Evaluation of wild barley introgression lines for agronomic traits related to nitrogen fertilization

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Abstract In the coming decades, climate change and resources constraints will make profitable and economically reliable agriculture more and more challenging. To evaluate the potential of exotic alleles to maintain performance under low nitrogen input, we investigated a set of 41 introgression lines (S42ILs) originating from the hybridization of the German spring barley ‘Scarlett’ and the Israeli wild barley ‘ISR42-8’. These lines were assessed in field trials for yield, yield components, grain protein content and chlorophyll content during growing seasons 2015 and 2016 in two different test sites in Germany under low and high nitrogen supply levels, N_0 and N_1 . Our analyses revealed 17 regions for putative quantitative trait loci (QTL), linked to one or multiple traits, across all chromosomes. In particular, lines S42IL_119 and S42IL_121 exhibited an enhanced thousand grain weight of 7% and 9% under N_1 and N_0 , respectively. In addition, six QTL were found for grain number per ear

leading to a decline of grain number of up to 20%. Furthermore, three new QTL for chlorophyll content could be identified on chromosomes 1H and 2H. The present study revealed QTL effects of wild barley introgressions in a spring barley elite background, especially under low nitrogen. The selection for nitrogen efficient lines with beneficial exotic alleles represents the first step towards the development of spring barley cultivars genetically adapted to nitrogen limitations.

Keywords Quantitative trait locus (QTL) · Barley · *Hordeum vulgare* ssp. *spontaneum* · Introgression lines · Nitrogen stress

Introduction

A major challenge of agriculture is the economic, ecological and sustainable production of crops irrespective of the existing conditions (Christen 2000). Since all crop characteristics result from the combination of environmental and genetic factors they both have to be taken into consideration to improve crop yield. Environmentally, plant growth and yield formation are influenced by various determinants, such as drought, nitrogen or salt stress. These extreme conditions can cause prematurity and devastating yield losses. Drought stress, threatening plants especially in

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regions with intense heat and high salinity, can be combated by strategies such as irrigation and rhizosphere colonizing microorganisms (Yang et al. 2009). The common method to counteract limited nitrogen supply is nitrogen (N) fertilization. Since crops obtain nutrients from water-soluble compounds in the ground, such as nitrate, the amount of required fertilizer also depends on the availability of mineralized N in the soil (Stark and Brown 1987), application time and N use efficiency of the genotype (Delogu et al. 1998). The consecutive application of large amounts of nitrogen not only increases costs for farmers but also the risk of ground water pollution. To reduce nitrogen burdens, the German Fertilizer Ordinance (Düngeverordnung, DüV) was tightened several times lowering the permitted average N balance over 3 years. Thus, there is need for an efficient nitrogen management strategy. Therefore, using more N efficient cultivars could lead to more stable productivity under limiting conditions and, hence, these cultivars can play a major role in crop rotations.

Barley is one of the oldest cultivated crops in the world with a high adaptive capacity compared to other major cereals (Rawson et al. 1988; Delogu et al. 1998; Garthwaite et al. 2005). Nevertheless, modern elite barley cultivars are marked by restricted genetic biodiversity due to high selection pressure (Russell et al. 1997; Ellis et al. 2000). To overcome these genetic constraints, wild alleles of barley relatives that are adapted to harsh conditions have been introduced (Brown et al. 1988; Dubcovsky et al. 1996; Matus et al. 2004; Hori et al. 2005; Garthwaite et al. 2005; Schnaithmann and Pillen 2013) resulting in positive effects for diverse agronomic traits (Mano and Takeda 1997; Talamé et al. 2004; Xue et al. 2009; Saade et al. 2016; Merchuk-Ovnat et al. 2018; Pham et al. 2019; Wiegmann et al. 2019). In particular, Schmalenbach et al. (2008) reported on an increase in thousand grain weight and ear number when exotic alleles were introgressed from wild barley (*Hordeum vulgare* ssp. *spontaneum*, *Hsp*) into spring barley (*Hordeum vulgare* ssp. *vulgare*). Therefore, the utilization of existing wild barley collections may help to breed new cultivars with enhanced agronomic performance in marginal sites and/or under low-input N fertilization strategies.

Molecular biology techniques facilitate the targeted introgression of wild barley alleles. In this regard the wild barley introgression library S42IL was

constructed. After an initial cross of the German spring barley cultivar ‘Scarlett’ and the Israeli wild barley accession ‘ISR42-8’, the amount of exotic genome was diminished by repeated marker assisted backcrossing resulting in lines with either a single small introgression or multiple introgressions (Von Korff et al. 2004). After selecting a set of lines representing the whole exotic genome in the background of the elite parent the agronomic performance of this set can be evaluated by phenotypic assessment and QTL analyses (Hayes et al. 1993; Toojinda et al. 1998). This way, the genetic basis of trait variation can be revealed. Until now, exotic introgressions in S42IL and its precursor population S42 could be associated with a broad spectrum of traits like threshability (Schmalenbach et al. 2011), root and shoot related parameters (Hoffmann et al. 2012; Naz et al. 2012), grain parameters and yield related traits (Schnaithmann and Pillen 2013; Honsdorf et al. 2017; Arifuz-zaman et al. 2014), nutrient accumulation (Reuscher et al. 2016; Soleimani et al. 2017) as well as drought stress tolerance traits (Honsdorf et al. 2014a, b) in greenhouse trials. Furthermore, field trials showed associations of exotic introgressions with yield and yield-related parameters (Von Korff et al. 2006; Schmalenbach et al. 2009; Saal et al. 2011), powdery mildew and leaf rust resistance (Schmalenbach et al. 2008), malting quality (Schmalenbach and Pillen 2009) and growth phenology (Wang et al. 2010). So far, little is known about the influence of exotic barley alleles on nitrogen utilization under contrasting N fertilization levels in the field.

The first step in breeding for higher yield and yield stability is to analyze physiological determinants of yield elements (Araus et al. 2002). However, assessing the impact of different amounts of nitrogen fertilizer on yield is difficult due to diverse compensatory effects. Among others, late plant organs, such as grains, reveal efficient nutrition utilization in the field. Abeledo et al. (2003) showed that in barley cultivars grain yield is associated with thousand grain weight, grain number m⁻² and biomass. Thus, subdividing major traits, such as yield, into secondary parameters and the evaluation of their performance under different N fertilization management conditions can assist breeders to select genotypes that are modified for nitrogen adaption.

In this study we examined the effect of two different nitrogen strategies on 41 introgression lines

(ILs) of S42IL during 2015 and 2016 at two different test sites in Germany (Merbitz and Morgenrot). For this purpose, yield and yield components as well as grain protein content and chlorophyll content were evaluated and associated with exotic introgressions potentially harboring beneficial alleles for future barley breeding.

Materials and methods

Plant material

For the present study the S42IL library was used, established by introgressive hybridization of wild type alleles from ISR42-8 into the gene pool of spring barley cultivar ‘Scarlett’ (Von Korff et al. 2004). Pre-ILs (BC2) were backcrossed again (BC3) with the recurrent parent ‘Scarlett’ followed by repeated rounds of two to four selfings, as described in Schmalenbach et al. (2011), resulting in a population of 83 S42ILs (Honsdorf et al. 2017). Each S42IL carries one (occasionally multiple) small wild barley genetic introgression, which usually overlaps with the neighboring S42IL. In total, the 83 S42IL library covers 94.5% of the ‘ISR42-8’ genome (Honsdorf et al. 2017). Thereof, a set of 41 S42ILs, covering 75.3% of the exotic genome, was selected for field experiments.

Experimental sites

The field experiments were conducted for 2 years (2015 and 2016) in ‘Morgenrot’ (51° 47′ 19.2″ N 11° 12′ 14.5″ E) and ‘Merbitz’ (51° 36′ 38.6″ N 11° 53′ 27.8″ E), Germany, giving rise to a total of four environments. The soil types in ‘Morgenrot’ and ‘Merbitz’ were loam (pH: 7.0) and sandy loam (pH: 6.9), respectively. Both locations represent very dry areas of Germany with an average temperature of 8.8 °C and a precipitation of 550 mm per year in Morgenrot and 9.5 °C and 450 mm in Merbitz.

Experimental setup

A randomized split-plot design with 41 S42ILs and the population reference parent ‘Scarlett’ was applied at both experimental sites (see Online Table S1). All genotypes were cultivated with (N_1) or without (N_0)

nitrogen fertilization in three replications. For the N_1 treatment, calcium ammonium nitrate was applied at BBCH 31 (Lancashire et al. 1991). Prior to sowing, available soil mineral N content was measured. Based on local agriculture practices, site-specific fertilization with calcium ammonium nitrate aiming for a total difference of 40 kg N ha⁻¹ between N_0 and N_1 levels was applied at both test sites. At both locations the plot size was 4.0 m × 1.2 m with 252 plots. The trials were sown in spring with a seed density of 300 grains m⁻². Crop management treatments (i.e. growth regulators, herbicides, insecticides, fungicides) were applied according to site-specific recommendations.

Phenotypic evaluation

In total, nine yield-related and nitrogen-related traits were evaluated. The S42IL plots were phenotyped as described in Table 1.

Statistical analysis

Analysis of variance (ANOVA) was conducted for each trait by fitting a linear mixed model in SAS software version 9.2 (procedure MIXED; SAS Institute 2008) to check for significant genotype and treatment effects. The following model was applied:

$$Y_{ijkl} = \mu + G_i + T_j + E_k + B_l + G_i \times T_j + B_l \times T_j + \varepsilon_{ijkl}$$

where μ is the general mean of trait Y, G_i is the fixed effect for each of the $i = 42$ genotypes (41 S42ILs plus ‘Scarlett’), T_j is the fixed effect for each of the $j = 2$ N levels, E_k is the fixed effect for each of the $k = 4$ environments, B_l is the random block effect, $G_i \times T_j$ is the fixed interaction effect between i th genotype and j th treatment, $B_l \times T_j$ is the random interaction effect between l th block and j th N level and ε_{ijkl} is the random error effect of Y.

Least squares means (LSMeans) were calculated for factors G and $G \times T$.

The SAS procedure CORR was applied to calculate Pearson’s correlation coefficients between all traits based on LSMeans across replications and years for each nitrogen treatment. In addition, the autocorrelation of a trait between N_0 and N_1 was calculated.

The SAS procedure VARCOMP was applied to calculate variance components. The broad-sense

Table 1 Assessed quantitative traits

Parameter	Abbreviation	Unit	Instrument	Measurement
Ears per m ²	EAR	Number	Counting	Based on the average number of ears counted in a 0.5 m row per plot
Grains per ear	GEA	Number	MARVIN ^a	Average number of grains per ear based on 10 ears
Thousand grain weight	TGW	g	MARVIN ^a	Average weight of 1000 grains extrapolated from a sample of 10 ears
Grain length	GRL	mm	MARVIN ^a	Average length of grain from 10 ears
Grain width	GRW	mm	MARVIN ^a	Average width of grain from 10 ears
Grain area	GRA	mm ²	MARVIN ^a	Calculated average surface based on grain length and grain width
Grain yield ^b	YLD	dt/ha	Harvester	Grain weight calculated from harvesting the whole plot ($\approx 5 \text{ m}^2$)
Grain protein content ^c	GPC	% of dry matter	Foss Infratec Grain Analyser	Measured average value of 300 g grain per plot
Chlorophyll content ^d	SPAD	SPAD index	SPAD-502Plus	Measured average of 10 flag leaves at BBCH 61 (Lancashire et al. 1991)

^aMARVIN seed analyzer (GTA Sensorik GmbH, Neubrandenburg, Germany)

^bHarvest data in Merbitz 2015 absent due to extensive lodging

^cOnly in Morgenrot

^dOnly in Merbitz

heritability for each trait was estimated across all environments according to Holland et al. (2003):

$$H^2 = 100 \times \frac{\sigma_G^2}{\sigma_G^2 + (\sigma_{GE}^2/e) + (\sigma_{GT}^2/t) + (\sigma_{GET}^2/et) + (\sigma_\varepsilon^2/etr)}$$

where σ_G^2 is the variance component of the genotype (G), σ_{GE}^2 is the variance component of genotype $\times$ environment, σ_{GT}^2 is the variance component of genotype $\times$ nitrogen level, σ_{GET}^2 is the variance component of genotype $\times$ environment $\times$ nitrogen level and σ_ε^2 is the experimental error variance component with e, t and r being the number of environments, treatments and replications, respectively.

QTL detection

To test the significance of genotypic differences between individual S42ILs and the recurrent parent ‘Scarlett’ a post hoc Dunnett test was performed (Dunnett 1955). The presence of a QTL was accepted, if a S42IL revealed a significant LSMeans difference from ‘Scarlett’ with $p < 0.05$ within a single treatment or across both treatments. If significant lines were carrying overlapping or flanking introgressions showing similar effects in the same direction (based on the

genetic map of Honsdorf et al. 2017), a single QTL was assumed. The relative performance (RP) of a S42IL, describing the deviation from ‘Scarlett’ in %, was calculated by the following equation:

$$RP(IL) = 100 \times \frac{LSMeans(S42IL) - LSMeans('Scarlett')}{LSMeans('Scarlett')}$$

Results

Descriptive statistics

In Table 2 all measured trait statistics are listed comparing the S42IL population and ‘Scarlett’. As the genetic background of S42ILs originates from ‘Scarlett’, the majority of the 41 S42ILs exhibited similar phenotypes. Only a few genotypes varied strongly in their trait expression compared to ‘Scarlett’. Coefficients of variation were highest for EAR and YLD. Remarkably, only for EAR, YLD and GPC strikingly higher means were found under N₁. Broad-sense heritabilities were high with $H^2 > 0.75$ for all traits except GPC ($H^2 = 0.22$, Table 3).

Table 2 Descriptive statistics

Trait	Genotype	N ₀					N ₁				
		N	Mean	Min	Max	CV	N	Mean	Min	Max	CV
EAR	S42ILS	473	779.16	400.00	1360.00	19.60	488	831.97	368.00	1712.00	21.86
	Scarlett	12	844.00	576.00	1136.00	18.56	12	830.67	416.00	1296.00	30.83
GEA	S42ILS	473	22.74	10.60	28.20	9.49	488	22.92	7.50	29.00	11.12
	Scarlett	12	23.45	20.60	25.70	7.11	12	23.48	19.50	25.90	8.45
TGW	S42ILS	473	47.90	34.84	56.73	7.43	488	47.43	36.56	56.12	7.42
	Scarlett	12	48.14	42.55	53.06	5.83	12	47.31	42.09	54.33	8.56
GRL	S42ILS	473	8.15	5.63	10.88	9.04	488	8.23	6.33	10.56	8.06
	Scarlett	12	7.87	6.09	8.70	12.15	12	8.18	7.00	8.70	7.89
GRW	S42ILS	473	3.83	3.38	4.20	4.38	488	3.81	3.24	4.20	4.64
	Scarlett	12	3.89	3.64	4.10	4.00	12	3.81	3.52	4.00	4.10
GRA	S42ILS	473	21.36	14.91	27.87	10.65	488	21.44	16.03	26.82	9.85
	Scarlett	12	20.99	16.37	23.84	12.51	12	21.35	17.61	23.59	10.19
YLD	S42ILS	350	59.95	29.67	77.02	16.24	365	62.96	38.24	81.62	16.60
	Scarlett	9	60.97	40.45	71.83	20.20	9	63.51	49.90	78.62	15.40
GPC	S42ILS	228	10.38	8.80	12.90	7.95	242	11.40	9.80	13.38	5.41
	Scarlett	6	10.19	9.10	11.22	8.81	6	11.09	10.60	11.80	3.71
SPAD	S42ILS	246	46.52	32.90	56.90	10.71	246	46.60	31.70	57.00	11.24
	Scarlett	6	47.37	37.80	53.00	11.40	6	46.38	38.30	56.00	12.75

N, Min, Max and CV correspond to the number of observations, minimum, maximum and coefficient of variance [%], respectively

Table 3 Heritabilities

Trait	σ_G^2	σ_E^2	σ_T^2	σ_{GT}^2	σ_{GE}^2	σ_{GET}^2	σ_e^2	H ² (%)
EAR	2320.88	2631.97	1260.28	0.00	0.00	1640.39	22813.46	66.76
GEA	1.23	1.62	0.00	0.00	0.86	0.00	2.23	79.97
TGW	1.56	7.29	0.02	0.10	0.48	0.00	4.90	80.66
GRL	0.04	0.43	0.00	0.00	0.00	0.00	0.13	86.42
GRW	0.00	0.03	0.00	0.00	0.00	0.00	0.01	78.03
GRA	0.17	5.07	0.00	0.01	0.04	0.00	0.79	78.20
YLD	6.44	108.94	5.94	0.00	5.63	0.00	16.71	75.37
GPC	0.01	0.00	0.39	0.00	0.02	0.00	0.39	22.05
SPAD	1.60	31.13	0.06	0.15	0.00	0.28	8.04	78.21

σ_G^2 , σ_E^2 , σ_T^2 , σ_{GT}^2 , σ_{GE}^2 , σ_{GET}^2 and σ_e^2 correspond to the genotype, environment, treatment, genotype $\times$ treatment, genotype $\times$ environment, genotype $\times$ environment $\times$ treatment, and error variance component, respectively

ANOVA

The nitrogen level had a significant ($p < 0.05$) effect on EAR, GRW, YLD and GPC (Online Table S2). Under nitrogen limitation (N₀) EAR, YLD and GPC were significantly reduced compared to N₁ whereas

GRW was significantly increased under N₀ (Table 2). For all traits significant genotype main effects were observed. Significant Genotype $\times$ Treatment effects only occurred for GRW.

Trait correlations

Correlations between traits, separately for N_0 and N_1 , are displayed in Online Table S3. In the following, the correlation coefficient under N_0 is described first, followed by N_1 data. Under both nitrogen levels YLD was positively correlated with EAR ($r = 0.47$ and 0.19), but showed a negative correlation with GPC ($r = -0.35$ and -0.29). Under N_1 the correlations between grain components were generally a bit stronger than under N_0 . TGW revealed moderate correlations with GRL ($r = 0.30$ and 0.29), GRW ($r = 0.53$ and 0.67) and GRA ($r = 0.72$ for both). Interestingly, we observed a higher correlation of GRA with GRL ($r = 0.81$ and 0.82) than with GRW ($r = 0.23$ for both). Low positive correlations were detected for SPAD with TGW ($r = 0.31$ and 0.25) and GRA ($r = 0.34$ and 0.11).

QTL detection

To detect S42ILs significantly differing from ‘Scarlett’ we carried out a mixed model ANOVA followed by a post hoc Dunnett test. Taking all traits together, 21 lines were significantly deviating from ‘Scarlett’, leading to a total number of 45 putative QTL (Table 4, Online Table S4). Some S42ILs showed significant effects for several traits simultaneously (Table 5). Hereafter, each trait will be described in detail.

Grain yield (YLD)

Nine QTL could be found for this trait across all chromosomes. In general, all S42ILs showed 7–13% lower yields across both fertilization conditions.

Remarkably, most significant differences were found across both nitrogen levels or under N_1 . The *Hsp* alleles present in S42IL_148 led to the highest yield losses of 13% under N_1 level.

Additionally, five noticeable lines (S42IL_109, S42IL_122, S42IL_123, S42IL_135 and S42IL_136) showed an interesting tendency to increase yield across both nitrogen levels of 3–4 dt/ha. Under N_1 treatment, S42IL_122 even had an increased yield by 6 dt/ha.

Thousand grain weight (TGW)

Five QTL were detected on chromosomes 3H, 4H, 5H and 6H. While S42IL_119 and S42IL_121 with main introgressions on 4H showed an increase in TGW by 6–9%, most other S42ILs showed a decrease in TGW compared to the malting barley variety ‘Scarlett’. Except for S42IL_176, the reducing effect was stronger under nitrogen level N_0 .

Grain surface area (GRA), grain length (GRL) and grain width (GRW)

A number of QTL were found for grain shape with five for GRA, seven for GRL and eight for GRW. For GRA, the *Hsp* alleles at all QTL provoked an increase and the strongest effect was observed in line S42IL_121, where trait performance was increased by 10.5% under N_0 treatment.

All *Hsp* alleles at QTL regions for GRL differed positively from ‘Scarlett’, while all QTL regions for GRW showed a decrease. S42ILs 102, 121 and 143 showed strong increases in GRL across and within both treatments with values of up to +15.2% (S42IL_143). In the remaining lines the *Hsp* alleles resulted in increasing values across both treatments and within N_0 compared to ‘Scarlett’ varying from 4 to 9%. The differences between ‘Scarlett’ and the significant lines for GRL were most pronounced under N_0 conditions.

Ear number (EAR)

We observed significant effects for S42IL_119, which carries introgressions on chromosomes 3H and 4H leading to a significant decrease of ears per square meter.

Grains per ear (GEA)

In total, six QTL were identified with *Hsp* alleles decreasing GEA significantly. The strongest effect compared to ‘Scarlett’ was found at QGea.S42.2H.a (S42IL_107 and S42IL_109) and QGea.S42.2H.b (S42IL_110) for N_1 with 17% less grains per ear.

Table 4 List of selected QTL for yield components across and within N levels

Trait	QTL	Line	Pos. of main introgression (chromosome, in cM) ^a	N level	LSMean	Dev. ^b	RP % ^c	Candidate gene			
YLD (dt/ha)											
QYld.S42.1H	QYld.S42.1H	S42IL_143	1H 82.6–112.3	N ₁	56.9	– 6.6	– 10.4	Ppd-H1 ^{*a} sdw1/HvGA20ox ₂ ^{*d} [sdw1/HvGA20ox ₂ ^{*d}] Vrn-H1 ^{*c} ; [HvELF3 ^{*f}] [sdw1/HvGA20ox ₂ ^{*d}] HvCO1 ^{*g}			
				Across	56.4	– 5.8	– 9.3				
				Across	57.1	– 5.1	– 8.2				
	QYld.S42.2H	S42IL_107	2H 12.5–41.2	Across	57.1	– 5.1	– 8.2				
				QYld.S42.3H.b	S42IL_140	3H 86.2–148.2	N ₀		54.6	– 6.4	– 10.5
							Across		56.6	– 5.7	– 9.2
	QYld.S42.4H	S42IL_121	4H 51.9–81.2				N ₀		53.9	– 7.1	– 11.6
				N ₁	56.4	– 7.1	– 11.2				
				Across	55.1	– 7.1	– 11.4				
	QYld.S42.5H	S42IL_176	5H 81.3–140.1	N ₁	57.1	– 6.4	– 10.1				
				Across	56.7	– 5.5	– 8.9				
				QYld.S42.6H.a	S42IL_148	6H 0.3–11.3	N ₁		55.2	– 8.3	– 13.1
Across	55.1	– 7.1	– 11.4								
QYld.S42.7H	S42IL_134	7H 37.6–68.4	N ₀				54.5	– 6.5	– 10.7		
			N ₁	56.8	– 6.7	– 10.6					
			Across	55.6	– 6.6	– 10.6					
TGW (g)											
QTgw.S42.3H	QTgw.S42.3H	S42IL_111	3H 43.1–55.2	Across	49.7	1.9	4.2	[sdw1/HvGA20ox ₂ ^{*d}] Vrn-H1 ^{*c} ; [HvELF3 ^{*f}]			
				QTgw.S42.4H.a	S42IL_119	4H 35.9–81.2	N ₀		51.0	2.9	6.0
							N ₁		50.8	3.5	7.4
	Across	50.9	3.2				6.7				
	QTgw.S42.4H.a	S42IL_121	4H 51.9–81.2	N ₀	52.7	4.5	9.4				
				Across	51.0	3.2	6.7				
				QTgw.S42.5H	S42IL_176	5H 81.3–140.1	N ₀		45.2	– 2.9	– 6.0
	N ₁	44.1	– 3.2				– 6.8				
	Across	44.7	– 3.0				– 6.3				
	GRA (mm ²)										
	QGr.S42.4H	S42IL_121	4H 51.9–81.2	N ₀	23.2	2.2	10.5		[sdw1/HvGA20ox ₂ ^{*d}]		
				Across	22.7	1.6	7.5				
GRL (mm)											
QGrL.S42.1H.a	QGrL.S42.1H.a	S42IL_102	1H 0.2–62.3	N ₀	8.6	0.8	8.9	[sdw1/HvGA20ox ₂ ^{*d}]			
				N ₁	8.8	0.6	7.3				
				Across	8.7	0.7	8.7				
	QGrL.S42.1H.b	S42IL_143	1H 82.6–112.3	N ₀	9.1	1.2	15.2				
				N ₁	8.9	0.7	8.5				
				Across	9.0	1.0	12.5				
	QGrL.S42.4H.a	S42IL_119	4H 35.9–81.2	N ₀	8.4	0.5	6.3				
				Across	8.4	0.4	5.0				
				QGrL.S42.4H.a	S42IL_121	4H 51.9–81.2	N ₀		8.7	0.8	10.1
	Across	8.6	0.6				7.5				

Table 4 continued

Trait	QTL	Line	Pos. of main introgression (chromosome, in cM) ^a	N level	LSMean	Dev. ^b	RP % ^c	Candidate gene
GRW (mm)								
	QGrw.S42.1H.a	S42IL_102	1H 0.2–62.3	N ₀	3.7	– 0.2	– 4.1	Vrn-H2 * ^h Vrn-H1 * ^e ; [HvELF3 * ^f]
				Across	3.7	– 0.1	– 6.5	
	QGrw.S42.1H.b	S42IL_143	1H 82.6–112.3	N ₀	3.8	– 0.1	– 3.1	
				Across	3.7	– 0.1	– 6.5	
	QGrw.S42.4H	S42IL_124	4H 110.2–115.2	N ₀	3.8	– 0.1	– 3.2	
				Across	3.7	– 0.1	– 6.5	
	QGrw.S42.5H	S42IL_176	5H 81.3–140.1	N ₀	3.8	– 0.1	– 3.4	
				N ₁	3.7	– 0.1	– 2.8	
				Across	3.7	– 0.1	– 6.5	
				EAR				
QEar.S42.4H	S42IL_119	4H 35.9–81.2	Across	708.7	– 128.7	– 15.4		
GEA								
	QGea.S42.2H.a	S42IL_107	2H 12.5–41.2	N ₀	19.7	– 3.8	– 16.2	Ppd-H1 * ^a
				N ₁	19.5	– 4.0	– 17.0	
				Across	19.6	– 3.9	– 16.6	
	QGea.S42.2H.a	S42IL_109	2H 33.9–62.7	N ₀	19.9	– 3.5	– 15.0	HvFT4 * ^b ; HvCEN * ^c
				N ₁	19.5	– 4.0	– 17.0	
				Across	19.7	– 3.8	– 16.2	
	QGea.S42.2H.b	S42IL_110	2H 89.5–97.8	N ₀	20.5	– 3.0	– 12.8	[HvCEN * ^c]
				N ₁	19.5	– 4.0	– 17.0	
				Across	20.0	– 3.5	– 14.9	
SPAD								
QSpad.S42.1H	S42IL_142	1H 122.1–132.7	N ₀	41.2	– 5.3	– 11.3		
QSpad.S42.2H.a	S42IL_107	2H 12.5–41.2	Across	50.1	3.9	8.4	Ppd-H1 * ^a	
QSpad.S42.2H.b	S42IL_110	2H 89.5–97.8	N ₁	53.5	7.5	16.3	HvCEN * ^c	
			Across	50.3	4.1	8.9		
QSpad.S42.5H	S42IL_176	5H 81.3–140.1	Across	42.4	– 3.8	– 8.2	Vrn-H1 * ^e ; [HvELF3 * ^f]	

^aHonsdorf et al. (2017)^bDeviation from Scarlett LSMeans^cRelative performance = deviation from Scarlett LSMeans in %*^aTurner et al. (2005)*^bFaure et al. (2007)*^cComadran et al. (2012)*^dJia et al. (2015)*^eYan et al. (2003)*^fFaure et al. (2012), Zakhrebekova et al. (2012)*^gGriffiths et al. (2003)*^hYan et al. (2004)

[]Candidate genes on sub-introgression

Table 5 Outstanding lines with several QTL within N₀ and N₁, as well as across N₀ and N₁ treatments

Line	QTL number within N ₀	QTL number within N ₁	QTL number across N ₀ and N ₁
S42IL_107	2	3	4
S42IL_121	5	2	5
S42IL_134	3	3	4
S42IL_137	4	2	4
S42IL_143	4	2	4

Chlorophyll content (SPAD)

Four SPAD QTL, located at chromosomes 1H, 2H and 5H, were found. Two of them, associated with S42IL_107 and S42IL_110, led to higher SPAD values of up to 16%. By contrast, S42IL_142 and S42IL_176 showed chlorophyll reduction compared to ‘Scarlett’ across and within treatments.

Discussion

In the present study we evaluated 41 introgression lines (S42ILs) that arose from interbreeding the German elite spring barley cultivar ‘Scarlett’ with the Israeli wild barley accession ‘ISR42-8’. Each S42IL carries a specific exotic chromosome segment bearing the potential for several trait improvements important for further barley breeding. Thus far, a number of studies on S42IL performance under greenhouse and field conditions have been carried out (Schmalenbach et al. 2008, 2009; Wang et al. 2010; Saal et al. 2011; Schmalenbach et al. 2011; Hoffmann et al. 2012; Schnaithmann and Pillen 2013; Honsdorf et al. 2014a, b; Naz et al. 2014; Reuscher et al. 2016; Honsdorf et al. 2017; Soleimani et al. 2017). However, only two of these focused on nitrogen stress, conducted in a hydroponic system (Hoffmann et al. 2012) and in greenhouse. The latter revealed QTL for chlorophyll content, grain parameters and nitrogen/carbon content (Schnaithmann and Pillen 2013). The only field-based nitrogen stress trial was conducted with S42, the precursor population of S42IL (Saal et al. 2011). Therefore, we conducted field experiments with S42IL at two test sites, Merbitz and Morgenrot, in Germany with two different nitrogen treatments each to assess total grain yield, yield components as well as grain shape and N related traits under field conditions.

Under N fertilization (N₁) conditions the traits YLD, GPC and EAR showed a significant ($p < 0.05$) increase compared to low nitrogen supply (N₀). These results are in accordance with several nitrogen related studies (Baethgen et al. 1995; Hussain et al. 2006; El-Habbal et al. 2010). Although all presented QTL regions revealed significant effects across both N treatments some were clearly stronger either under N₀ or N₁. In the following, the major QTL identified for different trait complexes are discussed.

Grain yield

Nine QTL reducing grain yield could be detected (Table 4, Online Table S4). None of them were found in the nitrogen stress trial of Schnaithmann and Pillen (2013) under greenhouse conditions. In particular, significantly high losses were found in S42ILs, which carry an introgression at the *sdw1/HvGA20ox₂* locus, possibly affecting YLD because of stronger lodging (Kuczyńska et al. 2013). Final grain yield is the product of genetic control by a multitude of QTL from different chromosome regions, huge environmental impacts and yield-forming traits (Larson et al. 1996; Slafer 2003). Naz et al. (2014) described an extensive root system for S42IL_176 compared to ‘Scarlett’ in a greenhouse experiment. Astonishingly, the enlarged root dry weight showed no beneficial effect on grain yield in field conditions. On the contrary, S42IL_176 significantly revealed 6 dt/ha lower YLD compared to ‘Scarlett’.

Schnaithmann and Pillen (2013) described three significant yield increasing QTL under higher nitrogen level. However, unfortunately, determining grain yield in greenhouse experiments seems to lead to strong limitations for field transferability as they could not be confirmed in the present study. However, in our study two relevant yield-increasing S42ILs (S42IL_122 and S42IL_123) with an overlapping

introgression from 91 to 100 cM on chromosome 4H and two (S42IL_135 and S42IL_136) with an overlapping introgression from 78 to 111 cM on 7H could be found. Although statistically not significant, they indicate promising yield effects across both nitrogen treatments. The fact that S42IL_137, which also shares the 7H introgression, showed no yield-increasing effect can be explained by the presence of the yield-reducing QTL on 3H. For S42IL_135 a significant grain yield increase under N₁ was also reported in the greenhouse study of Schnaithmann and Pillen (2013).

In 2015, both test sites were affected by less precipitation in May and June compared to 2016 (Online Table S5). Heavy rainfall events followed in July and August. In these circumstances the 41 S42ILs showed an average grain yield loss of 29.1% in 2015 compared to 2016. One line, S42IL_123, combined good grain yield and high crop stability of 59.7 dt/ha and 70.7 dt/ha in 2015 and 2016 in Morgenrot, respectively. Since the yield loss in S42IL_123 was only about 15.6% between the years, this line might be a valuable choice for breeding programs concerning drought management. Similar observations were reported by Honsdorf et al. (2014b). In greenhouse experiments investigating the impact of early drought stress on the S42IL population S42IL_123 showed higher biomass, photosystem II efficiency, chlorophyll content and tiller number under water limited conditions as compared to ‘Scarlett’. With regard to increasing heat and drought periods due to climate change, it will be necessary to rely on more tolerant genotypes. For this purpose, the respective introgression on 4H represents an interesting target.

The parameter grain yield is genetically very complex, which sometimes leads to a lack of statistical outcomes. Therefore, it is advisable to split grain yield into its agronomic determinants, i.e. ears per square meter (EAR), grains per ear (GEA) and thousand grain weight (TGW).

Grain parameters and yield components

Assessing the impact of nitrogen fertilization on yield and yield components is often difficult because of compensation effects. GRA and, hence, TGW, is based on GRL and GRW and these parameters are able to compensate each other as Ramya et al. (2010) reported for bread wheat. The link between GRL,

GRW, GRA and TGW is very strong in cereals (Groh et al. 2001; Ayoub et al. 2002; Breseghello and Sorrells 2007). Except for one greenhouse trial of Schnaithmann and Pillen (2013), no measurement of the grain parameters GRA, GRL and GRW were conducted for the S42IL population in field experiments.

Although S42IL_143 showed the highest increase in GRL compared to ‘Scarlett’ no QTL was found for TGW. The reason for that might be the opposing effect for GRW, which was detected in this line. S42IL_102 showed a similar performance with a slightly stronger reduction in GRW and a comparably weaker increase in GRL than S42IL_143, which also resulted in no QTL for TGW. An explanation for this observation might be that GRW had a higher impact on TGW ($r = 0.53\text{--}0.67$) than GRL ($r = 0.29\text{--}0.30$) (Online Table S3). Considering the correlation of GRL, GRW and TGW, our results coincide with the findings of Backes et al. (1995). In accordance to these findings, Schnaithmann and Pillen (2013) observed a significant increase in both lines for GRL, but not in TGW. Since TGW seems to be more complex but is highly related to the grain shape, it might be advantageous to use the manifold results of GRA, GRL and GRW, which revealed more QTL information.

S42IL_119 and S42IL_121 contain overlapping introgressions between 51.9 and 81.2 cM on chromosome 4H with a positive TGW performance compared to ‘Scarlett’. We could further narrow down the relevant genomic region to 57–81 cM as S42ILs 118 and 123 did not show the increasing effect on TGW. In addition, both S42IL_119 and S42IL_121 displayed another favorable *Hsp* effect of showing an increase in GRL and GRA but no significant decrease in GRW, resulting in higher TGW compared to ‘Scarlett’. This could be explained by introgressed wild alleles specifically affecting GRL. Hence, no compensatory impact of GRL and GRW was observed for S42IL_119 and S42IL_121 but rather an improvement in TGW.

Another outstanding attribute was found in the performance of S42IL_121 for GEA. In accordance with the negative correlation ($r = -0.27$ to -0.34) between TGW and GEA, it is known that less GEA leads to higher TGW (Kjær and Jensen 1996). Three S42ILs (111, 119 and 121) had significantly higher TGW, but only in S42IL_121 this was not significantly associated with a lower GEA. This newly found

favorable connection influenced by the *Hsp* introgressions in S42IL_121 could be used for further yield development processes.

Grain protein content is known to be very dependent on nitrogen fertilization management as was also true for this study ($p < 0.01$). However, no QTL could be identified for GPC, indicating genotype-independent effects. We assume that the low heritability ($H^2 = 0.22$) for GPC might have impeded QTL detection.

Chlorophyll content (SPAD)

Spaner et al. (2005) and Izsáki and Németh (2007) verified a high correlation of SPAD with N content in leaves. Chlorophyll quantification through sensor technique is used as a non-destructive method in sustainable agriculture and also an effective tool for selection. Unexpectedly, no significant difference in chlorophyll content in the flag leaves were found between N_0 and N_1 , but nevertheless showed trend towards higher content under N_1 level ($p = 0.13$). Compared to the greenhouse trials of Schnaithmann and Pillen (2013) and Honsdorf et al. (2014b) three new S42ILs were detected with significantly differing chlorophyll content from ‘Scarlett’. Significantly lower chlorophyll values were specifically found in S42IL_176, which also showed significantly slower plant development and smaller TGW than ‘Scarlett’, carrying a main introgression on chromosome 5H and sub-introgressions on 1H and 3H. Due to similar results of introgression lines overlapping with S42IL_176 on chromosome 1H between 126 and 131 cM (S42IL_128 and S42IL_142) this sub-introgression seems the most promising genomic region carrying the responsive QTL. In contrast, the lines overlapping with S42IL_176 on 5H (S42IL_126 and S42IL_127) show no effect on SPAD values and TGW. In contrast, S42IL_110 and S42IL_107, having overlapping introgression on 2H, revealed significantly higher chlorophyll values and faster development.

Conclusions

The present study with the wild barley introgression population S42IL points out that exotic *Hsp* introgressions lead to high variation in agronomically

important traits as compared to the elite control parent ‘Scarlett’. Our analyses over four environments revealed various consistent QTL for yield-related traits. In total, 45 QTL were detected for 9 different traits. Especially in the low N treatment (N_0) we found promising lines that significantly differed from ‘Scarlett’. Based on this, it seems to be important to adjust breeding concepts for optimal results. At first glance, the performance of the introgression lines mainly revealed adverse QTL, which is probably a result of recurrent phenotypic selection in elite barley for decades. Although many *Hsp* allele effects turned out to be undesirable, these findings can provide valuable information about genomic regions involved in trait expression with potentially beneficial alleles being present in further genetic resources. Nonetheless, some introgression lines showed beneficial effects on agronomic traits. In particular, introgressions on 2H (89.5–97.8 cM), 3H (43.1–55.2 cM) and 4H (35.9–81.2 cM) showed a comparably lower yield reduction through an improved grain morphology under N_0 as well as N_1 . To reveal useful *Hsp* effects, it might be advantageous to select for several traits under nitrogen stress rather than under optimal nitrogen fertilization as was already suggested by Weltzien and Fischbeck (1990) and Basford and Cooper (1998). In our study, S42ILs 109, 122, 123, 135 and 136 for grain yield and S42ILs 119 and 121 for grain components appeared to be good candidates to increase trait performance under low nitrogen supply. Thus, for future barley breeding under low nitrogen fertilization it might be promising to use selected genotypes of the S42IL library.

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Author contributions SZ and BK performed the experiments. SZ and AM analyzed the data and wrote the manuscript. OC, KP and AM conceived and designed the experiments.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights No human or animal material was used. The research conducted complied with all institutional and national guidelines.

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