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Assessment of Morphological Diversity and Population Structure in Barley Landraces and Advanced Breeding Lines

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ABSTRACT: Phenotypic diversity in a panel of 143 barley genotypes including Iranian and exotic landraces as well as cultivars and advanced breeding lines was evaluated by using 29 categorical (ordinal, nominal, and binary) descriptors and 9 quantitative traits used in variety registration. Four categorical characteristics were monomorphic among the genotypes, while the remaining traits harbored a great diversity in terms of morphological traits. Diversity index of 25 polymorphic traits ranged from 0.06 (hairiness of lower leaves sheaths) to 0.69 (spiculation of inner lateral nerves of dorsal side of lemma) with an average of 0.37. Cluster analysis using neighbor joining (NJ) algorithm based on Jaccard's similarity matrix of 25 polymorphic categorical traits grouped all the genotypes according to their number of ear rows (NER) (six- and two-rowed) except four pairs of individuals. The putative relevance of phenotypes with NER, seasonal growth habit (SGH), and origin of samples was investigated through graphical mosaic pattern, of which phenotypes of 10 traits showed possible association. The results show that nine quantitative traits could be considered in developing new barley varieties.

Keywords: *Hordeum vulgare* L., categorical descriptors, quantitative traits, barley landraces and cultivars, graphical mosaic pattern

INTRODUCTION

The 'landrace' term was emerged once 'modern' crop varieties were available to farmers (Berg, 2009). Whilst newly-bred varieties must be morphologically uniform in expression of characteristics as mandated by variety registration criteria (Cooke & Reeves, 2003), landraces, on the other hand, are genetically heterogeneous making them adaptable to local growing conditions and environmental fluctuations. Although the advent of varieties promised higher yields to meet the demand of growing population, they were influenced by environmental changes, whilst simultaneously posed a threat on survival of landraces which were conserved during generations by local farmers and now in world-wide gene banks. However, results of testing for value for cultivation of 159 Spanish landrace-derived lines in multi-environmental trials shows their superiority in terms of agronomic traits over 26 in comparison with cultivars, though indicating their negative attributes of being late matured, and prone to lodging due to longer heights (Yahiaoui *et al.* 2014).

Overall, a suggested definition for an autochthonous landrace as "a variety with a high capacity to tolerate biotic and abiotic stress, resulting in a high yield stability and an intermediate yield level under a low input agricultural system" (Zeven, 1998) envisage them as an invaluable gene pool for crop breeding as well as association mapping for identifying responsible genomic regions for specific adaptation (Bellucci *et al.* 2013). Now, in spite of being the fourth most cereal crop with total production of more than 144 million tons in 2014 (FAOSTAT, 2014), cultivated barley (Hordeum vulgare L.) suffers from bottlenecks of narrow genetic base resulted from domestication process and breeding activities (Peel & Rasmusen, 2000). The importance of landraces is better conceived with global environmental change and food security issues, as they are rich in genetic resource for developing, for instance, disease-resistant and drought-resilient elite varieties (reviewed by Newton *et al.* 2010).

The objective of this study was to evaluate diversity of a panel of Iranian and exotic barley landraces as well as cultivars and advanced breeding lines in terms of morphological characteristics comprising categorical descriptors and quantitative traits currently used in technical tests for barley variety registration. Moreover, population structure of this panel was investigated through their relevance with morphological traits.

MATERIALS AND METHODS

A. Plant materials

A set of 143 barley landraces and advanced breeding lines was compiled from Plant Genetic Resources and Cereals Research Departments of Seed and Plant Improvement Institute (SPII), Iran. The accessions were geographically originated from eight countries (Iran, Egypt, China, US, England, India, Pakistan, and Algeria) (Table 1). The heterogeneous genetic nature of landraces was alleviated by ear-to-row pure line selection method during 2008-09 in Dryland Agricultural Research Institute (DARI).

B. Phenotyping

The genotypes were planted in the experimental field of Seed & Plant Certification & Registration Institute (SPCRI) under lattice square (12×12) design with two replications in autumn of 2013 and 2014 and were harvested in the following year. The seeds of winter and facultative type accessions were soaked in water for 24 hours and then stored at 2°C for 40 days prior to cultivation in order to relieve their vernalization requirements. A total of 38 morphological traits comprising 9 quantitative traits and 29 categorical characteristics (descriptors) were measured.

Table 1: Distribution of 143 barley landraces, cultivars and advanced breeding lines used in this study according to their origin, number of ear rows (NER) and seasonal growth habit (SGH).

Origin/Country		Two-rowed			Six-rowed			Sum
		W	S	F	W	S	F	
Iranian landraces		8	21	9	29	1		68
Exotic landraces	China	2	8	2	9	2		23
	Egypt		1		8	2	1	12
	Algeria	1	1					2
	Denmark		1					1
	England		1		1			2
	Ethiopia		1					1
	India				1			1
	Pakistan		2					2
	Russia		3					3
	Spain		1		1			2
	USA		2					2
Cultivars and Advanced breeding lines		2	13		7	2		24
	Sum	13	55	11	56	7	1	143

Abbreviations: W (winter type); S (sring type); F (facultative type); IL (Iranian landraces); EL (Exotic landraces); ABL (advanced breeding lines)

Among the categorical traits, 14 characteristics were ordinal (a visual scale of the expression intensity of a trait), 6 nominal (like color or shape of an organ) and 9 binary (presence or absence of a phenotype). The traits were chosen from UPOV's Distinctness, Uniformity, and Stability (DUS) test guideline in barley (UPOV 1994), CPVO's DUS test protocol (CPVO 2012), India's DUS test guideline (PPV & FRA 2011), and characteristics used by Wang *et al*. (2012). Ear density (ED) of genotypes was scored (very lax to very dense) as an ordinal characteristic and was also measured by counting number of grains per one cm of ear (Table 2). Although not recorded, the records of seasonal growth habit (SGH) i.e. 62 spring, 68 winter, 12 facultative types, and one genotype with unknown growth type and number of ear rows (NER) of genotypes i.e. 80 two- and 63 six-rowed were provided by DARI.

C. Statistical analyses

The states of expression (phenotypes) of categorical variables were treated as binary (Crossa & Franco, 2004) for calculating Jaccard distance coefficient ($Jxy=(b+c)/(a+b+c)$) between pairs of individuals, where a is the number of common phenotype between individuals x and y, b is the number of phenotypes present in x and absent in y, and c is the number of phenotypes present in y and absent in x. The matrix of dissimilarity was used as entry for cluster analysis using unweighted neighbor-joining (uNJ) algorithm using DARwin software v.6 (Perrier and Jacquemoud-Collet 2006).

Table 2: List of 29 morphological characteristics (descriptors) and 9 quantitative traits used in this study according to categorical type (ordinal, nominal, binary), and distribution (number and frequency) of their phenotype in 143 barley samples.

Abbr.	Type of variable	Trait	Phenotype (Number, frequency of varieties)/(range of variable)
KCAL	O	Kernel: color of aleurone layer	O: whitish (72,0.5), weakly colored (19,0.13), strongly colored (52,0.36)
PGH	O	Plant: growth habit	Erect (6,0.04), semi-erect (74,0.52), intermediate (59,0.41), semi prostrate (4, 0.03)
LLHL	B	Lowest leaves: hairiness of leaf sheaths	Absent (138, 0.97), present (5,0.03)
FLAC	B	Flag leaf: anthocyanin color of auricles	Absent (58,0.41), present (85,0.49)
FLIA	O	Flag leaf: intensity of anthocyanin color of auricles	absent or very weak (58,0.41), weak (49,0.34), medium (35,0.24), strong (1,0.01)
FLA	O	Flag leaf: attitude	erect (77,0.54), semi-erect (58,0.41), horizontal (8,0.06)
FLGS	O	Flag leaf: glaucosity of sheet	weak (36,0.25), medium (92,0.64), strong (15,0.1)
AACT	B	Awns: anthocyanin color of tips	Absent (28,0.2), present (115,0.8)
AIAC	O	Awns: intensity of anthocyanin color of tips	absent or very weak (28,0.2), weak (82,0.57), medium (25,0.17), strong (8,0.06)
GACN	O	Grain: anthocyanin color of nerves of lemma	O: absent or very weak (126,0.88), weak (11,0.08), medium (5,0.08), very strong (1,0.01)
NER	B	Ear: number of rows	two (80,0.56), six (63,0.44)
ESh	N	Ear: shape	tapering (9,0.06), parallel (134,0.94)
ED	O, C	Ear: density	O: Very lax (21,0.15), lax (69,0.48), medium (43,0.3), dense (9,0.06), very dense (1,0.01) C: 2.1 to 12.6 grains per cm with an average of 4.4 ± 2.4
GRHT	B	Grain: rachilla hair type	short (7,0.05), long (136,0.95)
RCFS	O	Rachis: curvature of first segment	absent or very weak (55,0.38), weak (88,0.62)
EDSS	B	Ear: development of sterile spikelets	Full (80,1.00), monomorphic in all 80 two-rowed varieties
SSA	N	Sterile spikelet: attitude (in mid-third of ear)	parallel to weakly divergent (5,0.06), divergent (75,0.94)
MSLG	O	Median spikelet: length of glume and its awn relative to grain	shorter (6,0.04), equal (90,0.63), longer (47,0.33)
GH	B	Grain: husk	present (143,1.00), monomorphic in all 143 varieties
GSLN	O	Grain: spiculation of inner lateral nerves of dorsal side of lemma	O: absent or very weak (16,0.11), weak (53,0.37), medium (48,0.34), strong (18,0.13), very strong (8,0.06) B: Absent (16,0.11), present (127,0.89)
GHVF	B	Grain: hairiness of ventral furrow	Absent (143,1.00), monomorphic in all 143 varieties
AL	O	Awn: length (compared to ear)	short (5,0.03), medium (31,0.22), long (107,0.75)
AR	O	Awn: roughness	O:smooth (9,0.06), intermediate (30,0.21), rough (104,0.73)
SSTS	N	Sterile spikelet: tip shape	pointed (35,0.44), rounded (45,0.56)
CT	N	Collar: type	recurred (66,0.46), cup (77,0.54)
GC	N	Grain: color	yellow (106,0.74), green (3,0.02), black (34,0.24)

Abbr.	Type of variable	Trait	Phenotype (Number, frequency of varieties)/(range of variable)
RLFS	O	Rachis: Length of first segment	Short (26,0.18), medium (90,0.63), long (26,0.18), very long (1,0.01)
GDL	B	Grain: disposition of lodicules	Clasping (143, 1.00), monomorphic in all 143 varieties
SGH	N	Seasonal growth habit	Winter type (68,0.48), Facultative (12,0.08), Spring type (62,0.44)
SW	C	thousand-seed weight	29.3 to 56.6 g with an average of 44.4 ± 6.4
RL	C	Radicle length	9.4 to 20.1 cm with an average of 16.6 ± 1.9
CL	C	Coleoptile length	3.5 to 5.8 cm with an average of 4.7 ± 0.4
FLL	C	First leaf length	10.4 to 20.1 cm with an average of 16.5 ± 1.4
TSL	C	Total seedling length (first leaf plus radicle)	23.9 to 39.1 cm with an average of 33.1 ± 2.7
TEE	C	Time of ear emergence	65.75 to 79 days with an average of 72.6 ± 2.8
PH	C	Plant: height	77.5 to 105 cm with an average of 89.1 ± 4.7
EL	C	Ear: Length	4.7 to 108 cm with an average of 8.4 ± 1.1

Abbreviations: B (binary variable); O (ordinal variable); N (nominal variable); C (continuous variable)

Diversity index (DI) of categorical traits was calculated using $DI = 1 - \sum_{i=1}^{n} P_i^2$ formula, wherein p_i represents frequency of i^{th} phenotype of trait. In addition, association of morphological traits with NER and SGH groups was tested using Fisher's exact chi-square test for binary traits as well as nominal, and Mann-Whitney U test for ordinal traits. Microsoft Excel was used for showing the relevance of phenotypes with NER, SGH, and origin of samples by drawing graphical mosaic pattern.

RESULTS AND DISCUSSION

A. Morphological description of barley panel

The two-year range and mean values of nine quantitative traits i.e. ear length (EL), ear density (ED), time of ear emergence (TEE), plant height (PH), thousand-seed weight (SW), total seedling length (TSL), coleoptile length (CL), radical length (RL), first leaf length (FLL) were represented for diverse panel of barley samples (Table 2). Among 29 categorical traits, all barley accessions had similar phenotypes for four characteristics. In that respect, all 80 two-rowed genotypes had sterile spikelets with full development (EDSS). In addition, the grains of all 143 genotypes were husked (GH), hairless in ventral furrow (GHVF), and bearing clasping lodicules (GDL).The phenotypes of remaining 25 categorical traits were distributed across barley accessions, though in some cases the frequency of phenotypes was very low, for instance, samples with haired sheaths of lowest leaves (ch. LLHL) constitute only three percent of barley population indicating it's low polymorphism (Table 2). Diversity index (DI) of characteristics ranged from 0.06 (hairiness of lower leaves sheaths, LLHL) to 0.69 (spiculation of inner lateral nerves of dorsal side of lemma, GSLN) with an average of 0.37 (Table 3). Jaccard distance coefficient between pairs of individuals ranged from 0 (for four pairs of genotypes i.e. 4,14; 66,123; 55,111; 75,102) to 0.93 (for genotypes 95 and 122) with an average of 0.61. Dendrogram of 143 barley samples derived from unweighted neighbor joining (NJ) algorithm based on Jaccard's distance matrix of 25 polymorphic categorical traits clustered genotypes into two distinct groups of six- and two-rowed samples, while leaving four aforementioned genotype pairs not distinct which had similar phenotypes (Fig. 1).

B. Association of traits with NER and SGH grouping

The association of morphological traits with NER and SGH grouping as the major sources of population structure in barley, were evaluated between two and six-rowed samples (NER group), as well as winter and spring accessions (SGH group). Four out of nine quantitative traits namely ear length (EL), ear density (ED), thousand-seed weight (SW), and coleoptile length (CL) were significantly ($P \leq 0.01$) differed between NER groups as revealed by t-test (Fig. 2). In addition to these traits, first leaf length was also significantly ($P \leq 0.01$) differed between SGH groups (data not shown). Accordingly, the association of ordinal characteristics and nominal and binary traits was evaluated by respective Mann-Whitney U test and Fisher's exact tests. All characteristics showed significant differences between NER groups ($P \leq 0.05$) except for four traits i.e. glaucosity of flag leaf sheet (FLGS), anthocyanin color of lemma nerves (GACN), awn length (AL), and ear length (EL). However, the number of non-significant ordinal traits increased to nine between SGH groups (spring vs. winter types).

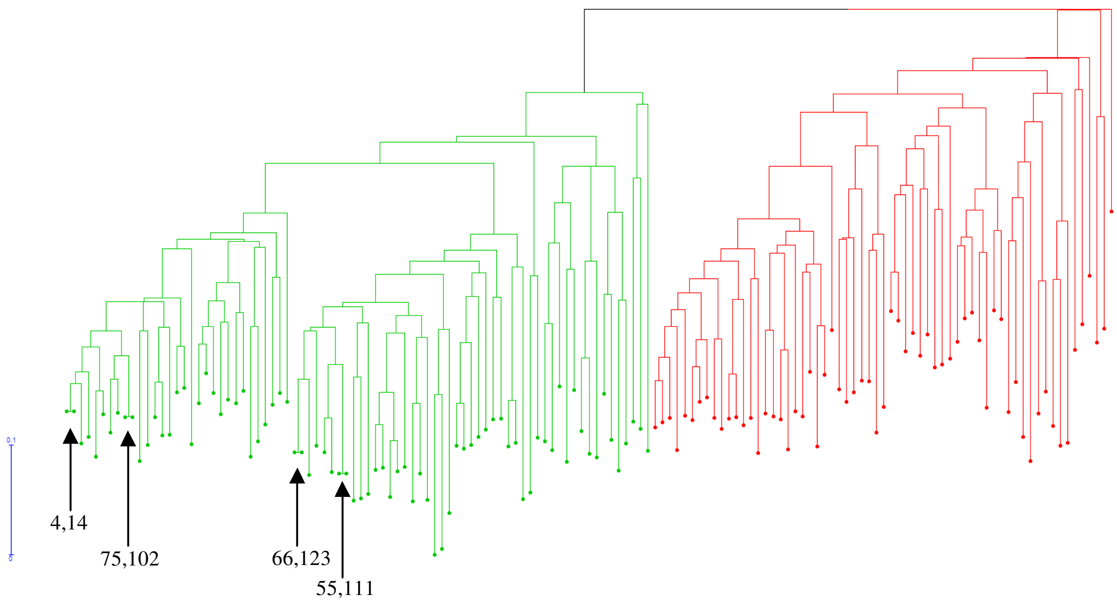

Fig. 1. Dendrogram of 143 barley genotypes derived from unweighted neighbor joining (NJ) algorithm based on Jaccard's distance matrix of 25 polymorphic categorical traits. The genotypes were clustered into two distinct groups of six- (red) and two-rowed (green) samples except four genotype pairs (denoted by arrow) which were not distinct (Jaccard's distance=0).

In that respect, values of plant growth habit (PGH), awn roughness (AR), length of first segment of rachis (RLFS), color of grain aleurone layer (KCAL), hairiness of lower leaves sheaths (LHLL), and rachilla hair type of grain (GRHT) were also non-significant between SGH groups. Moreover, values of tip shape (SSTS) and attitude of sterile spikelets (SSA) in 80 two-rowed samples did not differ between SGH types (Table 3).

On the other hand, the association of phenotypes of categorical traits with NER, SGH, and origin i.e. Iranian and exotic landraces, as well as cultivars and advanced breeding lines were shown by box colors after sorting phenotypes in each trait. The created mosaic pattern showed putative relevance of each phenotype with each grouping. For instance, within three grouping structure, it was obvious that the vast majority (~ 89%) of six-rowed barley samples were winter type; similarly there were two-rowed samples that constituted nearly all (90%) of samples with facultative and spring types. However, no clear relevance was found between origin of samples and either of NER and SGH grouping. As regards association of phenotypes, it was shown that the vast majority of 58 samples without anthocyanin color of auricles in flag leaf (FLAC) or with very weak intensity (FLIA) were winter type and six-rowed genotypes. Similarly, 26 out of 28 samples without anthocyanin color in awn tips (AACT) and two extremes of anthocyanin intensity (very weak and strong) (AIAC) were winter type and six-rowed. All 39 genotypes (except one) with smooth and intermediate roughness in awn (AR) were two-rowed and almost spring type samples. Nearly all genotypes with recurred collar (CT) were spring type and two-rowed barleys. Six-rowed samples constituted all 47 samples (except one) which the length of their glume and its awn in median spikelet (MSLG) was longer than grain. Twenty-four out of 26 genotypes which their length of first segment of rachis (RLFS) was short along with all 55 samples bearing very weak (or absent) curvature in first segment of rachis (RCFS) were six-rowed barleys. When ear density (ED) was considered as ordinal trait, the majority of winter type and two-rowed samples had very lax to lax density in ear (Fig. 3). Despite finding some relevance between phenotypes with NER and SGH groupings, no association was detected with origin of samples.

It was found that SGH (winter- and spring-types) and NER (two- and six-rowed samples) are the two primary determinants of structure in barley germplasm (Rostoks *et al.* 2006; Hamblin *et al.* 2010; Wang *et al.* 2012). The existence of this structure is due to domestication of barley over thousand years and adaptation of genotypes to different climatic zones and is also reinforces because of its high rate of self-pollination (Hamblin *et al.* 2010). In present study, categorical traits could differentiate all 143 barley genotypes (except 4 genotype pairs) and delineate them in terms of NER grouping (Fig. 1). This indicates that the weight of row-type is of a considerable value among all traits, though it's DI (0.37) was not the highest value and was equal to DI mean.

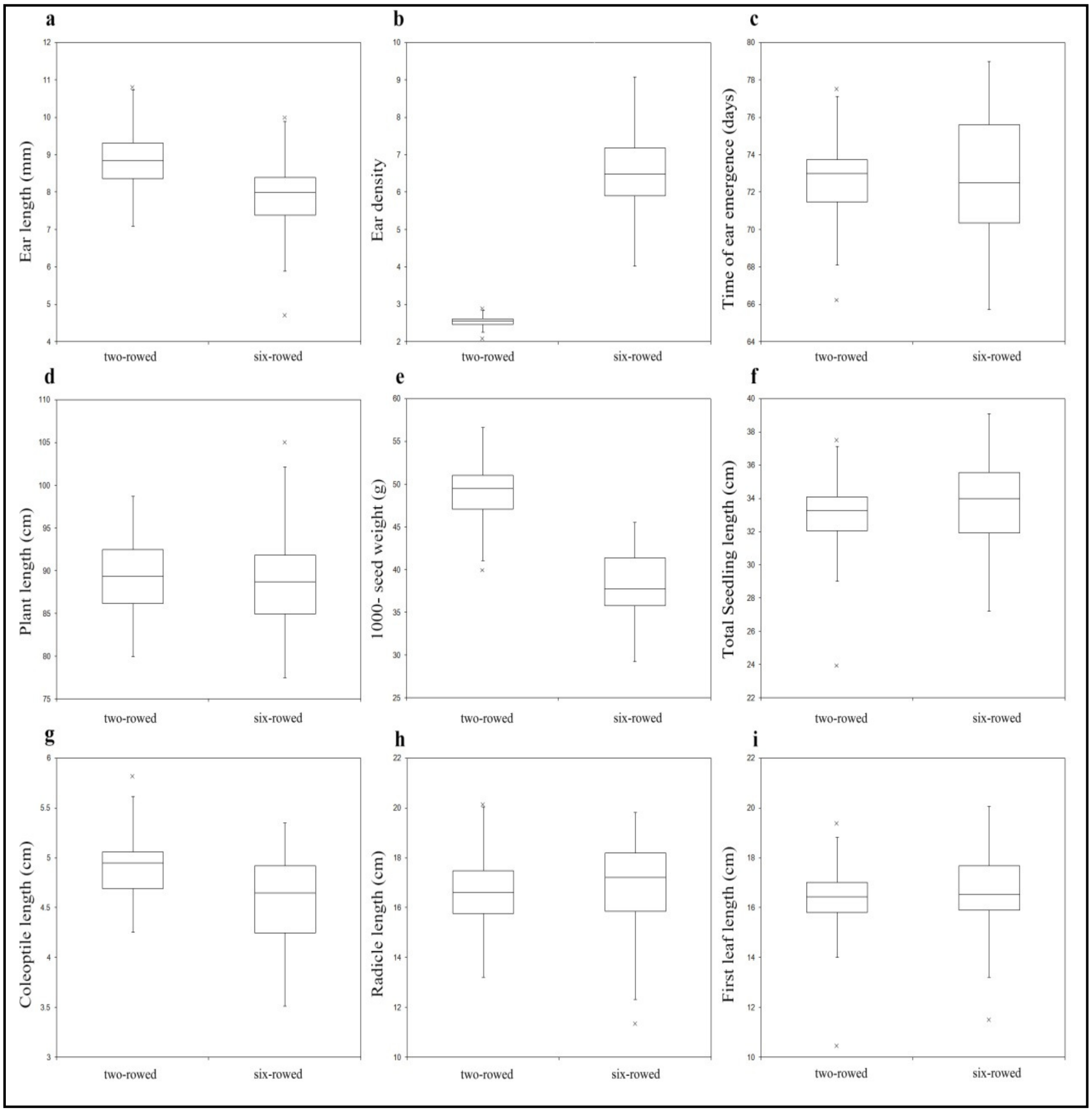

Fig. 2. Box plots of nine quantitative DUS traits grouped by the number of ear rows (NER). The 143 barley genotypes were differentiated into two- and six-rowed groups by cluster analysis on categorical traits using NJ tree. Student t test was used to estimate the significance of difference between NER means across two years. Four traits (a, b, e, g) were significantly associated with two- and six-rowed groups. Legends, a: ear length; b: ear density; c: time of ear emergence; d: plant height; e: thousand-seed weight; f: total seedling length; g: coleoptile length; h: radical length; i: first leaf length.

Present study gave insights into genetic structure of a panel of barley landraces and advanced breeding lines in terms of morphological descriptors and quantitative traits. While the identity of 143 barley samples were described by morphological traits which are currently used in DUS testing, quantitative traits used in present study provided part of agronomic characteristics for testing the value for cultivation and use (VCU) of new varieties in many countries (Cooke & Reeves, 2003). For instance, genotypes with longer coleoptile (3.5 to 5.8 cm in our 143 samples) may be correlated with vigorous seedling emergence, as is shown in wheat (Rebetzke *et al*. 2007) and suitable for deep sowing in areas with lower precipitation along with other traits like plant height and time of ear emergence.

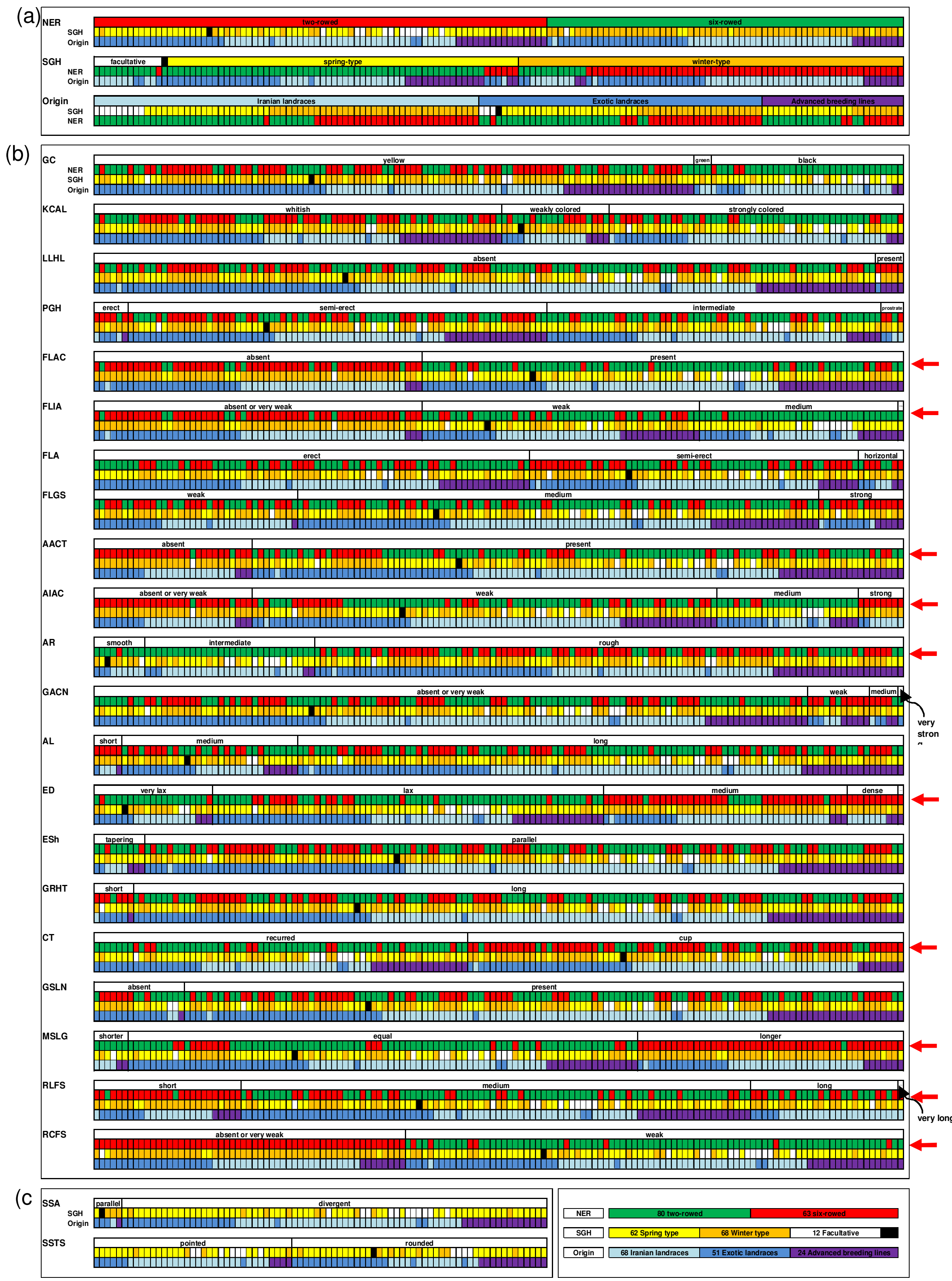
(a)
NER
SGH
Origin
two-rowed
six-rowed
SGH
NER
Origin
facultative
spring-type
winter-type
Origin
SGH
NER
Iranian landraces
Exotic landraces
Advanced breeding lines
(b)
GC
yellow
green
black
KCAL
whitish
weakly colored
strongly colored
LLHL
absent
present
PGH
erect
semi-erect
intermediate
prostrate
FLAC
absent
present
FLIA
absent or very weak
weak
medium
FLA
erect
semi-erect
horizontal
FLGS
weak
medium
strong
AACT
absent
present
AIAC
absent or very weak
weak
medium
strong
AR
smooth
intermediate
rough
GACN
absent or very weak
weak
medium
very strong
AL
short
medium
long
ED
very lax
lax
medium
dense
ESh
tapering
parallel
GRHT
short
long
CT
recurred
cup
GSLN
absent
present
MSLG
shorter
equal
longer
RLFS
short
medium
long
very long
RCFS
absent or very weak
weak
(c)
SSA
SGH
Origin
parallel
divergent
SSTS
pointed
rounded
NER
80 two-rowed
63 six-rowed
SGH
62 Spring type
68 Winter type
12 Facultative
Origin
68 Iranian landraces
51 Exotic landraces
24 Advanced breeding lines

Fig. 3.

In Fig. 3. Mosaic pattern of NER (two-rowed in green, six-rowed in red), SGH (spring type in yellow, winter type in orange, facultative in white, not assigned or NA in black), and origin (Iranian landraces in blue, exotic landraces in dark blue, advanced breeding lines in purple) representing their putative association with phenotypes of categorical traits in 143 (box b) and 80 two-rowed barley samples (box c). Mutual association between three groups is also given (box a). Possible relevance between phenotypes and groupings are indicated by red arrow. Abbreviations: NER (number of ear rows); SGH (seasonal growth habit); GC (grain color); KCAL (color of kernel aleurone layer); LLHL (hairiness of lower leaves sheaths); PGH (plant growth habit); FLAC (anthocyanin color of auricles in flag leaf); FLIA (intensity of anthocyanin color of auricles in flag leaf); FLA (attitude of flag leaf); FLGS (glaucosity of sheet in flag leaf); AACT (anthocyanin color in awn tips); AIAC (intensity of anthocyanin color in awn tips); AR (awn roughness); GACN (anthocyanin color of nerves of lemma); AL (awn length); ED (ear density); ESh (ear shape); GRHT (rachilla hair type of grain); CT (collar type); GSLN (spiculation of inner lateral nerves of lemma); MSLG (length of glume and its awn relative to grain); RLFS (length of first segment of rachis); RCFS (curvature of first segment of rachis); SSA (attitude of sterile spikelets); SSTS (tip shape of sterile spikelets).

Table 3: Diversity index (DI) of morphological traits and their Association with number of ear rows (NER) and seasonal growth habit (SGH) groupings based on Fisher's exact chi-square test (for binary and nominal traits) and Mann-Whitney U test (for ordinal traits). The maximum and minimum values of DI are shown in bold font.

Characteristic (abbreviation)	DI	Grouping	
		NER	SGH
		P	
Grain: color (GC)	0.33	< 0.05	< 0.05
Kernel: color of aleurone layer (KCAL)	0.5	< 0.05	ns*
Lowest leaves: hairiness of leaf sheaths (LLHL)	**0.06**	< 0.05	ns*
Plant: growth habit (PGH)	0.47	< 0.05	ns*
Flag leaf: anthocyanin color of auricles (FLAC)	0.37	< 0.05	< 0.05*
Flag leaf: intensity of anthocyanin color of auricles (FLIA)	0.59	< 0.05	< 0.05
Flag leaf: attitude (FLA)	0.45	< 0.05	< 0.05
Flag leaf: glaucosity of sheet (FLGS)	0.43	ns	ns
Awns: anthocyanin color of tips (AACT)	0.27	< 0.05	< 0.05*
Awns: intensity of anthocyanin color of tips (AIAC)	0.55	< 0.05	< 0.05
Awn: roughness (AR)	0.4	< 0.05	ns
Grain: anthocyanin color of nerves of lemma (GACN)	0.2	ns	ns*
Awn: length (AL)	0.33	ns	ns*
Ear: density (ED)	0.6	< 0.05	< 0.05
Ear: number of rows (NER)	0.37	---	< 0.05*
Ear: shape (ESh)	0.1	ns	< 0.05
Grain: rachilla hair type (GRHT)	0.1	< 0.05	ns*
Collar: type (CT)	0.37	< 0.05	< 0.05
Grain: spiculation of inner lateral nerves of dorsal side of lemma (GSLN)	**0.69**	< 0.05	< 0.05*
Median spikelet: length of glume and its awn relative to grain (MSLG)	0.4	< 0.05	< 0.05
Rachis: curvature of first segment (RCFS)	0.36	< 0.05	< 0.05
Rachis: Length of first segment (RLFS)	0.48	< 0.05	ns
Seasonal growth habit (SGH)	0.48	< 0.05	---
Sterile spikelet: tip shape (SSTS)	0.37	---	ns
Sterile spikelet: attitude (SSA)	0.1	---	ns
Mean	0.37		

*Significantly associated traits with SGH types in study of Wang *et al.* (2012); Abbreviations: NER (number of ear rows); SGH (seasonal growth habit)

Therefore, this might provide information for subsequent development of new varieties as a number of lines are promising for release. While this helps conservation of landraces which have been replaced by modern varieties over recent years, encourages the utilization of their desired economic traits and regions conferring specific adaptation to specific climatic zones in breeding programs which has been roughly disregarded by plant breeders.

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