

## Relationships Between Some Hordein Components and Quality Properties in Two Tunisian Barley Varieties as Influenced by Nitrogen Fertilisation

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**Abstract:** Two Tunisian barley varieties, Martin and Rihane, differing by their degrees of adaptation to the environmental conditions and grain yield potential, were grown under different levels of nitrogen (N) fertilisation. The effect of nitrogen supply on the hordein components (expressed in mg per albumen) was determined by Nu-PAGE electrophoresis system using the MOBS running buffer and densitometric analysis. Increasing nitrogen fertilisation increased the protein content by increasing the amount of C-hordeins. The sub-fraction BIII of high molecular weight B-hordeins appeared, in the present study, as the best criterion for distinguishing between the two barley varieties genetic adaptation, and its quantification could be recommended for the understanding of the quality properties of the barley response to different environmental conditions particularly nitrogen fertilisation which appears to be the main origin of differences in the protein aggregation mode of different B-hordein sulphur-rich sub-fractions.

**Keywords:** barley; hordein components; Nu-PAGE; adaptation; N fertilisation

Barley improvement is mainly based on the high yielding varieties selected. Fifteen barley varieties were selected in the last century in Tunisia (DEGHAIS *et al.* 1999). The variety Rihane, officially registered in 1987, has contributed significantly to the barley national production (EL FELAH *et al.* 1991), but a greater influence of weather and site conditions characterises this variety. However, the old varieties, such as Martin introduced in 1931, are still grown because they easily adapt to stress, especially in marginal unpredictable environments. However, quality properties of barley depend on the quality and the amount of proteins, and the balance between different protein components. The balance and the level of protein expression are highly influenced by N fertilisation (MARTIN *et al.* 1992). In the barley grain, the major N pool

is represented by the storage proteins which are soluble in concentrated alcohol solutions and are called "hordeins" (GIESE *et al.* 1983). Hordeins can be divided into four groups, A, B, C and D. The B- and C-hordeins are the main storage protein groups and are encoded by separate loci, called Hor-2 and Hor-1, respectively, located on the short arm chromosome 5 (JENSEN *et al.* 1980). According to SHEWRY *et al.* (1994), they belong respectively to the sulphur-rich and the sulphur-poor prolamin families and are also homologous respectively to Low Molecular Weight subunits of glutenins (LMW-GS) and to  $\omega$ -gliadins of wheat. Like LMW-GS, B-hordeins form aggregates stabilised by intra and intermolecular disulphide bonds (SHEWRY *et al.* 1994).

Using N determination, Nu-PAGE and densitometric measurements, we have investigated, in

the present work, the quantitative response of the main storage components, D-, C-, and B-hordeins of two Tunisian barley varieties grown under increasing levels of N fertilisation conditions. We have given a particular attention to assessing the effect of N supply on the sulphur-rich B-hordein sub-fractions BIII-, BII- and BI-hordeins in the aim to understand the relationship between these hordein components and the quality properties of adaptation which characterises each variety.

## MATERIAL AND METHODS

The barley grains of the varieties Martin and Rihane were provided by the Genetic Laboratory of the Tunisian National Institute of Agronomic Research. These two varieties were grown in two plots at the experimental station of Cherfech, 30 km north-west of Tunis. The rates of 0, 40, 80, 120 kg per ha of N fertiliser (ammonium nitrate, 33.5% of N) per cycle of growth were applied at four different stages (before the beginning of the 3-leaves, tillering, stem elongation, and ear emergence stages) according to the following patterns: 0 (0,0,0,0); 40 (20,20,0,0); 80 (20,20,20,20); 120 (40,40,20,20).

Fifteen random samples per treatment (5 replications  $\times$  3 repetitions) were mixed to produce about 1.5 kg of seeds. Sampling of the mixed seeds was made using a laboratory divider to obtain homogeneous samples of about thousand grains each. Ten grams were milled for Kjeldahl protein determination ( $P = 5.75 \times N$ ). Albumen was isolated according to EYNARD and LAURIERE (1998) and milled just prior to use. Electrophoretic analysis of total

albumen proteins was done according to LAEMMLI (1970) on ready-made Novex 4–12% Bis-Tris-HCl polyacrylamide gradient gels (Introgen CA, USA) using the recommended MOBS [3-(N-morpholino) propane sulfonic acid] running buffers of pH 7.7. The images of stained gels were numerated on a normal office scanner and analysed using the Wilbert-Lourmat Bio 1D software (France), taking into account both the whole surface and the intensity of each protein band.

## RESULTS AND DISCUSSION

### N content, grain and albumen dry weights

As illustrated in Figure 1, split and delayed N applications during the growth period of barley plants before ear emergence increase nitrogen and protein content in both varieties mainly at the highest levels of N fertilisation (120 kg/ha) but the increase is more important in variety Martin than in variety Rihane and is accompanied by a decrease of the grain dry weight (GDW) and, consequently, of the albumen dry weight (ADW) in both varieties (Figure 1).

Similar results were obtained with wheat varieties by BRUCKNER and MOREY (1988), indicating a negative relation between N content and the grain weight when intensifying N supply at early stages of growth. So, it was suggested that partitioning N fertiliser with multiple applications would minimise the effect of environment, particularly when the greater part of N supply was applied at early stages of the plant development.

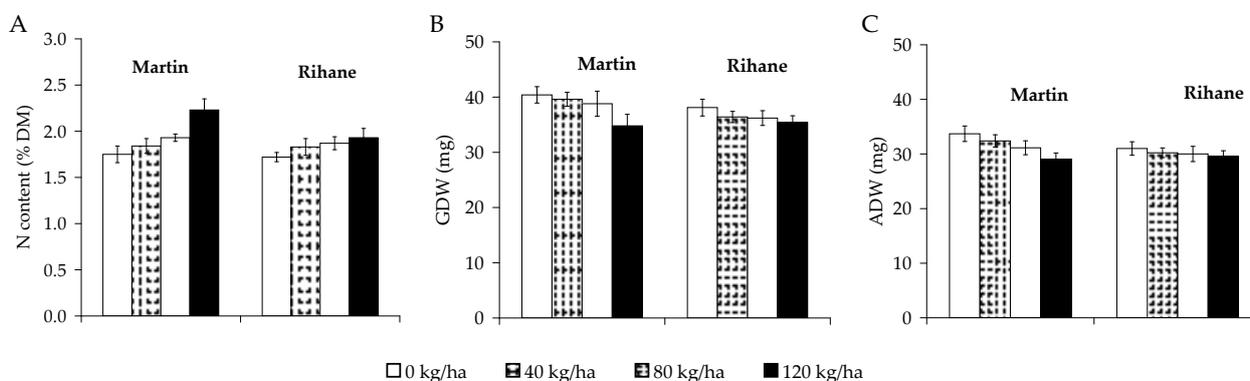


Figure 1. Nitrogen content (% dry matter), GDW and ADW of varieties Martin and Rihane as influenced by N fertilisation. Values are the averages ( $\pm$  standard deviation) of 3 repetitions per treatment (value of each repetition is the average of 5 replications) at 0.05 significant levels

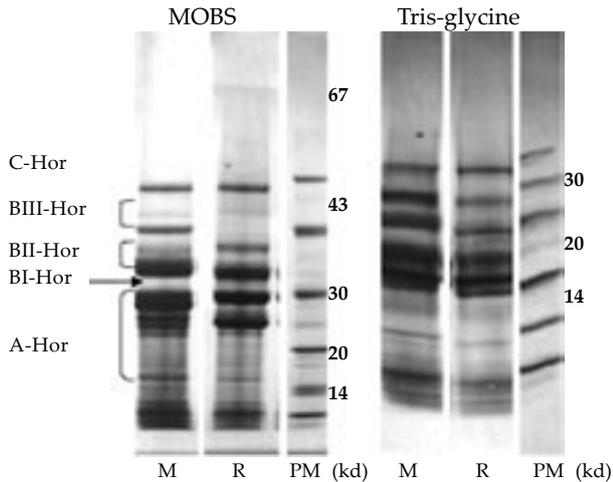


Figure 2. Electrophoresis patterns of the hordein components of varieties Martin (M) and Rihane (R) separated by Nu-PAGE. The buffer gels were Bis-Tris-HCl and the running buffers varied and were MOBS (proteins were reduced with antioxidant) and Tris-Glycine (proteins were reduced with 2-mercaptoethanol 5%)

Protein markers (PM) were phosphorylase (94 kd), bovine serum albumine (67 kd), ovalbumine (43 kd), carbon anhydrase (30 kd), trypsin I of soya (20 kd) and  $\alpha$ -lactoglobuline (14 kd)

### Nu-PAGE

Nu-PAGE was done by a modified SDS-PAGE technique of LAEMMLI (1970) in which the Tris-Glycine running buffer did not give a good separation on ready-made 4–20% polyacrylamide gradient gels, as illustrated in Figure 2. The Nu-PAGE system using running buffer MOBS with antioxidant addition showed a better separation of different hordein components on 4–12% polyacrylamide Bis-Tris-HCl gradient gel and separated well the major components of storage proteins D-, C- and B-hordeins of high and medium molecular weights, while Nu-PAGE system using MES running buffer separated well the A-hordein components of low molecular weights (BETTAIEB-BEN KAAB & EL FE-LAH 2003).

In addition, Figure 2 shows that C-hordein components of 45 to 80 kd were separated into four polypeptides bands in variety Martin and into three ones in variety Rihane. The B-hordein components extracted from the varieties Martin and Rihane comprised five and three bands respectively and could be divided into three sub-fractions BI, BII and BIII-hordeins with molecular weights increasing from 30 kd to 45 kd. Previous studies conducted on barley cultivars by FAULKS *et al.* (1981) are in agreement with our findings. The authors divided the B-hordein components into three sub-groups B1, B2 and B3, ranged in three classes called I, II and III of molecular weights varying from 35 kd to 46 kd and corresponding respectively to BI, BII and BIII-hordeins employed in the present work. The literature on the effect of N fertilisation on the quantities of B1, B2 and B3 sub-fractions was

mainly focused on the influence of genotype on the changes affecting the B-hordein components under various N levels and showed that the individual locus Hor-2 consisting of multiple genes encoding the subunits B1, B2 and B3 might be under separate regulatory control (FAULKS *et al.* 1981). Therefore, protein components would be accumulated in barley grain according to the linear law proper to each sub-group with varying N nutrition (LAURIERE *et al.* 1992).

In the present study, Nu-PAGE has constituted, in addition to the quantitative evaluation, a genetic identification of the two barley varieties and has shown a high degree of polymorphism of B- and C-hordein electrophoresis patterns of the old variety Martin as compared to the recent variety Rihane. In a previous work, the high polymorphism of B- and C-hordeins was found with a high adaptability to the environmental conditions of local Tunisian barley cultivars (BETTAIEB-BEN-KAAB & ATTIAS 1992).

### Effect of N fertilisation on hordein components

Figure 3 shows the changes of the main fractions of hordein components D-, C-, B- and A-hordeins. The minor D-hordein did not significantly change in either variety. The accumulation of C-hordeins fraction (represented by thick line) increased with intensifying N fertilisation in both varieties. However, the effect of N supply was more important in variety Martin than in variety Rihane and revealed varietal-dependence of the C-hordeins, particularly at the highest level of N fertiliser (Figure 3). Consequently, the C-hordein fraction seemed, in

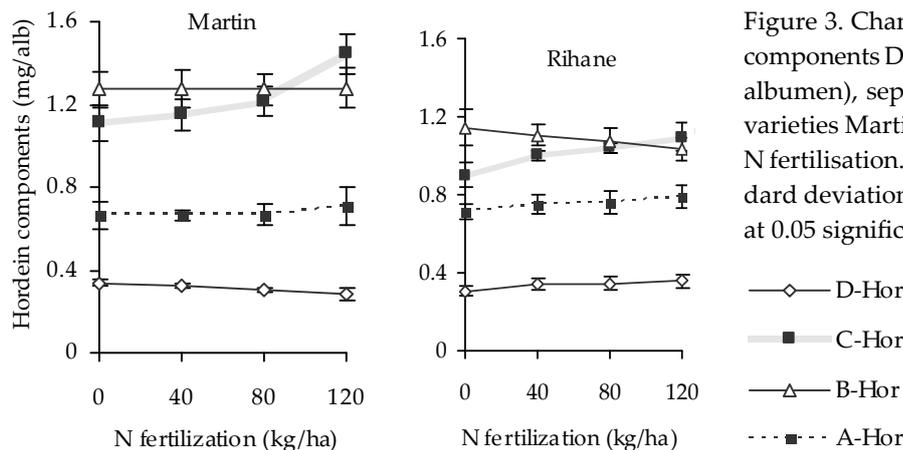


Figure 3. Changes of the amounts of hordein components D-, C-, B- and A-hordeins (mg per albumen), separated by Nu-PAGE MOBS, of varieties Martin and Rihane, as influenced by N fertilisation. Values are the averages ( $\pm$  standard deviation) of 5 repetitions per treatment at 0.05 significant levels

the present work, to act as the main nitrogen pool at the highest levels of N fertiliser. These findings are in agreement with previous studies described in literature (GIESE *et al.* 1983; WIESER & SEILMEIER 1998; DANIEL & TRIBOI 2000).

Accumulation of B-hordeins in the albumen of variety Martin was not significantly affected by N supply compared to variety Rihane in which the accumulation of this fraction in albumen decreased significantly (Figure 3). Previous studies conducted by WIESER and SEILMEIER (1998) on 13 wheat varieties support our findings about variety Rihane and suggest that the constancy of B-hordeins amounts of variety Martin under all N treatments will be probably at the origin of its high adaptation to the stress conditions.

The changes of different B-hordein sub-fractions BI-, BII- and BIII-hordeins under different levels

of N fertilisation are represented in Figure 4. The results show that the BI-hordeins sub-fraction presented a low protein quantity per albumen in both varieties, while the quantity of the BIII-hordeins sub-fraction (represented by thick line) was much greater, particularly in the variety Martin. Intensifying levels of N fertiliser did not affect the quantity of the sub-fraction BII-hordeins in either variety. On the contrary, the quantity of the sub-fraction BIII-hordeins behaved differently with intensifying N fertilisation as shown in Figure 4.

These results indicate that the major components BIII of high molecular weight B-hordeins appear to be, in the present work, a better prediction parameter to explain the differences in the behaviour between the two varieties in the degree to which they can adapt to various environmental conditions. The important quantity and the constancy of these

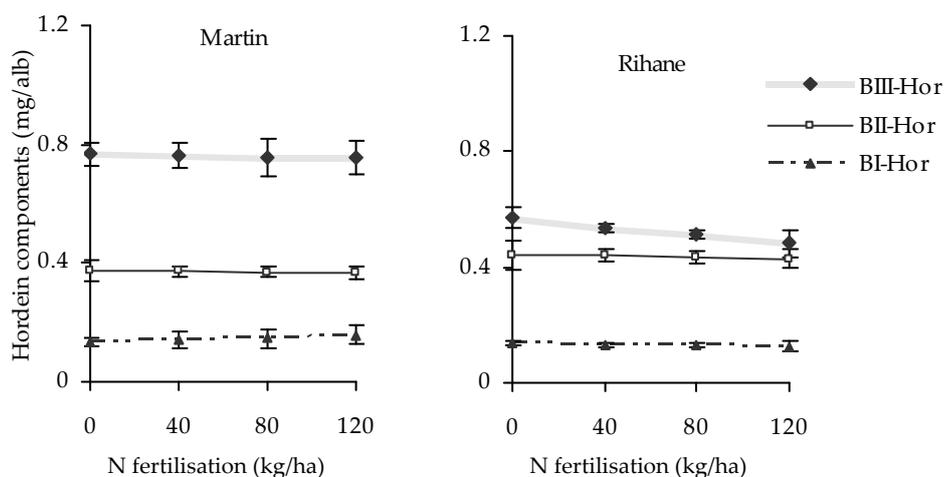


Figure 4. Changes of the amounts of B-hordein components BI, BII, BIII-hordeins (mg per albumen), separated by Nu-PAGE of varieties Martin and Rihane, as influenced by N fertilisation. Values are the averages ( $\pm$  standard deviation) of 5 repetitions per treatment at 0.05 significant levels

storage protein components under all N supply could favour the wide range of adaptability which characterises the old variety Martin. But it is premature to consider it as a genetic marker for the recognition of the barley varieties possessing this characteristic. Therefore, it appears necessary to investigate more precisely the protein aggregation of different B-hordein components, mainly those of the BIII-hordeins in both varieties, especially as the environmental factors, particularly N fertilisation and the maturation conditions, appear to be the main source of differences in the protein polymerisation mode and polymer distribution (JIA *et al.* 1996a,b).

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### References

- BETTAIEB-BEN KAAB L., ATTIAS J. (1992): Electrophoresis heterogeneity of the hordein of five barley (*Hordeum vulgare* L.) cultivars from Tunisia. *Comptes Rendus de l'Académie des Sciences, Série III*, **314**: 141–146.
- BETTAIEB-BEN KAAB L., EL FELAH M. (2003): Amount of hordein components separated by Nu-PAGE of two Tunisian barley varieties as influenced by nitrogen fertilisation. *Annales de l'INRAT*, **76**: 3–19.
- BRUCKNER P.L., MOREY D.D. (1988): Nitrogen effects on soft red winter wheat yield, agronomic characteristics and quality. *Crop Science*, **23**: 152–157.
- DANIEL C., TRIBOI E. (2000): Effects of temperature and nitrogen nutrition on the grain composition of winter wheat: effects on gliadin content and composition. *Journal of Cereal Science*, **32**: 45–46.
- DEGHAIS M., EL FELAH M., GHARBI M.S., ZARKOUNA T., CHAKROUN M. (1999): Les acquis de l'amélioration génétique des céréales en Tunisie. *Annales de l'INRAT*, **72**: 3–30.
- EL FELAH M., CHALBI N., EL GAZZAH M. (1991): Analyse de l'adaptation à l'aridité de quelques ressources génétiques locales d'orge (*Hordeum vulgare* L.) comparativement à des variétés améliorées. In: AUPELF-UREF (ed.): L'amélioration des plantes pour l'adaptation aux milieux arides. John Libbey, Eurotext, Paris: 197–209.
- EYNARD L., LAURIERE M. (1998): The combination of Indian Ink staining with immuno-chemiluminescence detection allows precise identification of antigens on blots. Application to study of glycosylated barley storage proteins. *Electrophoresis*, **19**: 1394–1396.
- FAULKS A.J., SHEWRY P.R., MIFLIN B.J. (1981): The polymorphism and structural homology of storage proteins (hordein) coded by the Hor-2 locus in barley (*Hordeum vulgare* L.). *Biochemical Genetics*, **19**: 841–858.
- GIESE H., ANDERSEN B., DOLL H. (1983): Synthesis of major storage protein, hordein, in barley. *Planta*, **159**: 60–65.
- JENSEN J., JØRGENSEN J.H., JENSEN H.P., GIESE H., DOLL H. (1980): Linkage of the hordein loci Hor-1 and Hor-2 with the powdery mildew resistance loci M1-1k and M-1-a on barley chromosome 5. *Theoretical and Applied Genetics*, **58**: 27–31.
- JIA Y.Q., FABRE J.L., AUSSENAC T. (1996a): Effects of growing location on response of protein polymerisation to increase nitrogen fertilisation for common wheat cultivar soisson: Relationship with some aspects of the breadmaking quality. *Cereal Chemistry*, **73**: 526–532.
- JIA Y.Q., MASBOU V., AUSSENAC T., FABRE J., DEBAEKE P. (1996a): Effects of nitrogen fertilization and maturation conditions on protein aggregates and the breadmaking quality of soisson, a common wheat. *Cereal Chemistry*, **7**: 123–130.
- LAEMMLI U.K. (1970): Cleavage of structural proteins during the assembly of the heads of bacteriophage T4. *Nature (London)*, **227**: 680–685.
- LAURIERE M., DAVID E., LANDRY J., SARRAFI A. (1992): Influence de la nutrition azotée sur l'accumulation des protéines du grain d'orge. In: 9<sup>ème</sup> Congrès International des céréales et du pain. Paris. 1–5 juin 1992.
- MARTIN B.J., SUTTON K.H., MOYLE T.H., HAY R.L., GILLESPIE R.N. (1992): Effect of nitrogen fertilizer on yield and quality of six cultivars of autumn-sown wheat. *New Zealand Journal of Crop and Horticultural Science*, **20**: 273–282.
- SHEWRY P.R., MILES M.J., TATHAM A.S. (1994): The prolamin storage proteins of wheat and related cereals. *Progress in Biophysics and Molecular Biology (Oxford)*, **61**: 37–59.
- WIESER H., SEILMEIER W. (1998): The influence of nitrogen fertilization on quantities and proportions of different protein types in wheat flour. *Journal of the Science of Food and Agriculture*, **76**: 49–55.

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## Souhrn

BETTAIEB-BEN KAAB L., EL FELAH M., LAURIERE M. (2005): **Vliv hnojení dusíkem na vztah mezi některými složkami hordeinu a kvalitativními vlastnostmi u dvou tuniských odrůd ječmene.** Czech J. Genet. Plant Breed., 41: 11–16.

Dvě tuniské odrůdy ječmene, Martin a Rihane, liší se stupněm adaptace k prostředí a výnosovým potenciálem, byly pěstovány při různých úrovních dusíkatého hnojení. Vliv dávky dusíku na složky hordeinu (vyjádřených v mg na albumen) byl stanoven při použití elektroforetického systému Nu-PAGE s pufrům MOBS a denzitometrickou analýzou. Zvýšení dávek dusíku při hnojení vedlo ke zvýšení obsahu bílkovin s následným zvýšením množství C-hordeinu. Nejlepším kritériem pro rozlišení genetické adaptace obou odrůd ječmene se jevila subfrakce BIII vysokomolekulárního B-hordeinu, jejíž kvantifikace je vhodná pro stanovení kvalitativních složek odezvy reakce ječmene na různé faktory prostředí, zejména hnojení dusíkem, které je hlavním původcem rozdílů ve způsobu agregace různých subfrakcí B-hordeinu bohatých na síru.

**Klíčová slova:** ječmen; složky hordeinu; Nu-PAGE; adaptace; hnojení dusíkem

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