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# Effects of N fertilisation, year and prolamin alleles on gluten quality in durum wheat (*Triticum turgidum* L. ssp. *turgidum*) landraces from Spain

E. Aguiriano[1], M. Ruiz[1]*, R. Fité[1] and J. M. Carrillo[2]

[1] *Centro de Recursos Fitogenéticos, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA). Autovía de Aragón Km 36, Apdo. 1045, 28800 Alcalá de Henares. Spain.*

[2] *Departamento de Biotecnología, ETSI Agrónomos, Universidad Politécnica de Madrid (UPM), Ciudad Universitaria, 28040 Madrid. Spain.*

## Abstract

A subset of durum wheat Spanish landraces, previously evaluated for yield at low and high nitrogen (N) levels, was analysed for quality, protein content (P) and sodium dodecyl sulphate sedimentation (SDSS) test. The evaluation was carried out at the two N rates and in two years. The influence of prolamin alleles at the *Glu-1*, *Glu-3*, *Glu-B2* and *Gli- 1* loci on quality parameters was also studied. The non significant Variety-by-Year or Variety-by-N interactions suggested that year and N affected all the varieties in a similar manner. Year and N effects were larger than variety effect for P, which increased with N. In contrast, variety genotype exhibited a stronger influence on SDSS test, which was not affected by year and fertilizer. Variety effects on P did not reflect the variety differences for SDSS test. A high positive influence of some prolamin alleles on quality parameters was detected, mainly for SDSS values. No correlation between yield and P was detected in the landraces adapted to low N. Based on the results of yield and quality evaluations, four landraces with high yield and high gluten strength were pre-selected for low N production.

**Additional key words**: cereals, germplasm, gliadins, glutenins, low-N, quality breeding.

## Resumen

**Efectos del abonado N, año y alelos de prolaminas en la calidad del gluten en variedades locales españolas de trigo duro (*Triticum turgidum* L. ssp. *turgidum*)**

Se ha evaluado la calidad (contenido en proteína, P, y test de sedimentación con dodecil sulfato sódico, SDSS) de un grupo de variedades locales españolas cuyo rendimiento había sido estudiado previamente para alto y bajo abonado nitrogenado (N). Los parámetros de calidad se han analizado para las dos dosis de N y en dos años. También se ha estudiado la influencia en la calidad de los alelos de prolaminas de los loci *Glu-1*, *Glu-3*, *Glu-B2* and *Gli-1* loci. La existencia de interacciones no significativas Variedad × Año ó Variedad × N indicaron que el año y el N afectaron a todas las variedades de una forma similar. Los efectos del año y del N fueron mayores que el efecto de la variedad para P, el cual aumentaba con el nivel de N. Por el contrario, la variedad influyó más en el test SDSS que no se vio afectado por el año ni el abonado. Los efectos de las variedades en P no reflejaron las diferencias en el SDSS. Se detectó una influencia alta y positiva de algunos alelos de prolaminas en los parámetros de calidad, principalmente en los valores del SDSS. No se detectaron correlaciones entre el rendimiento y P en las variedades adaptadas a bajo N. En función de los resultados se han seleccionado cuatro variedades locales con alto rendimiento y fuerza del gluten para producción con bajo N.

**Palabras clave adicionales**: cereales, bajo-N, germoplasma, gliadinas, gluteninas, mejora de la calidad.

* Corresponding author: mruiz@inia.es
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Abbreviations used: CRF-INIA (Plant Genetic Resources Centre - Spanish National Institute for Agricultural and Food Research and Technology), HMW (high-molecular-weight), LMW (low-molecular-weight), N (nitrogen), P (protein content), SDSS test (sodium dodecyl sulphate sedimentation test), Y (year).

# Introduction

High yield and good quality are necessary features in today´s durum wheat market. Both aspects respond positively to nitrogen (N) fertilization. However, there are situations, as in marginal areas or for ´organic´ production, where reduced N use is desired for environmental and economic reasons. Identification of wheat genotypes that maintain quality and yield with minimal fertilizer input has a particular relevance. Pasta quality depends mainly on grain protein, clearly influenced by environment and correlated negatively with yield, and on the seed storage prolamin proteins. Some studies have shown that particular allelic combinations of prolamins are responsible for differences in gluten strength among cultivars (Carrillo *et al*., 1990; Kaan *et al*., 1993). Hence, durum wheat quality can vary widely in response to environmental and genotypic factors.

Local varieties possess a fair stability of production under sustainable farming systems. The potential of such germplasm to reduce input use is considerable as a source of genes for yield stability, stress tolerance and end-use quality. Also, these varieties usually display higher genetic diversity than modern cultivars. The ability to tap into that diversity depends on identifying those accessions containing alleles of interest (Boggini *et al*., 1997). The evaluation of landraces for quality and yield at low N rate can help to pre-select genotypes adapted to reduced N application.

In a previous study, a set of durum wheat landraces was evaluated for grain yield at two N levels to identify genotypes adapted to low nitrogen production (Ruiz *et al*., 2008). The allelic variation at seven prolamin loci involved in quality was also analysed (Aguiriano *et al*., 2008). The objectives of the present work were to evaluate the same landraces for quality at two N levels and in two different years, and to study the influence of prolamin alleles on quality parameters.

# Material and methods

## Material

Fifty Spanish durum wheat (*Triticum turgidum* L. ssp. *turgidum*) landraces from 27 provinces were selected from the wheat collection maintained in the Plant Genetic Resources Centre at the Spanish National Institute for Agricultural and Food Research and Technology (CRF-INIA). The Bank number, local name, and geographical region of the landraces was described in Aguiriano *et al*. (2008). This sample set was evaluated during two seasons (2001-2002 and 2003-2004) and at two N rates in 2004. The N experiment was described by Ruiz *et al*. (2008). The varieties were sown in a randomised-complete-block design under rainfed conditions at Alcalá de Henares, Madrid. Four cultivars were used as test varieties: 'Senatore Capelli', 'Cocorit-71' and 'Yavaros-79' (described by Ruiz *et al*., 2008) and 'Don Pedro', released in 1990 and cultivated in the South of Spain.

## Quality evaluation

The prolamin composition of the landraces was reported in Aguiriano *et al*. (2008). HMW glutenin alleles at *Glu-1*, and B-LMW glutenin alleles at *Glu-3* and *Glu-B2* were identified with the nomenclature of Payne and Lawrence (1983) and Nieto-Taladriz *et al*. (1997), respectively. Gliadin alleles at *Gli-1* were designated following Kudryavtsev *et al*. (1996).

Gluten strength was estimated by SDSS test, according to Dick and Quick (1983). Grain protein (P) at 14% moisture was measured by a near-infrared reflectance analyser. The data were analysed by a two-way ANOVA. Differences between means and relationships between variables were tested using Student´s t-test and Pearson correlation coefficients, respectively.

# Results

## Quality parameters

Analyses of variance of grain protein and SDSS test showed no significant Variety-by-Year and Variety-by N fertilizer interactions (Table 1). Variety, year and N rate affected significantly grain protein (P), with N showing the greatest effect (Table 1). Ninety two percent of the varieties had higher P at high N than at low N level. The best test variety at low N was the old cultivar Senatore Capelli with a P of 14.4 %. Fourteen landraces had P values higher than 14.0 % at low N level, which is considered a «good» value for durum wheat quality.

Variety was the only significant effect on SDSS over years and N rates (Table 1). Senatore Capelli had the best values in all the experiments (50.5 to 67.2 mm) typical of «very high» gluten strength. Yavaros and Don

**Table 1.** Statistical significance and range of variation of grain protein (P) and sodium dodecyl sulphate sedimentation (SDSS) test over years and nitrogen (N) fertilisation rates

| Treatment | P (%) | | SDSS (mm) | |
|---|---|---|---|---|
| | Mean | Range | Mean | Range |
| 2002 | 14.6a | 12.9-16.5 | 40.5 | 22.5-70.0 |
| 2004 | 14.1b | 11.9-16.2 | 40.6 | 21.5-74.5 |
| Low-N | 13.5a | 11.8-16.6 | 42.7 | 18.0-78.7 |
| High-N | 14.2b | 12.8-15.8 | 41.5 | 21.5-69.7 |
| ANOVA | | | | |
| F-Variety (Y) | 2.57** | | 11.64** | |
| F-Year | 10.96** | | 0.00 NS | |
| F-Variety (N) | 3.76** | | 26.66** | |
| F-Nitrogen | 51.71** | | 3.92 NS | |
| V × Y | 0.59 NS | | 3.86 NS | |
| V × N | 0.08 NS | | 0.51 NS | |

NS., *,**: non significant, significant at P=0.05 and P=0.01, respectively. Between years and N rates means followed by a different letter are significantly different at P ≤ 0.05

Pedro showed values from 37.5 to 43.5 mm, which is in agreement with the «high» gluten strength of these cultivars. Twenty-two landraces had «high» SDSS values (>43.5 mm) and ten «very high» (>50 mm) at low N rate.

The accessions were classified in two groups by their geographical region: North (latitude > 41º 5´ 16´´ N) and South of Spain (latitude ≤ 41º 5´16´´ N). These two wide geographical areas have significant environmental differences in solar radiation and temperature. For P, both groups showed similar values over years and fertilizer. In contrast, landraces from the South had higher SDSS values than those from the North in both years and N rates (Table 2). Varieties were classified according to their convar. *durum* (27 entries) or *turgidum* (18 entries). No significant differences were observed between both groups (results not shown).

**Table 2.** Statistical significance of grain protein (P) and sodium dodecyl sulphate sedimentation (SDSS) test means between the North and South geographical groups

| | P | | SDSS | |
|---|---|---|---|---|
| | North | South | North | South |
| Low-N | 13.7 | 13.4 | 38.6a | 47.0b |
| High-N | 14.5 | 14.1 | 38.3a | 45.5b |
| Two-year average | 14.6 | 14.3 | 37.6a | 44.4b |

Means in the same row followed by a different letter are significantly different at P ≤ 0.05

## Parameter correlations

Correlations between SDSS test and P were significant only in year 2002 ($r = 0.296$, $P \leq 0.05$). Significant correlations ($r \geq 0.428$, $P \leq 0.01$) were detected between the four P values from the four experiments (in the two years and at both N levels). The four SDSS values were also significantly correlated ($r \geq 0.760$, $P \leq 0.01$). Correlations between P and yield (yield data in Ruiz *et al*., 2008) were calculated at the two N levels. No significant correlation was detected at low N while a significant negative correlation was found at high N level ($P \leq 0.05$). In previous work (Ruiz *et al*., 2008), these varieties were classified according to their yield response to N fertilizer in low-N varieties (negative response), high-N varieties (positive response) and indifferent-N varieties (indifferent response). The analysis for each subgroup indicated that correlations between P and yield were negatively significant only for high-N varieties at high N.

## Effects of prolamin alleles on quality parameters

Due to the observed P dependence upon environmental conditions (N and year, Table 1) only the allelic variants with means significantly different in more than one experiment were considered. P values at low and high N level, respectively, for the alleles *Glu-A1a* (13.7 and 14.6) and *c* (13.7 and 14.2) were higher than for allele *b*

(12.9 and 13.8). For the rest of the loci, *Glu-A3new-1* (14.5 and 15.0) was better than *e* (13.4 and 13.9), *Gli-A1new-3* (13.9 and 14.8) than *e* (12.8 and 13.6), and *Gli-B1b* (14.1 and 14.8) than *new-6* (12.5 and 13.3).

Analyses of the SDSS data for all the allelic variants of prolamins are shown in Table 3. Since no significant year effect was evident from ANOVA (Table 1) the average of the two seasons 2002 and 2004 was studied.

For HMW glutenin subunits, landraces with *Glu-A1b* had lower SDSS values than those with allele *a*. For *Glu-B1*, allele *b* had the highest mean value but the differences were not statistically proved because of the high variance of the landraces of this group. *Glu-B1new-1* had higher values than *a*, *d*, *e* and *q* in the two years and in the N experiments, although the differences were not statistically evident in the latter. Allele *a* was associated with lower values than *d* and *e* at low N level and in both years.

Regarding LMW glutenin subunits encoded at *Glu-A3*, allele *f* was associated with the best SDSS values, but differences were not statistically significant due to the high variability of the group. Allele *new-1* had higher values than *a*, *b* and *e* in both years and in the N experiments although the differences were not significant in the latter. At *Glu-B2*, allele *a* resulted better than *b* at both N rates. At *Glu-B3*, allele *a* performed significantly better than *h* and *new-1* at low N, than *b* and *h* at high N, and than *b* and *new-1* in both years. The new alleles *new-6* and *new-9* showed the best values (not statistically proven). For the gliadins encoded at *Gli-A1*, significant differences were obtained only at high N level. In this experiment, allele *b* performed better than *f* and *new-3*. At *Gli-B1*, allele *a* had significantly lower values than *b* and *c* at high N level and in both years. At low N, alleles *b* and *c* were associated with significantly better SDSS values than *new-1*.

# Discussion

The absence of significant Variety-by-Year or by-N interactions (Table 1) suggested that year and N affected all the varieties in a similar manner. This stability is desirable if selecting for use in diverse environments. Ames *et al*. (2003) obtained the same result for the SDSS test but they found a significant V × N interaction for P. In contrast, Lerner *et al*. (2006) detected no significant V × N interaction for P, and significant Y × V interaction for P and SDSS. Boggini *et al*. (1997) found significant genotype-environment interaction for both quality parameters. The wide ranges of variation found for P and SDSS test were expected because the landraces analysed varied greatly in geographical origin and agro-morphological characters (Ruiz *et al*., 2008).

## Quality parameters

Although P was affected significantly by genotypic variation, the environmental effect (Y and N) was greater (Table 1). These results were in agreement with Boggini *et al*. (1997), Abad *et al*. (2004) and Lerner *et al*. (2006). In general grain protein responded positively to increasing levels of N fertilizer in agreement with Ames *et al*. (2003) and Abad *et al*. (2004).

Varieties had a large effect on SDSS test in both years and N rates, but year and fertilizer did not affect this quality parameter (Table 1). Ames *et al*. (2003) detected a positive influence of N on SDSS values, whereas Abad *et al*. (2004) observed that the N effect depended on location and year. Lerner *et al*. (2006) found significant effects of Y and fertilizer but V was the most important factor for SDSS. The significant correlations found among all the P and among all the SDSS values, in spite of the different environmental conditions (Y and N), indicated that both parameters were under genetic control. The high correlation obtained for SDSS values confirms the strong influence of variety genotype on this test. Variety effects on P did not reflect the variety differences for SDSS test. These results suggested that loci controlling both factors were different and increased P did not significantly influence gluten strength. Negative correlation between yield and P were detected at high N only in high-N varieties. The absence of correlation between yield and P for the varieties adapted to low N (low- and indifferent-N varieties) allows simultaneous selection for yield and P together with high quality.

## Effects of prolamin alleles on quality parameters

Very few significant differences were found between prolamin alleles for P. There was a negative influence of the alleles *Glu-A1b*, *Glu-A3e*, *Gli-A1e* and *Gli-B1new-6*, and a positive influence of *Glu-A1a* and *c*, *Glu-A3new-1*, *Gli-A1new-3* and *Gli-B1b*. In contrast, Kaan *et al*. (1993) found a negative effect of *Glu-A1c* and no significant effect of *a* and *b*. These authors also found no significant differences between the *Glu-B1b*, *d*, *e* and *f* as in the present work.

The ranking of prolamin alleles obtained in relation to SDSS test was similar in the four experiments (Table 3). The influence of alleles *a* (subunit 1) and *b* (2*) at *Glu-A1* has not often been studied since most durum wheats possess allele *c* (Null). In this work, allele *b* showed a negative influence while allele *a* improved SDSS values (Table 3). In the varieties analysed, allele *b* appeared together with the *Gli-B1new-1* or *new-6* and *Glu-B3new-1*, which conferred poorer quality than other alleles at the same loci (Table 3). For instance, *Glu-A1a* was more frequently linked to *Gli-B1c* (γ-45) and *Glu-B3a*, both associated with good quality (Table 3). These results confirmed those of Kaan *et al*. (1993) who found a positive influence of *Glu-A1a* and a relationship between this allele and the presence of γ-45. On the other hand, Brites and Carrillo (2001) obtained no significant differences between *b* and *c*, whereas Raciti *et al*. (2003) observed that allele *b* had better gluten quality than *c*. In the present work, it seems that the remaining allelic composition at *Gli-B1-Glu-B3* is too prejudicial to allow compensation. It is known that *Glu-A1* is genetically independent of *Gli-B1* and *Glu-B3*, so the association found (linkage disequilibrium) could be affected by a selection process or confer some selective advantage.

For *Glu-B1* alleles, a positive contribution of *new-1*, *d* (6+8) and *e* (20x+20y), and a negative contribution of *a* (7) were observed. Allele *new-1* controlled two subunits with mobility lower and higher, respectively, than that of subunit 8* (Aguiriano *et al*., 2008). Only two varieties were analysed having allele *b* (7+8), but its presence was associated with the highest values, supporting the results of Boggini *et al*. (1997) and Lerner *et al*. (2006). The positive effects of *d* and *b* on SDSS were also detected by Turchetta *et al*. (1995), Raciti *et al*. (2003) and Martinez *et al*. (2005). Contrary to these results, several studies have observed a negative contribution of allele *e* to gluten quality (Ruiz and Carrillo, 1995; Turchetta *et al*., 1995; Brites and Carrillo, 2001). However, Raciti *et al*. (2003), Vazquez *et al*. (1996) and Ciaffi *et al*. (1991) found that allele *e* had similar quality to *d*. Nevertheless, the varieties with allele *e* analysed in this study frequently possessed allele *a* at *Glu-B3* and *c* at *Gli-B1*, which had a clear positive influence on quality (Table 3).

Regarding LMW glutenin subunits, *Glu-B2a* was better than *b* at both N levels (Table 3). In general, no significant differences between both alleles have been shown in other studies (Ruiz and Carrillo, 1996; Brites and Carrillo, 2001) although Martinez *et al*. (2005) found a positive effect of *a* on the mixograph test. Alleles at *Glu-A3* and *Gli-A1* loci, both tightly linked, showed very few significant differences, mainly at low N level (Table 3). The *Glu-A3new-1* and *f* were associated with the highest values, and *a*, *b* and *e* with the lowest. Allele *f* was analysed only in two varieties, both having the *Glu-B3a* related to high gluten strength. Very few reports have studied the relations between *Glu-A3* alleles and quality. Ruiz and Carrillo (1995) and Vazquez *et al*. (1996) found no influence of *Glu-A3* on SDSS test. In contrast, Carrillo *et al*. (2000) observed that allele *a*, more common in wheat cultivars, was superior to *f*, and both better than *b* and *e*. The superiority of *new-1* with respect to the «good» allele *a* may be a valuable find for quality breeding that should be confirmed with further study. In agreement with this result, the best allele at *Gli-A1*, allele *g*, was linked to *Glu-A3new-1*. At *Glu-B3*, allele *a* was clearly superior with respect to *h*, *new-1* and *b*. Alleles *a* and *b* are usually present, respectively, in the glutenin patterns LMW-2 and LMW-1 which are associated with high and low gluten quality (Carrillo *et al*., 1990; Ciaffi *et al*., 1991).

For *Gli-B1*, the results were in agreement with the tight linkage between *Glu-B3* and *Gli-B1*. Hence, *Gli-B1b* and *c* (γ- 45 and linked to *Glu-B3a*) were associated with higher SDSS values than *new-1* (γ-44 and linked to *Glu-B3new-1*) and *a* (γ-42 and linked to *Glu-B3b*). This result is consistent with the better quality of landraces from the South than from the North of Spain (Table 2), taking into account that *Glu-B3a* and *Gli-B1c* were more frequent in the former group and *Glu-B3new-1* in the latter (Aguiriano *et al*., 2008).

The same durum wheat landraces studied in this work were previously evaluated for grain yield at low N production (Ruiz *et al*., 2008). Based on the results of both evaluations, four landraces were selected, all of them low-N varieties from the South of Spain, with high yield and high gluten quality at low N level. These varieties pre-selected, after confirmation through more extensive trials, could be a reservoir of widely adapted germplasm for sustainable low-input production.

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**Table 3.** Means and standard deviation of the sodium dodecyl sulphate sedimentation (SDSS) test values for the prolamin alleles at both Nitrogen (N) fertilizer levels and the two-year average

| Locus | Low- N | High -N | Two-year average |
|---|---|---|---|
| *Glu-A1* | | | |
| *a* | *46.9 ± 12.2a* | *43.8 ± 10.2* | *44.4 ± 10.6a* |
| *b* | *37.1 ± 7.8b* | *37.6 ± 8.8* | *35.7 ± 8.8b* |
| *c* | *43.5 ± 13.1* | *43.0 ±12.4* | *41.9 ± 10.9* |
| *Glu-B1* | | | |
| *a* | 31.8 ± 4.3a | 32.7 ± 2.3bd | 29.5 ± 4.2b |
| *an* | 26.0 ± 11.3 | 25.3 ± 5.3cd | 28.4 ± 7.6 |
| *b* | 54.3 ± 15.2 | 53.5 ± 15.6 | 54.4 ± 10.7 |
| *d* | 44.1 ± 12.2b | 42.0 ± 10.4ac | 41.7 ± 10.6c |
| *e* | 44.0 ± 11.1b | 43.0 ± 11.3ab | 41.4 ± 7.5c |
| *f* | 42.3 ± 14.6 | 41.8 ± 16.8 | 40.8 ± 15.3 |
| *new-1* | 49.8 ± 7.4 | 51.0 ± 9.2 | 53.1 ± 1.8a |
| *q* | 39.2 ± 1.0 | 41.7 ± 3.4 ac | 37.8 ± 1.6bc |
| *Glu-A3* | | | |
| *a* | 43.0 ± 8.0 | 41.7 ± 8.9 | 38.9 ± 7.7a |
| *b* | 34.3 ± 7.5 | 35.9 ± 6.0 | 35.0 ± 8.3a |
| *e* | 34.6 ± 13.9 | 35.9 ± 12.6 | 37.5 ± 12.0a |
| *f* | 66.1 ± 17.9 | 61.1 ± 12.2 | 64.0 ± 11.7 |
| *new-1* | 49.5 ± 10.3 | 45.7 ± 5.3 | 51.8 ± 3.0b |
| *new-2* | 49.5 ± 13.4 | 43.0 ± 14.1 | 41.0 ± 10.6 |
| *Glu-B2* | | | |
| *a* | 47.3 ± 9.9a | 45.9 ± 10.4a | 44.8 ± 9.6a |
| *b* | 40.2 ± 12.3b | 39.2 ± 10.4b | 38.8 ± 10.7b |
| *Glu-B3* | | | |
| *a* | 49.5 ± 11.1a | 47.0 ± 11.4a | 45.0 ± 11.5a |
| *b* | 35.5 ± 4.9 | 36.0 ± 2.8b | 32.9 ± 2.3b |
| *d* | 37.2 ± 9.3 | 36.5 ± 8.5 | 41.0 ± 8.0 |
| *h* | 36.9 ± 5.5b | 35.8 ± 5.6b | 34.7 ± 8.3 |
| *i* | 34.5 ± 12.7 | 35.5 ± 9.9 | 37.4 ± 13.3 |
| *new-1* | 39.2 ± 1.3b | 41.7 ± 4.2 | 37.8 ± 2.0b |
| *new-6* | 44.5 ± 10.5 | 43.7 ± 14.3 | 46.7 ± 11.3 |
| *new-9* | 49.5 ± 13.4 | 42.5 ± 13.4 | 44.8 ± 16.0 |
| *Gli-A1* | | | |
| *b* | 44.6 ± 12.9 | 44.1 ± 11.1a | 42.3 ± 12.0 |
| *c* | 41.1 ± 8.9 | 39.4 ± 9.7 | 37.9 ± 8.3 |
| *e* | 36.3 ± 15.0 | 36.7 ± 11.5 | 36.1 ± 9.1 |
| *f* | 41.0 ± 2.8 | 37.8 ± 0.4b | 41.0 ± 9.2 |
| *g* | 47.5 ± 10.5 | 44.8 ± 11.4 | 47.3 ± 10.9 |
| *new-3* | 37.0 ± 4.2 | 34.8 ± 2.5b | 36.1 ± 3.7 |
| *Gli-B1* | | | |
| *a* | 35.5 ± 3.5 | 36.0 ± 2.0a | 32.9 ± 1.6a |
| *b* | 47.0 ± 4.7a | 45.0 ± 7.4 | 46.5 ± 7.1b |
| *c* | 47.2 ± 12.7a | 44.1 ± 12.5b | 42.9 ± 11.8b |
| *new-1* | 38.4 ± 8.4b | 40.1 ± 8.9 | 37.9 ± 8.9 |
| *new-6* | 32.2 ± 9.9 | 33.2 ± 8.1 | 34.6 ± 10.6 |
| *new-7* | 48.5 ± 12.2 | 44.1 ± 9.9 | 43.0 ± 11.2 |
| *new-8* | 37.5 ± 6.1 | 36.7 ± 7.8 | 41.5 ± 9.4 |

Means in the same column and locus followed by different letters are significantly different at $P \leq 0.05$

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