Identification of quantitative trait loci for mineral elements in grains and grass powder of barley

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ABSTRACT. Mineral elements in barley (*Hordeum vulgare*) play an important physiological role in global human health. In this study, quantitative trait loci (QTLs) for concentration of nine mineral elements in barley grain and grass powder were detected in a population of 193 recombinant inbred lines of the barley cross Ziguangmangluoerling x Schooner and the parents. We observed large genetic variation contributing to element concentrations in both grains and grass powder. The mean K, Ca, and Fe concentrations in grass powder were 6.67, 12.00, and 4.58 times that of regenerating barley grains. In grains, 17

QTLs that accounted for 6.36-64.08% of the phenotypic variation in Zn, Mg, Ca, K, Na, Mn, Fe, and P concentrations were identified. In grass powder, seven QTLs were identified; these accounted for 6.03-21.86% of the variation in Ca, Zn, Mg, K, Fe, and Cu concentrations. These QTLs affecting elements in grain and grass powder are so far unreported in barley. To our knowledge, QTLs with pleiotropic effects for three elements were also identified for the first time in barley. The *qK1/qMg1/qCa1* region between markers Bmag0211 and GBMS0014 on chromosome 1H was shown to have large additive effects for Mg, Ca, and K concentrations in grains. These additive effects indicated that the high element (Mg, Ca, Zn, Mn, and K) alleles were contributed by Ziguangmangluoerling. These results will further our understanding of the genetic basis of mineral elements and help us develop markers linked with mineral elements for marker-assisted selection breeding in barley.

Key words: Quantitative trait locus; Mineral element; Grain; Grass powder; Recombinant inbred lines; Barley

INTRODUCTION

Mineral elements found in crop grain play an important role in human health (Huang et al., 2015; Kashian and Fathivand, 2015). The WHO recommends consuming <2.00 g Na and more than 3.51 g K per person per day (Drewnowski et al., 2015a). Deficiencies of Ca, Fe, and Zn in the diet are a key problem of global health (Myers et al., 2014; Drewnowski et al., 2015b). Trace elements, such as Fe, Zn, Cu, and Mn, are essential for maintaining health, immunity, growth, production, and reproduction of animals (Santos et al., 2015). Eight single nucleotide polymorphism loci have been shown to be significantly associated with concentrations of Ca, Cu, K, Na, and Zn in brown rice (Huang et al., 2016). The barley (*Hordeum vulgare*) genome encodes a subset of 10 metallothioneins (MTs) that is specifically involved in Cu detoxification (Schiller et al., 2014). Zn starvation was found to lead to up-regulation of MT1a, whereas Cu starvation up-regulated MT2a, which has two Cu-responsive elements in the promoter (Schiller et al., 2014). A major reason for the outbreak of many chronic human diseases has been suggested to be the transition of the staple food from ancient brown rice and barley to modern polished rice and wheat fine flour, resulting in loss of intake of mineral elements (Zeng et al., 2013).

Barley grass powder and grains are very important for human health, especially their contents of bioactive compounds, including the polyphenols, beta-glucan, superoxide dismutase, lutonarin, and saponarin. However, mineral elements also possess multiple health benefits. Fe-S clusters are very important for origin of life especially acetyl-CoA, DNA, and RNA world (Fuss et al., 2015). Low Ca absorption is associated with osteoporosis and fracture (Chang et al., 2015). Mg intake around 400 mg/day is associated with a lower incidence of colorectal cancer in postmenopausal women (Gorczyca et al., 2015). Lower Na and higher K intake as well as antiobesity were associated with lower the risk of hypertension (Chen et al., 2015). Barley grass powder of young green barley leaves possesses properties that help blood flow, digestion, and general detoxification (Lahouar et al., 2015). The grass powder also has antioxidant and anti-inflammatory activity, and prevention of chronic diseases may be related to the bioactive compounds superoxide dismutase, lutonarin, and saponarin (Lahouar et al., 2015).

The order of element concentrations (mg/kg) in barley grains is K (4900) > P (4125) > Mg (1425) > Ca (625) > Fe (50.1) > Zn (26.8) > Mn (15.9) > Cu (4.94) (Hejcman et al., 2013). In grass powder, it is K (49,753) > Ca (8688) > Mg (5847) > Fe (29.2) > Se (26.4) > Zn (20.5) > Cu (7.2) > Mn (5.2) (Hui and Tian, 2010). Breeding barley cultivars with high concentrations of K, Ca, Fe, and Zn is an effective and economic means of solving element deficiencies and, thus, potentially mitigate some global health problems.

Quantitative trait loci (QTL) analysis has proven useful for identifying and localizing favorable alleles in barley. Alleles have been identified for grain water uptake (Cu et al., 2016), spring drought (Fiust et al., 2015), malt extract (Wang et al., 2015a), limit dextrinase (Wang et al., 2015b), semi-dwarf genes (Li et al., 2015a), and dwarfing gene *btwd1* (Ren et al., 2014). The concentrations of mineral elements in barley are genetically complex traits, and there is large genotypic variation in the primary barley gene pool. The genetics of mineral elements in barley appear to be quantitatively inherited. However, QTL analysis information of element concentrations in grass powder and barley grains is unreported so far. In this study, QTLs and concentrations of nine mineral elements in grass powder and regenerating barley grains were determined, using ICP-AES analysis. This may not only provide a better understanding of the genetic mechanisms governing mineral element concentration in grains and grass powder, but could also benefit genetic improvement of Fe, Zn, and Ca contents in barley functional foods by pyramiding favorable QTLs.

MATERIAL AND METHODS

Plant materials

A barley RIL population consisting of 193 F_8-derived lines was developed from a cross between two two-rowed barley varieties, Ziguangmangluoerling and Schooner. The two parents differ significantly in element concentration in both grains and grass powder. Ziguangmangluoerling is a two-rowed, hull-less barley landrace with high concentrations of P, Zn, Mn, Mg, Ca, and Cu, and low Fe, Na, and K in the grains. The grass powder of this variety has high concentrations of Fe, Cu, and K, and low concentrations of P, Zn, Mn, Mg, Ca, and Na. Schooner is a two-rowed, hulled malting barley cultivar with high concentrations of Fe, Na, and K, and low concentrations of P, S, Zn, Mn, Mg, Ca, and Cu in the grains. In contrast, the grass powder contains high concentrations of P, Zn, Mn, Mg, Ca, and Na, and low concentrations of Fe, Cu, and K.

Determination of mineral element concentrations

ICP-AES determination and analysis conditions were carried out as previously described (Zeng et al., 2009, 2010). The 193 barley RILs and their parents (Schooner and Ziguangmangluoerling) were planted in Yanhe town (1638 m above sea level) of Yuxi prefecture in Yunnan Province, China in the fall of 2012-2013. The phenotypic value of each line in a test environment was taken as the average of two replicates. Barley grass was cut and dried in the elongation stage, the character of regeneration barley all developed normally in the field with watering and fertilizing except those that had a plant height <15 cm. From each of the 193 RILs and their parents, 10 g grains and 10 g grass powder were ground into powder, followed by dry storing at 4°C. Samples were digested using a wet method and any

residues were dissolved using hydrochloric acid to convert elements into the inorganic ion state as follows: Subsequently, 0.5 g each sample was added to 5 mL nitric acid and 1 mL chloric acid, heated until the solution was clear, and steamed to nearly dry on an electric hot plate. To dissolve the residue, 5 mL hydrochloric acid (1:1) was added, followed by transfer to a 50 mL volumetric flask. This was repeated three times per sample while processing a blank sample at the same time. Analyses were then performed by plasma emission spectrometer. This method proved to be simple, rapid, highly sensitive, and accurate, and can be used to assay several elements at the same time. The recovery ratio obtained by standard addition method ranged between 93.5 and 110.2%, and the relative standard deviation ranged from 1.7 to 4.6%. The solution was analyzed for eight elements (P, K, Ca, Mg, Fe, Zn, Cu, Na, and Mn) in both grains and grass powder, using ICP-AES (Zeng et al., 2010). The SPSS 16.0 software was used to perform descriptive statistics of the RIL population and to investigate the mineral element concentrations.

DNA extraction, primer selection, and polymerase chain reaction (PCR)

Total genomic DNA was extracted from fresh leaves of the F_9 RIL plants using the CTAB method (Rogers and Bendich, 1988), with minor modifications. We used 604 sequence primers, to make a genetic linkage map of seven chromosomes consisting of 775 simple sequence repeat (SSR) marker loci representing six populations (Varshney et al., 2007). These SSR markers were synthetized by Beijing Dingguo Changsheng Biotechnology Co. Ltd. A 10-µL reaction mixture containing 1.5 µL PCR buffer (20 mM Tris, pH 8.0, 50 mM KCl, 2.5 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, and 50% glycerol), 2 µL DNA, 0.5 µL forward and reverse primers, 0.4 µL each dNTPs, 0.2 µL Taq polymerase, and 4.9 µL ddH_2O. The PCR was done on a Biometra T gradient PCR machine (Goettingen, Germany) using a protocol consisting of an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 40 s; annealing at 55°C (depending on the individual SSR primer) for 40 s; and extension at 72°C for 1 min, followed by a 5-min final extension at 72°C. The PCR products were separated on 8% polyacrylamide gel by electrophoresis for 2 h at 180 V. Bands were detected using a silver-staining method (Varshney et al., 2007).

QTL analysis

To screen polymorphism between Ziguangmangluoerling and Schooner, 604 SSR markers distributed across all seven barley chromosomes were used. A set of 180 polymorphic SSR markers were used to genotype the 193 RILs and the two parents, which were then used to identify the linkage groups. PCR products that had the same band size as Ziguangmangluoerling were denoted 0, those that had the same band size as Schooner were denoted 2, heterozygotes were denoted 1, and all others were denoted -1. Genetic linkage groups were identified using the QTL IciMappingV3.3 software (http://www.isbreeding.net; Meng et al., 2015). The genetic map spanned 2671 cM providing an average density of one SSR marker per 14.84 cM based on the Ziguangmangluoerling x Schooner RILs, which contained 180 SSR markers with an average of 26 markers per linkage group (unpublished data). The QTLs and genetic effects associated with the mineral element concentrations were identified using inclusive composite interval mapping analysis using the IciMappingV3.3 software (Wang, 2009). A threshold value of LOD ≥ 2.5 was used to identify the presence of a putative QTL (Wang, 2009) and estimated genetic parameters. The percentage phenotypic variance (i.e., genetic contribution)

explained by each QTL, and its additive effects were estimated. QTLs were named according to McCouch et al. (1997), but in alphabetic order for QTLs on the same chromosome.

RESULTS

Variation in element concentration in the parents and RILs

Compared with Schooner, a two-rowed and hulled malting barley cultivar, Ziguangmangluoerling is a two-rowed, hull-less barley landrace with high grain concentrations of P, K, Mg, Ca, Fe, Zn, Mn, and Cu, and low concentration of Na; In its grass powder, the concentrations of Fe, Cu, and K are high, whereas P, Zn, Mn, Mg, Ca, and Na are low (Table 1). The phenotypic differences in mineral element concentrations in grains and grass powder between Ziguangmangluoerling, Schooner, and the RILs are presented in Table 1.

Table 1. Genetic variation in mineral concentrations in grains and grass powder for the 195 RILs and the two parents.

Trait	Product	Parent (mg/kg)		RIL population					
		ZGM*	SCH*	Mean	Range	CV (%)	Variance	Skewness	Kurtosis
P	Barley grains	2772.0	1867.00	2585.6	5601.1	40.4	108,800.0	0.86	0.45
	Grass powder	3645.0	3890.00	4187.9	3270.0	11.6	23,700.0	0.07	1.43
K	Barley grains	4690.0	3889.00	4801.7	8499.0	38.4	340,000.0	-0.11	-0.35
	Grass powder	32,200.0	22,900.0	31,828.0	44,900.0	28.1	797,8000.0	0.00	-0.65
Mg	Barley grains	1364.0	917.2	1248.6	1797.6	31.5	15,480.0	-0.53	-0.54
	Grass powder	2015.0	3020.0	2470.7	2190.0	15.3	14,330.0	-0.17	0.19
Ca	Barley grains	582.8	461.3	567.7	1086.6	41.5	55,474.9	-0.19	-0.80
	Grass powder	6250.0	6320.0	6786.5	7690.0	2.0	188,100.0	0.47	-0.19
Na	Barley grains	157.6	165.9	189.9	605.8	55.1	10,936.3	0.87	1.67
	Grass powder	3040.0	6630.0	4327.6	14,160.0	43.5	354,300.0	1.54	5.86
Fe	Barley grains	58.7	42.8	52.9	235.0	59.1	976.9	1.55	6.38
	Grass powder	252.0	248.0	241.3	405.1	22.9	3057.2	0.19	2.62
Zn	Barley grains	50.8	25.9	39.5	66.8	39.5	242.6	-0.00	-0.86
	Grass powder	37.1	69.2	56.8	68.9	23.2	173.4	0.24	0.30
Cu	Barley grains	16.1	11.2	14.1	67.4	72.9	106.0	2.35	7.87
	Grass powder	42.7	11.9	10.5	14.7	15.0	2.5	1.62	8.76
Mn	Barley grains	19.7	17.1	29.1	114.2	85.2	613.7	1.67	1.88
	Grass powder	29.1	69.4	41.3	52.3	17.2	50.7	0.82	0.18

*ZGM = Ziguangmangluoerling, SCH = Schooner.

The frequency distributions of macroelement concentrations (P, K, Mg, Ca, and Na) in regenerating barley grains and grass powder for RILs derived from Ziguangmangluoerling x Schooner are presented in Figure 1 and Table 1. The concentrations of P and Mg in grains were both found to be highest in Ziguangmangluoerling, followed by the RILs and Schooner. However, the concentrations of K in grass powder was higher in Ziguangmangluoerling and RILs compared to in Schooner. The concentrations of Mg and Na in grass powder were both found to be highest in Schooner, followed by the RILs and Ziguangmangluoerling. The concentration of Na in grass powder was approximately twice as high in Schooner compared to that observed in Ziguangmangluoerling, whereas the RILs showed an intermediate Na concentration. The concentrations of K and Na in grains were both found to be highest in RILs, followed by the Ziguangmangluoerling and Schooner. The concentrations of P and Ca in grass powder were both found to be highest in RILs, followed by the Ziguangmangluoerling and Schooner.

Two (P, Mg, and Ca) or three peak (K) continuous distributions were observed in the RIL population for four macroelements in grains except Na (single peak). However, single

peak continuous distribution were observed in four macroelements (P, Mg, Ca, and Na) in grass powder, except K (two peak), and with the exception of Na, the average P, K, Mg, and Ca concentrations in both grains and grass powder were close to average of Ziguangmangluoerling and Schooner, i.e., their mid-parent value (Figure 1). This indicates that all the five elements were quantitative traits controlled by multiple genes, which satisfies the demands for QTL analysis.
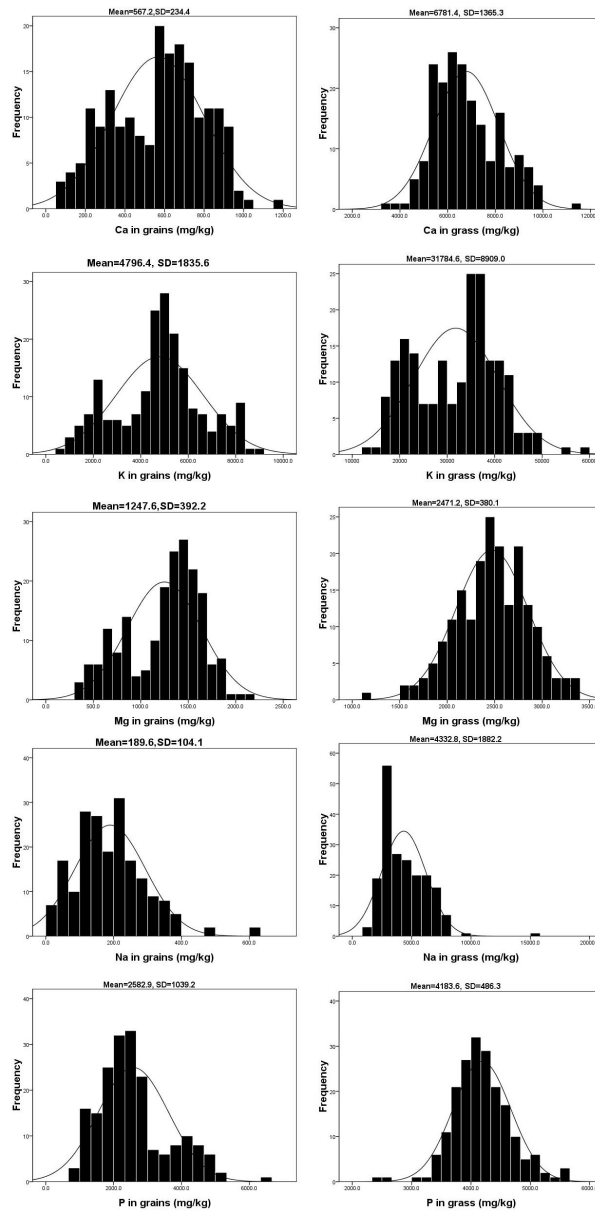
Figure 1. Frequency distributions of the concentrations of macroelements P, K, Mg, Ca, and Na in barley grains and grass powder in 193 RILs derived from Ziguangmangluoerling x Schooner.

The frequency distributions of the microelement concentrations (Fe, Zn, Cu, and Mn) in regenerating barley grains and grass powder for RILs derived from Ziguangmangluoerling x Schooner are presented in Figure 2 and Table 1. The concentrations of Fe, Zn and Cu in grains were found to be highest in Ziguangmangluoerling, followed by the RILs and Schooner. However, the concentrations of Fe and Cu in grass powder were both found to be highest in Ziguangmangluoerling, followed by the RILs and Schooner. The concentrations of Zn and Mn in grass powder were approximately twice as high in Schooner compared to that observed in Ziguangmangluoerling, whereas the RILs showed an intermediate Zn and Mn concentration. The concentrations of Mn in grains were found to be highest in RILs, followed by the Ziguangmangluoerling and Schooner.

Single-peak (Fe and Cu) or two-peak (Zn and Mn) continuous distributions in grains and single-peak (Fe, Zn, Cu and Mn) continuous distributions in grass powder were observed in the RIL population for all four microelements (Figure 2). The average concentrations of Fe, Zn, and Cu in the grains and the average concentrations of Fe, Zn, and Mn in grass powder were close to their respective mid-parent values (Figure 2), indicating that these three elements were quantitative traits controlled by multiple genes and, thus, satisfied the demands for QTL analysis.
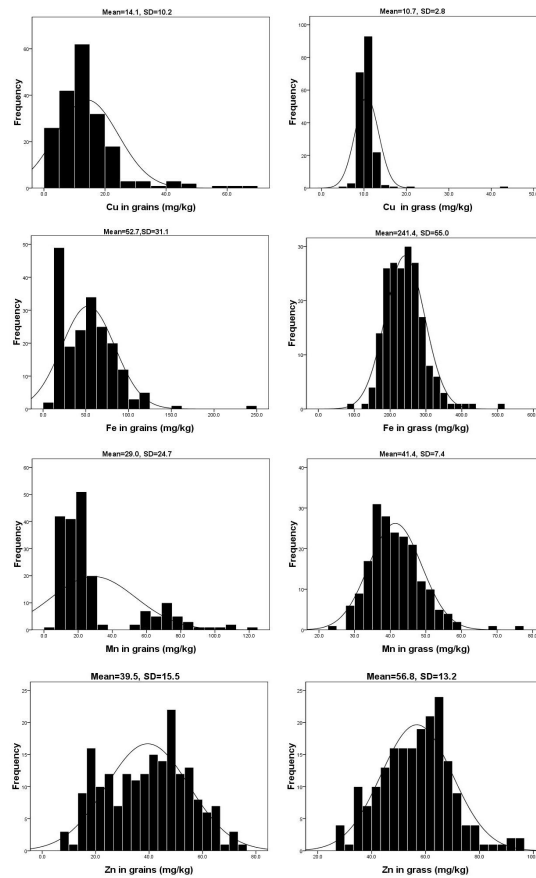
Figure 2. Frequency distributions of concentrations of microelements Fe, Zn, Cu, and Mg in barley grains and grass powder in 193 RILs derived from Ziguangmangluoerling x Schooner.

Detection of QTL for element concentrations in regenerating barley grains

We identified 17 QTLs for eight element concentrations (P, K, Mg, Ca, Na, Fe, Zn, and Mn) in regenerating barley grains were unreported so far. The average phenotypic variances explained (PVE) by these 17 QTLs ranged from 6.36 to 64.08%, as identified in the RIL population (Table 2). Specifically, the first co-localizations of QTLs for *qK1/qMg1/qCa1* region for Bmag0211-Gbms0014 were identified on chromosome 1H; the average PVE of *qK1*, *qMg1*, and *qCa1* were from 34.28 to 62.51%, indicating that they were major QTLs for macroelements (K, Ca, and Mg) with a potential health effect. QTLs *qCa1*, *qCa6*, and *qCa7* for Ca were identified on chromosomes 1H, 6H, and 7H in RILs with favorable alleles, all from the parent Ziguangmangluoerling, which was shown to have the largest effects for Ca concentrations in grains. The average PVE of *qCa1*, *qCa6*, and *qCa7* were from 62.51 to 62.34%, indicating that they were major QTLs for Ca concentrations in grains. In addition, QTLs *qMg7-1* and *qZn7-2* for Mg and Zn concentrations, respectively, were identified on chromosome 7H in the RILs with favorable alleles all from the parent Ziguangmangluoerling. The average PVE of *qMg7-1* and *qZn7-2* were 49.41 and 44.21%, respectively, indicating that they were major QTLs for Mg and Zn concentrations in grains. QTL *qNa4* for Na concentration was identified on chromosome 4H in RILs with favorable alleles from the parent Schooner. The average PVE of *qNa4* was 12.31%, indicating that it was major QTL for Na concentrations in grains.

Table 2. QTLs for element concentration in barley grains identified in a population of 195 RILs.

Trait	QTL	Chr.[a]	Marker interval	Interval (cM)	LOD[b]	PVE[c] (%)	Additive effect
P	*qP7*	7H	Bmag0914-Gbm1464	10.46	2.75	6.36	-265.93
K	*qK1*	1H	Bmag0211-Gbms0014	60.73	3.84	34.28	1090.82
	qK7	7H	Hvcabg-Ebmac713	60.74	3.02	6.42	-481.71
Mg	*qMg1*	1H	Bmag0211-Gbms0014	60.73	4.04	55.37	302.57
	qMg7-1	7H	Bmac0156-Bmag0007	71.62	2.74	49.41	277.61
	qMg7-2	7H	Bmac0273-Ebmac0764	26.45	2.90	7.07	106.56
Ca	*qCa1*	1H	Bmag0211-Gbms0014	60.73	4.34	62.51	189.89
	qCa6	6H	Bmag0174-Gbm1270	91.33	4.60	64.08	-191.23
	qCa7	7H	Bmac0156-Bmag0007	71.62	5.30	62.34	186.78
Na	*qNa4*	4H	Bmag0808-Bmag0740	16.19	4.53	12.31	36.66
	qNa7	7H	Bmag0507-Bmag0321	3.32	3.35	7.58	29.70
Fe	*qFe2*	2H	Bmag0571-Bmag0378	41.29	3.34	7.88	-9.43
Zn	*qZn4*	4H	Hvbammgb84-Bmag0808	17.72	3.43	7.37	4.22
	qZn7-1	7H	Bmag0385-Ebmac755	30.50	4.05	8.66	-5.23
	qZn7-2	7H	Bmac0156-Bmag0007	71.62	3.02	44.21	10.33
Mn	*qMn7-1*	7H	Bmag0914-Gbm1464	10.46	4.47	9.63	-7.77
	qMn7-2	7H	Bmac0162-Gbm1303	17.73	4.18	9.30	7.67

[a]Chromosome, [b]Genome-wide log of odd (LOD) threshold of 2.5 was determined by a 1000-permutation test at P < 0.05, [c]Phenotypic variance explained (PVE) by each QTL.

Detection of QTLs for element concentrations in barley grass powder

Seven QTLs for six element concentrations (K, Mg, Ca, Fe, Zn, and Cu) in barley grass powder were unreported so far. The average PVE of these seven QTLs ranged from 6.03 to 21.86% (Table 3). Specifically, QTL *qCa3* for Ca and QTL *qK5* for K concentration were identified on chromosomes 3H and 5H in RILs with favorable Ca alleles from Schooner but K alleles from Ziguangmangluoerling. The average PVE of *qCa3* and *qK5* were 21.85 and 16.45%, respectively, indicating that they were major QTLs for Ca and K concentrations in grass powder.

Table 3. QTLs for element concentration in barley grass powder identified in the RIL population.

Trait	QTL	Chr.	Marker interval	Interval (cM)	LOD	PVE (%)	Additive effect
K	qK5	5H	Gbms0032-Gbm5008	31.38	3.42	16.45	0.38
Mg	qMg7	7H	Hvwaxy4-Gbm1326	11.27	3.02	8.79	0.01
Ca	qCa2	2H	Bmag0378-Gbm1119	13.39	3.35	6.03	0.03
	qCa3	3H	Hvltppb-Gbm1159	5.81	10.76	21.85	-0.06
Fe	qFe3	3H	Gbm1159-Bmag0138	55.45	3.03	7.33	-15.05
Zn	qZn6	6H	Gbm1270-Ebmac0806	6.81	4.35	9.93	-4.61
Cu	qCu2	2H	Bmag0125-Gbm1309	36.40	4.07	10.69	0.52

DISCUSSION

Element concentration variation in regenerating barley grains and grass powder

There is very large genetic variation contributing to element concentration in regenerating barley grains and grass powder (Table 1). The element concentrations of regenerating barley grains in the 193 RILs and their parents were in turn K > P > Mg > Ca > Na > Fe > Zn > Mn > Cu, whereas in grass powder they are K > Ca > Na > P > Mg > Fe > Zn > Mn > Cu. The average K, Ca, and Fe concentrations in grass powder were 6.67, 12.00, and 4.58 times that found in regenerating barley grains. The average concentrations for nine elements in grains were similar to those previously reported (Newman and Newman, 2008). P, K, Mn, and Cu in grass powder were also similar to those reported previously (Yang et al., 2016). However, the Na, Ca, Mg, Fe, and Zn concentrations in barley grass powder for the 193 RILs and their parents were higher than those reported by Yang et al. (2016). The grain Fe and Zn concentrations of 92 *H. spontaneum* genotypes were found to average 743 and 1739 mg/kg, respectively, whereas 140 ± 5.1 mg/kg was found in Yunpi 6 (the highest cultivar of grain Fe concentration) and 98.7 ± 5.1 mg/kg was found in Supi 4 (the highest cultivar of grain Zn concentration), respectively (Yan et al., 2012). Previously, the mineral elements contents (mg/kg) of barley grass have been shown to be K (27,000) > Na (13,000) > Ca (4740) > Mg (4110) > P (2350) > Fe (820) > Mn (42.0) > Zn (13.0) > Cu (12.0) (Lin, 2005). The mean concentrations (mg/kg) of eight elements in 628 brown rice landraces were found to be P (3480) > K (2540) > Mg (1480) > Ca (157) > Zn (32.8) > Fe (32.0) > Cu (13.6) > Mn (13.2) (Zeng et al., 2010). The K, Ca, and Fe concentrations in grass powder were found to be 10.63, 30.19, and 25.63 times that of brown rice. An increased intake of K might be beneficial for hypertension control in humans populations (Noh et al., 2015). Dietary Ca/P ratio is one of the indicators that can predict bone health (Arai and Sakuma, 2015). An increased Ca intake is associated with reduced blood pressure, reduced rate of bone loss, and a moderate risk reduction of fracture (Shiraki, 2015). Fe and n-3 long-chain polyunsaturated fatty acid have been associated with cognition, especially in children with good reading comprehension (Sørensen et al., 2015). Total Fe bioavailability in the US diet is 15.1-15.5% (Armah et al., 2015). Healthy dietary of the most major for modern humans will be the polished rice or wheat flour add grass powder (Zeng et al., 2016b).

Genetic approaches are currently being applied in barley, aiming to biofortify functional foods with essential mineral elements that are commonly lacking in human diets such as Fe, Zn, and Ca. However, current efforts to develop barley cultivars with high mineral content associated with good agronomic performance are still very restricted. Therefore, barley grass powder could be an important functional food to solve the global mineral malnutrition problem.

QTL detection and pleiotropy for eight element concentrations in regenerating barley grains

We identified 17 QTLs for eight element concentrations (P, K, Mg, Ca, Na, Fe, Zn, and Mn) in barley grains that were unreported so far. These explained from 6.36 to 64.08% of the phenotypic variance in the RIL population in this study (Table 2). Eight major QTLs were identified on chromosomes 1H, 6H, and 7H in the RILs, and explained from 12.31 to 64.08% of the phenotypic variance. These QTLs were shown to have the largest effects for four element concentrations in grains, i.e., QTLs *qK1*, *qZn7-2*, *qMg7-1*, *qMg1*, *qCa1*, *qCa6*, and *qCa7* explained 34.28-64.08% of the total phenotypic variation in four element concentrations and the favorable alleles at these loci were contributed by Ziguangmangluoerling (Mg, Ca, and Zn) and Schooner (K). A comparison of the QTLs identified in this study with previously reported concentrations of these four elements in crop grains revealed that same four elements were shown to have large effects. For instance, QTLs *qK8-1*, *qK9-1*, *qMg5-1*, *qCa1-1*, and *qZn8-1* explained 14-19% of the total phenotypic variation in four element concentrations and the favorable alleles at these loci were contributed by wild rice (*Oryza rufipogon*) (Garcia-Oliveira et al., 2009). The *qK6.1/qCa6/qZn6/qMn6/qCu6* region for major QTLs was a 29.9-kb region flanked by RM19410 and Si2944, with the enhancing alleles derived from Milyang 46 (Yu et al., 2015). In maize, the *NRAMP* genes are likely responsible for the observed natural variation in maize grain Zn and Fe concentrations (Jin et al., 2015).

The associated QTLs for *qK1/qMg1/qCa1* region for Bmag0211-GBMS0014 were identified for the first time on chromosome 1H in barley. The average PVE of *qK1*, *qMg1*, and *qCa1* were 34.28, 55.37, and 62.51%, respectively. This indicates that they were major QTLs for macroelements K, Ca, and Mg with potential health effects. It is noteworthy that the QTL in the Bmag0211-GBMS0014 region on chromosome 1H that affected the K, Mg, and Ca concentrations in the RIL population was an important QTL with a large additive effect and genetic background independence. This finding merits confirmation in other populations and fine-mapping for map-based cloning. The additive effects indicated that the alleles for higher element concentrations were contributed by Ziguangmangluoerling, and that these may be the most important components for enhancing element concentrations through methods of genealogical improvement. Significant additive genetic effects as well as parental mean effects have previously been detected for P, K, Ca, and Na (Fernandes Santos and Boiteux, 2015). Co-localization of QTLs for element concentration has previously been found in various species, such as rice (Yu et al., 2015) and in maize grain (Simić et al., 2012). Co-localization of QTLs for the *qP3/qMg3/qZn3* region was shown to have the largest effects for P, Mg, and Zn contents, whereas the *qK6.1/qCa6/qZn6/qMn6/qCu6* region was found to be responsible for five of seven mineral elements in milled rice in RIL populations (Yu et al., 2015). Three QTLs for Fe/P, Zn/P, and Mg/P were co-localized on chromosome 3 in maize, coinciding with the SSR marker bnlg1456 (Simić et al., 2012). Co-localization of QTLs for functional components has been previously found in functional rice, including resistant starch and total alkaloid content in both brown and polished rice (Zeng et al., 2016a). Co-localization of these QTLs may be due to pleiotropy or linkage (Li et al., 2015b). Six QTLs with pleiotropic effects were found between markers Bmag353 and GBM1482 on chromosome 4H in barley (Chen et al., 2014). These results show that there was at least partial genetic overlap between K, Mg, and Ca concentrations in the RIL population, which is similar to co-localizations of QTLs for functional components. The present study will be useful for genetic improvement programs

aiming to increase the concentrations of K, Mg, and Ca in barley grains. Cultivar improvement can be obtained with relative ease by molecular breeding. The molecular mechanisms for the larger additive effects observed in K, Mg, and Ca concentrations await deeper investigation.

Detection of QTLs for eight element concentrations in barley grass powder

Seven QTLs for K, Mg, Ca, Fe, Zn, and Cu concentrations in barley grass powder were unreported so far. These explained from 6.03 to 21.86% of the phenotypic variance in the RIL population in this study (Table 3). The average PVE of *qCa3* and *qK5* were 21.85 and 16.45%, respectively, indicating that they were major QTLs for the Ca and K concentrations in grass powder. Comparisons of the QTLs identified in this study with previously reported K concentrations in maize and tobacco leaves revealed that K concentrations were shown to have larger effects. For instance, the highest LOD score of 15.52 was detected for K concentration in leaves of an intermated B73 x Mo17 maize population, explaining more than 20% of the phenotypic variance (Zdunić et al., 2014). The K content has been shown to be higher during the early stages of tobacco growth; however, some tobacco seedling genes constituted an important gene-regulatory network under early low-potassium stress (Li et al., 2016). Future research should focus on a comprehensive analysis of the gene-regulatory network of K and Ca concentration in barley grass.

Conflicts of interest

The authors declare no conflict of interest.

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