



RESEARCH ARTICLE

MALTING QUALITY AND GENETIC DIVERSITY IN BRAZILIAN ELITE BARLEY GERMPLASM

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ABSTRACT

Barley is a cereal crop with several end uses such as animal feed and brewing, depending on the quality of the malt. Brazil produces barley mainly for brewing using as feed only the discards from the malt industry. Genotypes of barley generally have low genetic diversity as the breeding programs intend to improve the malting quality, reducing available sources for improving quality. This study investigated the malt quality (Kolbach index,  $\beta$ -glucans,  $\alpha$ -amylase and diastatic power) and the genetic diversity among 11 Brazilian barley cultivars and inbred lines grown in seven different locations in southern Brazil, using molecular markers and the quality data aiming to find the potential of Brazilian barley to qualify for the international market and identifying variability for further breeding progress. BRS Cauê, PFC 2007057 and BRS Korbel showed very good values for the malt quality parameters, being promising genotypes for quality improvement. The molecular markers and the quality data showed genetic diversity among the genotypes studied suggest potential gains in breeding barley for brewing in Brazil.

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INTRODUCTION

Brazil produces barley (*Hordeum vulgare* L.) mainly for brewing. The cultivation of this cereal is concentrated in the southern region in the states of Paraná, Santa Catarina and Rio Grande do Sul (Minella, 2013). The productivity in the 2015/2016 season was approximately 2,600 kg/ha in a cultivated area of 103,000 ha (CONAB, 2016). Local production is supplying only 30% of the national industry demand, being the deficit coming from importation (Minella, 2014). The brewery industry is responsible for 1.7% of the Brazilian gross national product (GNP). Malt quality of barley for brew must be in accordance with several pre-set parameters according to European Brewery Convention (EBC), American Society of Brewery Chemist (ASBC), Institute of Brewing (IOB) and the Middle European Brewery Analysis Commission (MEBAK).

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Some of these parameters are wort viscosity, protein content, soluble nitrogen, Kolbach index,  $\beta$ -glucanase quantity,  $\alpha$ -amylase activity, diastatic power and free amino nitrogen. Ideal malt has to meet an equilibrium of these parameters. Genetic improvement of malt quality and keeping stable the expression of all these parameters results in loss of variability among elite lines of cultivated challenging having to work with a narrow genetic base seeking genetic combinations with the lowest possible divergence, since the quality of the malt can be damaged by high genetic variability (Hayes et al., 2003). In other hand, barley presents a genome ( $2n = 2x = 14$ ) of 5.3 billion base pairs and 5.1 Gb genes (Hayes et al., 2003; The International Barley Genome Sequencing Consortium, 2012), allowing large gene exploration. Gene searching must be linked to an efficient use of the available germplasm, which depends on the characterization of the potential of each genotype for using in the genetic breeding program (Iorczeski et al., 2011). The progress in any breeding program is dependent on the genetic diversity available (Poehlman and Slepier, 1995). Characterization and diversity evaluation is

fundamental to the organization of the germplasm in order to identify promising parents for crossing (Mohammadi and Prasanna, 2003). The development a malting barley cultivar is considered to be more difficult and complex than other cereals once, besides the normal steps of breeding, promising lines must be submitted to rigorous evaluations of malt quality, which is indispensable for the industry approval of a new cultivar (Caierão, 2008).

Methodological approaches to determine the genetic diversity via molecular markers to aid the breeding of barley for malting quality are deficient in Brazil. The use of markers can decrease the difficulties in selecting genetic similar lines, since this analysis allows the detection of minor differences in the genome. Microsatellite markers or SSR (Simple Sequence Repeats) are commonly used in studies of genetic diversity and genetic mapping, because they are abundant in the genome, are multi-allelic, high informative and easily detectable. The high polymorphism makes them one of the best options for use in the characterization of genotypes, especially in related germplasm with low variability (Zhang and Li, 2010). Many studies report its use successfully for this purpose (Hayes *et al.*, 2003, Kroth *et al.*, 2005, Tahernezhad *et al.*, 2010, Amabile *et al.*, 2013). Therefore, this study purposed to determine the malt quality of Brazilian barley genotypes cultivated in different locations and environments and evaluate the genetic diversity among them, using micromalting analyzes and microsatellite markers, intending to identify promising sources for use in brewery industries and in the genetic breeding program, guiding future crossings aiming malt quality.

## MATERIALS AND METHODS

### Field experiment and harvesting

Seven cultivars and four elite inbred lines of barley included in the 2013 Cultivation and End Use Value (VCU3) Test bred by the Barley Breeding Program of Embrapa Wheat, located in Passo Fundo/Rio Grande do Sul/Brazil (Table 1) were compared to the international standard cv. Scarlett.

**Table 1. Studied cultivars and lineages of barley and its genealogy**

Number	Genotype	Genealogy
1	BRS Brau	MN 698/3/BRS 195/Schooner/Embrapa 129
2	BRS Cauê	BRS Borema/BRS 195
3	BRS Elis	BRS 195/Scarlett
4	MN 610	PFC 85104/PFC 85106
5	MN 743	MN 681/Gimpel
6	MN 6021	Dominique/Quilmes Ayelen
7	PFC 2007020	PFC 2002025/Prestige
8	PFC 2007052	BRS Lagoa/BRS Elis
9	PFC 2007057	BRS 195/Barke
10	PFC 2007103	PFC 200043/Barke
11	BRS Korbel	BRS Sampa/Danuta
12	Scarlett	Amazone/Br.2730e//Kym

The field experiment was conducted in 2012 at three locations (Bagé, Passo Fundo and Victor Graeff) in the Rio Grande do Sul state (RS)/Brazil and four locations (Candói, Guarapuava, Pinhão and Teixeira Soares) in Paraná state (PR)/Brazil. The trials were sown in June in the RS sites and in May and June in the PR locations. The conduction of the field tests in each place was performed according to the technical specifications

for the crop (Reunião Nacional de Pesquisa de Cevada, 2011). In each site, three replicates for each sample were collected. The harvested grains were dried to 12% moisture, cleaned, classified by a sieve of 2.5 mm and maintained in a dry chamber with controlled temperature. Homogeneous samples of 250 grams of grain per genotype/location were prepared and sent for malt quality analyzes three months after harvesting.

### Micromalting analyzes for malt quality

The micromalting analyzes were conducted by the Versuchs- und Lehranstalt fuer Brauerei (VLB) in Berlin and by the Anheuser-Busch InBev in the United States of America. The variables analyzed were the Kolbach index (%),  $\beta$ -glucans (ppm mg/l),  $\alpha$ -amylase (dextrin unit - DU) and diastatic power (Windisch-Kolbach - WK). The analysis methods were based at EBC and ASBC standard malt controls. Results obtained were subjected to the analysis of variance and normality and were analyzed by Tukey test at 1% probability. Individual ANOVA's were performed for micromalting data as a dependent variable using the SAS program (SAS Institute, 2004 – version 9.1).

### Genetic variability

Genetic diversity among cultivars for micromalting analyzes was determined by the Euclidean distance. The accessions were grouped by UPGMA method developed by Sokal & Michener (1958) and the program used was the NTSYS version 2.1 "Numerical Taxonomy System of Multivariate Analysis System" (Rohlf, 1998). Microsatellite (SSR) molecular marker analyzes were performed at the Laboratory of Biotechnology of the Embrapa Wheat, Passo Fundo/RS/Brazil. The extraction of the DNA from leaf tissue was based on CTAB method as described by Doyle & Doyle (1990). Microsatellite primers designed for barley and available in the literature were synthesized. Nineteen markers were used for genetic diversity (Box 1): Bmac0032, HVM40, HVM4, HVCMA, HVDHN7, scssr07759, scssr08623, scssr01846, scssr08238, scssr04163, scssr15334, scssr09041, scssr03906, scssr03907, HvSMEi843, HvSMEi845, HvSMEi846, HvSMEi868, HvSMEi1326 (Liu *et al.*, 1996; Ramsay *et al.*, 2000; Moralejo *et al.*, 2004). Some of them are associated to malt quality such as HVM4, HvSMEi1326, HvSMEi868, scssr01846, scssr03907, scssr04163a, scssr07759, scssr09041 and scssr15334.

Amplifications of PCR (polymerase chain reaction) were performed at 15  $\mu$ l solution containing 0.2  $\mu$ M of each primer (forward and reverse), 0.35 mM of each dNTP, 2.5 mM of MgCl<sub>2</sub>, 0.75 U of Taq polimerase, buffer 1X and 80 ng of DNA from each genotype studied. Reactions were conducted in a thermocycler GeneAmp 9700 Thermal Cycler (Applied Biosystems) using the following program: one cycle at 95 °C for 3 min; 10 cycles of 94 °C for 45 s [60 °C for 45 s, decreasing 1 °C per cycle until 55 °C], 72 °C for 45 s; 25 cycles of 94 °C for 45 s, 50 °C for 45 s, 72 °C for 45 s; and one cycle of 72 °C for 5 min. The DNA ladder marker was 100 bp. Agarose gels were visualized in GelDoc + XR (Bio-Rad) equipment. Presence and absence of each allele for each marker were initially analyzed. Genotypes were considered as operational taxonomic units and bands as a binary character. To determine the genetic diversity a dendrogram of genetic distance according to Nei (1972) was generated using the NTSYS program and the UPGMA clustering method.

## RESULTS AND DISCUSSION

A mean of each malt quality parameter obtained for each cultivar in all grown locations was done and all were compared by Tukey test 1% (Table 2). The data of malt quality at brewing industry are interpreted differently for each variable and different designations were given according to quality levels. Thereby, a classification scores (CS) was proposed (Table 2) aiming to the interpretation of the data.

**Table 2. Means for each quality parameter of the barley genotypes grown in field. Proposed classification scores (CS) according the different levels of industrial limits for acceptance for malting. Embrapa Trigo, Passo Fundo/RS, 2013**

Genotypes	Kolbach index(%)	CS	$\beta$ -glucan (mg/l)	CS	$\alpha$ -amylase (DU)*	CS	Diastatic power (WK) <sup>1</sup>	CS
BRS Brau	43.70 a	VG	342.86 d	NS	60.37 bc	G	356.05 bcde	S
BRS Cauê	43.566 a	VG	568.43 abcd	NS	66.32 ab	VG	457.37 a	S
BRS Elis	43.686 a	VG	564.43 abcd	NS	60.94 abc	G	342.05 cde	S
MN 610	35.86 c	NS	843.43 a	NS	42.14 d	NS	296.97 e	S
MN 743	38.13 bc	G	739.14 ab	NS	51.56 cd	G	413.97 abc	S
MN 6021	42.43 ab	VG	651.71 abc	NS	55.73 bcd	G	308.95 de	S
Scarlett	43.39 a	VG	574.00 abcd	NS	74.55 a	VG	413.63 abc	S
PFC 2007020	40.39 abc	VG	486.86 bcd	NS	49.45 cd	G	414.57 abc	S
PFC 2007052	38.13 bc	G	733.71 ab	NS	60.81 abc	G	346.91 cde	S
PFC 2007057	40.43 abc	VG	738.14 ab	NS	62.32 abc	VG	426.89 ab	S
PFC 2007103	40.13 abc	VG	704.43 ab	NS	58.75 bc	G	407.46 abc	S
BRS Korbel	44.09 a	VG	362.86 cd	NS	67.86 ab	VG	380.13 bcd	S

Means followed by the same letter in the column do not differ statistically by Tukey test at 1%. Classification scores for Kolbach index: NS = Not satisfactory (<38%), G = Good (38 to 40%), VG = Very Good (up to 40.1%);  $\beta$ -glucans: NS = Not satisfactory (>200 ppm mg/L), S = Satisfactory (<200 ppm mg/L);  $\alpha$ -amylase: NS = Not satisfactory (<45), B = Good (45 to 61.5), VG = Very Good (>61.6); Diastatic power: NS = Not satisfactory (<249); S = satisfactory (250 to 700), VG = Very Good (701-799). \*DU = dextrin unit; WK = Windisch-Kohlbach.

BRS Cauê, PFC 2007057 and BRS Korbel had similar malt quality to the standard cv. Scarlett, showing that national genotypes can present a quality profile comparable to that required by the international market. None of these genotypes have Scarlett in its genealogy, showing the potential of the Brazilian germplasm in breeding for quality. Other genotypes also showed reasonable malt quality, but MN 610 presented satisfactory levels only for one (diastatic power) of the four malt quality parameters evaluated. The Kolbach index represents how much of the total nitrogen existing in the grain was dissolved, indicating the percentage of nitrogen released from the endosperm by proteolytic enzymes (Kunze, 1999; Schoerper, 2009). The amount of  $\beta$ -glucans is directly related to the viscosity and therefore is related to problems in the filtration steps during the beer production process (Aastrup and Erdal, 1980). The  $\alpha$ -amylase enzymes hydrolyze the starch reducing it to smaller chains, thereby causing a decreasing in the viscosity. Its activity is measured by the time needed to break down the starch. The diastatic power evaluates the potential of degradation of  $\alpha$ -amylase and  $\beta$ -amylase on starch (Barclay & Bamforth *et al.*, 1993). Low values in this analysis may indicate difficulties in the brazing step of the brewery and, in contrast, high values may influence the degree of fermentation (Zschoerper, 2009).

In the case of malting barley, climate and the interaction between the genetic content of each cultivar and the environment are strong factors that influences to obtain a good quality malt (Miralles *et al.*, 2011). In order to know the behavior of each genotype in a certain cultivated area, it is essential to study and analyze its performance according to the genotype *versus* environment interaction (Kang, 1990). It was possible to observe that variables of malt quality showed significant interaction between genotype and environment (Table 3). Variance analysis showed location/environment

influenced all evaluated variables for malt quality. The influence in all analyzes can be explained by the fact that genotypes were selected by the breeders for traits such as high productivity and wide adaptability. It is known that these traits are determined by multiple genes presenting quantitative inheritance and are tightly influenced by environment, which hinders the determination of barley malt quality performance and the improvement for malt quality traits (Falconer, 1981; Paterson *et al.*, 1991; Ramalho *et al.*, 2004).

For the genotype *versus* environment interaction, Victor Graeff were the location where genotypes showed better malt quality performance. Relatively low genetic variability regarding to malting quality may occur due to low heritability of the characters (Han *et al.*, 1997). Kaeppeler and Rasmusson (1991) showed that the heritability for the genes encoding  $\alpha$ -amylase activity varied from 37 to 65% in F<sub>2</sub> and F<sub>5</sub> progenies, respectively. These data confirm those data presented by Ullrich *et al.* (1997) and Hayes *et al.* (2003) that reported quantifying the changes in malting quality is a difficult and laborious process due to the complex inheritance of this character. Similarly, Amabile *et al.* (2013) also report that the magnitude of genetic variation present in cultivars used in breeding programs of barley is controversial and more studies of the genetic diversity of malt quality are essential. For the molecular markers and micromalting genetic diversity, genotypes were evaluated by Euclidean distance and Nei 72, respectively. Both analyzes aimed to identify the diversity among genotypes and to assist the genetic breeding program, especially during the parental selections for crossings and back-crossings. Regarding the diversity of the Brazilian genotypes evaluated, the micromalting data resulted in three main groups (Figure 1) and the molecular markers resulted in five groups (Figure 2). The best performance genotypes for malt quality BRS Cauê and BRS Korbel were classified in the same group and PFC 2007057 grouped separately in the micromalting analysis. Molecular markers, in other hand, grouped all three genotypes separately. MN 610 presented low similarity to others genotypes according the micromalting data, however in the molecular marker data the similarity turns bigger. Genotypes that grouped in same groups for both analyzes are shown in Table 4.

Diversity results shows 50% of correlation among both analyzes, micromalting and molecular marker, as six of the

twelve genotypes grouped equally. However, the bigger quantity of groups in the molecular marker analysis emphasizes the efficiency in differentiate the germplasm. Despite the higher correlation observed, it is known that is difficult to relate morphological data with genetic data in cases where a limited number of molecular markers was used (Semagn, 2002).

**Table 3. Values of F test and variance analysis for the interaction between genotypes and location of cultivation/environment Embrapa Trigo, Passo Fundo/RS, 2013**

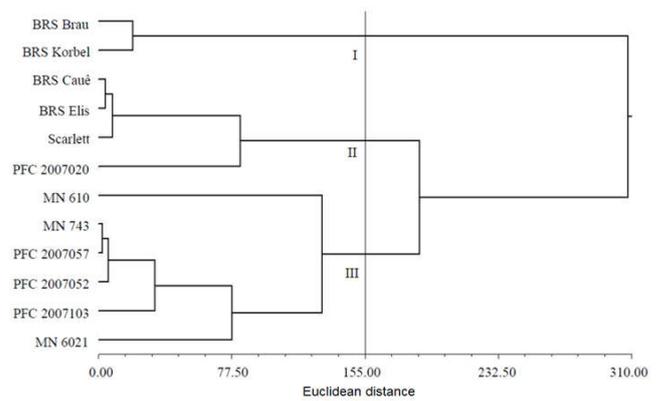
Quality variables	F test	Variation coefficient
Kolbach index(%)	8.79	6.22*
$\beta$ -glucan (mg/L)	10.22	26.5*
$\alpha$ -amylase (DU) <sup>1</sup>	19.49	13.03*
Diastatic power (WK) <sup>2</sup>	14.79	10.36*

Significance <0.0001. \*Genotype/location significant interaction. <sup>1</sup>DU = dextrin unit; <sup>2</sup>WK = Windisch-Kohlbach.

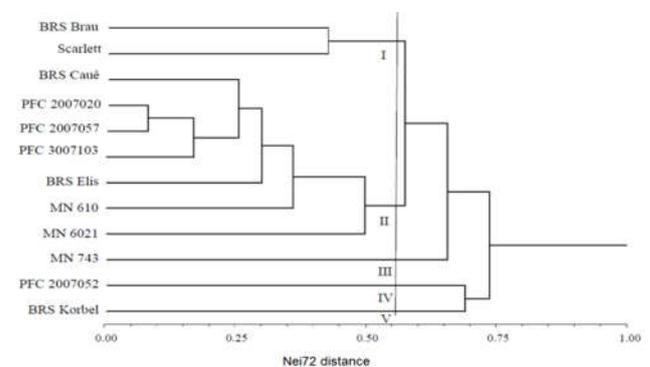
**Table 4. Distribution of genotypes across groups formed in the micromalting and molecular markers diversity analyzes. Embrapa Trigo, Passo Fundo/RS, 2014**

Genotype	Genotypes Groups	
	Malting data	Molecular marker data
<b>BRS Brau</b>	<b>I</b>	<b>I</b>
<b>BRS Cauê</b>	<b>II</b>	<b>II</b>
<b>BRS Elis</b>	<b>II</b>	<b>II</b>
MN 610	III	II
<b>MN 743</b>	<b>III</b>	<b>III</b>
MN 6021	III	II
Scarlett	II	I
<b>PFC 2007020</b>	<b>II</b>	<b>II</b>
PFC 2007052	III	IV
PFC 2007057	III	II
PFC 2007103	III	II
<b>BRS Korbel</b>	<b>I</b>	<b>I</b>

In bold: genotypes that are in the same group for both dendrograms



**Figure 1. Dendrogram obtained by UPGMA clustering method based on Euclidean distance using the NTSYS program, considering the micromalting data in barley genotypes. Embrapa Trigo, Passo Fundo/RS, 2014**



**Figure 2. Dendrogram obtained by UPGMA clustering method based on the distance of Nei 72 through the NTSYS program, considering the data of microsatellite markers in barley genotypes. Embrapa Trigo, Passo Fundo/RS, 2014**

In the early 1990s, as discussed by Heffner *et al.* (2009), the marker-assisted selection was still considered a very expensive

#### Box 1. SSR molecular markers used for the evaluation of genetic diversity in barley genotypes

SSR	Forward sequence	Reverse sequence	Reference
Bmac0032	CCATCAAAGTCCGGCTAG	GTCGGGCTCATACTGAC	
HVM40	CGATCCCCTTTCCAC	ATTCTCCCGTCCACTC	
HVM4	AGAGCAACTACCAGTCCAATGGCA	GTCGAAGGAGAAGCGGCCCTGGTA	Liu et al. (1996); Ramsay et al. (2000)
HVCMA	GCCTCGGTTTGGACATATAAAG	GTAAGCAAATGTTGAGCAACG	
HVDHN7	TTAGGGCTACGGTTCAGATGTT	ACGTGTCTCTCGCTGCTG	
scsrr07759	GCAACTCCTCATCATCTCAGG	CAACAGCCAGAAGGTCTACG	
scsrr08623	AACATTTACACCCAATCTAATCC	ACAGTAGAAGCTAGCCTTGG	
scsrr01846	GGCTCGGTAATAAGTAGC	AGCCGAGCATGTAATCACC	
scsrr08238	CAGCAGCAGATCAAATCAGG	TACTTCTCTTGGCCTTGG	
scsrr04163	GAAGAAACAACCCAATCTCC	AGGATCGTACGAAGAACAGC	Liu et al. (1996)
scsrr15334	GGGAGCCGTAAGTAAGAACC	CGACCTCTGAATCTCAAATCC	
scsrr09041	CATGTCAGTGGGGTTCTAGC	TCTACTGGACCTGCTGACC	
scsrr03906	ACCATGTCTTCCCAAGC	GGAAGTGGACGAAGAATCC	
scsrr03907	CTCCCATCACCATCTGTC	GACATGGTTCCCTTCTTCTC	
HvSMEi843	TCAGGAAAGAAGGAAAGTGA	TGACAGTTCAGACGAACCTCA	Moralejo et al. (2004)
HvSMEi845	CTGCTCTAAGATTCGCTGAT	AACAGTGCACATGGTACAAA	
HvSMEi846	ACGGACAAAGATTTCGGT	CTCATCTTGACGCTCAC	
HvSMEi868	CTGCAAGAAGCCAAGAATAC	ATTGGGAGTGTAGGAGACT	
HvSMEi1326	CCTTACTCCAATCCACTG	CCATCTGTCAATCTCAACT	Ramsay et al. (2000)

According Paux *et al.* (2012) phenotypic and genotypic association becomes very efficient when the phenotypic history throughout the years and the available data in databases is used for correlations with molecular data. They also report an economic gain because the only additional cost will be the molecular markers analysis.

tool for breeding programs. Nevertheless, currently, these costs have decreased and have facilitated large-scale marker-assisted selection genotyping in breeding programs (Eathington *et al.*, 2007). Estimates genetic distance between germplasm is needed in a breeding program, because it allows the breeder an efficient selecting of parents for crossings, especially when

they need to identify individuals genetically contrasting or similar (Cruz and Regazzi, 1997; Bered, 1999). In addition, breeders must properly assess the genetic diversity for malt quality optimizing the genetic breeding program, decreasing time and efforts in the efficient choosing of the parental. Therefore, results obtained of this kind of analyzes are directly applicable in a breeding program and its applicability is increasing in numerous research institutions.

## Conclusions

Malt quality determines the barley end use in the Brazilian market, or for brewing or for animal feed. Some of the national genotypes showed a “Scarlett pattern”, being promising genotypes for the international market of brew such as BRS Cauê, PFC 2007057 and BRS Korbel. Environment also influenced the performance of the evaluated genotypes, being Victor Graeff the best location for malt quality. The molecular diversity analyzes showed that there is variability among the Brazilian germplasm, despite the phenotypic malt quality similarity, being good sources for the genetic breeding of the cereal.

## REFERENCES

- Amabile, R.F., Faleiro, F.G., Vieira, E.A., Peixoto, J.R., Capettini, F. and Ribeiro Júnior, W.Q. 2013. Genetic diversity of irrigated barley based on molecular and quantitative data and on malting quality. *Pesquisa Agropecuária Brasileira* 48: 748-756.
- Bered, F. 1999. Variabilidade genética: ponto de partida para o melhoramento de plantas. In: Sacchet AMOF (ed.) *Genética, para que te quero?* Editora UFRGS, Porto Alegre, p. 99-104.
- Caierão, E. 2008. Cevada. In: Barbieri R L *Origem e evolução de plantas cultivadas*. 1 ed. Embrapa Informação Tecnológica, Brasília, p. 289-310.
- Cruz, C.D. and Regazzi, A.J. 1997. Modelos biométricos aplicados ao melhoramento genético. Editora UFV, Viçosa, 390 p.
- Doyle, J.J. and Doyle, J.L. 1990. Isolation of plant DNA from fresh tissues. *Focus* 12: 13-15.
- Eathington, S.R., Crosbie, T.M., Edwards, M.D., Reiter, R.S. and Bull, J.K. 2007. Molecular markers in a commercial breeding program. *Crop Science*, 47: S-154-S-163.
- Falconer, D.S. 1981. *Introdução à genética quantitativa*. Editora UFV, Viçosa, 279 p.
- Han, F., Romagosa, I., Ullrich, S.E., Jones, B.L., Hayes, P.M. and Wesenberg, D.M. 1997. Molecular marker-assisted selection for malting quality traits in barley. *Molecular Breeding* 3: 427-437.
- Hayes, M.P., Castro, A., Marquez-Cedillo, L., Corey, A., Henson, C., Jones, L.B., Kling, J., Mather, D., Matus, I., Rossi, C. and Sato, K. 2003. Developments in Plant Genetics and Breeding. In: Hintum TV, Knüpffer, H and Sato K. *Diversity in Barley (Hordeum vulgare)*. 1.ed. Elsevier, Amsterdam, p. 201-226.
- Heffner, E.L., Sorrells, M.E. and Jean-Luc Jannink, J.L. 2009. Genomic Selection for Crop Improvement. *Crop Science* 49: 1-12.
- Iorczeski, E.J., Albuquerque, A.C.S., Bonow, S., Consoli, L., Torres, G.A.M., Brammer, S.P. 2011. Pré-melhoramento de cereais de inverno: uma abordagem para ampliar a base genética. In: Lopes MA, Fávero AP, Ferreira MAJ da F, Faleiro FG, Folle SM, Guimarães EP. *Pré-melhoramento de plantas: estado da arte e experiências de sucesso*. Embrapa Informação Tecnológica, Brasília, p. 379-400.
- Kaeppler, H.F. and Rasmusson, D.C. 1991. Heritability, heterosis, and maternal effects of alpha-amylase activity in barley. *Crop Science*, 31:1452-1455.
- Kroth, M.A., Ramella, M.S., Tagliari, C., Francisco, A. and Arisi, A.C.M. 2005. Genetic similarity of brazilian hull-less and malting barley varieties evaluated by RAPD markers. *Scientia Agricola* 62: 36-39.
- Liu, Z.W., Biyashev, R.M. and Saghai-Marooof, M.A. 1996. Development of simple sequence repeat DNA markers and their integration into a barley linkage map. *Theoretical and Applied Genetics*, 93: 869-876.
- Mohammadi, S.A. and Prasanna, B.M. 2003. Analyses of genetic diversity in crop plants – Salient statistics tools and considerations. *Crop Science*, 43: 1235-1248.
- Moralejo, M., Swanson, J.S., Muñoz, P., Prada, D., Elía, M., Russell, J.R., Ramsay, L., Cistué, L., Codesal, P., Casas, A.M., Romagosa, I., Powell, W. and Molina-Cano, J.L. 2004. Use of new EST markers to elucidate the genetic differences in grain protein content between European and North American two-rowed malting barleys. *Theoretical and Applied Genetics* 110: 116-125.
- Miralles, D.J., Arisnabarreta, S. and Alzueta, I. 2011. Desarrollo ontogênico y generación del rendimiento. In: Miralles DJ, Benech-Arnold RL and Abeledo G. *Cebada cervicera*. Gráfica, Buenos Aires, p.1-34.
- Nei, M. 1972. Genetic distance between populations. *American Naturalist* 106: 283-292.
- Paterson, A.H., Tanksley, S.D., Sorrells, M.E. 1991 DNA markers in plant improvement. *Advances in Agronomy* 46: 39-90.
- Poehlman, J.M. and Sleper, D.A. 1995. *Breeding Field Crops*. 4<sup>th</sup> ed. Iowa State University, Press, Iowa, 473 p.
- Ramsay, L., Macaulay, M., Ivanissevich, S.D., Maclean, K., Cardle, L., Fuller, J., Edwards, K.J., Tuveesson, S., Morgante, M., Massari, A., Maestri, E., Marmiroli, N., Sjakste, T., Ganal, M., Powell, W. and Waugh, R. 2000. A simple sequence repeat-based linkage map of barley. *Genetics Society of America*, 156: 1997-2005.
- Paux, E., Sourdille, P., Mackay, I. and Feuillet, C. 2012. Sequence-based marker development in wheat: Advances and applications to breeding. *Biotechnology Advances* 30: 1071-1088.
- Ramalho, M.A.P., SANTOS, J.B. and PINTO, C.A.B.P. 2004. *Genética na Agropecuária*. Editora UFPA, Lavras, p. 255-289.
- Rohlf, J.F. 1998. NTSYS pc: Numerical Taxonomy and Multivariate Analysis System. Versão 2.0 Applied Biostatistics Inc, New York, 31 p.
- SAS Institute Inc 2004. SAS/STAT® 9.1 User's Guide. SAS Institute Inc., Cary, 5124 p.
- Semagn, K. 2002. Genetic relationships among ten endod types as revealed by a combination of morphological, RAPD and AFLP markers. *Hereditas* 137: 149-156.
- Sokal, R.R. and Michener, C.D. 1958. A statistical method for evaluating systematic relationships. *The University of Kansas Scientific Bulletin, Kansas*, 30 p.
- Tahernezhad, Z., Zamani, M.J., Solouki, M., Zahravi, M., Imamjomeh, A.A., Jafaraghaei, M. and Bihamta, M.R. 2010. Genetic diversity of Iranian *Aegilops tauschii* Coss. using microsatellite molecular markers and morphological traits. *Molecular Biology Reports* 37: 3413-3420.

- The international barley genome sequencing consortium 2012. A physical, genetic and functional sequence assembly of the barley genome. *Nature* 491: 711-716.
- Ullrich, S.E., Han, F. and Jones, B.L. 1997. Genetic complexity of the malt extract trait in barley suggested by QTL analysis. *Journal of the American Society of Brewing Chemists* 55: 1-4.
- XXVIII Reunião Nacional de Pesquisa de Cevada, 2011. Indicações técnicas para a produção de cevada cervejeira nas safras 2011 e 2012. Embrapa Trigo, Passo Fundo, 99 p.
- Zhang, G. and Li, C. 2010. Genetics and Improvement of Barley Malt Quality. Zhejiang University, Hangzhou and Heidelberg, 296 p.

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