Natural Variation in Grain Selenium Concentration of Wild Barley, *Hordeum spontaneum*, Populations from Israel

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Abstract Wild barley (*Hordeum spontaneum*), the progenitor of cultivated barley, is an important genetic resource for cereal improvement. Selenium (Se) is an essential trace mineral for humans and animals with antioxidant, anticancer, antiarthropathy, and antiviral effects. In the current study, the grain Se concentration (GSeC) of 92 *H. spontaneum* genotypes collected from nine populations representing different habitats in Israel was investigated in the central area of Guizhou Province, China. Remarkable variations in GSeC were found between and within populations, ranging from 0 to 0.387 mg kg^{-1} among the 92 genotypes with an average of 0.047 mg kg^{-1}. Genotype 20_C from the Sede Boqer population had the highest GSeC, while genotype 25_1 from the Atlit population had the lowest. The mean value of GSeC in each population varied from 0.010 to 0.105 mg kg^{-1}. The coefficient of variation for each population ranged from 12% to 163%. Significant correlations were found between GSeC and 12 ecogeographical factors out of 14 studied. Habitat soil type also significantly affected GSeC. The wild barley exhibited wider GSeC ranges and greater diversity than its cultivated counterparts. The higher Se grain concentrations found in *H. spontaneum* populations suggest that wild barley germplasm confer higher abilities for Se uptake and

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accumulation, which can be used for genetic studies of barley nutritional value and for further improvement of domesticated cereals.

Introduction

The essential trace mineral selenium (Se) is of fundamental importance to human and livestock health [1–3]. Se enters the food chain mainly through plants. In many countries, soils are often low in available Se; hence, the food systems are deficient in Se [4–6]. Based on surveys of plasma or serum Se levels, Combs [4] estimated that from half a billion to a billion people worldwide may suffer from Se deficiency.

Cereals, meat, and fish are the main sources of Se in most diets [4]. In foods, the Se exists in different chemical forms, with Se being generally more bioavailable from plants than from animal foodstuffs [7, 8]. More than 80% of the total Se in seleniferous corn, wheat, and soybean consists of L-(1)-selenomethionine [9], which is the most appropriate supplemental form of Se in major staple foods [10]. As a main source of calorie intake, cereal-based foods are extensively consumed in the developing world [11]. However, cereals are inherently very poor in both concentration and bioavailability of microelements such as Zn, Fe, and Se in the seeds, particularly when grown in microelement-deficient soils [8, 11, 12]. Cereal crops such as wheat, barley, rye, and oats are non-Se-accumulators, rarely accumulating more than 0.1 mg Se kg^{-1} dry weight [13]. Developing cereals that are genetically enriched in micronutrients and proteins and improving their bioavailability (biofortification) using genetics and genomics tools are considered as promising and cost-effective approaches to reducing malnutrition [6, 14–17]. Moreover, the existence of large genetic variation in grain micronutrients is essential for a successful breeding program aimed at the development of new micronutrient-rich plant genotypes.

Wild barley, *Hordeum spontaneum* C. Koch, is the progenitor of cultivated barley, *Hordeum vulgare* L. ssp. *vulgare* [18]. It is widely distributed throughout the eastern Mediterranean rim and western Asian countries [18]. The center of diversity for *H. spontaneum* and the primary site of its domestication are considered to be the Fertile Crescent of the Near East [18–20]. In Israel, *H. spontaneum* is abundant, occupying an extraordinarily large diversity of habitats ranging from the mesic Mediterranean to the xeric southern steppes [21]. Several decades of studies on genetic resources of wild cereals (wheat and barley) for crop improvement, conducted at the Institute of Evolution at the University of Haifa, Israel, have revealed that the vast genetic resources of wild cereals have great potential for improving the narrowing genetic base of domesticated cereals [18, 21–26]. Moreover, barley has a self-fertile, diploid ($2n=2x=14$) genetic system and can therefore serve as a model species for genetic and physiological studies in Triticeae species [27]. In particular, barley cannot be grown in soil with excess Se without the risk of Se toxicity because it is a Se-sensitive plant [18]. Therefore, wild barley is an optimized model species for improving grain Se content in cereals via gene introgression. The objectives of the present study were to analyze the variation in grain Se concentration (GSeC) in Israeli *H. spontaneum* populations and to choose a set of donor parents for the breeding of Se-enriched barley cultivars.

Materials and Methods

Plant Materials

Ninety-two genotypes from nine populations of the wild barley *H. spontaneum*, representing different geographical zones in Israel (Fig. 1 and Table 1), were used in the present study. All nine populations were selected from the Gene Bank Collection of the Institute of Evolution at the University of Haifa: six of them originated from mesic habitats in the north and center of Israel, with mean annual rainfall of >500 mm, Mt. Hermon

Fig. 1 Geographical location of Israeli *Hordeum spontaneum* populations used in the present study. *1*, Mt. Hermon; *10*, Maalot; *20*, Sede Boqer; *22*, Mehola; *24*, Akhziv; *25*, Atlit; *26*, Caesarea; *32*, Ein-Zukim; *37*, "Evolution Canyon"
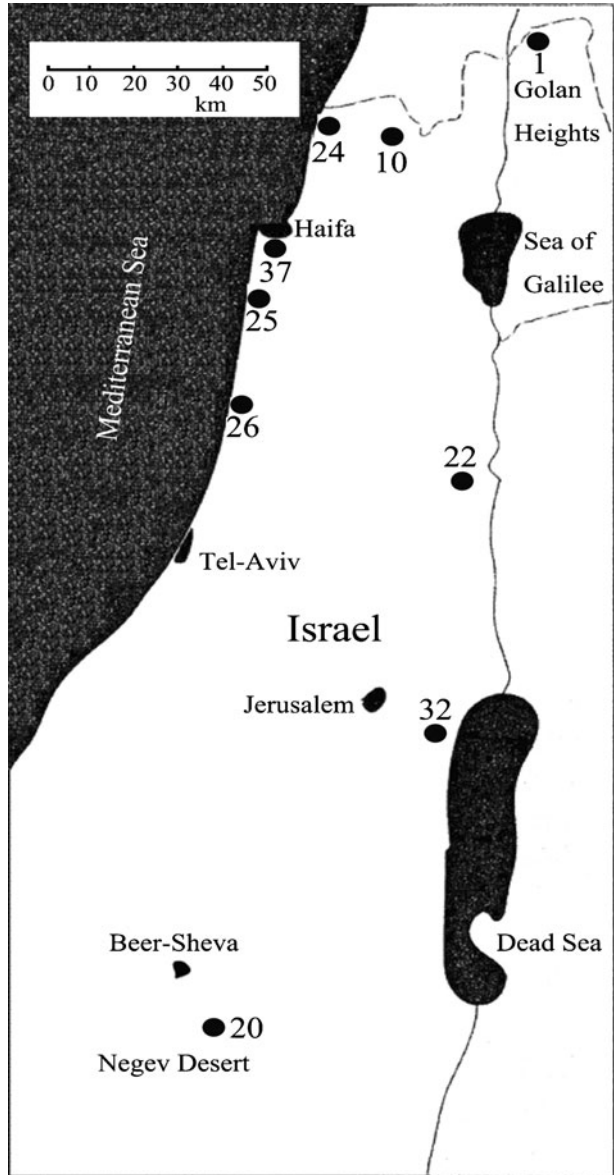
Table 1 Ecogeographical Factors of the Nine *Hordeum spontaneum* Populations in Israel

ID[a]	Population	Ln	Lt	Al	Tm	Ta	Tj	Td	Tdd	Ev	Rn	Rd	Hu14	Huan	So
1	Mt. Hermon	35.75	33.28	1,530	11	20	1	19	6	160	1,400	70	52	58	1
10	Maalot	35.27	33.00	500	17	23	8	15	10	150	785	55	50	64	2
20	Sede Boqer	34.78	30.87	450	19	26	9	15	13	168	91	15	36	53	6
22	Mehola	35.48	32.13	−150	22	30	13	17	13	180	270	39	34	53	3
24	Akhziv	35.10	33.05	10	20	26	12	13	10	130	620	56	60	67	3
25	Atlit	34.95	32.70	50	20	26	13	13	9	133	500	48	65	72	4
26	Caesarea	34.90	32.50	10	20	26	13	13	9	130	540	48	65	72	4
32	Ein-Zukim	35.44	31.74	−200	24	31	15	16	12	210	100	20	35	52	6
37	Evolution Canyon	34.58	32.43	90	22.5	28	13	15	9	142	600	48	65	67	1

Geographical variables: Ln=longitude (decimal), Lt=latitude (decimal), and Al=altitude (meters). Temperature variables: Tm=mean annual temperature (°C), Ta=mean August temperature (°C), Tj=mean January temperature (°C), Td=mean seasonal temperature difference (°C), Tdd=mean daily temperature difference (°C), and Ev=mean annual evaporation. Water variables: Rn=mean annual rainfall (millimeters), Rd=mean number of rainy days, Hu14=mean humidity at 1400 hours (percent), Huan=mean annual humidity (percent), and Dw=mean number of dewy nights in summer (millimeters). Dummy soil variables: So=soil types, *1* terra rosa, *2* rendzina, *3* alluvium, *4* sandy loam, *6* loess (desert soil)

[a] Population IDs and selected environmental data are based on [21]; Ein-Zukim data is based on [63]

(population 1), Maalot (10), Akhziv (24), Atlit (25), Caesarea (26), and "Evolution Canyon" (37); and the other three were from xeric habitats in Israel's central and southern regions, with mean annual rainfall of <300 mm, Sede Boqer (20), Mehola (22), and Ein-Zukim (near the Dead Sea; 32; Fig. 1 and Table 1). Ten barley cultivars from five provinces in China—Yangnongpi 5, Yancheng 01094, and Supi 4 from Jiangsu Province; Ganpi 3 from Gansu Province; Baoshan 8640-1, Yunpi 2, and Yunpi 6 from Yunnan Province; E32380 from Hubei Province; and Zhexiu 13 and Zhexiu 33 from Zhejiang Province—were used as controls. All cultivars were provided by the Hangzhou National Barley Improvement Center in China.

A randomized complete block design with three replications was used. Seeds of the 92 wild barley genotypes and ten barley cultivars were sown in a field of loess soil under natural rainfall conditions at an experimental station in the central area of Guizhou Province, China (latitude 26°25′, longitude 106°40′, altitude 1,096 m, mean annual rainfall 1,500 mm, soil Se content 0.20 mg kg^{-1}, and pH 6.6) in mid November 2006. Fully mature spikes were harvested manually from May to June 2007.

Determination of Se in Barley Seeds

The barley caryopses were dehulled by removing awn, glumes, lemma, and palea. Se concentration in the barley grains was determined by hydride generation-atomic fluorescence spectrometry (HG-AFS) following a modified protocol [28] at the State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences. The grains were washed thoroughly with deionized water, dried at 70°C, pulverized, and passed through a 100-mesh (0.149 mm) sieve. For digestion, 0.100 g of each sample was digested in a 50-mL PTFE tube with 3 mL HNO$_3$ (GR grade, Merck) at room temperature for 3 h and then at 145°C for 8 h. After cooling to room temperature, the tube was opened, and 2 mL H$_2$O$_2$ (GR grade, Merck) was added. The mixture was evaporated to dryness at 70°C. The dried mixture was redissolved in 3 mL 5 M HCl (ACS grade, Fisher Scientific) and transferred

to a 25-mL volumetric glass tube (Pyrex). The final solution was prepared by heating it at 90°C in a water bath for 90 min and then diluting it to 25 mL with deionized water.

An atomic fluorescence spectrometer AFS-920 equipped with a sequential injection HG sampling system (Beijing Titan Instrumentals Co. Ltd., Beijing, China) was used for Se measurement. The Hg sampling system provides high sensitivity and rapid isolation of Se from the mixture. A Se hollow cathode lamp was used as the radiation source at 196.0 nm. The hydride was atomized with an argon–hydrogen flame. The operating parameters of the HG-AFS were optimized and are summarized in Table 2. Titan standard software was used for system control and data analysis.

Se concentration was expressed as milligram per kilogram dry weight. During the analysis, a reference substance (GSV-1 and GSV-3, Se concentration of 0.184 ± 0.01 and 0.140 ± 0.01 mg kg^{-1}, respectively), a blank, and duplicate samples (one duplicate per five samples) were used to ensure the accuracy and precision of the results (quality control). The measured values of GSV-1 and GSV-3 were 0.185 ± 0.01 ($n=6$) and 0.153 ± 0.003 mg kg^{-1} ($n=4$), respectively, which is consistent with the reference value. The blank was well below the detection limit (0.2 μg L^{-1}). The results of the duplicate samples were relatively good with a coefficient of variation (CV) in the range of 1.3% to 6.0%.

Statistical Analysis

JMP6.0 (SAS Institute) software was used to perform analysis of variance (ANOVA). Tukey–Kramer's honestly significant difference (HSD) test was used to compare means of all pairs (significance level, 5%). Spearman's rho correlation was used to analyze the multivariate correlations.

Results

Natural Variation in *H. spontaneum* GSeC

Large variations were observed in *H. spontaneum* GSeC among the 92 genotypes and nine populations, respectively (Fig. 2 and Table 3). GSeC among the 92 genotypes ranged from 0 to 0.387 mg kg^{-1}, with an average of 0.045 mg kg^{-1}. The genotype with the highest GSeC was 20_C, which originated in the Sede Boqer population, while that with the lowest

Table 2 Operating Parameters of the Hydride Generation-Atomic Fluorescence Spectrometry

Parameters	Settings
Selenium hollow cathode lamp	196.0 nm
Lamp current	75 mA
Negative high voltage of photomultiplier	265 V
Atomizer height	8 mm
Atomization temperature	200°C
Carrier HCL flow	0.72 mol/L (6%)
KHB_4 concentration	1% (*w/v*) (in 0.2% (*w/v*) NaOH solution)
Carrier argon flow	400 mL/min
Shield argon flow	800 mL/min
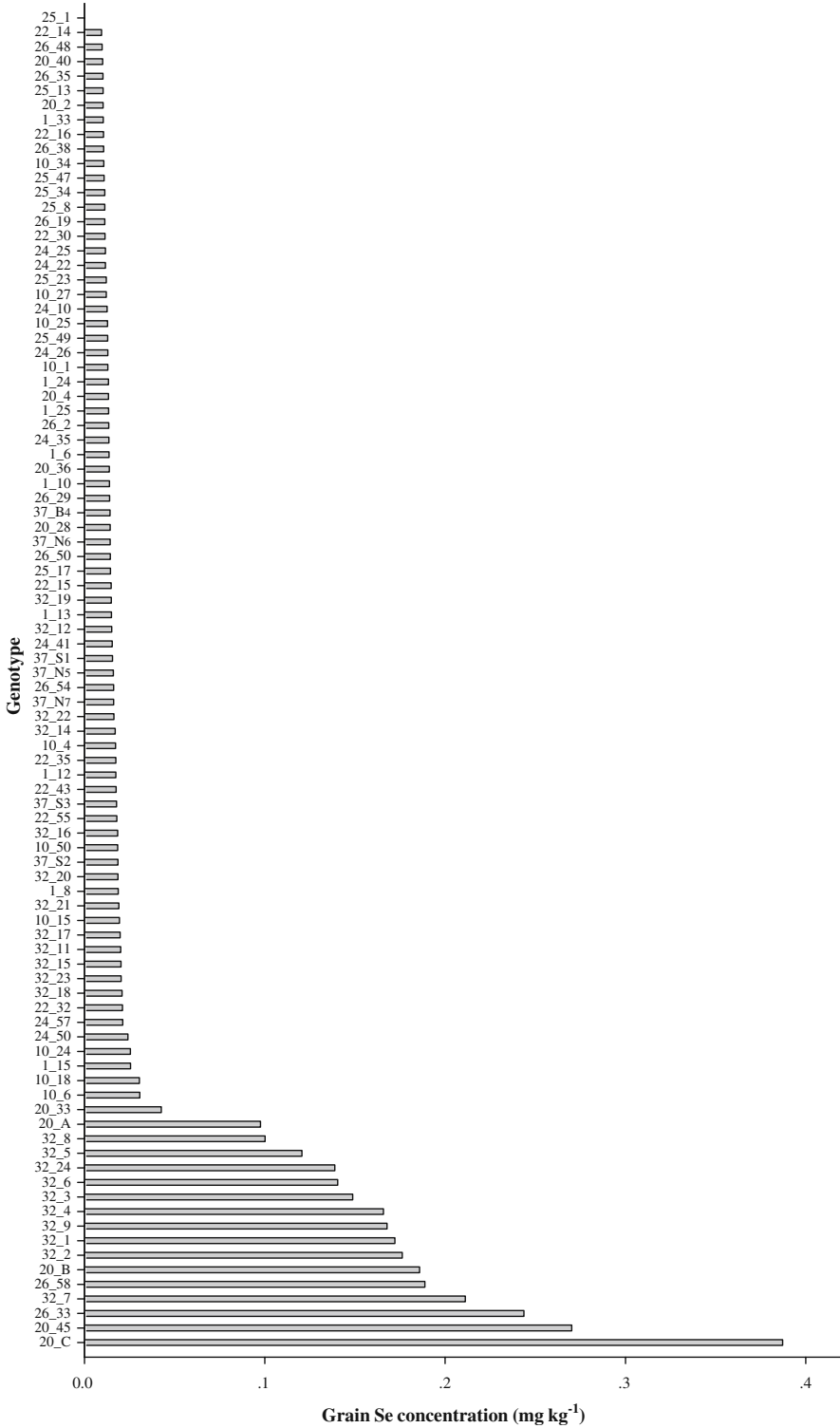
◄ **Fig. 2** GSeC of 92 *Hordeum spontaneum* genotypes

GSeC was 25_1, originating from the Atlit population. The mean value of GSeC in each population varied from 0.010 to 0.105 mg kg^{-1}. GSeC of the Sede Boqer population, which ranged from 0.010 to 0.387 mg kg^{-1} and averaged 0.105 mg kg^{-1}, ranked highest among the nine populations, while GSeC of the Atlit population, ranging from 0 to 0.014 mg kg^{-1} and averaging 0.010 mg kg^{-1}, ranked the lowest. One-way ANOVA indicated significant differences in GSeC among the nine *H. spontaneum* populations ($P \leq 0.001$). The population CV showed large differences among the nine populations, ranging from 12% for the "Evolution Canyon" population to 163% for the Caesarea population.

GSeC of Cultivated Barley as Compared with *H. spontaneum*

The GSeCs of the ten barley cultivars ranged from 0.000 to 0.144 mgkg^{-1} (Table 3). Baoshan 8640-1 from Yunnan Province, Ganpi 3 from Gansu Province, and Yancheng 01094 and Supi 4 from Jiangsu Province had the lowest value of 0.000 mg kg^{-1}, whereas Zhexiu 13 and Zhexiu 33 from Zhejiang Province had the highest values, 0.134 and 0.144 mg kg^{-1}, respectively. However, the highest GSeC in a cultivar, that of cv. Zhexiu 33, was far lower than the highest GSeC in a wild barley genotype, that of 20_C (0.387 mg kg^{-1}). A total of 11 *H. spontaneum* genotypes (three genotypes from Sede Boqer, six from Ein-Zukim, and two from Caesarea) had higher GSeCs than Zhexiu 33. The CV for the ten cultivars was 112%, which was also lower than the highest CV (163%)

Table 3 GSeC of Nine *Hordeum spontaneum* Populations and Ten *Hordeum vulgare* Cultivars

ID	Populations/variety	Samples	GSeC (mean±SD; mg kg^{-1})	Range (mg kg^{-1})	CV (%)
1	Mt. Hermon	9	0.015±0.004	0.010–0.026	31
10	Maalot	10	0.019±0.007	0.011–0.131	39
20	Sede Boqer	10	0.105±0.133	0.010–0.387	128
22	Mehola	8	0.015±0.004	0.010–0.021	27
24	Akhziv	8	0.015±0.005	0.012–0.024	29
25	Atlit	8	0.010±0.004	0.000–0.014	42
26	Caesarea	10	0.053±0.087	0.010–0.244	163
32	Ein-Zukim	22	0.080±0.072	0.015–0.211	90
37	Evolution Canyon	7	0.039±0.046	0.014–0.123	12
	Sum of Wild barley	92	0.045±0.070	0.000–0.387	12–163
Zj1	Yunpi 2	1×3	0.028±0.003		
Zj2	Yunpi 6	1×3	0.039±0.008		
Zj3	Zhexiu 12	1×3	0.134±0.012		
Zj4	E32380	1×3	0.073±0.013		
Zj5	Yangnongpi 5	1×3	0.077±0.005		
Zj6	Baoshan8640-1	1×3	0.000±0.000		
Zj7	Yancheng 01094	1×3	0.000±0.000		
Zj8	Zhexiu33	1×3	0.144±0.010		
Zj9	Ganpi 3	1×3	0.000±0.000		
Zj10	Supi 4	1×3	0.000±0.000		
	Sum of cultivars	10×3	0.050±0.056	0.000–0.144	112

in the Caesarea wild barley population. As expected, the cultivars exhibited a narrower GSeC range and less diversity than the wild barley.

Association of *H. spontaneum* GSeC with Ecogeographical Factors

Of the 14 correlations analyzed between GSeC and ecogeographical factors, 12 were statistically significant (Fig. 3). GSeC was negatively correlated with latitude, altitude, mean annual rainfall, mean number of rainy days, mean humidity at 1400 hours, and mean annual humidity and positively correlated with mean annual temperature, mean August temperature, mean January temperature, mean seasonal temperature difference, mean daily temperature difference, and mean annual evaporation, indicating that GSeC increased with temperature and evaporation and decreased with latitude, altitude, rainfall, and humidity, irrespective of longitude. Therefore, GSeC was mainly influenced by temperature and water conditions. One-way ANOVA showed that the mean GSeC of xeric genotypes was significantly greater than that of mesic genotypes ($P=0.0004$).

Moreover, nine *H. spontaneum* populations were derived from five soil types (Table 4), and one-way ANOVA indicated a significant difference in GSeCs among the *H. spontaneum* originating from those five soil types ($P=0.0003$). GSeC of *H. spontaneum* originated from loess was the highest, while that of *H. spontaneum* originated from alluvium was the lowest. Tukey–Kramer HSD test indicated that except for loess, the other four soil types—terra rosa, rendzina, alluvium, and sandy loam—gave similar GSeC values, and there was a significant difference in this parameter between loess and these other four soil types (Table 4).

Moreover, there were no significant correlations between GSeC and 1,000-grain weight for either wild *H. spontaneum* ($P=0.08$) or barley cultivars ($P=0.41$), indicating that higher GSeC is not necessarily related to small grain weight in barley.

Discussion

General Overview of Se in Food Plants

Se levels in food plants are generally low, with worldwide data on cereal and grain Se levels reporting <0.1 to 0.8 mg kg^{-1} [29]. However, cereals and their products make a major

Nonparametric: Spearman's ρ

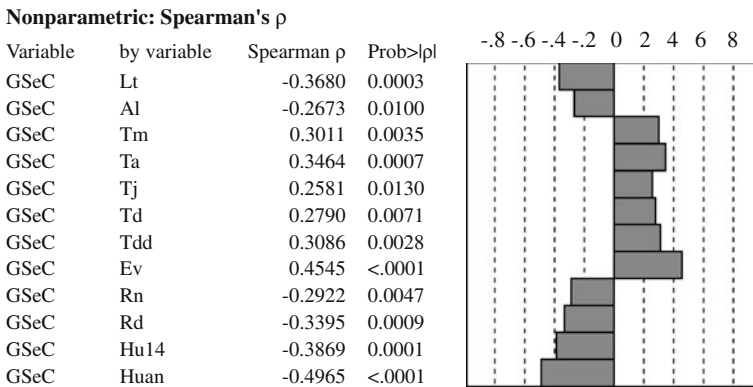| Variable	by variable	Spearman ρ	Prob>|ρ|
GSeC	Lt	-0.3680	0.0003
GSeC	Al	-0.2673	0.0100
GSeC	Tm	0.3011	0.0035
GSeC	Ta	0.3464	0.0007
GSeC	Tj	0.2581	0.0130
GSeC	Td	0.2790	0.0071
GSeC	Tdd	0.3086	0.0028
GSeC	Ev	0.4545	<.0001
GSeC	Rn	-0.2922	0.0047
GSeC	Rd	-0.3395	0.0009
GSeC	Hu14	-0.3869	0.0001
GSeC	Huan	-0.4965	<.0001

Fig. 3 Spearman's rho correlation between GSeC and ecogeographical data (Table 1) of the original sites of nine *Hordeum spontaneum* populations in Israel

Table 4 GSeC of *Hordeum spontaneum* Originated From Five Different Soil Types in the Collection Site in Israel

Soil type (in the collection site)	Samples	Population	Mean±SD (mg kg^{-1})	Range (mg kg^{-1})	CV (%)	Tukey–Kramer HSD test, $P<0.05$[a]
1, Terra Rosa	16	Mt. Hermon Evolution Canyon	0.016±0.004	0.010–0.026	23	b
2, Rendzina	10	Maalot	0.019±0.007	0.011–0.031	39	b
3, Alluvium	16	Mehola Akhziv	0.015±0.004	0.010–0.024	27	b
4, Sandy loam	18	Atlit Caesarea	0.034±0.067	0.000–0.244	196	b
6, Loess	32	Sede Boqer Ein-Zukim	0.088±0.094	0.010–0.387	107	a
ANOVA			$P\leq0.001$			

[a] Different letters stand for significant differences among soil types

contribution to the dietary intake of Se in the population worldwide, especially in developing countries such as China and India [11]. The World Health Organization and Food and Agriculture Organization normative requirement estimates of dietary Se are 0.04 and 0.03 mg day^{-1} for men and women, respectively [30]. The US, Canadian, and European recommended dietary allowance (RDA) for Se is 0.055 mg day^{-1} [31, 32]. In Australia and New Zealand, the RDA for adult men and women is 0.07 and 0.06 mg day^{-1}, respectively [33]. In the UK, the reference nutrient intake is set at 0.075 and 0.060 mg day^{-1} for adult males and females, respectively [34]. In many countries, rice and wheat are the most commonly consumed cereals. Nevertheless, Se levels in rice and wheat sold for domestic consumption are insufficient in some countries, for example, a mean Se level in rice of 0.05 mg kg^{-1} in Thailand and 0.02 mg kg^{-1} in China [2]. British wheat has low Se status [35], with Se levels in bread being approximately 0.039 to 0.048 mg kg^{-1} [2]. The average wheat GSeC in Scandinavian countries ranges from 0.007 to 0.018 mg kg^{-1} [36]. Aro et al. [37] reported mean wheat GSeCs of 0.001, 0.005, and 0.028 mg kg^{-1}, respectively, in three studied villages of Transbaikalian Russia. Most of the world's wheat falls within the ca. 0.002 to 0.060 mg kg^{-1} range [38]. Consequently, Se malnutrition afflicts quite a few people, especially those living in areas of very low Se availability in soils and extremely low Se concentrations in staple crops [4, 8]. On the other hand, the bioavailability of organic Se in plant and animal sources is higher than that of the inorganic selenate and selenite [31]. Moreover, Se in wheat grain is highly bioavailable compared to some fish and vegetables [32, 39, 40], and wheat is the most efficient Se accumulator of the common cereal crops [5]. Therefore, in recent years, researchers have focused on strategies to exploit genetic resources and develop genetically Se-enriched and protein-Se-enriched wheat using genomics tools [5, 6, 15, 31, 41–44].

Se in Barley

Surprisingly, reports on GSeC in barley have been scant in recent years. Barley is one of the principal cereal crops, the fourth largest in the world. It serves as a major animal feed crop and as human food it is used for malting, which in turn is used to make beer, whisky, and several other products such as bread, biscuits, and soups. In Chinese Tibet, barley was

grown mostly as a staple food for humans. Recently, however, interest in barley as a food grain is reviving due to heightened consumer awareness of good nutrition and increased interest in foods and food ingredients that are enriched in dietary fiber [45].

Barley is not only an economically important crop but it is also considered as a model species for genetic studies [46, 47]. Wild barley, as the progenitor of cultivated barley, is an important genetic resource that can be exploited in breeding programs for the introduction of resistance to a broad range of diseases and pests, tolerance to drought, salt, poor soils and climate factors, and key properties, e.g., malting content, kernel weight, size, color, and dormancy [22, 23, 48–52]. In the current study, a wide range and very high GSeCs were found in 92 genotypes of *H. spontaneum* from nine populations. GSeCs of 11 *H. spontaneum* genotypes were higher than that of the highest cultivar (Zhexiu 33) out of ten Chinese cultivars. The genotype of *H. spontaneum* with the highest GSeC (20_C) had 2.7 times the GSeC of cv. Zhexiu 33 (Table 3 and Fig. 2). Furthermore, in the current set of experiments, the grains were harvested from plants that had been grown in the same location, at the same time, under the same conditions. Therefore, the variation in GSeC revealed genetic differences resulting from the adaptation of *H. spontaneum* to different eco-environments. Our results demonstrate the huge potential of wild barley for improvement of GSeC. These high-GSeC *H. spontaneum* genotypes can be utilized as a set of donor parents for biofortification of barley cultivars and as optimized model plants for the improvement of cereal Se concentration via gene introgression, towards the development of Se-enriched cereals in the future.

The environment is a key contributor to the range of morphological variation found in various organisms [53]. Associations between the performance of wild barley populations and the ecogeographical conditions of their collection sites can shed light on the major driving forces contributing to the ecological fitness of these populations and, more specifically, to the development of their Se uptake and accumulation mechanisms. In the current study, Spearman correlation analysis showed that high temperature and evaporation and low latitude, altitude, rainfall, and humidity at the site in which *H. spontaneum* originated are favorable to wild barley having gained a strong ability to take up Se from the soil and to accumulate Se in its grains during evolution. This could explain why the Sede Boqer and Ein-Zukim populations, which originated in sites with the lowest latitude, altitude, and rainfall and highest temperature and evaporation, had the highest GSeCs, while the Mt. Hermon population, originating in the site with the lowest temperature and highest latitude, altitude, and rainfall, was ranked second lowest in GSeC of the nine populations. Soil type at the *H. spontaneum* collection site was also an important factor affecting its ability to take up Se and to accumulate Se in its grains. The report drew particular attention to the wide range of Se concentrations in different types of foods and revealed that the variations are due to differences in Se availability in the soil on which an animal is raised or a plant grown [2]. In the present study, nine *H. spontaneum* populations were derived from five soil types. GSeC of *H. spontaneum* originating from loess soil was highest and significantly higher than the GSeC in *H. spontaneum* originating from alluvium (lowest), terra rosa, rendzina, and sandy loam (Table 4). Our results suggest that loess has low Se availability and that the wild barley growing there has developed strong Se uptake and accumulation abilities. Se occurs in well-drained alkaline soils chiefly as selenates, which are highly available for absorption by plant roots. In contrast, in acidic, poorly drained soils, it is present mainly as selenides, which are not available to plants, and sometimes as elemental Se [54]. In reality, the loess soil in both Sede Boqer and Ein-Zukim was not true loess, but a desert soil which was very poorly drained. However, to support this hypothesis, additional studies are required to determine soil Se content and availability in the original sites from which the *H. spontaneum* populations were collected.

All of the results of this study clearly demonstrate that the evolutionary background of the environments has a strong effect on GSeC of wild barley. *H. spontaneum* is hermaphroditic and self-pollinating [55], with a fragile rachis that breaks apart after maturation [56], such that the spikelets become entangled in the remains of their dry mother plants, contributing to the development of local genotypes. The great biodiversity found among these genotypes is the result of adaptations to local environmental factors in each area and microhabitat. These factors include the amount of rain, temperature, type and depth of soil, slope direction, shade, presence of stones, rocks, salinity, and possibly also other factors [57]. A particular food may show an over tenfold difference in Se content, depending on where it was produced. Se levels in foods can vary enormously, not only between countries but also between regions within a country [2]. Stochastic environmental fluctuations create a mosaic of habitats in which variation takes place in a temporal rather than spatial dimension [58]. Therefore, the genetic population structure of barley is a mosaic. In the present study, though the Caesarea population originated in a mesic habitat, it ranked third out of nine populations in GSeC. The Mehola population, originating in a xeric habitat, ranked second lowest in GSeC. Our results indicate that large differences exist between and within populations. The CV of each of the nine populations ranged from 12% to 163%. In the Sede Boqer population, the CV was large (128%), and the GSeC of the highest genotype 20_C was 38.7 times higher than that of genotype 20_40. Thus, the genetic mosaic appears to reflect the underlying ecological heterogeneity, which derives from local and regional geological, edaphic, climatic, and biotic differentiations [59]. The genetic mosaicism of wild barley GSeCs is the basis of barley GSeC improvement. The high-GSeC genotypes selected in the present study will be used to breed for barley cultivars with high GSeC. Cultivated barley contains, on average, 40% *H. spontaneum* alleles [60]. Because *H. spontaneum* and cultivated barley are inter-fertile, *H. spontaneum* can be used to increase the genetic diversity of cultivated barley by crosses [61]. In the past, breeders were reluctant to use exotic germplasm in their breeding programs due to linkage drag of negative traits. However, modern genomic technologies have led to the development of marker-assisted selection (MAS) approaches that enable efficient transfer of only small chromosome segments carrying the target gene and therefore avoiding linkage drag of negative traits. For example, the stripe rust resistance gene *Yr15*, derived from wild emmer wheat, is being utilized in MAS wheat breeding programs in the USA (http://maswheat.ucdavis.edu/Index.htm). A few years ago, the high grain protein and mineral gene, *Gpc-B1* (*TtNAM-B1*), was cloned from wild emmer wheat [14]. The absence of the functional *TtNAM-B1* wild emmer allele in modern germplasm suggests a broad potential impact of this gene in breeding of cultivated wheat varieties [14]. Moreover, a homologous barley gene, designated *HvNAM-1* was shown to be responsible for the grain protein QTL located on barley chromosome 6H [62]. Therefore, the results presented in the current and previous studies demonstrate the potential of wild cereal populations as a rich source for grain protein and mineral content genes for crop improvement.

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