ORIGINAL PAPER

Adel H. Abdel-Ghani · Heiko K. Parzies ·
Ayed Omary · Hartwig H. Geiger

Estimating the outcrossing rate of barley landraces and wild barley populations collected from ecologically different regions of Jordan

Abstract The results of previous studies conducted at the University of Hohenheim and the International Center for Agricultural Research in the Dry Areas (ICARDA) indicated that the yielding ability and stability of barley (*Hordeum vulgare* L.) could be improved in environments with drought stress by increasing the level of heterozygosity. This would require increasing the outbreeding rate of locally adapted breeding materials. As a first step, we estimated the outcrossing rate of 12 barley landraces (*Hordeum vulgare* ssp. *vulgare*, in short *H. vulgare*) and 13 sympatrically occurring populations of its wild progenitor [*Hordeum vulgare* ssp. *spontaneum* (C. Koch), in short *H. spontaneum*] collected from semi-arid localities in Jordan during the 1999/2000 growing season. In each *H. vulgare* or *H. spontaneum* population 28–48 spikes were sampled, and up to six offspring (seeds) per spike (called a family) were used for PCR analyses. Collection sites covered high–low transects for rainfall and altitude in order to detect possible environmental effects on the outcrossing rate. Four microsatellite markers located on different chromosomes were used to genotype the samples for estimating the outcrossing rate. Low season-specific multilocus outcrossing rates (t_m) were found in both cultivated and wild barley, ranging among populations from 0–1.8% with a mean of 0.34%. Outcrossing rates based on inbreeding equilibrium (t_e), indicating outcrossing averaged across years, were two- to threefold higher than the season-specific estimates. Under high rainfall conditions somewhat higher—though not significantly higher—outcrossing rates were observed in *H. spontaneum* than in *H. vulgare*. The season-specific outcrossing rate in *H. spontaneum* was positively correlated ($r=0.67$, $P=0.01$) with average annual precipitation and negatively correlated ($r=0.59$, $P=0.05$) with monthly average temperature during flowering. The results suggest that outcrossing may vary considerably among seasons and that high precipitation and cool temperatures during flowering tend to enhance outcrossing. The rather low levels of outcrossing detected indicate that increased vigour due to heterozygosity has not been a major fitness advantage in the evolution and domestication of *H. spontaneum* and *H. vulgare*, respectively. Stable seed production to secure survival under extreme heat and drought stress may have been more important. Cleistogamy may be considered as an effective mechanism to warrant pollination even in drought-stunted plants with non-extruding spikes.

A. H. Abdel-Ghani · H. K. Parzies · H. H. Geiger (✉)
Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim,
70593 Stuttgart, Germany
e-mail: geigerhh@uni-hohenheim.de
Tel.: +49-711-4592644
Fax: +49-711-4592343

A. Omary
Faculty of Agriculture, Mu'tah University,
P. O. Box 7, Karak, Jordan

Introduction

Drought stress, caused by low and erratic rainfall, is one of the major constraints to barley production in the West Asia and North Africa (WANA) region. Low grain yield and even crop failure are common in this region, where locally adapted landrace populations of barley are predominantly cultivated. Heterozygosity has been demonstrated to be associated with drought tolerance in several crop species, including barley (Allard and Bradshaw 1964; Finlay 1964; Becker and Léon 1988; Mayer et al. 1995; Einfeldt 1999). An increased level of heterozygosity may in the long run be accomplished by developing synthetic or hybrid varieties from germplasm with enhanced outcrossing. Since barley is a self-pollinator with a predominantly cleistogamous flowering behaviour, breeding for high outcrossing would be a necessary first step to achieve this goal.

Outcrossing rates in plants are genetically controlled but are also influenced by environmental conditions. High rainfall and cool environmental conditions can promote

outcrossing in barley (Brown et al. 1978; Chaudhary 1980). Parzies et al. (2000) reported higher outcrossing rates under fluctuating rainfall conditions. Light intensity has been shown to modify the outcrossing rate in other self-pollinated species such as wheat (Demotes-Mainard et al. 1995) and rice (Li et al. 1996). Low population density reduced outcrossing in the cross-pollinated crop *Cuphea laminuligera* (Krueger and Knapp 1991).

Various methods employing genetic markers have been proposed to estimate outcrossing rates in mixed mating populations. Ritland and Jain (1981) and Brown et al. (1978) used progeny arrays (families) to estimate the outcrossing rate of maternal parents in barley; the presence of non-maternal alleles within a family was considered as evidence for an outbreeding event. Brown (1975) suggested assaying a large number of families with very few progeny each for the presence of heterozygote isoenzyme loci. When heterozygotes were present, more offspring of the particular family were tested to distinguish between solitary heterozygotes, originating from outcrossing in the parent generation, and segregating families, tracing back to a maternal plant outcrossed in an earlier generation. Ritland and Jain (1981) showed that when a multilocus model is used, a moderate number of individuals (6–10) per family is optimal for the detection of heterozygotes and subsequent inferences about outcrossing. The advantage behind multilocus estimates is that they allow the detection of outcrossing events even in families with identical alleles at a given locus if non-parental alleles occur at other loci. Enjalbert and David (2000) described a maximum-likelihood procedure to estimate the season-specific outcrossing rate in the parent generation and the average outcrossing rate in the parent and previous generations based on the analysis of multilocus heterozygosity in a single generation assumed to be in inbreeding equilibrium (Brown and Allard 1970). In this case, a simple equation exists between the outcrossing rate and Wright's within-population inbreeding coefficient F (Wright 1969).

Wild barley [*Hordeum vulgare* ssp. *spontaneum* (C. Koch) Thell; in short *H. spontaneum*] is the sole progenitor of cultivated barley (Harlan and Zohary 1966), while barley landraces (*Hordeum vulgare*. ssp. *vulgare*; in short *H. vulgare*) have evolved through natural as well as farmer-directed selection (Harlan 1975; Frankel et al. 1995). Natural populations of *H. spontaneum* and barley landraces from the Fertile Crescent display a high degree of variability in morphological and developmental traits, disease resistance, protein content and allozymes (Nevo et al. 1984; Weltzien 1988, 1989; Weltzien and Fischbeck 1990; Jaradat 1991, 1992).

Knowledge of the level and variation of outcrossing is needed for developing strategies of increasing heterozygosity in barley breeding populations. The major objective of the investigation reported here was therefore to obtain reliable estimates of outcrossing rate in barley landraces collected in situ at semi-arid sites in Jordan and to study the possible effects of climatic and weather factors on this characteristic. We were also interested in the adaptive significance of outcrossing in wild barley populations occurring in natural habitats neighbouring the fields from which the landrace samples were taken.

Materials and methods

Plant materials and eco-geography of the study area

In March 2000, a field trip was organized to locate *Hordeum spontaneum* populations growing in the vicinity (2–3 km) of barley landraces cultivated on farms where farmers preserve their own seeds. On subsequent missions to each collection site, about 50 individual spikes were taken at distances of about 1 m apart. In total, 13 *H. spontaneum* populations and 12 *H. vulgare* landraces were collected during May–June 2000 across the range of the distribution of the species in Jordan (Fig. 1). The collection sites covered high–

Table 1 Eco-geographical information on13 sampling sites of the barley landraces and *Hordeum spontaneum* populations

Region	District	Site	Abbrevation	Altitude (m)	Long-term average seasonal rainfall (mm) (October–May)	Seasonal rainfall in 1999/2000 (mm) (October–May)	Long-term average monthly temperature during flowering (°C)	
							March	April
Northern	Irbid	Sal	IS	490	478	361	12.2	16.6
	Ajloun	Samta	AS	1034	547	906	8.8	12.8
	Jarash	Om Qntara	JO	610	350	458	12.2	16.2
	Mafaraq	Manshia	MM	850	152	170	11.5	15.7
Central	Amman	Sahab	AS	878	275	91	11.7	15.9
	Salt	Yazidia	SY	885	600	471	6.3	10.5
	Ma'daba	Team	MT	785	358	336	11.5	15.3
	Dieban	Baraza	DB	715	270	304	10.9	14.7
Southern	Karak1	Faqo	KF	890	326	204	11.2	15.0
	Karak2	Mu'tah	KM	1,200	350	264	9.2	13.0
	Tafila	Al Bada	TA	1,200	250	131	10.2	14.5
	Shoubak	Ghair	SG	1,460	315	164	7.9	11.7
	Maán	Basta	MB	1,420	160	78	10.1	14.5

Fig. 1 Rainfall distribution and sampling sites in Jordan
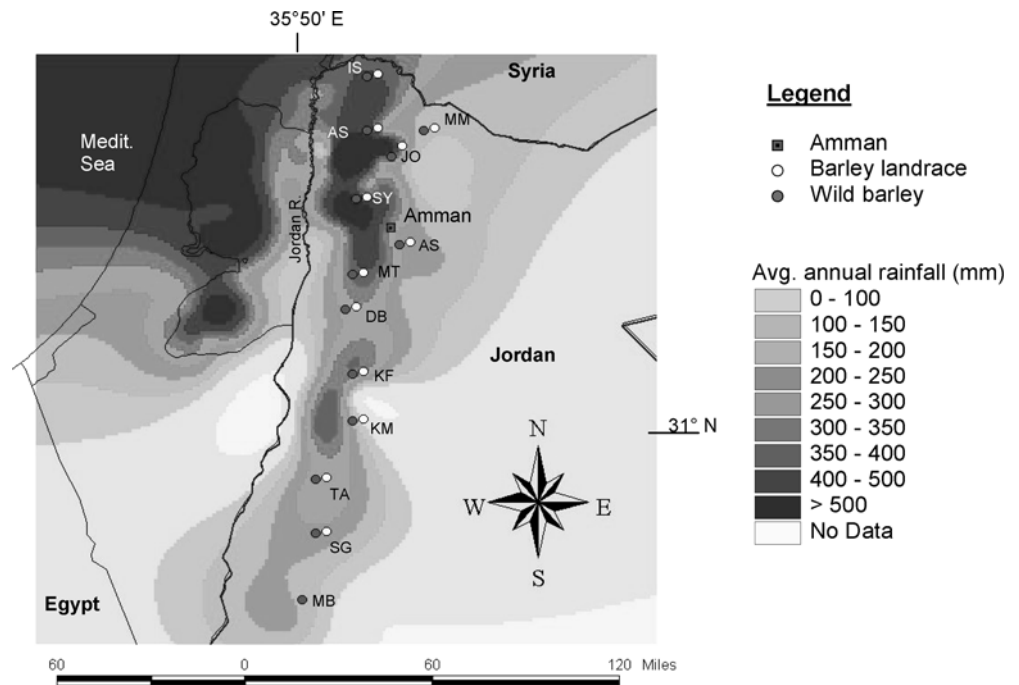
low transects for altitude, rainfall and temperature during flowering
(Table 1). The geographical position of each site was defined using a
map of the scale 1:3,50,000. A digital barometer-altimeter (model
AIR-HB-1L, Sokkia, Europe) was used to determine the altitude of
the site. Long-term average seasonal rainfall and monthly temper-
ature data were obtained from the Water Authority of Jordan and the
Jordan Meteorological Department, whereas the seasonal rainfall
data of 1999/2000 was obtained from local sources at the individual
collection sites.

DNA extraction

For each population 28–48 spikes were sampled, and from each
spike six seeds were germinated and transplanted to small pots. In
what follows, the progeny of an individual spike is called a family.
To break dormancy, we pre-chilled the seeds on a moist filter paper
at 4°C for 3 weeks. Seeds of H. spontaneum, which generally
showed a deeper dormancy, were additionally treated with 0.05%
giberellic acid. Leaves from individual barley seedlings were
harvested at the three-leave stage, stored directly at −80°C and
later dried for 48 h by lyophilization. Thereafter, total genomic DNA
was isolated from dried ground leaf material using the method
described by Saghai Maroof et al. (1984) with minor modifications.

PCR analyses

Four microsatellite markers (WMC1E8, Bmag 0125, Bmag 0353,
and Bmag 0222) were selected from a collection established at the
Scottish Crop Research Institute (SCRI 1999; Macaulay et al. 2001).
Markers were selected on the basis of: (1) specificity (i.e. single
copy); (2) distribution across the genome; (3) polymorphism
information content (PIC); (4) high reproducibility. Microsatellite
primer sequences, repeat motifs, expected product sizes and the
chromosomal locations of these primers are listed in Table 2. PCR
analyses were carried out in 20-μl volumes under the following
conditions: 20–50 ng template DNA, 250 nM of each primer,
200 nM dNTPs, 1 U Taq polymerase and 1.5 mM MgCl$_2$. The
reaction was performed for each primer pair following the
thermocycling programmes recommended by SCRI (1999). The
PCR products were run in 3.5% metaphore agarose gels at 120 V for
about 3 h and subsequently stained with ethidium bromide.

Estimation of outcrossing rates

Multilocus outcrossing rate (t_m) was estimated employing the MLTR
software (Ritland and Jain 1981; Ritland 1990). Estimation is based
on a multilocus mixed-mating (i.e. mixture of outcrossing and self-
fertilization) model assuming self-fertilization at a rate of s and
random mating (outcrossing) at a rate of $t=1−s$. MLTR is applicable to
highly polymorphic markers such as microsatellites, where an
arbitrary number of alleles per locus may occur. The banding pattern

Table 2 Chromosomal locations, repeat motifs, sequences and expected product sizes of the microsatellite primers used for the marker analyses

Microsatellite designation	Chromosomal location	Repeat motif	Forward primer (5′→3′)	Reverse primer (5′→3′)	Product size[a] (bp)
WMC1E8	1H	$(AC)_{24}$	TCATTCGTTGCAGATACACCAC	TCAATGCCCTTGTTTCTGACCT	197
Bmag 0125	2H	$(AG)_{19}$	AATTAGCGAGAACAAAATCAC	AGATAACGATGCACCACC	134
Bmag 0353	4H	$(AG)_{21}$	ACTAGTACCCACTATGCACGA	ACGTTCATTAAAATCACAACTG	119
Bmag 0222	5H	$(AC)_9, (AG)_{17}$	ATGCTACTCTGGAGTGGAGTA	GACCTTCAACTTTGCCTTATA	179

[a]In barley variety Blenheim

of each family depends on the outcrossing rate, the genotype of the parent plant and the gene frequency (p) in the parental pollen cloud. The likelihood procedure is an iterative two-step process, in which first the most likely maternal genotype of each family is inferred and thereafter outcrossing is estimated from deviation of this genotype from progeny arrays. According to Ritland and Jain (1981) and Shaw (1982) only a few—say two to four—polymorphic markers are sufficient to obtain reliable outcrossing rate estimates if an adequate family size—about six seedlings per spike—is chosen. The resulting (season-specific) multilocus outcrossing rate, as estimated by MLTRsoftware, is designated t_m.

A second estimation procedure is based on the equilibrium inbreeding coefficient (F_e) under partial self-ing:$F_e = (1 - t)/(1 + t)$ (Crow and Kimura, 1970). This equation can be transformed to:

$$t = \frac{1 - F_e}{1 + F_e}$$

F_e equals$1 - H_e/H_r$, where H_e is the equilibrium heterozygosity at a given locus, and H_r is the heterozygosity expected under random mating (Hardy–Weinberg equilibrium). For estimating the outcrossing rate, we replaced H_e by the observed heterozygosity, and H_r was computed from estimated allele frequencies (p_I) by the formula $H_r = \sum(1 - p_I^2)$ where summation runs over all alleles at a given locus. The foregoing parameters were calculated for each marker locus separately and then averaged across loci. The resulting estimate is designated t_e.

Results

Family structure

Because of the co-dominance of microsatellite markers, families could be classified into three groups. The first group consists of families in which all of the individuals of each family are exclusively homozygous for a certain allele; the second group comprises families in which one of the six progeny of each family is heterozygous; the third group consists of families showing a diallelic segregating pattern (Fig. 2). We considered homogenous
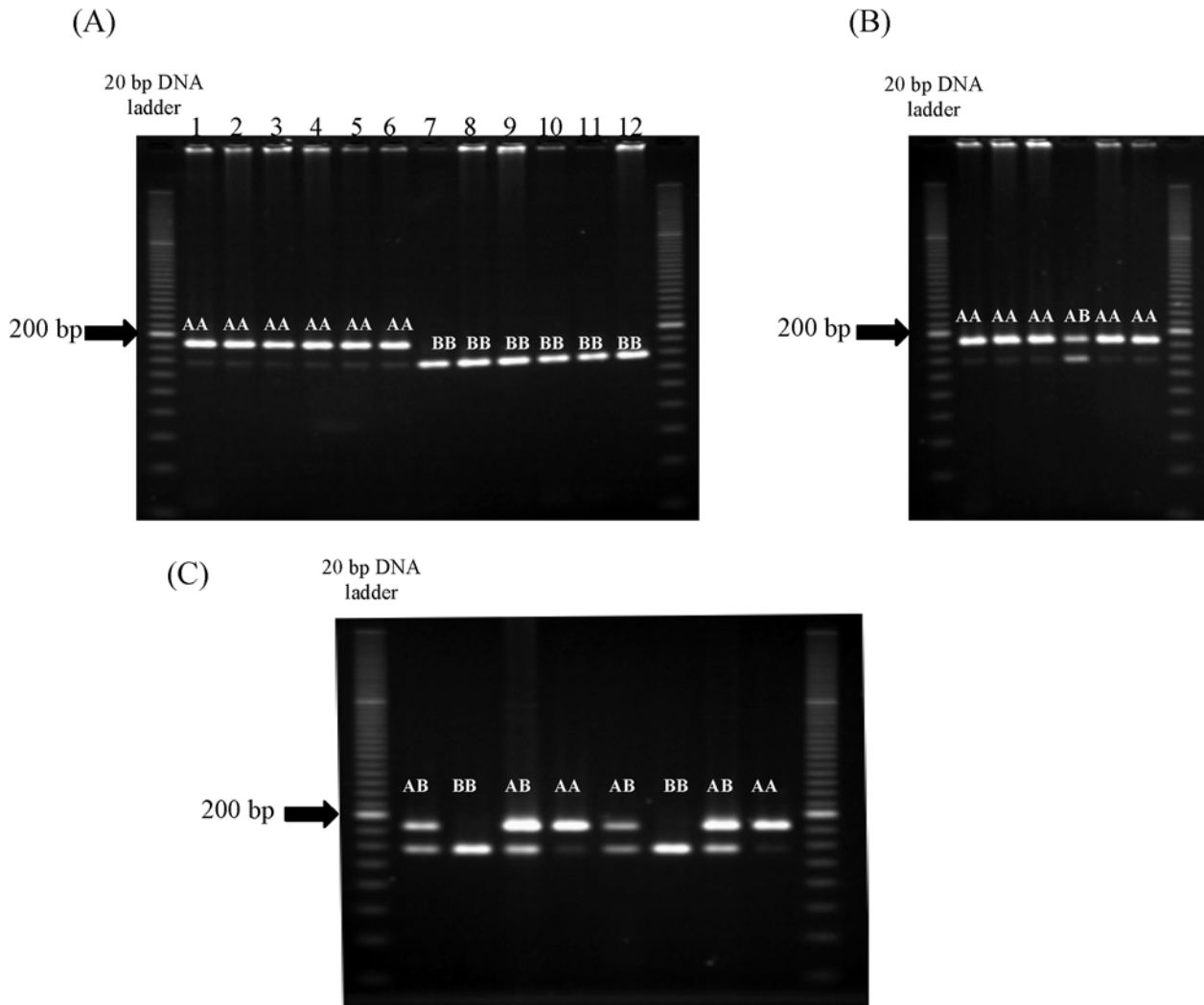

Fig. 2a–c Examples of the three possible banding patterns within families for microsatellite marker WMC1E8. **a** Two uniform families (*lanes 1–6* and *7–12*, respectively) with all of the individuals being homozygous for alleles A and B, respectively, **b** family, in which one individual out of six shows a co-dominant banding pattern, **c** family segregating for homozygous and heterozygous carriers of alleles A and B

Table 3 Estimates of the season-specific (1999/2000) outcrossing rate (t_m), the equilibrium inbreeding coefficient (F_e) and the equilibrium outcrossing rate (t_e) of 12 *Hordeum vulgare* and 13 *H. spontaneum* populations from different districts in Jordan

District	t_m (%)		F_e		t_e (%)	
	H. vulgare	*H. spontaneum*	*H. vulgare*	*H. spontaneum*	*H. vulgare*	*H. spontaneum*
Irbid	0.0	0.0	1.000	0.963	0.00	1.89
Ajloun	0.6	1.3	0.995	0.986	0.24	0.72
Jarash	0.0	0.6	1.000	0.979	0.00	1.07
Mafaraq	0.0	0.0	0.932	0.976	3.54	1.20
Amman	0.5	0.0	0.992	0.934	0.39	3.40
Salt	0.6	1.8	0.984	0.979	0.81	1.06
Ma'daba	0.0	0.6	1.000	0.993	0.00	0.35
Dieban	0.0	0.0	1.000	1.000	0.00	0.00
Karak1	0.6	0.0	0.964	1.000	1.82	0.00
Karak2	0.0	1.8	1.000	0.967	0.00	1.68
Tafila	0.0	0.0	0.995	0.936	0.26	3.32
Shoubak	0.0	0.0	1.000	1.000	0.00	0.00
Maan	–	–	–	1.000	0.00	0.00
Mean	**0.19**	0.47	**0.989**	0.978	**0.59**	1.13

families (group 1) to be progenies of selfed plants, whereas the presence of families in the second and third groups was taken as evidence of outcrossing events having occurred in the parent or a previous generation, respectively. Most families belonged to the first group (93.5%); only very few belonged to the second (1.6%) and third group (4.9%).

Outcrossing rates and inbreeding coefficients

Season-specific outcrossing rates ranged from $t_m=0$ to $t_m=1.8\%$ in the *H. spontaneum* populations and from $t_m=0$ to $t_m=0.6\%$ in the barley landraces (Table 3). The two six-rowed varieties *Rum* and *Acsad 176* had outcrossing rates of $t_m=0.6$ and $t_m=0\%$, respectively. Averaged across populations, the outcrossing rate tended to be higher in the *H. spontaneum* populations (0.47%) than in the landraces (0.19%) and the two local varieties (0.30%), although the differences were not significant. Mean inbreeding coefficients at inbreeding equilibrium (F_e) were close to one. Estimates of the outcrossing rate based on F_e were two- to threefold higher than the season-specific t_m estimates.

Correlations between outcrossing rate and environmental characteristics

Strong to intermediate positive correlations were detected between t_m and both seasonal and long-term average annual precipitation in *H. spontaneum* and barley landraces, respectively (Table 4). Moderate to low negative correlations occurred between t_m and monthly average temperature during the flowering period (March and April). No significant correlation occurred between t_e and rainfall or temperature. Neither t_m nor t_e displayed any significant association with relative humidity and altitude.

Discussion

Outcrossing rate estimates

The very low season-specific outcrossing rates of $t_m=0\%$ to $t_m=1.8\%$ estimated in the present study are within the range of most previously published t_m values for barley and other self-pollinated grasses. Chaudhary et al. (1980) reported a low average outcrossing rate in barley (0.35%) grown under the dry-land conditions of the Canadian prairies. Some authors have obtained slightly higher estimates than those reported in the present study. A

Table 4 Coefficients of correlation of the season-specific and equilibrium outcrossing rates (t_m and t_e, respectively) with climatic and weather characteristics in *H. vulgare* landraces and *H. spontaneum* populations

Species	Outcrossing rate	Long-term rainfall	Rainfall in 1999/2000	Long-term temperature	
				March	April
H. vulgare	t_m	0.50	0.36	−0.35	−0.33
	t_e	−0.39	−0.24	0.13	0.14
H. spontaneum	t_m	0.67**	0.59*	−0.60*	−0.62*
	t_e	−0.02	0.19	0.26	0.20

*, **Significant at 0.05 and 0.01 probability levels, respectively

mean outcrossing rate of 1.7% was reported by Parzies et al. (2000) for ten populations of barley landraces from different eco-geographical regions in Syria; this group used two co-dominant isozyme markers and employed the same statistical methodology as used in this study. Brown et al. (1978), using isozyme markers, reported a mean outcrossing rate of 1.6% (range: 0–9.6%) in 28 populations of *H. spontaneum* from Israel. Notably, 15 of these populations showed 0% outcrossing. Doll (1987) reported a 5% outcrossing rate in autumn-sown lines and a less than 0.5% outcrossing rate in spring-sown lines. Considering other self-pollinated cereals, Golenberg (1988) calculated an outcrossing rate for *Triticum dicoccoides* of between 0.7% and 0.18%. For *Avena barbata*, Kahler et al. (1980) reported an outcrossing rate of less than 1%, whereas other authors reported outcrossing rates of greater than 7% for this species (Marshall and Allard 1970; Allard et al. 1972).

The comparatively low outcrossing rates found in the present study for both barley landraces and *H. spontaneum* populations from Jordan may partly be due to the marker system used, since only morphological or isozyme markers were employed in previous studies. Furthermore, the sampling strategies varied among studies. For instance, Brown et al. (1978) used a two-step procedure: they first analysed a single seedling per family and, in a second step, they extended the analysis to four seedlings if the first seedling proved to be heterozygous.

The automatic selection theory of Fischer (1941, cited in Jain 1976) may provide a general explanation for the low outcrossing rates found in barley. Automatic selection may have discriminated against partially cross-pollinating genotypes and in favour of strictly self-pollinating genotypes because the latter may well contribute male gametes to the population's "pollen cloud" but in turn do not pick up any gametes from it. Thus, the frequency of genes favouring cleistogamy may have increased gradually over generations.

Influence of climatic conditions

Our results showed that the season-specific outcrossing rate estimates of *H. spontaneum* populations were associated with both long-term climatic and actual weather characteristics. Surprisingly, no significant correlation could be detected between the estimates of equilibrium outcrossing rate and any of the climatic or weather characteristics. Correlation coefficients showed a similar tendency for the landraces though none of the coefficients reached significance. Thus, high annual precipitation and cool temperatures during flowering time seem to enhance outcrossing, at least in wild barley populations. This may be explained by the observation that under severe drought and heat stress spikes usually remain within the flag sheath and, consequently, pollination and fertilization occur while the spike is still enclosed. Contrary to this, under high annual rainfall and cool temperatures during flowering, the spike emerges from the flag sheath before flowering, thus allowing for anther extrusion and cross-pollination among chasmogamous genotypes. In addition, under cool and moist conditions, higher outcrossing rates may be caused by extended pollen viability (Hammer 1977; Lansac et al. 1994; Gupta et al. 2000). Our results agree with the findings of Giles et al. (1974), Brown et al. (1978) and Chaudhary et al. (1980) who showed that cool and moist conditions promote outcrossing in barley.

Estimates of the equilibrium outcrossing rate suggest that, averaged across years, outcrossing may occur much more frequently than what we observed in the 1999/2000 growing season. The 1999/2000 growing season was extremely dry during the springtime at several of the collection sites. In any case, the discrepancy between the t_m and t_e estimates indicates considerable annual fluctuations of the outcrossing rate. Similar observations have been reported for barley populations under dry conditions in the Canadian Prairies (Chaudhary 1980) as well as in other self-fertilized species such as *Phaseolus lunatus* (Allard and Workman 1963), *Avena fatua* (Imam and Allard 1965) and in allogamous but self-fertile rye materials (Geiger and Schnell 1970).

Differences between cultivated and wild barley

The experimental design of the present study enabled us to compare the outcrossing rates of cultivated barley landraces with those of sympatrically occurring populations of wild barley over a wide range of environmental conditions in a supposed centre of origin of barley. No significantly different outcrossing rates were observed between *H. vulgare* and *H. spontaneum*. Hammer (1975, 1977, 1984), however, found that outcrossing-related floral traits such as stigma size, anther length and anther extrusion were reduced in most of the cultivated cleistogamous barleys in comparison to chasmogamous *H. spontaneum* accessions. A possible explanation for the results of the present study is provided by the *reproductive assurance* hypothesis (Jain 1976; Barrett et al. 1990) which claims that the conditions for cross-pollination are unfavourable when (1) a single seed establishes a new colony following long-distance dispersal; (2) plant density and, consequently, opportunities for cross-pollination are low; (3) severe water, cold and heat stress reduce the availability of pollen from other plants. This may have applied to barley under domestication, when plants have been moved to remote areas and initially grown under complete isolation at low density. Additionally, farmers probably have unconsciously opted for cleistogamy to avoid crop failure in the above-described stress situations.

All in all, the extremely low level of outcrossing detected under drought and heat stress conditions in the present study indicates that increased vigour due to heterozygosity has not been a major force in the course of evolution and domestication of barley. Survival through cleistogamy seems to have been more important. Cleistogamy may be considered to be an effective mechanism to warrant pollination even in drought-stunted plants with non-dehiscent spikes.

594

Consequences for crop improvement

Drought is the prevalent abiotic stress in barley growing areas in the WANA region. Intergenotypic diversity (heterogeneity) and intragenotypic diversity (heterozygosity) are potential buffering mechanisms against unpredictable stresses (Finlay 1964; Mayer et al. 1995; Einfeldt 1999). The present study reveals a very low level of outcrossing in natural and cultivated barley populations under arid and semi-arid conditions. To capitalize on these buffering mechanisms, the outcrossing behaviour needs to be increased by breeding. Geiger et al. (1994) suggested recurrent selection for outcrossing-related floral traits such anther extrusion and anther and stigma size as a possible approach. The barley gene bank of the International Center for Agricultural Research in the Dry Areas (ICARDA) contains a number of drought-adapted accessions showing various degrees of open-pollination (Abdel-Ghani et al. 2003). This material is presently being evaluated for its outcrossing behaviour as it is considered to be a valuable genetic resource for increasing the level of heterozygosity in stress-adapted barley gene pools and combines a high-yielding potential with superior drought tolerance.

References

Abdel-Ghani AH, Parzies HK, Ceccarelli S, Grando S, Geiger HH (2003) Evaluation of floral characteristics of barley in the semi-arid climate of north Syria. Plant Breed 122:273–275

Allard RW, Bradshaw A (1964) Implications of genotype-environmental interactions in applied plant breeding. Crop Sci 4:503–508

Allard RW, Workman PL (1963) Population studies in predominantly self-pollinated species. IV: Seasonal fluctuation in estimated values of genetic parameters in lima bean. Evolution 17:470–480

Allard RW, Babbel GR, Clegg MT, Kahler AL (1972) Evidence for coadaptation in *Avena barbata*. Proc Natl Acad Sci USA 69:3043–3048

Barrett SCH, Husband BC (1990) The genetics of plant migration and colonisation. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (eds) Plant population genetics, breeding and genetic resources. Sinauer, Sunderland, Mass., pp 254–277

Becker H, Léon J (1988) Stability analysis in plant breeding. Plant Breed 101:1–23

Brown AHD (1975) Efficient experimental designs for the estimation of genetic parameters in plant populations. Biometrics 31:145–160

Brown AHD, Allard RW (1970) Estimation of the mating system in open-pollinated maize populations using isozyme polymorphisms. Genetics 66:133–145

Brown AHD, Zohary D, Nevo E (1978) Outcrossing rates and heterozygosity in natural populations of *Hordeum spontaneum* Koch in Israel. Heredity 41:49–62

Chaudhary HR, Jana S, Acharya SN (1980) Outcrossing rates in barley populations in the Canadian prairies. Can J Genet Cytol 22:353–360

Crow JF, Kimura M (1970) An introduction to population genetics theory, 1st edn. Harper and Row, New York

Demotes-Mainard S, Doussinault G, Meynard JM (1995) Abnormalities in the male developmental program of winter wheat induced by climatic stress at meiosis. Agronomie 16:505–515

Doll H (1987) Outcrossing rates in autumn and spring-sown barley. Plant Breed 98:339–341

Einfeldt CHP (1999) Effects of heterozygosity and heterogeneity on yield and yield stability of barley in the dry areas of North Syria. PhD thesis, University of Hohenheim, Stuttgart

Enjalbert J, David J (2000) Inferring recent outcrossing rates using multilocus heterozygosity: application to evolving wheat populations. Genetics 156:1973–1982

Finlay KW (1964) Adaptation—its measurement and significance in barley breeding. In: Proc 1st Int Barley Genet Symp. PUDOC, Wageningen, The Netherlands, pp 351–359

Fischer RA (1941) Average excess and average effect of a gene substitution. Ann Eugen 11:53–63

Frankel H, Burdon J, Peacock J (1995) Landraces in transit—the threat perceived. Diversity 11:14–15

Geiger HH, Schnell FW (1970) Die Züchtung von Roggensorten aus Inzuchtlinien I. Selbstungsanteile in Polycross-Nachkommenschaften. Theor Appl Genet 40:305–311

Geiger HH, Hauser C, Oettler G, Wahle G (1994) Recurrent selection in self-pollinated cereals: theoretical background and first experimental results in triticale. In: Proc Eucarpia Symp Prospectives Cereal Breed Europe: New Methods Cereal Breed. Landquart, Switzerland, pp 39–40

Giles RJ, McConnell G, Fyfe JL (1974) The frequency of natural-cross fertilization in a composite cross of barley grown in Scotland. J Agric Sci 83:447–450

Golenberg EM (1988) Outcrossing rates and their relationship to phenology in *Triticum dicoccoides*. Theor Appl Genet 75:937–944

Gupta SK, Singh D, Sharma SC (2000) Genetic variability for allogamic traits in barley (*Hordeum vulgare* L.). Agric Sci Digest 20:1–4

Hammer K (1975) Die Variabilität einiger Komponenten der Allogamieneigung bei der Kulturgerste (*Hordeum vulgare* L.). Kulturpflanze 23:167–180

Hammer K (1977) Fragen der Eignung des Pollens der Kulturgerste (*Hordeum vulgare* L. s.l.) für die Windbestäubung. Kulturpflanze 25:13–23

Hammer K (1984) Blütenbiologische Untersuchungen an der Gaterslebener Wildgersten-Kollektion (*Hordeum* L. subgenus *Hordeum*). Kulturpflanze 32:79–88

Harlan JR (1975) Our vanishing genetic resources. Science 188:618–621

Harlan JR, Zohary D (1966) Distribution of wild wheats and barley. Science 153:1074–1080

Imam AG, Allard RW (1965) Population studies in predominantly self pollinated species VI. Genetic variability between and within natural populations of wild oats from differing habitats in California. Genetics 51:49–62

Jain SK (1976) The evolution of inbreeding in plants. Annu Rev Ecol Syst 7:469–495

Jaradat AA (1991) Grain protein variability among populations of wild barley (*Hordeum spontaneum* C. Koch) from Jordan. Theor Appl Genet 83:164–168

Jaradat AA (1992) Genetic diversity of four estrase loci in natural populations of *Hordeum spontaneum* C. Koch from Jordan. Theor Appl Genet 84:725–729

Kahler AL, Allard RW, Krazkowa M, Wehrhahn CF, Nevo E (1980) Association between isozyme phenotype and environment in the slender wild oat (*Avena barbata*) in Israel. Theor Appl Genet 56:31–47

Krueger SK, Knapp SJ (1991) Mating systems in *Cuphea laminuligera* and *Cuphea lutea*. Theor Appl Genet 82:221–226

Lansac AR, Sullivan CY, Johnson BE, Lee KW (1994) Viability and germination of the pollen of sorghum (*Sorghum bicolor* (L.) Moench). Ann Bot 74:27–33

Li HB, Zhang Q, Liu AM, Zou JS, Chen ZM (1996) A genetic analysis of low-temperature-sensitive sterility in *Indica japonica* rice hybrid. Plant Breed 115:305–309

Macaulay M, Ramsay L, Powell W (2001) A representative 'highly informative' genotyping set of barley SSRs. Theor Appl Genet 102:801–809

Marshall DR, Allard RW (1970) Maintenance of isozyme polymorphisms in natural populations of *Avena barbata*. Genetics 66:393–399

Mayer M, Gland A, Ceccarelli S, Geiger HH (1995) Comparison of doubled haploid lines and F_2 bulks for the improvement of barley in the dry areas of North Syria. Plant Breed 114:45–49

Nevo E, Beiles A, Gutterman Y, Storch N, Kaplan D (1984) Genetic resources of wild cereals in Israel and vicinity II. Phenotypic variation within and between populations of wild barley (*Hordeum sponataneum*). Euphytica 33:737–756

Parzies HK, Spoor W, Ennos RA (2000) Outcrossing rates of barley landraces from Syria. Plant Breed 119:520–522

Ritland K (1990) A series of FORTRAN computer programs for estimating plant mating system. J Hered 81:235–237

Ritland K, Jain S (1981) A model for the estimation of outcrossing rate and gene frequencies using *n* independent loci. Heredity 47:35–52

Saghai Maroof MA, Soliman K, Jorgensen R, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Scottish Crop Research Institute (SCRI) (1999) Barley SSR primer sequences: http://www.scri.sari.ac.uk/ssr/pdf

Shaw DV (1982) Optimum number of marker loci for estimating outcrossing in plant populations. Theor Appl Genet 61:231–235

Weltzien E (1988) Evaluation of barley (*Hordeum vulgare* L.) landrace populations originating from different growing regions in the Near East. Plant Breed 101:95–106

Weltzien E (1989) Differentiation among barley landrace populations from the Near East. Euphytica 43:29–39

Weltzien E, Fischbeck G (1990) Performance and variability of local barley landraces in near-eastern environments. Plant Breed 104:58–67

Wright S (1969) Evolution and genetics of populations, vol 2. The theory of gene frequencies. University of Chicago Press, Chicago