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EFFECTS OF NITROGEN AND IRRIGATION ON GLUTEN PROTEIN COMPOSITION AND THEIR RELATIONSHIP TO "YELLOW BERRY" DISORDER IN WHEAT (*TRITICUM AESTIVUM*)

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Abstract

In Mexico and the rest of the world, the presence of "yellow berry" (YB) in wheat grains (*Triticum aestivum*) has been related with poor quality, this defect is associated with low protein content in the grains. However, the quality of the wheat depends not only on the protein content, but also on the composition of the gluten proteins. The effect of the various agronomic factors on the composition of wheat gluten has been a subject of study worldwide. However, in Mexico, wheat quality still remains an issue, as there is a lack of knowledge regarding the optimal agronomic conditions to produce wheat with good-quality gluten. For this reason, the effects of nitrogen (N) rates and irrigations on the amount of gliadin subclasses, glutenin subunits (two main groups) and grain protein content as well as the relation of these proteins to the YB content in wheat grains were investigated. The experiment was conducted on arable farmland in the Valley of Empalme, Sonora, Mexico (27º 58' N, 110º 49' W; 10 m altitude), during the fall-winter period of 2009-2010. Tarachi, the hard wheat cultivar studied, was selected for its relative susceptibility to the presence of elevated YB content in mature wheat kernels. Three levels of N (75, 150 or 250 kg ha^{-1}) and three levels of irrigation (1, 2 or 3 auxiliary irrigations) were studied. Using a N rate of 150 kg ha^{-1} with 3 auxiliary irrigations, wheat with good-quality gluten was obtained. The results suggest that the YB disorder is primarily related to the amount of protein in the wheat grain.

Key words: Nitrogen fertilization, Irrigation, Gluten, Yellow berry.

Introduction

In Mexico, the evaluation of the grain quality of wheat by milling companies is based on the protein content, which is dependent on many factors, including the nitrogen (N) rates, the number of auxiliary irrigations, the cultivar, and the temperature (Daniel & Triboi *et al*., 2000; López-Bellido *et al*., 2008; Bahrani *et al*., 2009; Maqssod *et al*., 2012; Munir *et al*., 2012; Qamar *et al*., 2012; Saeed *et al*., 2013). Farmers commercializing wheat with low protein content receive financial penalties, resulting in a financial burden. A decline in protein content is primarily attributed to a low N rate during the fertilization process (Altenbach *et al*., 2011; Abedi *et al*., 2011). For this reason, large amounts of fertilizer are normally used, increasing production costs and the possible contamination of ground water by N leaching into the subsoil (Peña-Cabriales *et al*., 2001). The quality of the wheat depends not only on the protein content, but also on the composition of the gluten proteins (Dupont & Altenbach, 2003). The composition of the wheat gluten is complex, including high molecular weight and low molecular weight glutenin subunits (HMW-GS and LMW-GS) as well as the monomeric α-, β-, γ- and ω-gliadins (Shewry *et al*., 1986). The glutenins are responsible for the viscoelastic properties of wheat dough and the gliadins contribute to dough extensibility (Tatham & Shewry, 1985; Shewry *et al*., 1992).

The composition of the wheat gluten may be affected by several factors being more important the N fertilization, the irrigation and the cultivar. Saint *et al*., (2008) studied changes in the grain protein composition of winter wheat genotypes under different levels of N and water stresses, determining that the changes in the protein composition are related to a general increase in the protein content, regardless if is the result of a reduced irrigation schedule or an increased fertilization rate. Johansson *et al*., (2001) investigated the influence of cultivar types and N applications on the protein concentration and composition, as well as the amount and the size-distributions of the different protein components in 10 spring wheat cultivars (*Triticum aestivum* L.). They reported that an increased N rate significantly correlated with an increase in all protein components, gliadins and glutenins and that this increase in the protein components correlated significantly with an increase in the protein concentration and the bread volume. Triboi *et al*., (2000) studied the effects of environmental conditions (three N fertilization rates, two cultivars, two sites and two growing seasons) on the quantitative variation of wheat storage proteins. They found that the protein content increased with the supply of N to soil. As the grain protein content increased, the gliadin and glutenin contents and the gliadin to glutenin ratio also increased. In general, the literature suggests that the protein content in wheat grain increases with high N rates and low irrigation levels. Increasing the protein content increased the amount of the subunits of glutenin and the subclasses of gliadins. The effect of the various agronomic factors on the composition of wheat gluten has been a subject of study worldwide. However, in Mexico, wheat quality still remains an issue, as there is a lack of knowledge regarding the optimal agronomic conditions to produce bread wheat with good-quality gluten.

On the other hand, in Mexico and the rest of the world, the presence of "yellow berry" (YB) in the grains has been associated with poor quality. This defect is described as a low development of the endosperm, causing grains with yellowish spots that can cover the entire grain. This defect is associated with low protein content in the grains (Sharma *et al*., 1983). N deficiency in the soil is considered the most critical factor contributing to the presence of YB in wheat grain (Anderson, 1985). Lopez-Ahumada *et al*., (2010) reported that grains with YB have a higher starch content that normal grains. This difference in the starch content can be attributed to the low protein content of the grains with YB. This defect has been also related to reduced gliadins with increased albumins and globulins proportions (Gianibelli *et al*., 1990). Globally, YB disorder is considered a serious problem in durum wheat, bread wheat and triticale, significantly affecting the grain protein content and resulting in poor quality bakery products and pasta (Lookhart *et al*., 2003). The correlation between YB and the levels of different glutenin subunit main groups and gliadin subclasses that determine baking quality has yet to be determined.

The objective of this work was to determine the effects of the number irrigations and N fertilization rates on gluten protein composition (glutenin subunit main groups and gliadin subclasses) and grain protein content as well as the relationship between these effects and the presence of YB, in order economically produce good quality wheat gluten and to determine whether the presence of YB affects the quality of the wheat gluten or only the protein content.

In the present article, the effects of the N rates and the number of auxiliary irrigations on the relative amounts of the different gliadin subclasses and glutenin subunits and protein content and its relation to YB content on the mature wheat grain, is reported.

Materials and Methods

Growth and harvesting: The hard wheat cultivar Tarachi (*Triticum aestivum*) was studied. Selected for its relative susceptibility to high YB contents, this cultivar was grown and harvested under the conditions described below.

Site: The experiment was conducted on arable farmland in the Valley of Empalme, Sonora, Mexico (27º 58' N, 110º 49' W; 10 m altitude), during the fall-winter period of 2009-2010.

Crop management: The soil preparation consisted of bare fallowing with harrowing. The seed bed was 30 cm high with a separation between the beds of 90 cm. A pre-planting irrigation step was performed using an irrigation lamina of 24 cm. The wheat seeds were seeded on the wet double rows using a density of 80 kg ha^{-1}. Fifteen days after planting, an irrigation process with 14 cm lamina was applied to help the sprouting of the wheat. The following irrigations applied to the crop are named as auxiliary irrigations which were varied using an irrigation lamina 14 cm. The factors studied were the total N rate (75, 150 and 250 kg ha^{-1}) and the auxiliary irrigation number (1, 2 and 3). Urea was used (46-00-00) as the N source, and fertilization was performed during the tillering.

Experimental design: The experiment was designed using completely randomized block with a split plot arrangement and three replications. The main plots were the number of auxiliary irrigations (1, 2 and 3), and the subplots were the total N rates (75, 150 and 250 kg ha^{-1}). The experimental unit consisted of 4 furrows that were 6 m in length with 0.90 m of separation. To use the two middle furrows as a useful plot, 1 m at the beginning and the end of the furrow was eliminated. At maturity, the wheat grains were manually harvested and stored for later analysis.

Proteins extraction: The gliadins for the RP-HPLC analysis were extracted with 1 mL of 50% propanol from 300 mg of flour (Lookhart *et al*., 2003). The glutenins for the RP-HPLC analysis were extracted with 50% propanol containing 10% β-mercaptoethanol. Before glutenin extraction, gliadins were extracted and discarded (Lookhart *et al*., 2003).

Reverse-phase high-performance liquid chromatography (RP-HPLC): The gliadin subclasses and glutenin subunits were analyzed using the procedures reported by Lookhart *et al*., (2003). A Varian Prostar HPLC system (Palo Alto, CA) using a ZORBAX 300SB-CN column (Agilent Technologies, 4.6 mm in diameter and 250 mm in length, with 5 μm particle size) was used. The mobile phase was acetonitrile, and water containing 0.1% TFA. A step gradient was used in both cases and the acetonitrile concentration was increased in several steps, from 25 to 50% over 68 min for the gliadins and from 25 to 58% over 52 min for the glutenins. The column temperature was maintained at 70°C for the gliadins and 50°C for the glutenins. The flow rate was 0.5 mL/min. The detection was performed at 210 nm using a diode array detector (Varian, Palo Alto, CA).

Yellow berry content: The YB content was determined according to the following methodology; 100 g of mature wheat grains were weighed and those grains with white-yellowish spots covering an excess of 25% of the surface were manually separated and weighed, according to the Mexican norm NMX-FF-055-1984 (SECOFI, 1984). The YB content was expressed as a percent.

Protein content: The protein content was determined by the Dumas combustion method, using the N analyzer model FP528 (LECO, USA) with the AACC method 46-13 (Anon., 2000). N was multiplied by a factor of 5.7, and the protein content was expressed on a dry basis.

Statistical analyses: An analysis of variance (ANOVA) was performed using a level of significance of 95 %, followed by Tukey's test ($p \leq 0.05$) used to analyze the differences between the treatment means. The statistical analysis was performed using an SAS program (Anon., 2002).

Results and Discussion

Gliadins subclasses: Gliadins are monomeric proteins classified according to their mobility in acidic polyacrylamide gel electrophoresis into α-, β- γ-, and ω-gliadins (Metakovsky *et al*., 1984). Lookhart & Albers (1988) separated the gliadin subclasses of bread wheat by RP-HPLC. On the basis of hydrophobicity, the gliadins appeared in the following order of elution: ω-, α+β- and γ-gliadins. Figure 1 shows a representative RP-HPLC chromatogram of the gliadin subclasses of wheat grain for the Tarachi cultivar. Identification of the subclasses were based on the methods by Lookhart & Albers (1988). The areas under the curve of these peaks were corrected by the protein content to study the effects of the total N rate and the number of auxiliary irrigations on the different gliadin subclasses. In this study, the ω- and the α+β- gliadins had similar behavior, no significant differences ($p>0.05$) were observed when different N rates were applied. These results are consistent with Altenbach & Kothari (2007); they found that the transcription levels of the ω-gliadins changed little in the absence of N during the grain development, when plants were not supplied with N in the post-anthesis phase. As in this study, N was only supplied in the tillering phase. For the number of auxiliary irrigations, significant differences ($p\leq0.05$) were observed for the amounts of the ω- and the α+β- gliadins. The effects of the number of auxiliary irrigations on the ω- and the α+β- gliadins contents (Figs. 2 and 3). The amount of these subclasses increased with increasing number of irrigations. The Tukey test did not indicate significant differences between the groups corresponding with the 2 and 3 auxiliary irrigations. The lowest value of these gliadin subclasses content was for the group corresponding to the 1 auxiliary irrigation. This group had significant differences with both groups 2 and 3 auxiliary irrigations (Table 1). These results contrast with those found for the γ-gliadin subclass. Highly significant differences ($p\leq0.01$) for each factor (the N rates and the number of auxiliary irrigations) were present for this subclass. Figures 4 A and B show the effects of the N rates and the number of auxiliary irrigations on the γ-gliadin subclass. A low number of auxiliary irrigations and high N rates correspond with increased levels of γ-gliadin. The Tukey test indicated differences between the groups for each treatment and for each factor (Table 1). These results also indicated that the synthesis of the γ-gliadin subclass was favored with increased hydric stress and N rates. However, the syntheses of the ω- and α+β-gliadins were increased with increasing number of auxiliary irrigations, without being affected by the N rate. Several studies have demonstrated that the γ-gliadins differ significantly from the other gliadins in both molecular size and sequence, suggesting different synthesis conditions (Altenbach *et al*., 2010). The maximum transcription levels for the synthesis of the γ-gliadins were present for a shorter period (≈ 22 days post-anthesis) that for the rest of the gliadins (≈ 32 days post-anthesis) (Altenbach *et al*., 2007). The γ-gliadins may not have been affected by a large number of auxiliary irrigations, as these irrigations are related to the grain maturation time. A small number of irrigations decreased the grain maturation period, causing a decrease in the synthesis of the ω- and α+β-gliadins that require extended times to reach the maximal synthesis. At short time periods, the slow synthesis of the other gliadins would increase the proportion of the γ-gliadins. To obtain the maximum synthesis of ω- and α+β-gliadins, these results indicated that 2 auxiliary irrigations were needed. The increment of the different subclasses of the gliadins has been associated with a decreased quality of the gluten used for baking. Fido *et al*., (1997) studied the effects of the individual groups of the gliadins on the mixing properties of the doughs from both low and high protein flour, finding that the addition of the gliadin from all groups resulted in a decreased dough strength. Uthayakumaran *et al*., (2001) studied the effects of the α-+β-, γ-, ω- and total gliadins on the mixing and techno-functional properties of the doughs from both hard and soft flour, finding that the addition of all gliadin fractions resulted in decreased mixing times, peak resistances, maximum resistances to extension, and loaf height. The γ-gliadin subclass has been reported to have positive effects on bread quality (Van *et al*., 1992; Weegels *et al*., 1994) and often associated with a larger volume of loaf (Huebner *et al*., 1997). These characteristics would suggest that other parameters must be used to evaluate the influence of the gliadins on baking quality. Several authors have proposed using the gliadin/glutenin ratio as a parameter to characterize the technological properties of the baking flour (Weegels *et al*., 1996; Peña *et al*., 2005).

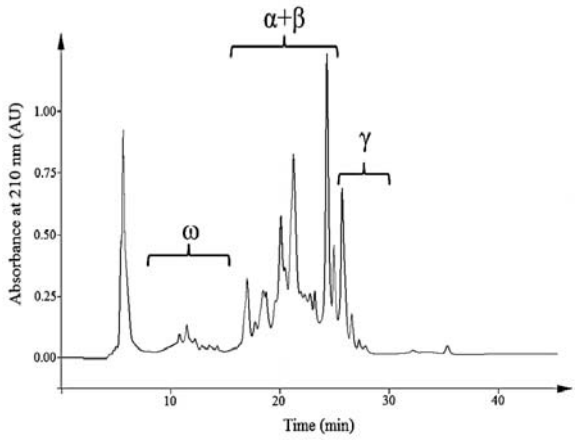

Fig. 1. Representative RP-HPLC chromatogram of the gliadins subclasses in the wheat grain, Tarachi cultivar.

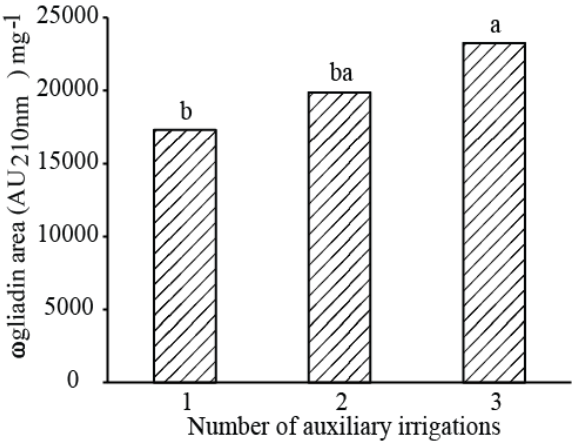

Fig. 2. Effects of the number of auxiliary irrigations on the ω-gliadin subclass (area under the RP-HPLC curve) of the wheat grain, Tarachi cultivar.

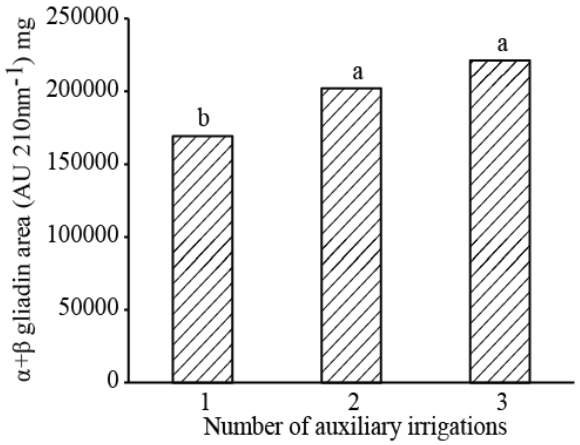

Fig. 3. Effects of the number of auxiliary irrigations on the α+β-gliadins subclasses (area under the RP-HPLC curve) of the wheat grain, Tarachi cultivar.

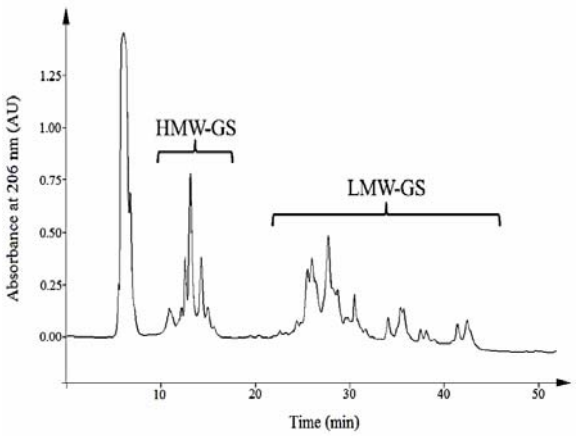

Fig. 5. Representative RP-HPLC chromatogram of the glutenins subunits in the wheat grain, Tarachi cultivar.

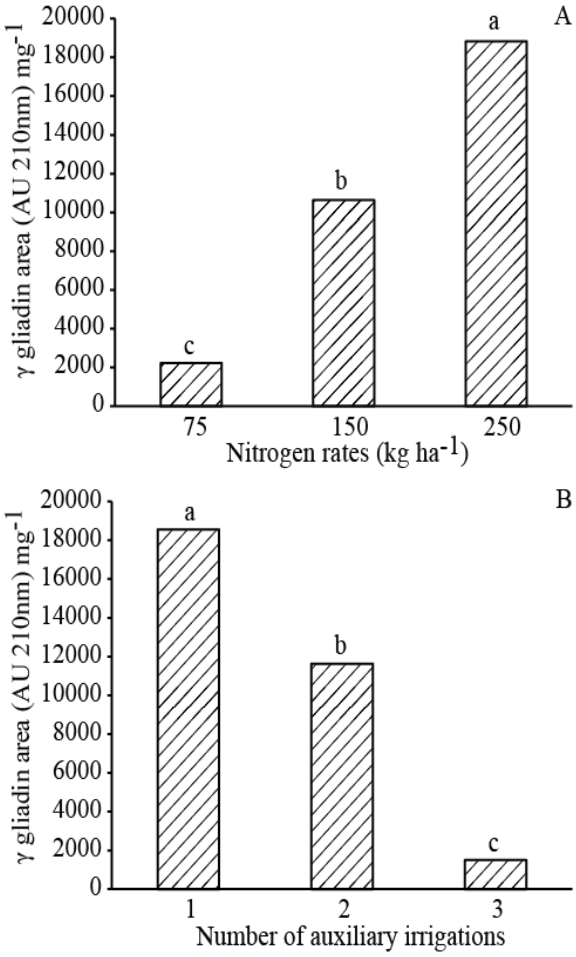

Fig. 4. Effects of (A) the nitrogen rates and (B) the number of auxiliary irrigations on the γ-gliadin subclass (area under the RP-HPLC curve) of wheat grain, Tarachi cultivar.

Table 1. Quantities (AU/mg) of the gliadins subclasses and the glutenin subunits with a correction for the total protein content for the wheat grain, Tarachi cultivar.

Variable		Gliadins subclasses			Glutenins subunits groups	
	Level	ω	α+β	γ	HMW	LMW
Nitrogen rates (kg ha^{-1})	75	21000^{a}	203648^{a}	2220^{c}	50572b	127194^{b}
	150	18917^{a}	203142^{a}	10634^{b}	63939^{a}	149340^{a}
	250	20508^{a}	185927^{a}	18829^{a}	65717^{a}	146458^{a}
Number of auxiliary irrigations	1	17295^{b}	169250^{b}	18557^{a}	30673b	101601^{b}
	2	19870^{ba}	202112^{a}	11626^{b}	69352^{a}	159588^{a}
	3	23261^{a}	221355^{a}	1499^{c}	80203^{a}	161803^{a}

Within columns, different letters indicate significant differences ($p \leq 0.05$) between mean (Tukey test)

Glutenin subunit groups: Glutenin is a group of polymeric gluten proteins. Glutenin molecules consist of glutenin subunits linked through disulfide bonds. These subunits are classified into two groups, high molecular weight and low molecular weight glutenin subunits (HMW-GS and LMW-GS) (Horvat *et al*., 2009). The representative RP-HPLC chromatogram of the subunits of the glutenins of the wheat grain used in this study (Fig. 5). The identification of the subunit groups was based on the methods by Weiser *et al*., (1998). Both the HMW-GS and LMW-GS presented highly significant differences ($p \leq 0.01$) for each factor (N rates and number of auxiliary irrigations). Both groups of subunits showed a similar behavior. At high N rates and a high number of auxiliary irrigations, the amount of these proteins (HMW-GS and LMW-GS) in the kernel of the mature wheat increased (Figs. 6 and 7). These results are consistent with Li *et al*., (2011),

reporting that for three bread wheat cultivars under irrigation, increased N levels promoted the accumulation of both HMW-GS and LMW-GS in the wheat grain. In the present study, Tukey's test indicated that the difference between the groups for the treatments of 250 and 150 kg N ha^{-1} was not significant (Table 1). At a treatment value of 75 kg N ha^{-1}, the lowest amounts of these groups of subunits were significantly different with respect to the other nitrogen treatments. For the number of auxiliary irrigations, Tukey's test showed no significant differences between groups for the treatments of 3 and 2 auxiliary irrigations (Table 1). The lowest value of these subunits was measured for the treatment of 1 auxiliary irrigation, which presented significant differences between groups of the other treatments. These results indicated that high N rates and a high number of auxiliary irrigations are needed to obtain the optimal amounts of the glutenin subunits. Among these subunits, the HMW-GS are especially important, because these proteins affect the variations in loaf volume. The HMW-GS are the main components of the gluten network responsible for retaining the air bubbles generated during the fermentation of dough, leading to a light, porous texture (Payne *et al*., 1984; Wieser & Zimmermann, 2000). The quality of the glutenins is dependent on the ratio of the HMW-GS/LMW-GS subunits (Southan & MacRitchie, 1999).

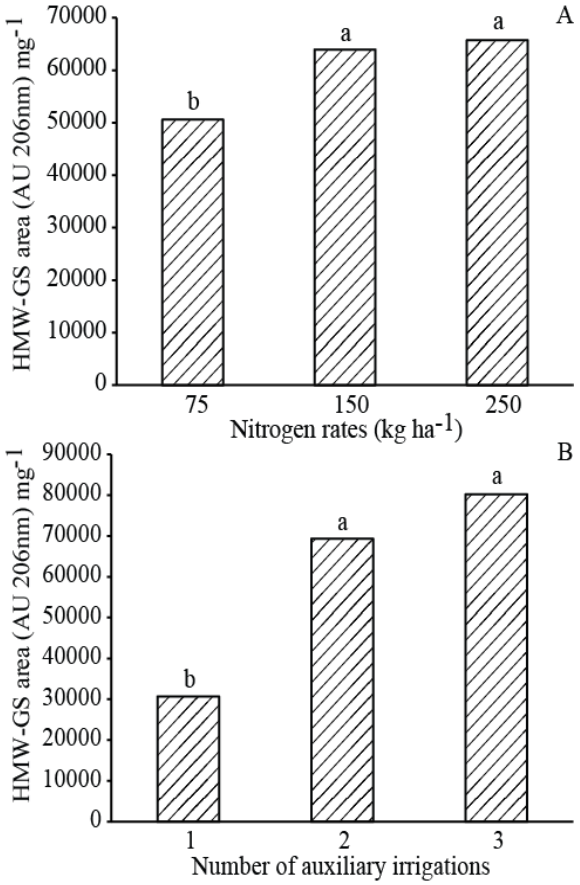

Fig. 6. Effects of (A) the nitrogen rates and (B) the number of auxiliary irrigations on the HMW-GS (area under the RP-HPLC curve) of the wheat grain, Tarachi cultivar.

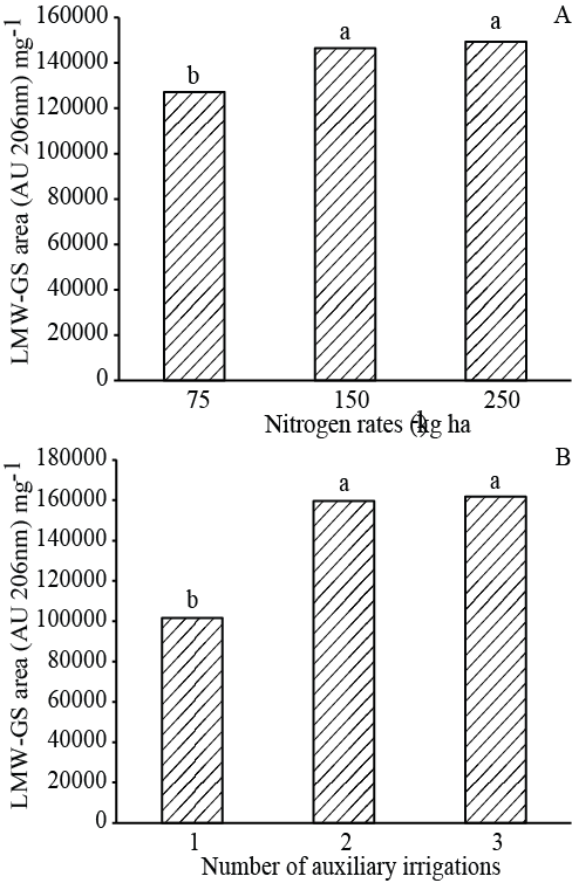

Fig. 7. Effects of (A) the nitrogen rates and (B) the number of auxiliary irrigations on LMW-GS (area under the RP-HPLC curve) of the wheat grain, Tarachi cultivar.

Quality parameters for the wheat grain and the gluten: The main quality parameter for bread wheat grain is the protein content. The interest of producing wheat with high-protein is due to its effect on the bread volume. At high protein content, higher loaf volumes are obtained (Stewart, 2003).With increasing rates of N, the protein content was increased (Table 2), this is explained, because there is an increased availability of N for the synthesis of aminoacids necessary for the protein synthesis. These results are consistent with Ejaz *et al*., (2002) who reported a linear increase in the protein percentage in bread wheat with increasing N rates. Table 2 shows that when increasing the number of auxiliary irrigations, the protein content tended to decrease. This behavior may be attributed to N leaching to the subsoil, reducing its availability. Peña *et al*., (2001) studied the N cycle and its agronomic and ecological implications, using isotopic techniques (^{15}N), and they found N losses of up to 90% emphasizing that greatest losses may occur by leaching and can be closely linked to water management. Table 1 shows that only γ-gliadin had similar behavior to that of the protein content (Table 2).

Table 2. Quality parameters for the grain and the gluten for the wheat grain, Tarachi cultivar.

Variable	Level	Protein content (%)	Yellow berry content (%)	GLI/GLU[d] ratio	HMW-GS/ LMW-GS ratio
Nitrogen rates (kg ha^{-1})	75	12.02[c]	39.19[a]	1.32249[a]	0.38730[a]
	150	14.88[b]	1.14[b]	1.14140[a]	0.41634[a]
	250	15.22[a]	1.04[b]	1.17449[a]	0.42558[a]
Number of auxiliary irrigations	1	14.61[a]	6.65[c]	1.55573[a]	0.30215[c]
	2	14.30[b]	10.89[b]	1.05230[b]	0.43318[b]
	3	13.218[c]	23.82[a]	1.03035[b]	0.49388[a]

Within columns, different letters indicate significant differences ($p \leq 0.05$) between mean (Tukey test)
[d]apparent calculated from areas under the curve of RP-HPLC analysis

Another important parameter related with the grain quality of wheat for bakery, is the YB content. As shown in Table 2, this phenomenon strongly depended on the number of auxiliary irrigations and the N fertilization. The YB content increased with increasing number of irrigations and decreasing N rates. These results are consistent with those reported by Solis and Diaz (2001) for durum wheat. It has been also reported that cultivar is another important factor contributing to the presence of the YB. Anderson *et al*., (1986) studied five bread wheat cultivars under similar growth conditions and reported a YB content ranging from 25.2-84.6%, indicating that some cultivars are more sensitive to this phenomenon that others. In our study, we found that the presence of this defect in the bread wheat grains can be solved by using a N rate of 150kg ha^{-1} and two auxiliary irrigations for the Tarachi cultivar. To avoid this YB problem, similar conditions may be useful for other cultivars.

In this study, high N rates and low numbers of auxiliary irrigations generated the highest values of total protein content. However, for the baking industry, it is important not only protein content, but also the quality of this protein. The most common wheat gluten quality parameters are the GLI/GLU and HMW-GS/LMW-GS ratios (Wieser *et al*., 2004), the insoluble polymeric protein (IPP) (Ciaffi *et al*., 1996) and the HMW-GS composition (GLU 1 score) (Payne, 1987).

The GLI/GLU ratio is an important parameter for determining the gluten quality. A low value of this ratio is considered to have high-quality gluten for breadmaking, as increased amounts of gliadin produce a decrease in the bread volume (Fido *et al*., 1997; Uthayakumaran *et al*., 2001). Table 2 shows that the GLI/GLU ratios (apparent, estimated by the area under the curve in the RP-HPLC determinations) were not affected by the nitrogen rates but were affected by the number of irrigations. The highest value was presented for 1 auxiliary irrigation, which showed differences between groups for 2 and 3 irrigations, this result can be explained because in this study, a low number of auxiliary irrigations increased the protein content and has been reported that as the grain protein content is increased, the gliadin and glutenin contents and the gliadin to glutenin ratio also is increased (Triboi *et al*., 2000). These results would suggest that to obtain a good gluten quality, 2 or 3irrigations would be necessary. However, under these conditions, low values of the protein content were obtained.

The HMW-GS/LMW-GS ratio is another important parameter to determine the gluten quality for breadmaking. A high value of this ratio represents a high-quality gluten, due at that HMW-GS is primarily responsible for generating the gluten network that retains the gas generated during the fermentation of the dough. As shown in Table 2, the effects of the N rates were not significantly different between the groups. However, the number of auxiliary irrigations had significant differences for each treatment. The highest value of the HMW-GS/LMW-GS ratio was for the treatment with 3 irrigations. Also, Table 2 shows an inverse behavior between the HMW-GS/LMW-GS ratio and the protein content by effect of number of irrigations. This indicating that an inverse relationship between the protein content and the gluten quality of wheat may be an effect of the number of irrigations. These results coupled with the GLI/GLU ratio suggest that the quality of wheat gluten depends only on the number of irrigations applied, regardless of the N rate, as long as these conditions provide for the needs of the plant. However, to obtain an acceptable amount of protein content and minimize the presence of the YB disorder, N fertilization is important. For this cultivar, a nitrogen rate of 150 kg ha^{-1} and 3 auxiliary irrigations were needed to obtain a grain and gluten of good quality.

Relationship between the YB content and the quality parameters for the wheat grain and the gluten: To characterize the interactions between the YB content and the content of the different groups of the glutenin subunits and the gliadin subclasses in the wheat gluten proteins and the quality parameters, a simple correlation test was performed. A strong negative correlation ($p \leq 0.01$) between the content of the YB and the total protein content in the mature wheat grain was found ($r = -0.8801$). This result is consistent with the reported by Behera *et al*., (2007) in bread wheat. The YB disorder is attributed to a decrease in and an abnormal protein synthesis that becomes more pronounced under the conditions of N deficiency. However, not correlation with the gluten protein content was observed, neither with the GLI/GLU and HMW-GS/LMW-GS ratios, which are the main quality parameters of the wheat gluten. These data suggest that the YB disorder relates mainly to the amount of protein in the wheat grain.

Conclusions

In this study, three main conclusions were found. First, to obtain a good quality of wheat grain and gluten a N rate of 150 kg ha^{-1} and 3 auxiliary irrigations were needed for this cultivar. This approach would suggest a potential for large savings for the farmers who usually apply nitrogen rates and auxiliary irrigations at levels above the optimal conditions found in this study. Second, the quality of the wheat gluten was only affected by the number of auxiliary irrigations. At high numbers of auxiliary irrigations, a higher quality of wheat gluten was observed. Third, the YB disorder was primarily related to low protein content.

Acknowledgments

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