Evaluation of morphological traits, genetic diversity and major resistance genes in barley subpopulations cultivated under organic and conventional farming systems

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Abstract. Most crop varieties currently grown in organic conditions have been bred for conventional farming, and are not adapted to increased environmental variability under organic farming conditions and unpredictable environmental fluctuations due to climate change. This can be mitigated by the use of heterogeneous material, increasing genetic diversity and enabling adaptation to local conditions. The objective of this study was to determine the effects of several generations of cultivation in parallel under organic and conventional farming systems on the genetic diversity, morphological traits and frequency of major disease resistance genes as indicators of adaptation to the farming system in heterogeneous spring barley populations with differing levels of diversity. Populations in differing generations originating from crosses between two, three, 10 and 15 parental genotypes were cultivated in organic and conventional farming systems for three, four or 10 generations, thus forming subpopulations in each environment. These subpopulations were genotyped, and tested for morphological traits in both farming systems. A significant effect of cultivation environment on tillering capacity ($p < 0.05$) was found for all tested populations and in several cases for plant height, ear length and grain number per spike, indicating some adaptation trends. In the short term, genetic diversity parameters were not decreased in the later generation populations in comparison to the initial populations with the exception of observed heterozygosity, as expected for a self-pollinating species. No clear differences in genetic diversity parameters between populations cultivated under either organic or conventional condition for several generations were identified.

Key words: genetic diversity, genotyping, heterogeneous populations, morphological traits, organic farming, spring barley.

INTRODUCTION

During the previous century, global dynamic changes have significantly affected society - changes in technology, societal structure and interests, food choice and many other aspects. These changes have increased demand for food, and led to the

intensification of agriculture and plant breeding, which has had profound consequences for the environment. These include loss of biodiversity, climate change, changes in agricultural practices, diminishment of arable land area. To mitigate these challenges, paradigm shifts in agriculture (Döring et al., 2015), plant breeding (Litrico & Violle, 2015; Raggi et al., 2016), legislation etc. are required to develop new strategies to ensure sustainable agriculture and food security. While increased production volumes of organic farming systems is important within the wider agricultural sector, organic farming can also provide other benefits such as decreased use of chemicals, increased genetic diversity in farmers' fields, and enable more close connections between producers and consumers (Risku-Norja & Mikkola, 2009).

In previous decades, plant breeding has focused on creating high yielding varieties for a limited number of crop species, developed for high-input monocultural agricultural farming systems with highly controlled conditions including a limited number of weeds, high nutrient availability and feasible reduction of pests and diseases (Lammerts Van Bueren et al., 2011; Döring et al., 2015). Most crop varieties currently grown in organic conditions have been bred for conventional farming systems (Lammerts Van Bueren et al., 2011). In most cases, they have not been specifically adapted to the increased environmental variability under organic farming conditions as well as to unpredictable environmental fluctuations due to climate change. Therefore, genotypes selected for high-input farming systems may not have the capacity to buffer lower inputs and respond to novel stress factors, not only in low-input, but also in high-input agricultural systems (Murphy et al., 2007). Within the Baltic states, the most important traits for barley varieties used for organic farming include disease resistance (Bankina & Gaile, 2009), as well as yield and quality (Leistrumaitė et al., 2009). Therefore, Latvian barley breeding for organic farming systems is particularly focused on these traits.

To increase genetic diversity and the resilience of agricultural systems, cultivation of heterogeneous populations of self-pollinating species has been suggested. In contrast to the currently dominating uniform varieties, populations are subjected to natural selection and can respond and adapt to particular environmental conditions (Wolfe et al., 2008). Although cultivation of these heterogeneous populations in a single environment can be expected to decrease genetic diversity due to natural selection eliminating unsuitable genotypes and genetic drift, diversity is sustained by cultivation of populations in different environments, leading to differentiation of populations into subpopulations (Goldringer et al., 2001). Natural selection and competition among plants within a population affects genetic diversity and agro-morphological traits. Agro-morphological traits in populations have been shown to change due to natural selection in particular environments as rapidly as in four generations (Enjalbert et al., 2011). The first traits to respond to natural selection in cereals are earliness (Verhoeven et al., 2008; Rhoné et al., 2010; Raggi et al., 2016) and plant height (Enjalbert et al., 2011; Raggi et al., 2016). In case of disease pressure in the environment, plants with disease resistance prevail in the populations (Enjalbert et al., 2011).

A range of strategies are available for increasing the within-field genetic diversity in agricultural systems. One approach to create genetically diverse populations is by crossing varieties with each other and then optionally to pool the seed from a number of crosses. Previous studies with populations created by crossing as little as two parents (simple populations) (Ločmele et al., 2017; Mežaka et al., 2017), and populations arising from cross combinations using several parents (complex populations) have been reported

(Ločmele et al., 2019). Creation of composite cross populations (CCPs) involving bulks of diallel crosses between a larger number of parents was first described by Harlan & Martini (1929), and they have been used in various studies of genetic and agro-morphological diversity in barley and wheat since then (Harlan & Martini, 1938; Suneson, 1956).

A limited number of studies have investigated population evolution in organic and conventional farming systems. Winter barley populations maintained for 13 generations and screened by molecular markers showed adaptation to particular growing environments (Raggi et al., 2017). In a number of studies of winter wheat populations created in the UK (Döring et al., 2015), different conclusions have been made. In research performed over 11 generations on adaptation of populations to particular farming system, no changes in allele frequencies were reported (Knapp et al., 2013). In a study of changes of weed competitive traits in populations, early vigour was improved after five years of cultivation in organic conditions in comparison to conventionally grown populations (Bertholdsson et al., 2016). Comparison of seedling traits in populations grown in parallel in organic and conventional conditions over ten years found that CCPs cultivated in organic conditions had longer, thicker and deeper root systems, indicating the ability to uptake nutrients from deeper soil layers, whereas in the CCPs cultivated in conventional conditions, almost no changes were observed (Vijaya et al., 2019). Assessment of the populations after five years cultivation in conventional and organic conditions showed that changes in number of tillers, kernel weight and kernel number were not affected by the generation or cultivation conditions (Bertholdsson et al., 2016) and that, to maintain sufficient diversity, the population size should be not less than 12,000–15,000 individuals (Brumlop et al., 2019). Yield stability depends on the genetic background of the populations: in conventional conditions, the population containing a high yielding genetic background was more stable, but in organic conditions the population containing a broader genetic background provided better stability (Weedon & Finckh, 2019). Our previous study on simple populations of spring barley over the course of six generations cultivated in organic and conventional conditions did not detect any differences in genetic diversity or allele frequencies (Mežaka et al., 2017). The effect of cultivation in conventional and organic growing conditions on CCP grain quality and plant morphology has also been studied in wheat and no significant effect of farming system on these traits has been reported (Brumlop et al., 2017).

The objective of this study was to determine the effects of several generations of cultivation in parallel under organic and conventional farming systems on the genetic diversity, morphological traits and frequency of major disease resistance genes and adaptation of the population to the farming system in three types of heterogeneous populations - simple (2 parents), complex (3–4 parents) and composite cross populations (diallel crosses of 10–15 parents).

MATERIALS AND METHODS

Plant material
Three types of spring barley (*Hordeum vulgare* L.) populations (based on number of parents used for crosses and crossing methods) were used in this study: (1) simple

populations (SP), created by crossing two parents, (2) complex populations (CP) involved cross combinations consisting of three to four parents and (3) composite cross populations (CCP) involved bulked diallel crosses between either 10 parents (CCP1 and CCP2) or five male sterile parents crossed to 10 pollinators each (CCP3). Two simple populations, two complex populations and three composite cross populations were used in the study (Table 1). Simple and complex populations originated from the breeding program for organic farming and were maintained after the selection of individual plants for breeding purposes was done, but CCPs were created for this study. A more detailed description of the simple populations P/I and A/Dz can be found in Mežaka et al. (2017).

Table 1. Population characteristics

Population denomination	Type of population	Pedigree/parents, year of crossing/ seasons of cultivation[1]	Analyses and generations[2]
P/I	Simple	Primus/Idumeja, **2004/10**	Morphological, SSR (F_{10}, F_{13})
A/Dz	Simple	Anni/Dziugiai, 2004/10	Morphological, SSR (F_{10}, F_{13})
CP-1	Complex	Irbe/PR-5108//PR-5999+PR-6000 (hulless barley), 2011/3	SSR and *mlo11* (F_4, F_6)
CP-2	Complex	KZ14-52/PR-4121//PR-4311, **2011/3**	Morphological and *mlo11, Mla18* (F_3, F_6)
CCP-1	Composite cross	Bulked dialell crosses among group of 10 parents: PR-4814, PR-5105, PR-3605, PR-5135, PR-4871, PR-5279, BZ-14-92, 'Balga', 'Jumara', 'Mik 1', **2013/3**	Morphological, SSR and *mlo11* (F_2, F_4)
CCP-2	Composite cross	Bulked dialell crosses among group of 10 parents:PR-5506, PR-5228, PR-5779, PR-6000, PR-5415, No.51, Irbe, Pirona, CDC Freedom, CDC Fibar (hulless barley), **2013/3**	SSR and *mlo11** (F_2, F_4)
CCP-3	Composite cross	Bulked crosses of 5 male sterile parents each crossed to 10 pollinators: PR-4814, PR-5105, PR-3605, PR-4181, PR-4121, PR-4311, PR-5137, BZ14-12, Golf, Mik-1, **2013/3**	SSR and *mlo11, Mla18* (F_2, F_4)

[1]number of seasons of cultivation in parallel under organic and conventional environments, including 2016; [2]two generations, in which genotyping was performed; * no data available for F_4 conventional subpopulation.

Initial multiplication was done as follows: F1 seeds of simple and complex populations were multiplied under conventional growing conditions, F2 were multiplied in a winter nursery in Chile, and F3 - under organic conditions. For CCPs, F1 seeds from diallel crosses were bulked in equal numbers from each combination and multiplied in a winter nursery in Chile. After initial multiplication, seeds of the F2 generation for CCPs and F4 for the simple and complex populations were divided in two subpopulations and

cultivated in parallel under organic and conventional farming systems for a number of generations (3, 4 or 10) (for details about each population, see Table 1), thus forming organic and conventional subpopulations (hereafter indicated with 'O' and 'C'). Subpopulations were then tested in both organic and conventional farming systems to determine if adaptive changes could be identified (Fig. 1).
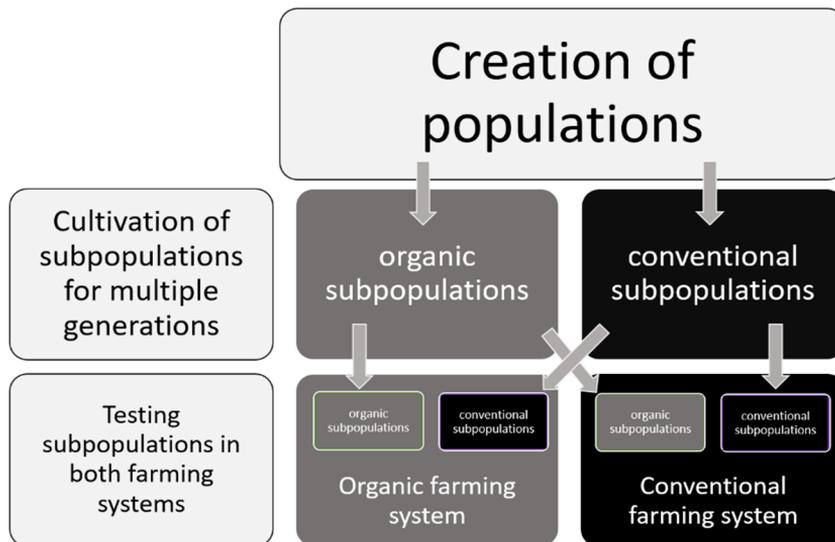
**Figure 1.** Schematic representation of experimental scheme.

Cultivation conditions

The cultivation of populations was carried out at the Institute of Agricultural Resources and Economics, Priekuli Research Centre (latitude 57°18'53.28" N, longitude 25°20'19.68" E, 120 m a.s.l.) between 2004–2016, under both conventional and organic crop management systems. The soil in all locations was sod-podzolic sand loam (Karklins, 2008). Minimum and maximum values of other soil properties are summarized in Table 2. Pre–crop in all C environments was potatoes, but in O environments - green manure or grain legumes. No fertilizers were used in O sites. In C

Table 2. Range of soil agrochemical properties in population cultivation fields during 2004–2016

Properties	Farming system	
	conventional	organic
pH KCL	5.3–6.4	5.1–6.0
Organic matter %	1.7–2.7	1.7–2.4
K_2O mg kg^{-1}	136–240	75–175
P_2O_5 mg kg^{-1}	133–196	90–208

sites mineral fertilizers were applied before sowing in order to obtain 5 t ha^{-1} grain yield, according to the results of soil property indicators. The amount of pure elements was: nitrogen (N) 80–95, phosphorus pentoxide (P_2O_5) 40–60, and potassium oxide (K_2O) 40–90 kg ha^{-1}. To restrict weeds in C sites, herbicide was applied, but in O sites harrowing at tillering (BBCH 21–29) was performed. No fungicide was applied in any of the cultivation years, but insecticide to restrict aphids (*Sitobion avenae* Fabr.) was applied in some years in C sites.

Meteorological conditions are summarized in Fig. 2 and presented according to the periods of cultivation: 2005–2016 for simple populations, 2011–2016 for complex populations and CCPs, and 2016, the year of sampling for genotyping and assessment of plant morphological traits. The weather conditions varied between 10-day periods in 2005, 2008, 2014 and 2015, when after rainy periods there was drought and vice versa. In some growth stages, the temperature delayed or accelerated plant development. Weather conditions were extreme in the year of phenotypic assessment (2016), when, after increased rainfall at the end of April, which delayed sowing, only 18% of the normal precipitation fell in May, delaying the development of plants. There was increased rainfall starting with the middle of June up to the end of August, peaking during the last 10-day period of June, when rainfall was 209% higher than the long-term average for this period. During this period, it was warmer than usual - the average air temperature surpassed the norm by 4.5 °C.
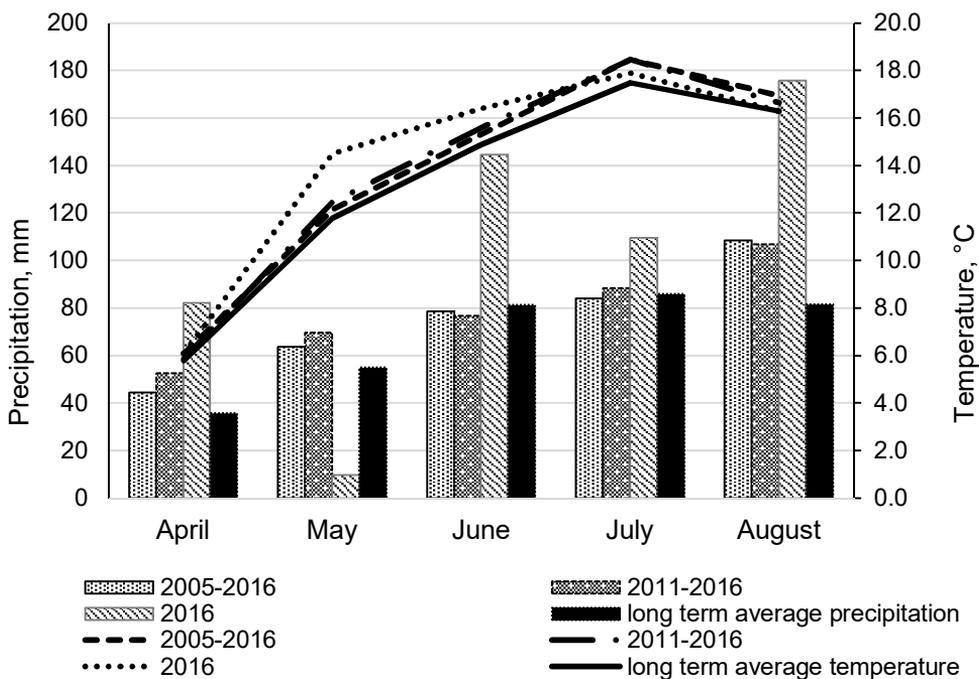
Figure 2. Sum of precipitation (bars) and average air temperature (lines) during barley vegetation periods in 2005–2016, 2011–2016, 2016 and long-term average over 1981–2010.

Phenotyping

Differences in plant morphology due to cultivation environment (O and C) were assessed for subpopulations of P/I, A/Dz, CP-2 and CCP-1. In 2016, both subpopulations (O and C) of each population were sown in both O and C sites next to each other in 12.3 m^2 plots, with a seed rate of 400 untreated seeds per m^2. Plant height, main ear length, number of productive tillers per plant (tillering) and number of grains in the main ear were measured in 100 randomly chosen plants per each subpopulation, collected during the maturity stage (BBCH 90).

SSR genotyping

Plant shoot samples from 96–100 randomly chosen individuals per population/subpopulation were collected for SSR marker genotyping. Each population was genotyped in two different generations: simple populations P/I and A/Dz (F_{10} and F_{13}), complex population CP-1 (F_4 and F_6), and composite cross populations CCP-1, CCP-2 and CCP-3 (F_2 and F_4) (Table 1). The initial generations for CP-1 (F_4) and the CCPs (F_2) were genotyped prior to dividing them into two subpopulations for cultivation under organic and conventional conditions, therefore these initial generations are not split by cultivation conditions. DNA was extracted using the modified protocol of Edwards et al. (1991). Nine simple sequence repeat (SSR) markers were multiplexed in three marker sets: set A (Bmag0125, Bmac0067, Bmac0032), set B (Bmag0135, WMC1E8, Bmag0173) and set C (Bmag0353, Bmac0093, Bmac0156) (Macaulay et al., 2001). The forward primer was synthesized with a 6-FAM, HEX or NED fluorescent label to allow visualisation of amplification products on a genetic analyser. Multiplex polymerase chain reactions (PCRs) were performed in a total reaction volume of 20 μl containing ca. 50 ng DNA, 4 μl 5x HOT FirePol® Blend Master Mix (Solis BioDyne, Estonia, final magnesium chloride ($MgCl_2$) concentration 2 mM), 0.2 μM of forward (labelled) and reverse primers. PCR conditions were as follows: 95 °C for 15 min, 40 cycles of 95 °C – 20 s, 58 °C (set A and B) or 60 °C (set C) - 40 s, 72 °C - 60 s, 72 °C - 10 min. PCR products were separated on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Waltham, USA), and genotyped using GeneMapper 4.0.

Major disease resistance gene genotyping

Markers linked to two major resistance genes (*Mla18, mlo11*), conferring resistance to barley powdery mildew (*Blumeria graminis* (DC.) Speer), were genotyped in populations where they were known to be present in the parental genotypes. The *Mla18* gene was genotyped in two populations, and the *mlo11* gene was genotyped in five populations (Table 1). PCR primers and conditions for *mlo11* and *Mla18* resistance gene analyses have been described previously (Kokina & Rostoks, 2008; Piffanelli et al., 2004). Briefly, PCR was performed in a 20 μl reaction consisting of ca. 50 ng DNA, final concentration of each primer - 0.5 μM, 1 x reaction buffer (Thermo Fisher Scientific, Lithuania), 2.5 mM $MgCl_2$, 0.2 mM dNTPs and 1 U of Hot Start *Taq* polymerase (Thermo Fisher Scientific, Lithuania). PCR products were visualized by agarose gel electrophoresis.

Statistical analysis

The group means and T-tests for plant morphological characters among populations were calculated in R version 4.0.3 (Team), 2013). Statistical differences of means of morphological traits of subpopulations grown at the same testing environment were calculated with Two Sample T-test if variances among groups were equal and Welch Two Sample t-test if variances were not equal ($p < 0.05$). Genetic diversity parameters calculated using GenAlEx 6.5 (Peakall & Smouse, 2012). In summary, total number of alleles and total number of alleles with a frequency over 0.05 were summed over all nine analyzed loci. The observed heterozygosity (Ho) (no. of heterozygous loci/number of samples), expected heterozygosity (He) (He = $1-\Sigma p_I^2$, where p_i is the frequency of the

i-th allele), number of effective alleles (Ne) (Ne = 1/(1-He)), mean information index (I) (I = -Σ$p_i$lnp_i) and were calculated for each locus, and subpopulations compared using a T-test. Matching multilocus genotype groups (shared by 2 or more individuals) were identified within each sub-population. Only individuals with exactly matching multilocus genotypes (including missing data) were considered to be included into one group.

RESULTS AND DISCUSSION

Populations respond to selective pressures in different environments by adaptation and evolution (Vijaya et al., 2019; Bocci et al., 2020). However, this is dependent on the presence of genetic and phenotypic diversity, and is influenced by mating system, selective pressure and other factors. This study assessed genetic and morphological changes in CCP, simple and complex barley populations cultivated in both organic and conventional farming systems for multiple generations, thus forming subpopulations in each environment. Subpopulations of four selected populations were then phenotyped in both organic and conventional conditions to assess if O subpopulations were more adapted to organic growing conditions than C subpopulations and vice versa.

Morphological traits
The effect of cultivation system on plant height was significant in four cases. Average plant height was significantly larger for the O subpopulation compared to the C subpopulation in both testing systems for the A/DZ population, as well as in the conventional testing system for the CP-2 population, but was lower in the organic testing system in the CCP-1 population (Fig. 3). Within the organic testing system, significant differences between average ear length in O and C subpopulations were observed for the population CCP-1 and, within the conventional system - for the population P/I (in both cases the average ear length was larger in the conventional subpopulation). Within the organic testing system, tillering differed significantly ($p < 0.05$) between all subpopulations; for CCP-1 the difference was also significant when tested in the conventional system. In the simple populations P/I and A/DZ, more tillers were found in conventional subpopulations, however the CP-2 and CCP-1 subpopulations had an increased number of tillers in organic subpopulations. Number of grains per ear did not significantly differ among subpopulations with the exception of the conventional testing system for the P/I population ($p < 0.05$) (Fig. 3). Coefficients of variation (data not shown) were generally higher for tillering (mean over the populations 38% while tested in O system and 25% in C system) and lower for plant height (12% and 10%, respectively) and with a trend of being higher under O testing system (mean over all traits 22% and 19%, respectively). No constant trends comparing variation of O and C subpopulations were identified.

One of the four measured plant traits, tillering, had significantly different mean values between both subpopulations of all four tested populations (also interpretable as effect of the cultivation environment) when tested in organic conditions. Subpopulations tested in conventional conditions showed no significant differences between number of tillers in simple and complex populations, but were significantly different in the CCP.

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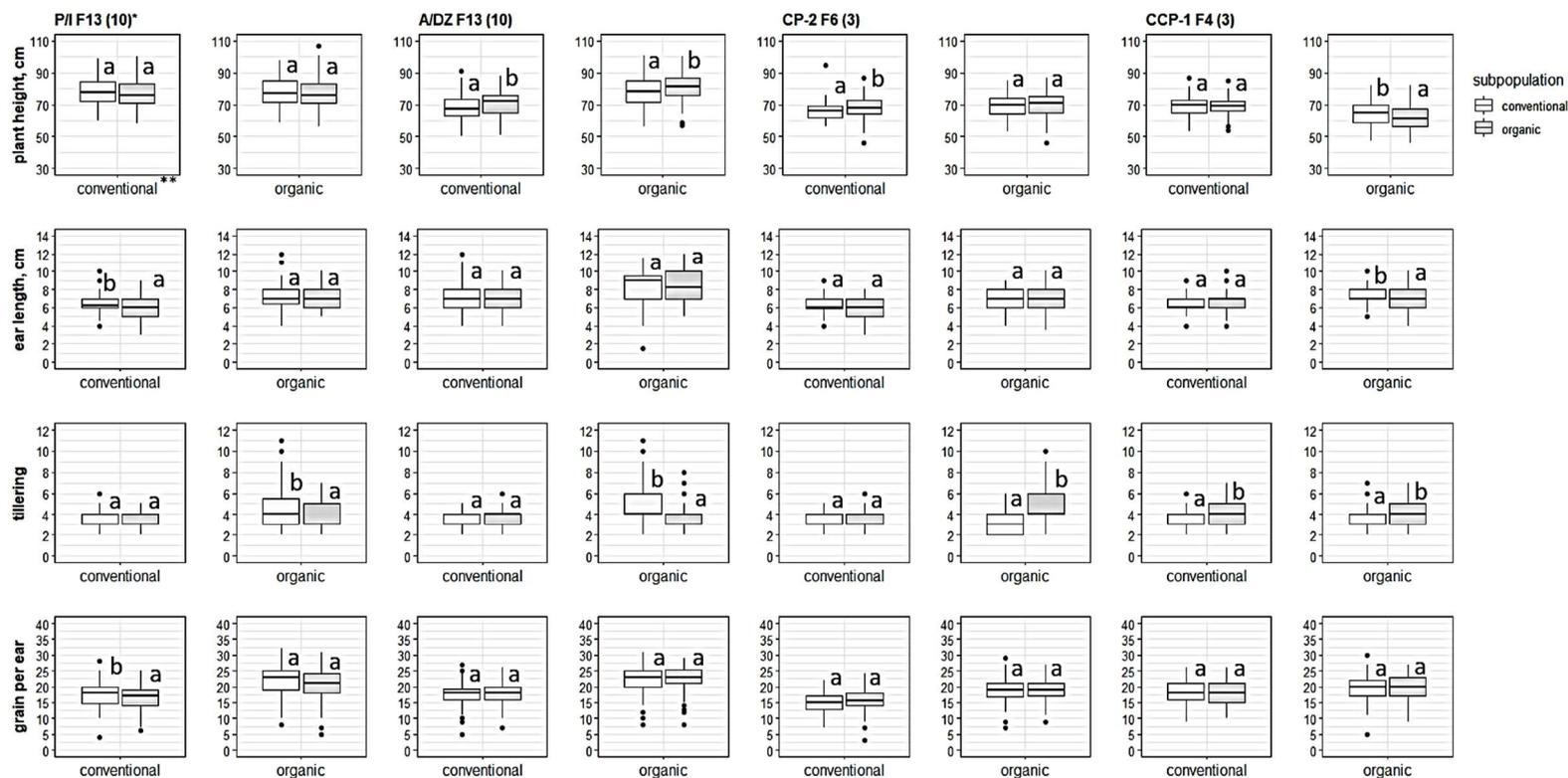
Figure 3. Plant height, ear length, number of tillers per plant, grain number per ear in organic and conventional subpopulations of four barley populations tested in both farming systems. Subpopulations tested in the given testing system indicated with the same letter do not differ according to *t*-test (*p* > 0.05). Horizontal lines within the boxes represent the median values, vertical lines indicate the 95% confidence interval of the median. The boxes represent first and third quartile boundaries. The dots represent outliers. *number in brackets represent number of seasons of cultivation. For description of populations refer to Table 1. ** refers to testing system.

The number of tillers was reduced in the simple organic subpopulations if compared to the conventional ones, while it was increased in the organic subpopulations of complex populations and CCP. It is possible that cultivation of the complex population and the CCP under organic farming conditions over a short term (three seasons) promoted multiplication of plants with better tillering capacity. However, in the case of simple populations, which were cultivated under the respective farming systems for a longer period, the opposite reaction was observed, which might be explained either by the specific reaction of the few genotypes involved (two instead of 10 for CCP) or by the effect of a longer cultivation time. Additional analyses planned for CCPs in F_9 can clarify this. But it should also be taken into account that tillering capacity is a trait with comparatively low heritability, affected to a greater extent by environmental conditions (Mežaka, 2018) which might also cause the observed differences. In contrast to our results, Brumlop et al. (2017) did not identify differences in tillering capacity in three wheat CCPs cultivated for six generations in conventional and organic farming systems.

Plant height is one of the first traits reported to respond in populations cultivated in differing conditions (Enjalbert et al., 2011; Raggi et al., 2016). Natural selection under organic conditions resulted in significantly shorter plants in the CCP-1 subpopulation compared to natural selection under conventional conditions, but resulted in taller plants in the simple population A/Dz cultivated in organic conditions compared to conventional conditions, for a comparatively longer time. The O subpopulation of CP-2 had increased plant height, which was significantly different under conventional testing conditions compared to the C subpopulation. The results indicate that a longer cultivation period (10 seasons) under organic environments can cause an increase in plant height, but over a shorter period (three seasons), plant height can either be increased or decreased. One of the reasons causing natural selection of taller plants could be the weed pressure present in the organic crop management system. With respect to mean ear parameters, shorter ears with less grain per ear were found in the O subpopulation of P/I tested in conventional conditions. However, Bertholdsson et al. (2016) and Brumlop et al. (2017) reported no significant differences in plant height and ear length in three wheat CCPs studied for six generations in conventional and organic farming systems. We can see a trend that differences between the subpopulations are more pronounced when tested in organic farming systems, compared to conventional systems, which can be explained by the smoothing effect of conventional management, ensuring an exogenous supply of nutrients and reducing biotic stress factors. This also confirms that adaptation of populations to specific growing conditions is more relevant for organic farming systems.

Brumlop et al. (2017) have questioned whether contrasting weather conditions over years influence the performance of populations more than differences in farming system. Over the course of our study, weather conditions varied between years and multiple deviations of temperatures and precipitation from long-term averages were observed. However, in this study, cultivation sites were close to each other, and both subpopulations received identical selection pressure in respect to weather conditions, therefore further studies are needed to confirm this.

Genetic analysis

The expected heterozygosity (He) and standard error (*SE*) was calculated over all analysed samples for each marker. The avearage values for each marker were - Bmag0125 (0.494, *SE*: 0.093), Bmac0067 (0.535, *SE*: 0.017), Bmac0032 (0.651,

SE: 0.071), Bmag0135 (0.350, *SE*: 0.162), WMC1E8 (0.295, *SE*: 0.142), Bmag0173 (0.543, *SE*: 0.231), Bmag0353 (0.551 *SE*: 0.040), Bmac0093 (0.539, *SE*: 0.064), Bmac0156 (0.454, *SE*: 0.209). Expected heterozygosity is equivalent to Polymorphism Information Content (PIC) for inbred lineages (Serrote et al., 2020), and the He/PIC values indicated that the markers were sufficiently informative for analysis of this germplasm. Markers with He/PIC values larger than 0.5 are classified as very informative, and with values between 0.25 and 0.50 as somewhat informative (Botstein et al., 1980).

Changes in genetic diversity between generations within populations

In some cases, the mean number of alleles within each genotyped population increased in advanced generations, compared to the initial generations genotyped. However, this was due to an increase of low frequency alleles, with a frequency of less than 0.05. No significant differences in the number of effective alleles or information index were observed between samples of one population taken from different generations. The observed heterozygosity was significantly ($p < 0.05$) increased in advanced generations in one of the simple (A/Dz) and the complex population CP1 subpopulation grown under organic conditions. A significant ($p < 0.05$) decrease in observed heterozygosity was observed in the advanced generations of all CCP populations as well as in the CP1 subpopulation grown in conventional conditions (Table 3). The number of matching multilocus genotype (shared by two or more individuals) groups is an indicator of the homogenization of the populations. As barley is predominantly self-pollinating, distinct lines sharing matching multilocus genotypes will be formed within populations. A larger number of matching multilocus genotype groups indicates a decrease in diversity within populations, as the number of genetically distinct individuals is decreased. Conversely, if all individuals within a population are genetically distinct, then no matching multilocus genotype groups are formed. In most subpopulations, there was not a substantial change in the number of multilocus genotype groups between initial and advanced generations. The exception was in the A/Dz conventional cultivation subpopulations and the CP1 advanced organic cultivation subpopulation. In both cases, there was a lower number of groups in the advanced generations, which corresponds to the previously reported increase in heterozygosity for these subpopulations. In the CCP populations, where a significant decrease in observed heterozygosity was observed between initial and advanced populations, the number of matching multilocus genotype groups was low, reflecting the increased complexity of the populations, involving crosses between a large number of parental varieties.

Changes in genetic diversity due to cultivation under different farming systems

In general, no significant differences in genetic diversity indices were observed between pairs of subpopulations cultivated in organic and conventional conditions. The observed heterozygosity was significantly ($p < 0.05$) increased in the complex population CP1 cultivated under organic conditions compared to conventional, but other genetic diversity indicators were similar between these two subpopulations (Table 3).

Table 3. Genetic diversity parameters

Population	Sub-population	N (generation) I	N (generation) Ad	Total Na I	Total Na Ad	Total Na ($f > 0.05$) I	Total Na ($f > 0.05$) Ad	Mean Ne (*SE*) I	Mean Ne (*SE*) Ad	Mean I (*SE*) I	Mean I (*SE*) Ad	Mean Ho (*SE*) I	Mean Ho (*SE*) Ad	No. of MMGs I	No. of MMGs Ad
P/I	O	95 (F_{10})	100 (F_{13})	27	37	16	17	1.633 (0.173)	1.829 (0.143)	0.525 (0.120)	0.713 (0.092)	0.012 (0.011)a	0.009 (0.002)a	15	14
	C	96 (F_{10})	100 (F_{13})	31	34	15	17	1.731 (0.165)	1.818 (0.149)	0.593 (0.106)	0.676 (0.105)	0.023 (0.014)a	0.002 (0.002)b	21	21
A/Dz	O	95 (F_{10})	100 (F_{13})	32	28	16	17	1.779 (0.192)	1.707 (0.159)	0.598 (0.140)	0.571 (0.114)	0.035 (0.019)a	0.185 (0.072)b	12	8
	C	96 (F_{10})	100 (F_{13})	17	15	26	15	1.649 (0.163)	1.676 (0.166)	0.466 (0.117)	0.508 (0.116)	0.005 (0.005)a	0.309 (0.081)b	25	6
CP-1	O	96 (F_4)	100 (F_6)	31	28	26	23	2.357 (0.333)	2.066 (0.281)	0.887 (0.146)	0.740 (0.140)	0.087 (0.015)a	0.422 (0.110)b	12	2
	C		100 (F_6)		23		18		1.795 (0.235)		0.562 (0.142)		0.010 (0.003)c		16
CCP-1	O	96 (F_2)	100 (F_4)	39	42	34	34	2.929 (0.395)	2.914 (0.330)	1.141 (0.128)	1.164 (0.122)	0.299 (0.044)a	0.075 (0.013)b	1	3
	C		100 (F_4)		39		34		2.941 (0.334)		1.162 (0.119)		0.074 (0.009)b		3
CCP-2	O	96 (F_2)	100 (F_4)	42	45	32	34	2.822 (0.508)	2.757 (0.446)	1.085 (0.154)	1.117 (0.140)	0.262 (0.047)a	0.137 (0.022)b	3	2
	C		100 (F_4)		41		34		2.667 (0.425)		1.080 (0.133)		0.079 (0.016)b		1
CCP-3	O	96 (F_2)	100 (F_4)	44	42	33	33	2.955 (0.405)	2.945 (0.395)	1.155 (0.144)	1.133 (0.141)	0.269 (0.043)a	0.081 (0.021)b	2	4
	C		100 (F_4)		45		34		3.112 (0.417)		1.193 (0.143)		0.079 (0.018)b		1

I – initial; Ad – advanced; O – organic; C – conventional; N – number of analyzed individuals; Na – number of alleles; Ne – number of effective alleles; I – information index; Ho – observed heterozygosity (values with different letters are significantly different ($p < 0.05$)); *SE* – standard error; MMGs - matching multilocus genotype (shared by 2 or more individuals) groups.

The observed heterozygosity was significantly ($p < 0.05$) higher in the in the P/I simple population advanced organic subpopulation compared to the advanced conventional subpopulation, however, average observed heterozygosity over the nine SSR loci was very low in both subpopulations (0.009 vs 0.002, respectively). No significant differences in genetic diversity indices were observed between the CCP subpopulations maintained under organic and conventional conditions for two seasons.

Genetic analysis of the simple populations in the 1st genotyping round (F_{10}) used in this study have been reported previously (Mežaka et al., 2017). The parental varieties of the complex and CCP populations were not genotyped, therefore the initial number of alleles in these types of populations was not known. As expected, overall population genetic diversity increased from the simple, to complex and CCP populations. However, analysis using SSR markers did not detect a proportional increase in genetic or allelic diversity compared to the number of parents used in the creation of these populations. The simple populations were created using two parental varieties, while the CCPs each had 10 parental varieties. Given the larger number of parental varieties, particularly for the CCPs, there was only a modest increase in the total number of alleles. This is probably a reflection of the lack of the genetic diversity of the parental material, where the parental varieties of one population share similar alleles. The total number of alleles slightly increased in most populations genotyped in advanced generations, compared to the initial generations. This increase was due to low frequency alleles, and may be due to stochastic fluctuations, or low levels of outcrossing or other sources of contamination. This reflects the low selection pressure on the populations, and the relatively large population sizes ensured that genetic drift did not affect the major allele composition over the relatively low number of generations. The observed heterozygosities in most of the analyzed populations had decreased after 2–3 generations, which is a result of the self-pollinating nature of barley, and the lack of selective pressure, particularly on heterozygous loci. As barley is a predominantly self-fertilizing species, it is not expected that the genetic diversity of the populations would be considerably increased by cross-pollination. Observed heterozygosity was significantly increased in advanced generations of two populations (A/Dz and CP1). The reason for the increase in observed heterozygosity in the two populations is not clear, and further investigation of these populations is needed (e.g. by genotyping of additional generations of these populations). CCP-3 did not have higher levels of observed heterozygosity or other genetic diversity indicators, despite having five male sterile parents, which would be expected to increase the observed heterozygosity due to increased cross pollination.

The maintenance of heterozygosity and the generation of new allelic combinations by recombination is dependent on outcrossing rates. While barley is a predominantly self-pollinating species, a number of studies have shown that outcrossing rates in barley are usually less than 1% (Kahler et al., 1975; Doll, 1987), but can be up to 5% in winter barley varieties (Doll, 1987). In this study, in most cases, the observed heterozygosity decreased in advanced generations, and no direct estimation of outcrossing rates was done. The rapid decrease in observed heterozygosity observed in the CCPs compared to other population types is due to the initial generations analyzed for each type of population - F_2 for the CCPs compared to F_{10} for the simple populations. However, observed heterozygosity increased in the advanced generations in two subpopulations (A/Dz F_{13} generation cultivated in conventional conditions and CP-1 F_6 cultivated in organic conditions), however the cause of this is not immediately clear. One possible

explanation could be differences between genotypes in flower morphology and timing of flowering, but this requires additional investigation to determine the basis of this increase in observed heterozygosity. In general, advanced generations in predominantly self-fertilizing barley, would be expected to have lower levels of observed heterozygosity. In the CCP populations, the number of matching multilocus genotype groups was low, which could be an indication of the increased complexity of the populations, involving crosses between a large number of parental varieties. However, genetic analysis of more advanced generations of the CCP populations is required to reveal if this level of diversity between individuals within populations is maintained.

No significant differences in genetic diversity parameters between the O and C subpopulations were observed, which again, is probably a result of low selective pressure and the small number of generations. Cultivation of subpopulations in organic and conventional conditions for a larger number of generations may increase the genetic diversity differences between them. However, other factors, such as selective pressures and environmental conditions can also affect the rate of differentiation between subpopulations. Subpopulations can also be supplemented with additional germplasm to increase genetic diversity, either from the same source as the original population, or with different germplasm. However, this will also have the effect of diluting any adaptive changes that have occurred in the subpopulations. Further research involving additional genotyping and phenotyping of the CCPs in more advanced generations will provide further data on this. In general, apart from a decrease in observed heterozygosity in advanced generations, as would be expected from a primarily self-pollinating species as barley, no large decrease in genetic diversity was observed in the analyzed populations. In fact, in some subpopulations, an increase in observed heterozygosity occurred, and the factors influencing this should be further investigated.

Comparison of major disease resistance gene frequency

During the course of the cultivation of the populations, powdery mildew (*Blumeria graminis* (DC.) Speer) infection was comparatively low, therefore there was no large selective pressure on these genes. The average powdery mildew score during the three cultivation seasons was 1.1 under O conditions and 2.6 under C conditions (scored from 0 (no infection) to 9 (heavy infection)), reaching 3.3 and 4.4, respectively, in the year 2015 with the highest infection level.

For the *Mla18* gene, there was not a large difference in the proportion of homozygous resistant and susceptible individuals in both populations after cultivation in both organic and conventional conditions. The proportion of heterozygous individuals decreased in the advanced generations in comparison to the initial populations. In contrast, the number of individuals homozygous for the *mlo11* resistance allele decreased in all populations, regardless of cultivation conditions. Results are summarized in Table 4.

Compared to the SSR genotyping results, a larger change in allelic frequencies was observed for the major resistance genes, despite low powdery mildew infection pressure. There was a tendency (in 5 out of 6 cases) that the subpopulations cultivated under conventional conditions had a higher proportion of resistant individuals, which could be a reflection of the higher average powdery mildew score in the conventional cultivation conditions. However, the disease pressure was not high, and the proportion of *mlo11* homozygous resistant individuals decreased in the C subpopulations in comparison to

the initial populations. This decrease in the proportion of *mlo11* resistant homozygotes in comparison to the *Mla18* gene may also be due to the pleiotropic effects of the recessive inheritance of the *mlo11* resistance gene, which has been reported to confer a yield penalty because of the necrotic lesions on plant leaves (Wolter et al., 1993). The reduction in the number of individuals homozygous for the *mlo11* resistance allele was more pronounced in the CCP populations, which could be due to the larger number of parental varieties for these populations, and subsequently, a larger amount of *mlo11* susceptibility alleles in the population. Another possibility is that the individuals possessing higher yield potential are *mlo*-susceptible and therefore the proportion of susceptible alleles increased.

Table 4. Percentage of individuals homozygous and heterozygous for resistant and susceptible alleles of the *Mla18* and *mlo11* markers in initial (I) populations and in subpopulations cultivated in organic (O) and conventional (C) conditions

Population	Gene	Subpopulation / Generation	Resistant	Susceptible	Heterozygous
CP-2	*Mla18*	I /F_3	27.3	49.5	23.2
		O /F_6	54.3	40.4	5.3
		C /F_6	61.9	34.8	3.3
CCP-3	*Mla18*	I/F_2	11.1	71.1	17.8
		O/F_4	77.9	17.9	4.2
		C/F_4	80.2	14.6	5.2
CP-2	*mlo11*	I /F_3	82.0	11.0	7.0
		O /F_6	81.2	18.8	0.0
		C /F_6	75.6	17.3	7.1
CP-1	*mlo11*	I/F_4	72.9	14.6	12.5
		O/F_6	21.9	62.2	15.9
		C/F_6	38.1	59.5	2.4
CCP-1	*mlo11*	I/F_2	80.6	10.8	8.6
		O/F_4	10.6	89.4	0.0
		C/F_4	11.0	87.9	1.1
CCP-2	*mlo11*	I/F_2	85.9	10.6	3.5
		O/F_4	18.9	81.1	0.0
CCP-3	*mlo11*	I/F_2	64.2	27.2	8.6
		O/F_4	6.3	92.4	1.3
		C/F_4	16.5	79.1	4.4

CONCLUSIONS

The aim of utilizing heterogeneous populations is to increase the genetic diversity in farmers' fields to buffer potential biotic and abiotic stresses, particularly in more variable organic farming conditions. The results of this study show that in the short term, most genetic diversity parameters are not decreased in the advanced generations in comparison to the initial generations. Observed heterozygosity was decreased in the CCPs between F_2–F_4 but was unexpectedly increased in two other populations between F_4–F_6, and F_{10}–F_{13}, respectively, indicating that in barley, which is predominantly self-pollinating, an increase of observed heterozygosity can occur, and the factors contributing to this should be further investigated. No clear differences in genetic diversity parameters between populations cultivated under either organic or

conventional condition for several generations were identified. The lack of clear differentiation between the O and C subpopulations is likely caused by a lack of strong selective pressure and the relatively short time-scale of the study. A significant effect of cultivation environment was found on tillering capacity in organic testing conditions for all tested populations, and in some cases for plant height, ear length and grain number per spike, indicating some adaptation trends.

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