value<0.0001.

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The PCA that included parameters of industrial quality and efficiency determinants of the use of N (Figure 4) indicated that the main component 1 (CP1) was conformed by the variables P (+), W (+), SDSS (+), TRE (+) and NabsT (+) and explained 31.4% of the variability observed, and the main component 2 (CP2) by L (+), NHI (+) and %Pro (+) accounted for 28.9% of the variability. The formation of groups according to the variety cycle was not observed, so that that factor did not present discriminative capacity for the studied variables.
Considering the variables associated to CP1, the alveographic parameter W and the sedimentation volume presented a high correlation with each other, being mainly determined by the ability to capture N by the crop (NabsT). According to CP2, extensibility (L) was strongly associated with %Pro, in agreement to the observations of Godfrey et al. (2010) and was determined by the NH1. This last fact evidences that the relationship between the extensibility and the efficiency in N recovery (REG) reported by Lerner et al. (2013) would be related to a greater capacity to partition N to harvestable destinations (NHI) and not to the capture of N from the soil (NabsT).

Protein composition

Of the loci coding for subunits of high molecular weight glutenins (HMWG), glu-B1 showed the highest variability followed by glu-A1, and the allelic variants 7 + 9 and 2* were the most frequent for the first and the second, respectively. In contrast, the glu-D1 locus was more homogeneous and the pair of subunits 5+10 were the most preponderant, except for the variety León, which presented the allele 2 + 12, of lower quality (Table 6). These results coincide with that reported by Lerner et al. (2009) for the Argentine collection of bread wheat.

For the subunits of low molecular weight glutenin (LMWG), we identified and grouped band patterns, and found a great variability in the electrophoretic mobility (Figure 5). Regarding the composition of gliadins, the CSS type was the most frequent and with lower quality when than CNN, according to Masci et al. (1991). Only four varieties presented introgression with rye (1B/1R) and were located in the low quality quadrants of the PCA, due to the above mentioned factors concerning the replacement of a group of LMWG and gliadins (ω-gliadins) by secalines (Martín and Carrillo, 1999). The ACA304 variety was the only one with overexpression of band 7 (7oe), and presented a good performance for the variables efficiency and quality (Table 6 and Figure 4). The Score glu-1, which ranks according to the HMWG composition, did not coincide strictly with the Quality Group (QG) classification; we found heterogeneity in the Score within each QG or vice versa (Table 6). This may be due to the influence of other protein fractions such as LMWG and gliadins (Branlard and Dardevet, 1985). The first accounts for about one-third of total protein in grains and 60% of total gluten (Shuaib et al., 2007) and have a significant influence on the final use of wheat flour (Wang et al. 2009), and are important in this case due to the great variability found in the band patterns.

In the PCA of Figure 6, shaped in the same way as in Figure 4, the varieties are grouped according to phenotypic attributes of use of N and industrial quality, which may also contribute to explain the lack of association between the Score and the QG. The varieties ACA201, Malevo, SY100 and SRM2331, belonging to QG 2 but possess Score 10, except for SY100 that has Score 8; presented high values in the CP1 and intermediate values in the CP2. This indicates that they had high baking capacity (SDSS, W) and tenacity (P), associated with intermediate NHI and high values of TRE and NabsT.

Yarará and ACA304 belong to QG 1 and have a Score 9 and 10 respectively. They presented positive values in CP 1 and CP 2. This indicates that they had high baking capacity and a balanced P/L ratio, in association with higher NHI than the previous. The variety 55CL, from QG 2 and Score 9, presented negative values in CP1 and intermediate values in CP2; as well as the varieties León and AGPFAST varieties, from QG3, which indicated a reduced baking capacity.

Although these genotypes presented an intermediate NHI, their TRE and NabsT values are very low which may partially explain this response. On the other hand, BAT112 (Score 9) was in the same group as the Carpincho and Baqueano varieties, from QG3 and with Score 6. These genotypes displayed intermediate values in CP1 and negatives values in CP2, thus indicating an intermediate baking capacity, but with reduced extensibility (L) due to low values of %Pro and NHI. An interesting case is that of variety Arex which, despite having Score 9, is in QG3.

This could be due to its low capacity to partition N to grain (NHI), which reduced the %Pro and the extensibility of the dough (negative values in CP2). On the other hand, varieties Meteoro, Torcaza and Rayo, despite having a high

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Table 5. Percentage of sum of squares (% SC) and p-value of the sources of variation (SV) considered for protein percentage in grain (%Pro), baking capacity (W), tenacity (P), extensibility (L) and volume of sedimentation (SDSS).

*p-value<0,05; **p-value<0,01; ***p-values≤0,0001. The variables expressed as percentage were transformed (√) for analysis. Prepared for this edition.
Score and being in QG1, were not located within the group of higher W (negative values in CP1). This could be explained by its high NHI and consequent increase of %Pro and extensibility (high values in CP2). Thus, imbalances can be produced in the proportions of each type of protein, leading to very extensible doughs and therefore affecting the final quality (Godfrey et al., 2010). In turn, the variety Proteo presented a very high %Pro but associated with a somewhat higher baking capacity, when compared to the previous ones (Table 6 and Figure 6). For all of the above, the ability of the crop to capture the N of the soil and to partition it to grain modified its content and protein balance, which would affect the expression of the genetic potential attributed to the allelic pattern of gliadins and glutenins.

**CONCLUSIONS**

Genotype was the main source of variation for the recovery efficiency of total N, grain and stover.

The ability to retranslocate N to harvestable destinations was affected by variety, N level and their interaction. It decreased with high doses of fertilizer in most of the genotypes.

The duration of the cycle affected the recovery efficiency of N due to changes in the recovery efficiency in stover, although differences between genotypes were also detected within each cycle.

The protein content in the grains and the extensibility of doughs were highly dependent on the level of N, while the baking capacity - main attribute of industrial quality - was determined to a greater extent by the genotype.

The relationship between extensibility and recovery efficiency of N in grain was related to the crop’s greater ability to partition N to harvestable destinations and not to the capture of N from the soil.

The score glu-1, which ranks according to HMWG alleles, did not strictly coincide with the Quality Group of the variety, evidencing the influence of other gluten protein fractions and their interaction with the environment.

The expression of the genetic potential attributed to the allelic composition of gliadins and glutenins could be regulated in part by attributes of efficiency in the use of N.

**ACKNOWLEDGEMENTS**

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Table 6. Allelic composition of high molecular weight glutenin (Glu-A1, Glu-B1, Glu-D1), type of gliadins (Gli), low molecular weight glutenins (LMWG), presence of introgression with rye (1B/1R), presence of overexpression of the Bx7 subunit (7oe), Quality Group (QG) and Score Glu1 (Score) for the analyzed varieties.

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Figure 5. Electrophoretic mobility patterns of low molecular weight glutenins (LMWG). CS: Chinese Spring (marker); P1: BIO2005; P2: BIO1003; P3: ACA201; P4: ACA801; P5: BAT102; P6: BAT111; P7: Carpincho; P8: Baqueano; P9: Atlax; P10: Meteoro; P11: SY100, SY200, SY300; P12: AGPFAST, León; P13: BAT112; P14: Yarará; P15: ACA304, SRM2331; P16: Torcaza, 55CL; P17: Malevo, Gladiador, Rayo; P18: Proteo; P19: Nutria.

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Figura 6. Biplot of the first and second principal component for total nitrogen recovery efficiency (TRE), total absorbed nitrogen (NabsT), nitrogen harvest index (NHI), protein percentage (% Pro), sedimentation volume (SDSS), baking capacity (W), tenacity (P) and extensibility (L) of 27 wheat varieties. The genotypes are represented by the points and the vectors represent the variables of use of nitrogen and industrial quality. The denomination of the variety, Quality Group (QG) and Score glu-1 (S) were included for the genotypes mentioned in the previous discussion.
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