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Changes in yield determinants and quality traits in wheat after 12 cycles of recurrent selection

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Abstract

The objective of this study was to test the genetic response to 12 cycles of a recurrent selection program for grain yield and related traits. During two seasons the 39 S-derived families (three/ C_0 – C_{12} populations) were evaluated under conventional (CT) and non-tillage (NT) systems. Grain and biomass yield were determined. The grain number per square meter and harvest index were also estimated. Flour protein content, sodium dodecyl sulphate sedimentation, and lactic acid solvent retention capacity were considered as end-use quality predictive tests. High molecular weight glutenin subunits (HMW-GS) variation was also analyzed. A significant increase in grain yield per selection cycle, estimated in 3.25 g m⁻² (1.3%) per year, was found under NT, but there was a not significant genetic progress under CT. The decrease in the percentage of protein in the most advanced selection cycles (11.0% vs. 9.7%) did not negatively affect its quality. HMW-GS variation analysis showed the erosion of extreme subunits related to gluten strength. After 24 years of recurrent selection, the grain yield was improved without affecting its industrial quality.

Keywords Wheat · Recurrent selection · Grain yield · Grain protein · HMW-GS

Abbreviations

CT	Conventional tillage
NT	Non-tillage
SDS	Sodium dodecyl sulphate sedimentation
SRC	Solvent retention capacity
HMW-GS	High molecular weight glutenin subunits
LMW-GS	Low molecular weight glutenins subunits
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide
	gel electrophoresis
RS	Recurrent selection

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Introduction

While some negative effects of non-tillage (NT) soil management can be seen (Herrera et al. 2013; Bankina et al. 2015), grain yields in conventional tillage (CT) and NT systems are comparable (Fernández et al. 2007; Tang et al. 2013). Lower yields under NT have led to the adoption of occasional strategic tillage in continuous NT farming systems; however, the impact in the long-term may be negligible or negative (Dang et al. 2015). These considerations agree with what was stated by Herrera et al. (2013), that many NT treatments had only some characteristics of well-established NT environments, that, according to Toliver et al. (2012) occurs after 10 years of NT. Maich and Di Rienzo (2014), comparing CT versus NT soil management, showed that only under CT a significant genetic progress was obtained. The grain yield obtained in the wheat crop cultivated under a newly implemented (6 year) NT soil management was 50% lower than those achieved in CT; this lower grain yield may have negatively affected the substantiation of a significant genetic progress in NT.

The increase in potential yields in wheat brought with it a decrease in the protein content of the grains. Moreover, a negative association has been observed between the SDS test (predictive test of the industrial quality of the flour) and the grain yield (Oelofse et al. 2010). However, the decrease in the amount of protein and the content of wet gluten could be compensated by an improvement in protein quality (Hristov et al. 2010). In this sense, the lactic acid solvent retention capacity (SRC) test is associated with the formation of the glutenin framework during baking and the strength of the gluten; therefore, it is useful to predict the breadmaking quality of wheat flour (Colombo et al. 2008).

Protein quality in wheat is mostly explained by allelic variation of the storage proteins glutenins and gliadins. Based on electrophoretic migration, glutenins are defined as high molecular weight glutenin subunits (HMW-GS) encoded at the *Glu-1* loci and low molecular weight glutenins subunits (LMW-GS) principally encoded by *Glu-3* loci. Allelic variation in loci encoding HMW-GS can be easily distinguished by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). This simplicity has facilitated the identification of specific HMW-GS and their contribution to breadmaking quality. This information, combined with the presence of the 1BL/1RS rye translocation, was used to create a Glu-1 quality score which is widely used for breadmaking selection and germplasm characterization in breeding programs (Bonafede et al. 2015).

The objectives of this study were to determine (1) genetic gain for grain yield and other agronomic traits after 12 cycles of recurrent selection, (2) the indirect effects on grain protein concentration, and (3) the genetic variation of HMW-GS.

Materials and methods

The recurrent selection program

The recurrent selection (RS) program was conducted in Campo Escuela de la Facultad de Ciencias Agropecuarias (Universidad Nacional de Córdoba), Córdoba (31° 29' S; 64° 00' W) in the central semiarid region of Argentina. The RS started in 1985 with an initial population (C_0) generated by crossing sixteen commercial varieties of bread wheat (Triticum aestivum L.) to obtain eighty-three F1 hybrid combinations. In each cycle of recurrent selection (each cycle is 2 years long because of evaluation and recombination), the selected material, in number no less than ten, out of a total of forty to fifty S₀ evaluated progenies, was crossed according to a complete diallel scheme. Throughout the 12 cycles of recurrent selection, the grain yield of the S₀ progenies became the main selection criteria. S₀ progenies were grown in single-row plots of 1.3 m long and 0.2 m row spacing, at a seeding rate of 100 grains per square meter, without replications and with regular controls. Check varieties were included at regular intervals for visual reference. The breeding program was conducted under rain-fed conditions,

without fertilization, and under CT until the ninth cycle of RS, from the tenth cycle of RS, NT conditions was used. Seed bulks of the selected progenies per cycle (C_0-C_{12}) were created and multiplied annually. Plants from these seed mixtures were grown at regular spaces in both dimensions (20 cm by 20 cm) during 2013. Three plants per cycle with satisfactory grain production for further plot evaluation were retained.

The genetic progress measurement

Experiments were established during two successive seasons (2015 and 2016). Sowing was done on 5th and 4th of May 2015 and 2016, respectively. Heading occurred in September and maturity at the end of October. The 39 S-derived families (no less than three generations of selfing) were evaluated under CT and NT systems in two fields, next to each other, located in the same site of the selection program. Single row plots 5 m long with row spacing of 0.20 m, at a seeding rate of 250 viable seeds by square meter were used. Because of restrictions to randomization, imposed by the tillage practices, two replicates in complete blocks where allocated within each field. Complete weed control was done by hand weeding.

At the plot level, grain and biomass yield $(g m^{-2})$ were determined. The grain number per square meter and harvest index (%) were also calculated. For each trait, a linear mixed model (regression) was fitted to the experimental data. The fixed terms of the model included the tillage factor (conventional tillage–non-tillage), the number of recurrent selection cycles as a covariable, and the interaction between them. This way of specifying the fixed part of the model allows the fitting of two regression lines: one for each tillage practice. The random part of the model included the effects of: year, blocks within year-tillage practice, family, and the random interactions among year, and fixed terms of the model. A significance level of 5% was used in order to interpret results.

Climatic condition

Residual soil moisture stress is the most common type of water stress experienced by rain-fed wheat in this zone, leading to post-heading water stress. The first year (2015) was characterized by a low total rainfall (73 mm), with a strong water deficit occurring at heading. The second year (2016), by contrast, was wetter (150.4 mm).

Quality testing

Three experimental lines per cycle were analyzed. The cycles evaluated were: C_0 , C_4 , C_8 and C_{12} . The methods of AACC 44-19 and 46-13, respectively, were used to

determine the moisture and protein content in the flour. The quality of the flour was determined by the method of AACC 56-11 (Lactic Acid SRC) and the sedimentation index in sodium dodecyl sulfate or SDS approved method 56–70 (AACC 2000). Data from cycles of RS were analyzed using ANOVA using Di Rienzo, Guzmán y Casanoves test (DGC) as the pair-wise comparison method. Significance level was 0.05. All data were analyzed using a statistical software package InfoStat (Di Rienzo et al. 2018).

HMW glutenin subunits scoring

SDS-PAGE analysis was used to identify HMW-GS constitution in experimental lines. Briefly, gliadins were extracted with 1.5 M dimethylformamide from wholemeal flour (single seed, two extractions per family). After a brief centrifugation, the glutenin pellets were solubilized to be separated by SDS-PAGE as before (Moiraghi et al. 2013). HMW-GS were designated as previously described (Payne and Lawrence 1983). The presence of the highly expressed Bx7 subunit for Glu-B1 locus was tested in lines scored as 7 + 8 by SDS-PAGE using PCR markers according to Butow et al. (2004).

Results

Grain yield and related traits

Table 1 summarizes the p values for the hypothesis of null slopes under CT and NT (first two columns). p values for the hypothesis of non-difference between intercepts under CT and NT are shown in the third column. A summary of linear regression lines fitted to each trait are shown in Fig. 1. Regarding the slopes, under NT they were significantly greater than zero for grain yield, harvest index and seeds

 Table 1
 Summary of p values for the hypothesis of null slopes and for the differences between intercepts of regression lines fitted on the number of recurrent selection cycles under conventional tillage and non-tillage

Trait	CT-slope p value	NT-slope p value	Intercept diff.(CT- NT) <i>p</i> value
Grain yield (g m ⁻²)	0.110832	0.000805	0.169036
Aerial biomass (g m ⁻²)	0.706761	0.356418	0.878521
Harvest index (%)	0.000711	0.000000	0.081514
Grains per square meter	0.366139	0.001025	0.056932

CT Conventional tillage, NT non-tillage

p values for the hypothesis of null slopes: first two columns. p values for the hypothesis of non-difference between intercepts: third column

per square meter (Table 1—2nd column). Under CT, the trait that showed a slope significantly greater than zero was the harvest index (Table 1—1st column). A significant increase of grain yield under NT, around 3.25 g m⁻² (1.3%) per year, was observed. On the other hand, under CT the estimated increase per year was about 1.54 g m⁻² (0.7%) which was nonsignificantly different from zero (Table 1, Fig. 1a).

Grain quality traits

Table 2 shows the mean values corresponding to the quantitative and qualitative variables analyzed. Statistically significant differences between means were found for grain yield and protein percentage. While a significant positive trend, for grain yield was found, as more cycles of recurrent selection were achieved, on the other hand, the percentage of proteins in the flour decreased. The two predictive quality tests (SDS and the lactic acid SRC) did not show a significant trend after 12 cycles of RS.

High molecular weight glutenin subunits

A reduction in the allelic frequencies of the HMW-GS was observed. Six subunits were observed in C₀, five subunits in C₄, five subunits in C₈ and three subunits (17 + 18 and 7 + 8 segregating in line 12-3) in C₁₂ (Table 3). Taking into consideration the original allelic variation observed in the parentals used in our study (Table 4), 2 + 12 (Klein Atlas, Marcos Juarez INTA, Leones INTA and Diamante INTA) was the first subunit eroded by RS followed by 7+9, 17+18, 70e+8 and 2*. The final fixed HMW-GS combination was 1, 7+8, 5+10 discarding extreme HMW-GS associated with very strong (70e+8) or weak (2+12, 7+9) gluten.

Discussion

Grain yield and related traits

During the evaluation of ten cycles of recurrent selection (Maich and Di Rienzo 2014), 6 years after replacing CT by NT (2005), only under CT the wheat showed a significant genetic gain for grain yield. In measuring the response to 12 cycles of recurrent selection, 10 years after replacing CT by NT, it was the NT soil management in which a significant change was in evidence. These results could be due to what was stated by Herrera et al. (2013), where many NT treatments had only some characteristics of well-established NT environments, which according to Toliver et al. (2012) occurs after 10 years of NT.

Considering that the largest contribution to the increased yield potential of modern wheat varieties came from the increase in harvest index (Zhang et al. 2012; Bustos et al.

Fig. 1 Regression lines on the number of recurrent selection cycles (NRSC), through conventional tillage ("c"—solid line) and non-tillage ("n"—dot dash line)



 Table 2
 Means values corresponding to grain yield and three variables associated with the gain quality after 12 cycles of recurrent selection in wheat averaged over conventional and non-tillage soil management

Cycle	Grain yield (g m ⁻²)	Protein (%)	SDS (ml)	Lactic acid SRC (%)
0	171.7c	11.0a	12.4	111.7
4	183.3c	10.2a	12.0	112.5
8	277.6a	9.2b	11.1	113.8
12	226.1b	9.7b	10.8	110.1
p value	0.0001	0.0006	0.0625	0.6306

Means with a common letter are not significantly different ($p \le 0.05$)

2013); our results under both, CT and NT soil management (Table 1, Fig. 1c), are coincident and were associated with the RS cycles attained, regardless of the environmental context in which the genetic progress was measured. On the order hand, our results did not show a significant trend in biomass production as dependent on the number of RS cycles (Table 1, Fig. 1b). Similarly to the conclusions of Royo et al. (2007), the genetic progress was due to changes

 Table 3
 HMW-GS composition observed in wheat genotypes considering different times in the recurrent selection program

Genotype	Glu-A1	Glu-B1	Glu-D1
C ₀₋₁	2*	7+9	5+10
C ₀₋₂	1	17+18	5 + 10
C ₀₋₃	1	70e+8	5 + 10
C ₄₋₃	1	70e+8	5 + 10
C ₄₋₁	1	7+8	5 + 10
C ₄₋₂	2*	7+8	5 + 10
C ₈₋₁	2*	7+8	5 + 10
C ₈₋₂	1	7+8	5 + 10
C ₈₋₃	1	70e+8	5 + 10
C ₁₂₋₁	1	7+8	5 + 10
C ₁₂₋₂	1	7+8	5 + 10
C ₁₂₋₃	1	17+18/7+8	5+10

in photosynthate partitioning within the plant (harvest index) rather than biological carbon sequestration as a whole. Similar to other results (Guarda et al. 2004; Shearman et al. 2005), under the NT soil management the number of grains

Table 4	nivi w-05 composition o	JUSEI VEU III WIIEat	genotypes used a	as parents ordered acco	fullig to release year

Variety	Year of release	Pedigree	HMW-GS composition ^a
'Olaeta Artillero'	1954	FLORENCE/APULIA//RELIANCE	2* 7* + 9 5 + 10
'Klein Atlas'	1963	KLLU/KL157//KLRE	1 7oe + 8 2 + 12
'Marcos Juárez INTA'	1971	SON64/KLRE	$5 + 10^{b}$
'Klein Fortín'	1971	KLAT'S'/KLIM'S	2* 70e+8 5+10
'Buck Namuncurá'	1971	RMAG/BPAM//BAGE/GRAL.ROCA	2*7+85+10
'Leones INTA'	1974	SON64/TZPP//NAI60=JAR'S'	1 7oe + 8 2 + 12
'Buck Cencerro'	1974	EUFCS/BQQ//BARU/3/BBOL/4/BMAN/3/BARU/ BQQ//RMAG	2* 7* +9 5+10
'Dekalb Tala'	1974	SON64/KLRE//MASSAUX5	1 7* +9 5+10
'Diamante INTA'	1975	SON64/TZPP//NAI/3/NAR59	17+82+12
'Cargill Trigal 700'	1977	CNO'S'/JAR'S'	$2 + 12^{(2)}$
'Victoria INTA'	1978	JAR'S'// <i>BB</i> /CNO	170e + 85 + 10
'Norking Pan 70'	1978	CNO/NAD//CHR	1 17 + 18 5 + 10
'Chasicó INTA'	1980	BMAN/3/SON64A//SKE/ANE	2*7+85+10
'Buck Pucará'	1980	BCIM/3/CAL/TOB//BB/CC	1 7oe + 8 5 + 10
'Cargill Trigal 800'	1981	SCOUT*5/AGENT//NAD/CO652643	$5 + 10^{(2)}$
'Las Rosas INTA'	1983	KLAT//INIA/BB/4/NP876/Pj62//CAL/3/BB	1 17 + 18 5 + 10

^aAccording to Gianibelli et al. (2002)

^bPartial information in this study. In italic BB, is the variety 'Bobwhite' carrying 1BL/1RS rye translocation

per unit land area contributed to the genetic gain of grain yield.

Grain quality traits

After 12 cycles of RS the highest grain yield achieved was accompanied by a decrease in the protein percentage. Similar results were obtained by Maich et al. (2017) after ten cycles of RS. The long-recognized negative relation between yield and protein content was fully substantiated. The absence of association between the predictive tests and the genetic progress achieved for grain yield questions the view that the amount and quality of the proteins necessarily go hand in hand.

High molecular weight glutenin subunits

A simple explanation of HMW-GS subunits fixation in the case of Ax1 and Dx5 + Dy10 subunits could be found in the higher frequency of these subunits in original panel of genotypes used in this recurrent selection program: 50% for Ax1 and 75% for Dx5 + Dy10. The situation is different for the fixed *Glu-B1* subunits combination 7 + 8 with an original frequency of 19% (varieties 'Buck Namuncurá', 'Chasicó INTA' and 'Diamante INTA'). A possible explanation could be the co-selection of an alternative locus linked to the *Glu-B1* 7 + 8 subunit favoring local adaptation and/or yield. A good candidate can be the 1BL.1RS rye translocation, highly appreciated among breeders because of the presence

of resistance genes to several diseases and pests (Rabinovich 1998; Vanzetti et al. 2011) combined with higher yield and better performance under abiotic stresses (Howell et al. 2014). Available pedigree data shows that even though varieties carrying subunits 7 + 8 do not carry the 1BL.1RS rye translocation, varieties 'Victoria INTA', 'Buck Pucará' and 'Las Rosas INTA' include 'Bobwhite' carrying 'Aurora' as 1BL.1RS donor (Rabinovich 1998). The genetic position of the *Glu-B1* locus at the central portion of 1BL chromosome arm would allow the occurrence of recombination events, creating chromosomes with the combination 1BL.1RS and *Glu-B1* subunits 7 + 8. Further studies scoring the 1BL.1RS rye translocation in selected recurrent selection lines will elucidate this hypothesis.

Conclusions

The genetic progress for grain yield under NT was mainly driven by improvement in the harvest index rather than aerial biomass production. The absence of association between the flour quality predictive tests and the genetic progress achieved for grain yield puts into question the association between quantity, and flour quality of proteins. A possible explanation of this result may come from the observed HMW-GS erosion of extreme subunits related to gluten strength. After 24 years of recurrent selection, the grain yield was improved without affecting its baking quality.

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