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Short communication. Efficiency of AFLP markers and seed storage protein electrophoresis to study the phylogeny of some *Hordeum* species

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Abstract

This study is focused on reconstructing the phylogeny of 60 accessions representing ten species of the genus *Hordeum* based on amplified fragment length polymorphism (AFLP) markers and SDS-PAGE of seed storage proteins. We obtained 339 polymorphic AFLP bands and 46 polymorphic protein bands from the SDS-PAGE of water soluble and non-soluble seed storage proteins. The phylogenetic tree deduced from AFLP analysis is concordant with that deduced from seed storage proteins electrophoresis. The studied taxa were clustered according to their genome type into two main groups representing the Old and New World's species. Inside each group the species were clustered according to their genome type. Highly significant cophenetic correlation coefficients obtained in both AFLPs (0.96) and seed storage proteins (0.89) indicate the reliability of the results. It can be concluded that both AFLP and SDS-PAGE are adequate techniques to study the *Hordeum* phylogeny.

Additional key words: genome; dendrogram; monophyletic; Old World species; New World species.

The genus *Hordeum* L. is a well defined, easily recognized and monophyletic plant group characterized by three one-flowered spikelets at each rachis node (Bothmer *et al*., 1995). It belongs to the tribe *Triticeae*, that represents a highly successful evolutionary branch of the grass family (*Poaceae*) (Bothmer *et al*., 2003) and comprises a vast number of genera and species. This numerous wild species are thus potential gene sources for cereal breeding.

The genus *Hordeum* contains 30-32 species and 40-45 taxa occurring in temperate areas of Eurasia, North and South Africa, Central and South America (Doebley *et al*., 1992; Bothmer & Komatsuda, 2011). Bothmer & Jacobsen (1985), based on life form, phytogeography, ecology, cytology, and compatibility in crosses, suggested a classification system including four sections and 31 species. Bothmer *et al*. (1986, 1987) defined four basic genomes according to the meiotic behaviour of different interspecific hybrids: genome I for *H. vulgare* and *H. bulbosum*; Y for *H. murinum*; X for *H marinum*; and H for the remaining *Hordeum* species. Doebley *et al*. (1992) stated that the evolutionary patterns in genus *Hordeum* are complex, including different breeding systems and various forms of polyploidy. Phylogeny of the genus *Hordeum* has been studied using different types of analyses: electrophoretic variation of isozymes (Jaaska & Jaaska, 1986; Jorgensen, 1986); mitochondrial and chloroplast DNA variation (Holwerda *et al*., 1986); RFLP (restricted fragment length polymorphism) technique (Baum & Bailey, 1991); repetitive DNA sequences using RFLP and in situ hybridization (Svitashev *et al*., 1994); fluorescence in situ hybridization (Bustos *et al*., 1996); chloroplast DNA sequences (Nishikawa *et al*., 2002); AFLP (amplified fragment length polymorphism) mar-

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Abbreviations used: AFLP (amplified fragment length polymorphism); ITS (internal transcribed spacer region); RFLP (restricted fragment length polymorphism); SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis); SNP (single nucleotide polymorphism).

kers and ITS (internal transcribed spacer region) sequence of the ribosomal RNA genes (El Rabey *et al*., 2002); or low and single copy nuclear markers (Zimmer & Wen, 2012).

The aim of the present study was addressing the phylogenetic relationships of 60 accessions belonging to the genus *Hordeum* from the Old and New World origin, using AFLP and seed storage protein electrophoresis.

Seeds of sixty accessions belonging to ten *Hordeum* species were supplied by different gene banks (Table 1). DNA was extracted from 15-days-old seedlings according to a modified CTAB method (Saghai-Maroof *et al*., 1984). AFLP markers were developed according to Vos *et al.* (1995). Seven primer combinations, namely E40/M38, E42/M38, E36/M41, E37/M40, E40/M40, E40/M41, and E37/M41 were constructed by MWG-Biotech GmbH, Germany according to Vos *et al*. (1995) and used in AFLP fingerprinting of the studied taxa. The sequence of these primers is as follows: E3: 5'-GACTGCGTACCAA TTCACC-3'; E37: 5'-GACTGCGTACCAATTCACG-3'; E40: 5'-GACTGCGTACCAATTCAGC-3'; E42: 5'-GACTGCGTACCAATTCAGT-3'; M38: 5'-GATG AGTCCTGAGTAAACT-3'; M40: 5'-GATGAGTCC GAGTAAAGC-3'; M41: 5'-GATGAGTCCTGAGTA AAGG-3'.

Both water soluble and non-soluble proteins were extracted and SDS-PAGE performed according to the method of Laemmli (1970) from seeds of the 18 accessions indicated in Table 1. These accessions were selected to represent the ten different barley species.

Both AFLP and protein gels were scored as 0/1 for absence/presence of the bands, respectively. The NTSYS PC2.0 software (Rohlf, 1998) was used for reconstructing phylogeny trees using both protein and AFLP data.

A total of 339 polymorphic AFLP bands were obtained from the AFLP analysis. These AFLP data were analyzed using the NTSYS-pc program. The resulting dendrogram had two main clusters, separating Old World's and New World's accessions (Fig. 1). The first cluster was divided into two groups; the first one contains *H. murinum* (genome Y) and *H. bulbosum* (genome I) taxa. The second group in this cluster contains all the accessions of *H. vulgare spontaneum* (genome I). The second cluster was also divided into two groups, the first one contains all species that have the H genome (*i.e., H. bogdanii, H. brevisbulatum, H. chilense, H. jubatum* and *H. pusillum*), whereas the second one contains *H. marinum,* which has the X genome (Fig. 1). High significant cophenetic correlation coefficient was obtained in the dendrogram based on the AFLP (r = 0.96), which indicates the reliability of the results.

SDS-PAGE of the water soluble and non-soluble seed storage protein generated 46 polymorphic protein bands. According to the dendrogram produced from the analysis of protein data, *Hordeum* taxa were divided into two clusters similar to the AFLP results (Fig. 2). The first cluster contains the Old World's accessions [genome I (*H. bulbosum* and *H. vulgare spontaneum*) and genome Y (*H. murinum*)]. The second cluster consists of the accessions representing the New World's species and the genome H (*H. bogdanii, H. brevisbulatum, H. chilense, H. jubatum* & *H. pusillum*) besides the genome X for the species *H. marinum* (Fig. 2). The reliability of these results was proven from the highly significant correlation of cophenetic coefficient for the protein dendrogram (r = 0.89). Moreover, the clustering of the *spontaneum* accessions is coincident with investigations carried out with SNP (single nucleotide polymorphism) markers (Shi-dong *et al.*, 2008).

Table 1. *Hordeum* accessions used in the current study for AFLP and protein analyses

Code No.	***Hordeum* species**	**Source**	**Origin**
1*	*H. m. murinum*	Nordic Gene Bank / 30886	Unknown
2*	*H. m. murinum*	Nordic Gene Bank / 30887	Unknown
3*	*H. vulgare* subsp. *spontaneum*	IPK / 9719	Libya
4*	*H. vulgare* subsp. *spontaneum*	IPK / 9721	Libya
5	*H. vulgare* subsp. *spontaneum*	IPK / 9840	Libya
6	*H. vulgare* subsp. *spontaneum*	IPK / 9823	Morocco
7	*H. vulgare* subsp. *spontaneum*	IPK / 9826	Morocco
8	*H. vulgare* subsp. *spontaneum*	SLU / 3139	Cyprus

Table 1 (cont.). *Hordeum* accessions used in the current study for AFLP and protein analyses

Code No.	*Hordeum* species	Source	Origin
9	*H. vulgare* subsp. *spontaneum*	SLU / 3140	Cyprus
10	*H. vulgare* subsp. *spontaneum*	SLU / 3141	Cyprus
11	*H. vulgare* subsp. *spontaneum*	SLU / 3142	Cyprus
12	*H. vulgare* subsp. *spontaneum*	SLU / 3883	Greece
13	*H. vulgare* subsp. *spontaneum*	SLU / 10288	Tadzhikistan
14	*H. vulgare* subsp. *spontaneum*	ICARDA / 180007	Jordan
15	*H. vulgare* subsp. *spontaneum*	ICARDA / 180008	Jordan
16	*H. vulgare* subsp. *spontaneum*	USDA / 41995	Israel
17	*H. vulgare* subsp. *spontaneum*	USDA / 406271	Israel
18	*H. vulgare* subsp. *spontaneum*	ICARDA / 181547	Saida (Libanon)
19	*H. vulgare* subsp. *spontaneum*	ICARDA / 181568	Rachaiya (Libanon)
20	*H. vulgare* subsp. *spontaneum*	USDA / 466024	Syria
21	*H. vulgare* subsp. *spontaneum*	USDA / 466627	Iran
22	*H. vulgare* subsp. *spontaneum*	USDA / 219796	Khuzestan (Iran)
23	*H. vulgare* subsp. *spontaneum*	USDA / 253933	Salahidin (Iraq)
24	*H. vulgare* subsp. *spontaneum*	USDA / 366431	Chekao (Afghanistan)
25	*H. vulgare* subsp. *spontaneum*	USDA / 212305	Mazarisharif (Afghanistan)
26	*H. vulgare* subsp. *spontaneum*	MPIZ / 2699	Turkmenistan
27	*H. bulbosum*	USDA / 106880	Turkmenistan
28*	*H. bulbosum*	USDA / 194460	Israel
29*	*H. bulbosum*	USDA / 205195	Aydin (Turkey)
30	*H. bulbosum*	USDA / 206372	Cyprus
31	*H. bulbosum*	USDA / 206443	Samsun (Turkey)
32	*H. bulbosum*	USDA / 240161	Israel
33	*H. bulbosum*	USDA / 206890	Eskisehir (Turkey)
34*	*H. bogdanii*	USDA / 269406	Kabul (Afghanistan)
35*	*H. bogdanii*	USDA / 314696	Kazakhstan
36	*H. bogdanii*	USDA / 440413	Kazakhstan
37	*H. bogdanii*	USDA / 440414	Kazakhstan
38	*H. bogdanii*	USDA / 499498	China
39	*H. bogdanii*	USDA / 499499	Gansu (China)
40	*H. bogdanii*	USDA / 499500	China
41	*H. bogdanii*	USDA / 499501	China
42*	*H. brevisubulatum*	USDA / 229448	Iran
43*	*H. brevisubulatum*	USDA / 531768	Tajikistan
44	*H. brevisubulatum*	USDA / 531769	Uzbekistan
45*	*H. marinum*	USDA / 240162	Libya
46	*H. marinum*	USDA / 200341	Israel
47*	*H. marinum*	USDA / 223324	Khuzestan (Iran)
48	*H. marinum*	USDA / 283418	Israel
49	*H. marinum*	USDA / 401364	Iran
50	*H. marinum*	USDA / 41409	Israel
51	*H. marinum*	ICARDA / 181278	Cyprus
52*	*H. chilense*	USDA / 531781	Rio Negro (Argentina)
53*	*H. chilense*	IPK / 972-89	Chile
54	*H. chilense*	IPK / 987-92	Alabama (USA)
55*	*H. pusillum*	USDA / 15654	Kentucky (USA)
56*	*H. pusillum*	USDA / 15663	USA
57	*H. jubatum*	USDA / 531782	Mexico (Mexico)
58*	*H. jubatum*	USDA / 566822	Mexico (Mexico)
59*	*H. jubatum*	IPK / 662-84	Bulgaria
60	*H. procerum*	ICARDA /181258	Sweden

*: Accessions used for protein analysis.

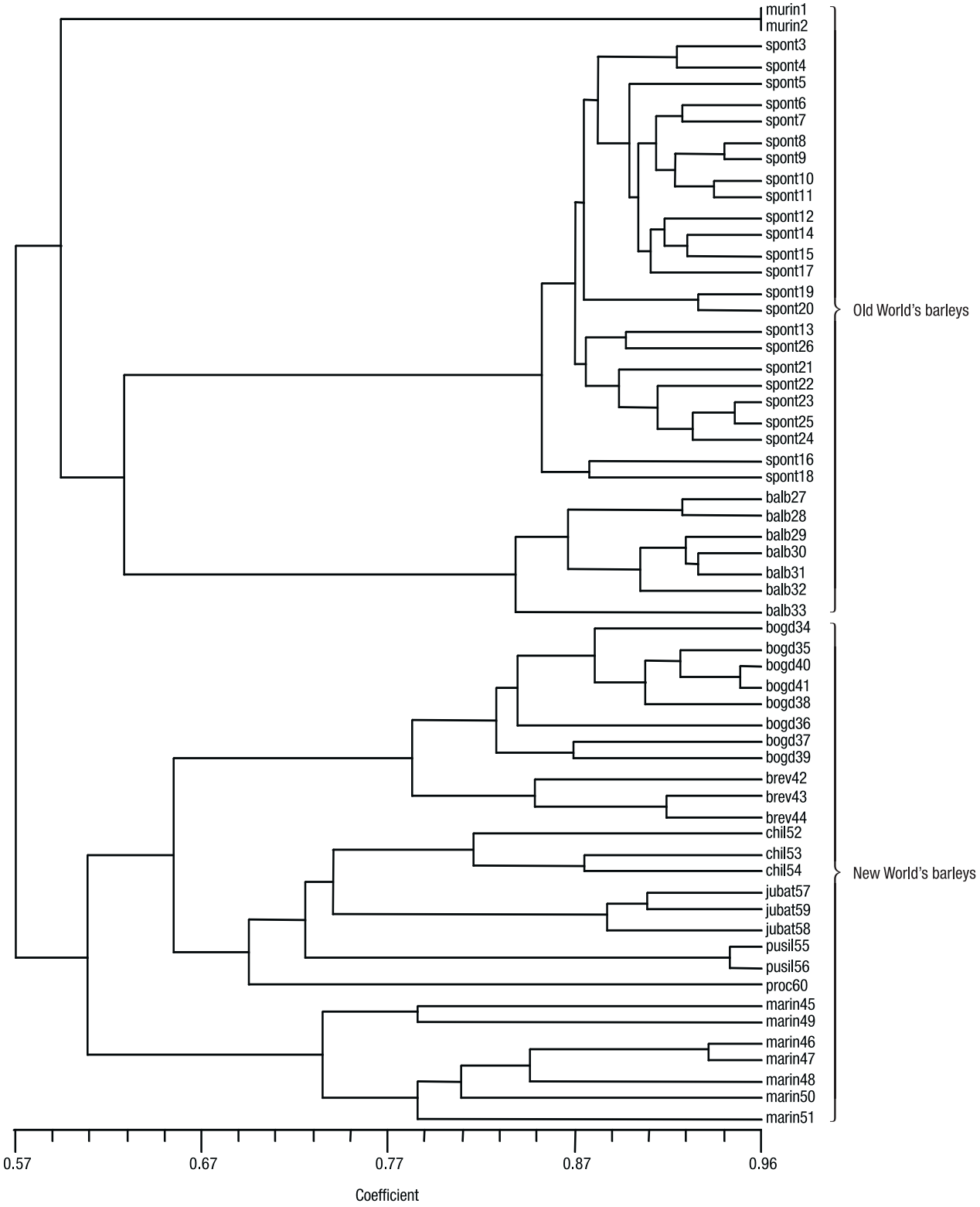

Figure 1. Phylogenetic relationships of the 60 *Hordeum* accessions under study based on AFLP data analysis using Dice's similarity coefficient and the UPGMA tree building method.

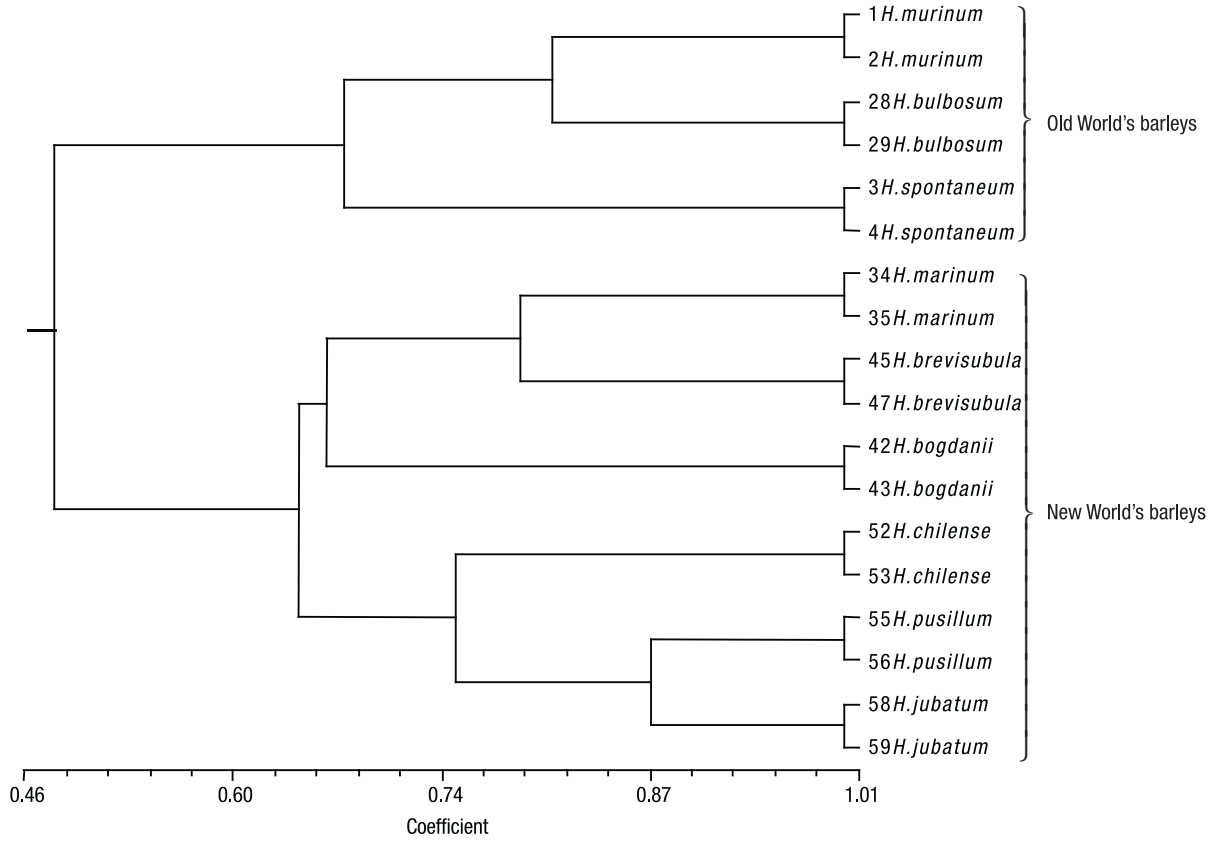

Figure 2. Phylogenetic relationships of 18 barley accessions representing the studied *Hordeum* taxa under study using both water soluble and water non-soluble protein data based on Dice's similarity coefficient and the UPGMA tree building method.

The phylogenetic analyses based on AFLP and protein data divided the studied taxa into two main groups representing the New and Old World's species. It was also noted that accessions of the same species were clustered together. This result is consistent with Bothmer *et al.'s* (1986, 1987) hypothesis concerning the four genome analysis of the interspecific hybrids in the genus *Hordeum*; genome I in *H. vulgare* and *H. bulbosum* (Old World), genome Y in *H. murinum* (Old World), genome X in *H. marinum* (Old World) and genome H in *H. brevisubulatum*, *H. bogdanii*, *H. pusillum*, *H. jubatum* and *H. chilense* (New World). Bothmer (1992) considered the wild *Hordeum* species as the secondary gene pool for barley breeding purposes due to its ability for genome elimination. Doebley *et al*. (1992), based on cDNA variations, and Svitashev *et al*. (1994) based on the use of repetitive DNA sequences using molecular hybridization techniques RFLP and in situ hybridization, obtained results consistent with the four genome hypothesis of Bothmer *et al.* (1986, 1987). In addition, the current results are also consistent with that of Saisho & Purugganan (2007) and Blattner (2009), concerning the four genome hypothesis. In conclusion, the phylogeny of the genus *Hordeum* species under study is consistent with that resulted from the AFLP data analysis and previous investigations.

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References

Baum BR, Bailey LG, 1991. Relationships among native and introduced North American species of *Hordeum*, based on chloroplast DNA restriction-site variation. Can J Bot 69: 2421-2426.

Blattner F, 2009. Progress in phylogenetic analysis and a new infrageneric classification of the barley genus

Hordeum (*Poaceae*: *Triticeae*). Breeding Sci 59(5): 471-480.

Bothmer R von, 1992. The gene pool of barley and preservation of wild species of *Hordeum*. IBPGR, Rome, Italy (5) pp: 32-35.

Bothmer R von, Jacobsen N, 1985. Origin, taxonomy and related species. In: Barley Monographs (Rasmussun D, ed.), pp: 19-56.

Bothmer R von, Komatsuda T, 2011. Barley origin and related species. In: Barley production, improvement and uses. Wiley-Blackwell Press, pp: 14-63.

Bothmer R von, Jacobsen N, Baden C, Jorgensen RB, Linde-Laursen I, 1995. An ecogeographical study of the genus *Hordeum*. 2nd edition IPGRI.

Bothmer R von, Flink J, Landström T 1986. Meiosis in interspecific *Hordeum* hybrids. I. Diploid combinations. Can J Genet Cytol 28: 525-535.

Bothmer R von, Flink J, Landström T, 1987. Meiosis in interspecific *Hordeum* hybrids. II. Triploid combinations. Evol Trends Plants 1: 41-50.

Bothmer R von, Sato K, Komatsuda T, Yasuda S, Fischbeck G, 2003. The domestication of cultivated barley. In: Diversity in barley (*Hordeum vulgare*) (von Bothmer R, van Hintum TH, Knupffer H & Sato K, eds), Elsevier Sci, Amsterdam. pp: 9-27.

Bustos de A, Cuadrado A, Soler C, Jouve N, 1996. Physical mapping of repetitive DNA sequences and 5S and 18S-26S rDNA in five wild species of the genus *Hordeum*. Chrom Res 4: 491-499.

Doebley J, Bothmer R von, Larson S, 1992. Chloroplast DNA variation and the phylogeny of *Hordeum* (*Poaceae*). Am J Bot 79: 576-584.

El Rabey H, Badr A, Schaefer-Pregl R, Martin W, Salamini F, 2002. Speciation and species separation in *Hordeum* L. (*Poaceae*) resolved by discontinuous molecular markers. Plant Biol 4: 1-9.

Holwerda BC, Jana S, Crosby WL, 1986. Organelle DNA variation in the genus *Hordeum*. Genetics 113 (1-2): 72.

Jaaska V, Jaaska V, 1986. Isoenzyme variation in the barley genus *Hordeum* L. I. Alcohol dehydrogenase and superoxide dismutase. Biochem Physiol Pflanzen 181: 301-320.

Jorgensen RB, 1986. Relationships in the barley genus (*Hordeum*): An electrophoretic examination of proteins. Hereditas 104: 273-291.

Laemmli UK, 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

Nishikawa T, Salomon B, Komatsuda T, Bothmer R von, Kadowaki K, 2002. Molecular phylogeny of the genus *Hordeum* using three chloroplast DNA sequences. Genome 45 (6): 1157-1166.

Rohlf FJ, 1998. NTSYSpc. Numerical taxonomy and multivariate analysis system, version 2.02c. Exeter Software, NY.

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW, 1984. Ribosomal DNA spacer length polymorphism in barley. Mendelian inheritance, chromosomal location and population dynamics. P Natl Acad Sci USA 81: 8014-8018.

Saisho D, Purugganan MD, 2007. Molecular phylogeography of domesticated barley traces expansion of agriculture in the Old World. Genetics 177: 1765-1776.

Shi-dong Y, Yu-ming W, Peng-fei Q, You-liang Z, 2008. Sequence polymorphisms and phylogenetic relationships of *hina* gene in wild barley from Tibet, China. Agric Sci in China 7(7): 796-803.

Svitashev S, Bryngelsson T, Vershinin A, Pedersen C, Säll T and Bothmer R von, 1994. Phylogenetic analysis of the genus *Hordeum* using repetitive DNA sequences. Theor Appl Genet 89: 801-810.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M, 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23 (21): 4407-4414.

Zimmer EA, Wen J, 2012. Using nuclear gene data for plant phylogenetics: progress and prospects. Mol Phylogenet Evol 65: 774-785.