Estimation of outcrossing rate in *Hordeum spontaneum* and barley landraces from Jordan

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Abstract
Previous studies conducted at the University of Hohenheim and ICARDA indicated that yielding ability and stability of barley (*Hordeum vulgare* L.) *could be improved in environments with drought stress by increasing the level of heterozygosity. This could be accomplished by developing synthetic varieties composed of germplasm with a high outcrossing rate. As a first step, we characterized the genetic structure of barley landraces and H. spontaneum populations collected from various semi-arid localities in Jordan to obtain reliable estimates of the amount and variation of outcrossing in this germplasm.*

Natural outcrossing was estimated in 13 populations of H. spontaneum and 12 barley landraces from collection sites that covered high-low transects for rainfall and altitude to detect possible environmental effects on outcrossing rate. Four microsatellite markers were used to estimate the outcrossing rate based on maximum likelihood methods and mixed mating models.

Low outcrossing rates were found in cultivated barley and its wild relative H. spontaneum ranging among populations from 0-1.8% with a mean of 0.32%. In H. spontaneum, somewhat higher, though not significant, outcrossing rates were observed than in H. vulgare populations under high rainfall conditions. A significant positive correlation was detected between outcrossing rate and average annual precipitation. However, a negative correlation occurred between outcrossing rate and monthly average temperature during flowering. Results suggest that high precipitations and cool temperatures during flowering may enhance outcrossing in cultivated and wild barley populations.

The rather low levels of outcrossing indicate that increased vigor due to heterozygosity has not been a major force 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 be more important. Cleistogamy may be considered as an effective mechanism to prolong pollen viability to warrant pollination even in drought-stunted plants with non-dehiscent spikes. Yet ICARDA's gene bank contains a number of drought-adapted accessions showing various degrees of open-pollination. This material is presently being evaluated for its outcrossing behavior. It is considered a valuable genetic resource for increasing the level of heterozygosity in actual barley gene pools and thus combining high yielding potential with superior drought tolerance.

Keywords*: Barley, Outcrossing rate, Microsatellites, Landrace, Plant genetic resources, Hordeum spontaneum*

2. Background and objectives of the study

Drought stress caused by low and erratic rainfall is considered as 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 predominantly locally adapted landrace populations of barley are cultivated. An increase of heterozygosity has been proposed by numerous authors as an approach to improve yielding ability and stability of barley under water stress conditions. Finlay (1964) reported that heterogeneous and partially heterozygous F_2 populations outyielded their homozygous parents substantially in variable stress environments of South Australia. He observed a 15.7% and 1.3% yield advantage in the lowest yielding environment (257 mm rainfall) and in the favourable high yielding environment (386 mm rainfall), respectively. These observations were confirmed in a study by Mayer et al. (1995) who found a superiority of F_2-derived bulks over completely homozygous doubled-haploid lines in barley for grain yield under low yielding drought conditions. This indicates the advantage of a heterogeneous and partially heterozygous population structure under drought stress. Einfeldt (1999) showed that in crosses among non-adapted barley lines yield increases due to heterozygosity were higher under severe drought stress conditions (270 mm rainfall) than under moderate drought stress conditions (380 mm). Similar results were obtained for F_2-bulks of crosses between non-adapted and locally adapted lines. An increased level of heterozygosity may be accomplished by developing synthetic varieties composed of germplasm with a high outcrossing rate. As a first step to pursue this strategy, we characterized the genetic structure of barley landraces (*Hordeum vulgare* ssp. *vulgare*) and wild barley (*H. vulgare* ssp. s*pontaneum*, in short *H. spontaneum*) populations collected from various semi-arid localities in Jordan, to obtain reliable estimates of the amount and variation of outcrossing in this germplasm.

3. Material and Methods

In March 2000, a field trip was conducted in order to locate *H. spontaneum* populations in the vicinity of cultivated barley landraces on farms, where farmers preserve their own seeds. On a subsequent mission at each collection site, individual spikes were taken at distances of about 1 m in each direction. In total, 13 *H. spontaneum* populations and 12 *H. vulgare* landraces were collected during May-June 2000 across the range of distribution of the species in Jordan (Table 1). The collection sites covered high-low transects for altitude, rainfall and temperature during flowering (Table 1). The eco-geographical information of collection sites is presented in Table 1. Furthermore, individual spikes of six-rowed local varieties (i.e. Rum and Acsad 176) were collected from the National Centre for Agricultural Research and Technology Transfer (NCARTT) seed production fields, to be used as checks in this investigation.

Table 1: Eco-geographical information of collection sites in Jordan

Region	Site	Latitude	Longitude	Annual rainfall (mm)	Altitude (m)	Temperature during flowering time	
						March	April
Northern	Irbid	° 32 ′39	° 35 ′49	478	490	12.2	16.6
	Ajloun	° 32 ′24	° 35 ′49	547	1034	8.8	12.8
	Jarash	° 32 ′17	° 35 ′56	350	610	12.2	16.2
	Mafaraq	° 32 ′24	° 36 ′05	152	850	11.5	15.7
Central	Amman	° 31 ′52	° 35 ′58	275	878	11.7	15.9
	Salt	° 32 ′05	° 35 ′46	600	885	6.3	10.5
	Ma'daba	° 31 ′44	° 35 ′45	358	785	11.5	15.3
	Dieban	° 31 ′34	° 35 ′43	270	715	10.9	14.7
Southern	Karak1	° 31 ′16	° 35 ′45	326	890	11.2	15.0
	Karak2	° 31 ′03	° 35 ′24	350	1200	9.2	13.0
	Tafila	° 30 ′47	° 35 ′35	250	1200	10.2	14.5
	Shoubak	° 30 ′32	° 35 ′35	315	1460	7.9	11.7
	Maán	° 30 ′13	° 35 ′31	160	1420	10.1	14.5

Four microsatellite primer pairs with high PIC values, were selected for outcrossing rate estimations. The reaction was performed for each primer pair following the programs recommended by the Scottish Crop Research Institute (SCRI; http://www.scri.sari.ac.uk/ssr/pdf). Multi-locus outcrossing rate (t%) was estimated using maximum likelihood methods based on the mixed mating model (Ritland and Jain, 1981). Analyses were conducted employing the MLTR-program supplied by K. Ritland (Ritland 1990).

3 Results and Discussion

4.1 Family banding patterns

Because of the co-dominance of microsatellite markers, homozygous and heterozygous individuals could be distinguished allowing that families (i.e. 3-6 offpring per spike) could be classified into three groups. The first group consisted of families with exclusively homozygous individuals, all showing the same allele. The second group consisted of families, homozygous for all but one individual, which was showing a co-dominant banding pattern. It can be assumed that this individual resulted from an outcrossing event in the parent generation. The third group consisted of families segregating for banding pattern as expected for selfed progenies of heterozygous parents. Most families belonged to the first group, whereas very few belonged to the second and third group as expected for a predominantly self pollinated crop.

4.2 Outcrossing rate estimates

The overall level of outcrossing ranged from 0 to 1.8% in *H. spontaneum* populations and from 0 to 0.60% in barley landraces. The two local varieties Rum and Acsad showed an average outcrossing rate of 0.60% and 0%, respectively. The overall means were higher in the *H. spontaneum* populations (0.47% ± 0.71) than in the barley landraces (0.24% ± 0.30) and the local varieties (0.3% ± 0.42), though not significantly. The high standard deviations of population means were not unexpected, since population means ranged widely and with most populations showing 0 outcrossing. This almost complete autogamy can mainly be explained by the flowering characteristics of barley. Normally flowers open only after the pollen has been shed onto the stigmata thus preventing cross-fertilization (Jain et al. 1979 and Hammer 1975). The extremely low estimates of outcrossing support earlier observations in various barley materials (Brown et al. 1978; Chaudhary et al. 1980; Parzies et al. 2000). Outcrossing rates of ten accessions of barley landraces from different eco-geographical regions in Syria were studied using two co-dominant isozymes (*Est*1 and *Est*2). The average outcrossing rate was 1.7% (Parzies et al. 2000). Similar results were obtained by Brown et al. (1978) who reported a mean outcrossing rate of 1.7% in 28 populations of *H. spontaneum* using isozyme marker, ranging from 0-9.6%. Notably, fifteen of these populations showed 0% outcrossing. Also, Chaudhary et al. (1980) reported a low average outcrossing rate in barley (0.35%) under dry-land conditions of the Canadian prairies. It can be concluded that barley in general displays a very level low of outcrossing.

4.3 Effect of environmental conditions on outcrossing rate

Regarding rainfall level, the collection sites were grouped into three types of environments, (a) humid regions, receiving more than 400 mm rainfall, (b) semi-arid regions receiving 200-400 mm, and (c) arid regions receiving less than 200 mm. Outcrossing estimates were higher in populations collected from humid sites than those collected from arid and semi-arid sites, though not significantly. The overall mean outcrossing rates in *H. spontaneum* were 1.03%, 0.40 % and 0 % for populations collected from humid, semiarid and dry sites, respectively. In barley landraces, outcrossing rates of 0.40%, 0.21% and 0% were observed for populations collected from humid, semi-arid and dry sites, respectively.

A strong to intermediate positive correlation was detected between outcrossing rate and average annual precipitation in *H. spontaneum* and barley landraces, respectively. Furthermore, a positive correlation was detected between outcrossing rate and relative humidity, and a negative correlation was calculated for the comparison between outcrossing rate and monthly average temperature during flowering period. Thus, high precipitation and cool temperatures during flowering time may enhance outcrossing in wild and cultivated barley populations. The differences in outcrossing rate among groups of populations is probably due to the fact that under sever drought and heat stress conditions spikes usually remain within the flag sheath and, as a consequence, pollination and fertilization will take place while the spike is still enclosed. These characteristics will decrease the possibility of outcrossing and advance the tendency towards autogamy. However, under favorable conditions, i.e. high rainfall and cool temperature during flowering, the peduncle will extend and the spike will emerge from the flag sheath before flowering occurs which may lead to the possibility of increased cross pollination. The present assumptions agree with findings of Brown et al. (1978), who found that populations collected from sub-humid regions had a mean outcrossing level about four times higher than those collected from dry regions. This is further in agreement with findings of Gils et al. (1974) and Chaudhary et al. (1980) who showed that cool and moist conditions promote natural outcrossing in barley.

Somewhat higher, though not significant outcrossing rates were observed in *H. spontaneum* than barley landraces under high rainfall conditions (> 400 mm annual rainfall). Results indicate that outcrossing related traits might have been subjected to unconscious selection due to cultivation during millennia of barley cultivation in the Fertile Crescent, and corresponds with findings of Hammer (1975, 1977 and 1984), who found that *H. spontaneum* has longer anthers than cultivated barley. Furthermore, outcrossing related components, such as stigma size, anther length and anther extrusion were reduced for most cleistogamous cultivated barleys compared to chasmogamous *Hordeum* species such as *H. bulbosum* (Hammer 1975 and 1984). The extremely low level of outcrossing detected in the present study indicates that increased vigour due to heterozygosity has not been a major force in the course of evolution of barley. Survival through cleistogamy seems to have been more important.

4.4 Outlook

Drought is the prevalent abiotic stress in barley growing areas in Syria and Jordan. Distribution and amount of rainfall vary greatly. The increase of intergenotypic diversity (i.e. heterogeneity) and of intragenotypic diversity (i.e. heterozygosity) are potential buffer mechanisms against such unpredictable drought stress conditions (Finlay 1964; Einfeldt 1999; Mayer et al., 1995). Outcrossing rate and consequently the level of heterozygosity can be increased by selecting for large protruding anthers and vigorous stigmata which may build up some pressure on lemma and palea, causing the opening of the floret and, in consequence, increasing the possibility of cross fertilization. It seems likely that recurrent selection for outcrossing-related floral traits is a possible approach to increase heterozygosity in barley (Geiger et al., 1994), since high variability in floral traits has been found in barley (Hammer 1975, 1984) and other autogamous species. For instance, genetic variability of floral characters was investigated in cultivated and wild rices (*Oryza sp.*) (Virmani and Athwal 1973) and revealed a wide variability in anther length, stigma length and percentage of exerted stigmata. Therefore, selection for high anther extrusion and longer anthers and stigmata may be a possible approach to improve outcrossing rate and, in consequence, improve yielding ability and stability based on higher heterozygosity.

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