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Prof. Dr. Dr. h.c. H.H. Geiger

Recurrent Selection for Increased Outcrossing Rates of Barley from Semi-arid Regions of Syria and Jordan

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Von
Aruna Nandety, M.Sc
aus Guntur – India

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Prodekan		Prof. Dr. Andreas Fangmeier
Berichterstatter,	1. Prüfer:	Prof. Dr.Dr.h.c. Hartwig.H. Geiger
Mitberichterstatter,	2. Prüfer:	Prof. Dr. Folkard Asch
	3. Prüfer:	Prof. Dr. Wilhelm Claupein

This PhD thesis is dedicated to my dad *Vijay Vardhan Rao Nandety*.

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List of Abbreviations

^{o}C	Degree Celsius
%	Percentage
kg	Kilogram
µg	Microgram
µl	Microlitre
bp	Base pair
A	Number of alleles
h	Hour
g	Gram
ng	Nanogram
mg	Milligram
mM	Millimolar
nM	Nanomolar
ha	Hectare
mm	Millimeter
min	Minute
DNA	Deoxyribonucleic acid
H_O	Observed Heterozygosity
ICARDA	International Center for Agricultural Research in Dry Areas
IPK	Institute of Plant Genetics and Crop Plant Research
PCR	Polymerase chain reaction
PIC	Polymorphic information content
SAS	Statistical Analysis Sofware
SSR	Simple Sequence Repeats
MLTR	Software for estimating multi-locus outcrossing rate
t_m	Multi-locus outcrossing rate
DTE	Days to ear emergence
GWT	Grain weight
SN	Seed number
WANA	West Asia and North Africa
FAO	Food and Agriculture Organization
RS	Recurrent selection
MARS	Marker assisted recurrent selection
dNTP	Deoxyribo nucleotide triphosphates
TBE	Tris-borate-EDTA
sec	Second
KV	Kilovolt
rfu	Relative fluorescence unit

1. Introduction

1.1 Barley

In low rainfall areas of the West Asia and North Africa (WANA) region, arid and semi-arid lands constitute the vast majority of the land area. The WANA region, as defined by the United Nations Food and Agriculture Organization (FAO), includes twenty countries stretching from Morocco to Afghanistan. It is characterized by hot dry summers and cool winters with annual precipitation below 1200mm. In the cereal-livestock production system which dominates in the WANA region, barley (*Hordeum vulgare* L.) is the key crop and is widely used as human food as well as feed for farmer's livestock. Although barley shows high adaptability to drought, the possibility of crop failure is high in semi-arid areas. The grain yield reaches 836 kg ha^{-1} on average (ranging from 283 to1342 kg ha^{-1}) in Jordan and 658 kg ha^{-1} (ranging from 160 to1500 kg ha^{-1}) in Syria (FAO 1997-2007).

1.2 Superior performance of heterozygous and heterogeneous genotypes under marginal conditions

It has been shown that heterozygosity enhances the level and stability of yielding performance in corn (Schnell and Becker 1986) and other crops such as sorghum (*Sorghum bicolor* L.) (Reich and Atkins 1970), rapeseed (*Brassica napus*) (Léon 1991) and faba bean (*Vicia faba* L.) (Stelling et al. 1994a). In sorghum, Haussmann et al. (2000) found that heterozygous entries of sorghum significantly out-yielded the homozygous entries for grain yield in both stress and non stress environments in a semi-arid area of Kenya. In barley, considerable increases of grain yield were observed in partially heterozygous F_2 populations compared to completely homozygous lines grown under drought conditions (Einfeldt et al. 2005, Mayer et al. 1995). Comparing homozygous lines in mixture and pure stand, the latter authors also observed a positive influence of heterogeneity. But the advantage of heterozygosity was much greater than that of heterogeneity.

1.3 Floral biology and outcrossing

Very low outcrossing rates were recorded in barley (less than 2%) because of predominantly cleistogamous flowering behavior (Giles et al. 1974, Brown et al. 1978, Doll, 1987, Parzies et al. 2000, 2008). Abdel-Ghani et al. (2004) found the outcrossing rate to be very low (0-1.8%) in drought adapted barley landraces and wild barley populations from Jordan. In various studies, ample quantitative variation was found for supposedly outcrossing related floral traits presuming that these traits might be related with the level of outcrossing (Virmani and Athwal 1973, Ceccarelli 1978, Doll 1987, Gupta 2000, Abdel-Ghani et al. 2003, 2005). Hence, Einfeldt et al. (2005) suggested that the genetic modification of these floral traits by means of recurrent selection may be a fast way to make the beneficial effects of heterosis available to low-income farmers.

Lodicule size differed markedly between cleistogamous and non-cleistogamous phenotypes in barley, pointing out that non-cleistogamous types have large-sized lodicules pushing the lemma and palea apart, causing the elongation of anther filaments and the release of pollen (Honda et al. 2005, Nair et al. 2010). Many authors found that this phenotypic difference results because of environmental conditions (Matsui et al. 2000 a, b, Rehman et al. 2004, Abdel-Ghani et al. 2005). Contrastingly, Turuspekov et al. (2004) found that the expression of cleistogamy is controlled by two-tightly linked genes. Recently, Nair et al. (2010) isolated the cleistogamous gene *Cly1* and found that this gene is expressed in the lodicules primordial cells and that the cleistogamous state in barley is recessive.

1.4 Recurrent selection using molecular markers

Recurrent selection (RS) is a cyclic breeding system aiming at a gradual increase in frequency of desirable alleles for a particular quantitative characteristic without a marked loss of genetic variability. The basic technique involves the identification of individuals with superior genotypes and their subsequent intermating to produce a new population.

A wide range of recurrent selection methods is being used for population improvement depending on the genetic units to be selected, the type of progeny to be evaluated, the intermating scheme and the genetic units to be intermated. In any method, the common goal is to improve the population mean performance while maintaining sufficient genetic variability in the population to warrant long-term selection response (Weyhrich et al. 1998). Recurrent selection has been used for various quantitative traits including grain yield, but no study has been reported until now on increasing heterozygosity. In this study, molecular markers are used to assess the genotypic constitution (homozygosity versus heterozygosity) of a plant as a criterion of outcrossing events in the parental generation or earlier. Selection for heterozygosity was performed over four RS cycles to accumulate alleles favoring natural outcrossing.

1.5 Objectives of the study

The general aim of present study was to create barley germplasm with increased heterozygosity. It was a project of the University of Hohenheim funded by the "Deutsche Forschungsgemeinschaft" (DFG). Source materials used for the research were obtained from the gene banks of the International Center for Agricultural Research in Dry Areas (ICARDA) and of the Institute of Plant Genetics and Crop Plant Research (IPK) in Germany. Previous studies had shown that heterozygous, heterogeneous barley materials are more stable than homozygous homogeneous materials and display increased grain and biomass yield in arid environments. Therefore, creating barley materials with increased heterozygosity can be expected to significantly improve the performance under those conditions.

The specific objectives of the study were:

1. To identify barley accessions displaying above-average heterozygosity in a genetically broad based collection of WANA-adapted genotypes. Marker-based RS for heterozygosity was performed over four generations to gradually increase the level of outcrossing in a population composed of those superior accessions. The key concept of the selection procedure was that plants which, according to their marker genotype, originate from outcrossing will inherit this characteristic to their offspring.

2. To compare the heterozygosity levels and the inferred outcrossing rates of the materials selected in each RS cycle in a final experiment under common environmental conditions and to analyse the relationships between heterozygosity and visually assessable flowering traits.

3. To estimate the population parameters response to selection, selection differential and realized heritability for the level of heterozygosity.

4. To provide barley materials with increased heterozygosity to practical plant breeding programs in the WANA region.

2. Literature Review

2.1 Heterozygosity in barley and other crops

Heterozygosity has been found to be an important prerequisite to improve the grain yield and stability in drought stress environments in several crop species (Allard and Bradshaw 1964, Allard and Hansche 1964, Mayer et al. 1995, Haussmann et al. 1998, Abdel-Ghani et al. 2004, Einfeldt et al. 2005). In the study of Einfeldt et al. (2005) to compare the effect of heterozygosity in on yield and yield stability by comparing barley F_2 populations and corresponding homozygous parent lines, the F_2 populations were found to have higher grain yield reflecting the positive effect of heterozygosity.

Kahler (1975) found that barley individuals that outcross more will leave more heterozygous progeny and have a higher probability of survival. In outbreeding species like maize (*Zea mays* L.) (Duvick 1999, Lee et al. 2003, Springer and Stupar 2007), and rye (*Secale cereale*) (Wahle and Geiger 1978), heterozyosity was found to have a strong positive effect in improving yield stability. In the partially allogamous species sorghum, Haussmann (1995) found that the heterozygous entries were highly superior to homozygotes in all environments for grain yield, stover yield and above–ground dry matter and slightly superior for harvest index. In faba bean, Stelling et al. (1994) found that the positive effects of heterozygosity and heterogeneity on yield and yield stability can be additively combined in F1-hybrid blends. In rapeseed, (Léon 1991) found the yield stability was improved slightly more by heterogeneity than by heterozygosity; while in barley, it has been concluded that heterozygosity is more important than heterogeneity in increasing the grain yield (Einfeldt et al. 2005). In autogamous wheat, hybrids displayed increased yield potential and yield stability due to heterosis (Jordaan et al. 1999).

2.2 Outcrossing rate and outcrossing related traits

Barley is a predominantly self-fertilizing cereal plant with a very low outcrossing rate (Brown et al. 1978, Mayer et al. 1995, Parzies et al. 2000, Abdel-Ghani et al. 2004). Season-specific outcrossing rates ranging from 0-1.8% were found in cultivated and

wild barley populations collected from different ecological regions of Jordan (Abdel-Ghani et al. 2004). An overall average outcrossing rate of 1.6% was recorded by Brown et al. (1978) in 26 populations of wild barley in Israel based on allozyme variation at 22 polymorphic loci. A similar level of outcrossing of 1.7% was obtained by Parzies et al. (2000) for 12 populations of barley landraces from Syria using isozyme markers. A somewhat higher range of season-specific outcrossing rate was obtained by Parzies et al. (2008) in six barley lines of ICARDA which differed in the level of anther extrusion.

Abdel-Ghani et al. (2004) reported significant genetic variation for floral traits such as anther extrusion, anther and stigma size and hypothesized that recurrent selection for these traits might be a possible way to enhance outcrossing in barley. However, no significant difference in outcrossing rates was detected between anther-extruding and non-extruding barley genotypes indicating that high anther extrusion is not a sufficient condition to cause increased outcrossing rates (Parzies et al. 2008). No research has been reported so far on attempts to increase the outcrossing rates in barley through recurrent selection. The only available study was by Kahler et al. (1975) in barley Composite Cross V (CCV), which was composed from intercrossing 30 varieties yielding F_1 hybrids. The latter were again pair-crossed, producing a single hybrid stock which was subsequently allowed to reproduce by natural mating for 28 generations. The levels of outcrossing rates obtained in generations F_8, F_{19} and F_{28} were 0.57%, 0.88% and 1.24%, respectively.

With regard to other self-pollinated cereal crops, Lawrie et al. (2006) found outcrossing estimates ranging from 0 to 3.5% in 35 Canadian spring wheat cultivars in two successive years, while two of the cultivars had a maximum of 10.6% and 6.3% displaying consistently elevated levels of outcrossing. Further, he found that the outcrossing estimates under greenhouse conditions were poor predictors for values observed under field conditions. In common oat (*Avena sativa* L.), Shorter et al. (1978) recorded a mean outcrossing rate ranging from 1.8 to 8.7% averaged across four generations. In the partially allogamous species rapeseed, Becker et al. (1992) found varying outcrossing rates between environments in Swedish spring variety, ranging on average from 12 to 47%, when tested at five locations in Sweden,

Denmark and Germany. In cultivated sorghum, Rabbi et al. (2009) found that multi-locus outcrossing rates ranged from 3.6 to 17.3% in two cultivars each from Kenya and Sudan. In wild sorghum populations representing four regions of Kenya, the mean outcrossing rate amounted to 49.7% (Muraya et al. 2009).

2.3 Recurrent selection

Recurrent selection was long established and is being extensively used in many cross pollinating species for improving quantitative traits. It has also been implemented in autogamous species during the past three decades although intermating selected genotypes is constrained by, cost and labour. In barley, it has been found that positive genetic gain for grain yield (Delogu et al. 1987, Parlevliet and Ommeren 1988) and for stronium content in the kernel (Byrne and Rasmusson 1974) could be achieved through recurrent selection. Hockett et al. (1989) reported that recurrent selection for outcrossing had a positive effect in selected male-sterile barley stocks for open-flowering (resulted in 78 – 98% seed set) as compared to unselected material (23 – 25% seed set).

In common bean (*Phaseolus vulgaris* L), another autogamous species, the families of third generation outyielded their respective second and third generation families using recurrent selection and found that the average realized gain per selection cycle amounted to 15% (Singh et al. 1999). In durum wheat (*Triticum turgidum* L.), Olmedo-Arcega et al. (1995) found that kernel weight increased by 2.8mg from cycle C0 to C2. In all the previous researches, recurrent selection was used to increase phenotypic traits, but no research has been done so far to directly increase the level of heterozygosity.

2.4 Microsatellite Markers

Microsatellites, also called simple sequence repeat (SSR) markers, are stretches of DNA consisting of tandemly repeated short units of 1-6 bp in length and are co-dominantly inherited (Tautz 1989, Ivandic et al. 2002). They have several advantages such as unambiguous designation of alleles, a relatively even dispersal across the genome, selective neutrality and high reproducibility. Microsatellites have been employed successfully in barley for estimation of outcrossing rates and in genetic

diversity studies (Abdel-Ghani et al. 2004, Parzies et al. 2008, Maestri et al. 2002, Brantestam et al. 2007, Jilal et al. 2008).

3. Materials and Methods

3.1 Plant materials

A large number of drought adapted barley accessions (lines and landraces) was compiled from collections of the gene banks of the International Center for Agricultural Research in the Dry Areas (ICARDA) and of the Institute of Plant Genetics and Crop Plant Research (IPK) in Germany. Furthermore, 12 highly diverse barley landraces and 11 populations of wild barley from Jordan from a previous DFG-funded project (Abdel-Ghani et al. 2004) were included (Table 3.1). The passport data of the genetic material are given in Appendix I.

Table 3.1 Composition of the base collection used in this study

Origin	Number of accessions	Row type
Jordan & Syria (ICARDA)	130	two-row & six-row
Jordan & Syria (IPK)	48	two-row
Landraces from Jordan	12	two-row
Wild barley populations	11	two-row
Total	201	

3.2 Chronological order of activities

The Chronological order of activities in the RS procedure experimented in this project is given in a flow chart (Fig.3.1)

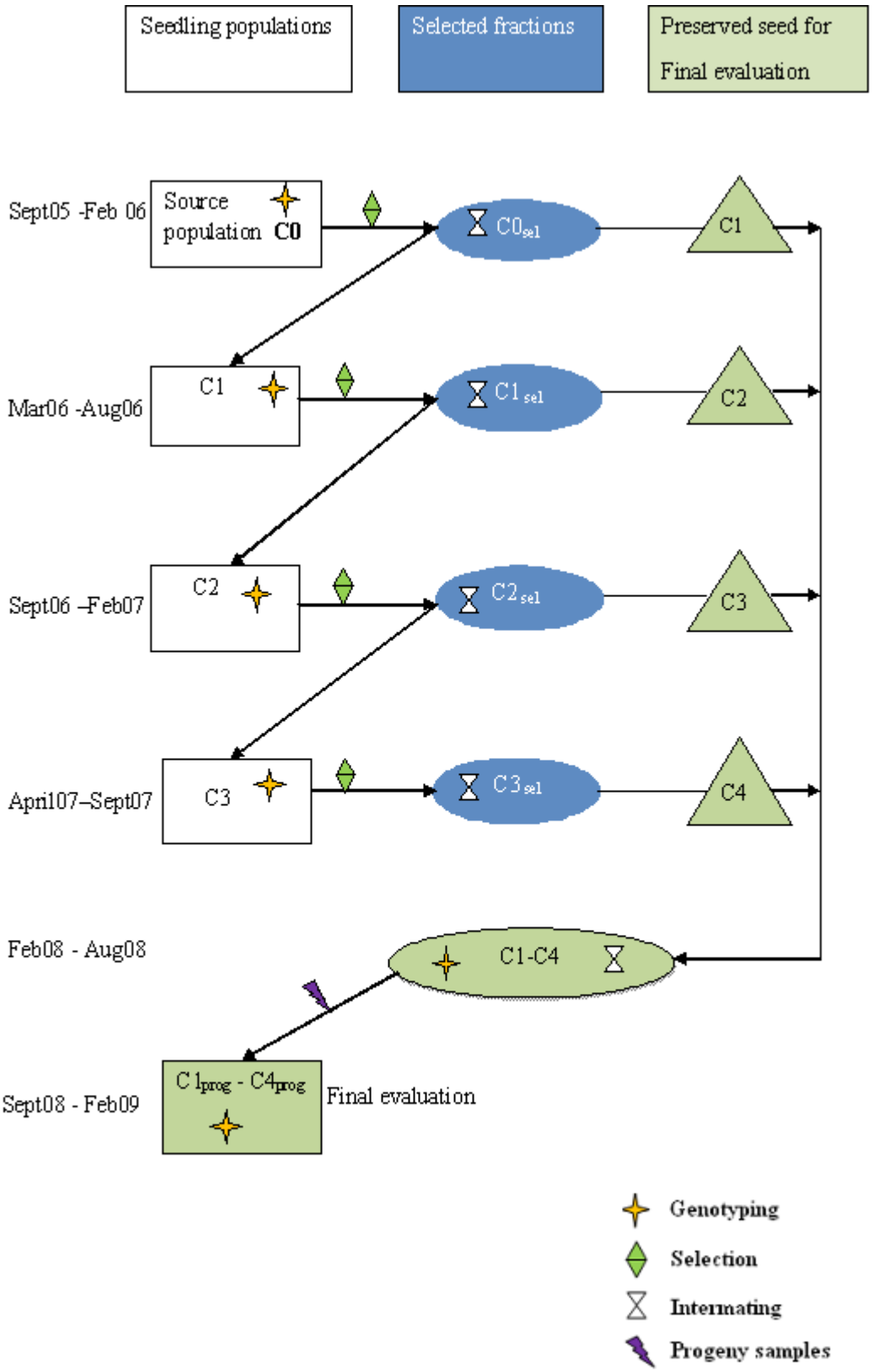

Fig 3.1 Flow diagram of chronological order of recurrent selection (RS) activities.

3.3 Recurrent selection procedure

During all the selection cycles as well as in the final evaluation experiment, plants were cultivated in a greenhouse at Hohenheim University, Stuttgart, Germany. The temperature in the greenhouse was not controlled due to the fact that the material has to be allowed to adapt to the fields of WANA region in future and also that there are no proper facilities to control the temperatures. A survey of the number of accessions and families per accession grown in the individual RS cycles is given in Table 3.2 (p.15).

3.3.1 First selection cycle

The first selection cycle was conducted between September 2005 and February 2006. The source population (C0) consisted of 15-18 individuals from each of the above 201 accessions, forming 569 bulks, each bulk consisting of 4 to 6 plants (3414 plants in total). Leaf tissue for DNA extraction was collected from seedlings 14 days after emergence. The seedlings were subjected to vernalization at 4ºC for 6 weeks. Marker analysis was conducted during the vernalisation period. One hundred and twelve plants belonging to 16 accessions, ranging 1 to 16 plants per accession, were selected on the basis of their marker genotype. These 112 plants were arranged in a crossing block in a randomized manner in the greenhouse and the temperature was between 0 and 10ºC. During anthesis, pollen movement was assured by employing a blowing device. To achieve air circulation in a revolving pattern, a plastic wall was placed at one side of the crossing block and a fan opposite to it (Fig. 3.2). Only 72 of the 112 plants survived. They descended from 16 accessions with a range of 1 to 12 families in each. Around 8000 seeds were harvested from these 72 plants in February 2006. These seeds were dried for 72h at 35ºC, threshed and cleaned. Six seeds from each of the 72 plants were preserved for final evaluation and the rest of the seed was used to establish population C1 for starting the next selection cycle (see flow diagram in Fig. 3.1).

3.3.2 Second selection cycle

Set I of population C1

The second selection cycle was conducted between March 2006 and August 2006. Because of strong dormancy of the majority of C1 seeds, population C1 had to be subdivided into two timely staggered sets (I and II).

Initially, about 4000 seeds (2 to 126 seeds per parent plant) were sown in trays, immediately after harvest (one week after drying). As none of the seeds germinated, they were taken out of the soil after one week and washed in water. They were treated with a fungicide (Landor®CT) and laid on moistened filter paper. The filter papers were placed in plastic boxes and subjected to a pre-chilling treatment for 4 days (ISTA, 1999). Then the seeds were subjected to changing temperatures of 20ºC with 8h of light during the day and 3ºC with16 h of darkness during night. Only 677 out of the 4000 seeds germinated whereas the rest remained dormant. The germinated seedlings formed Set I of population C1. Collecting leaf samples, vernalization and marker analysis were carried out as in the first RS cycle. Only 35 plants tracing back to 4 accessions were identified as heterozygous. These 35 plants were arranged in a randomized crossing block in the greenhouse as in the first RS cycle and the temperature was between 15 and 25ºC during flowering. About 10 to 180 seeds per plant, 2124 seeds in total were harvested in August 2006. Six seeds from each of the 35 plants were preserved for final evaluation and the rest of the seed was advanced to the next selection cycle constituting population C2.

Set II of population C1

Because of the small number of plants in Set I, resowing was done in May 2006, with the remaining 4000 seeds harvested from the selected 72 C0 plants to establish Set II. For this experiment, seed was left from 14 accessions. Set II comprised 14 accessions with 1 to 14 families per accession, and 6 to 64 plants per family amounting to 1796 plants in total.

Before resowing, the dried seeds were left in the paper covers and were subjected for one week to a changing temperatures every 12h between 25ºC with light and 13ºC with darkness. Seeds were then soaked in water overnight, dehulled and treated with

fungicide (landor® CT), kept on a filter paper moistened with GA_3 (0.05%) + KNO_3 (0.1%) solution and placed in plastic boxes. They were stored at 7ºC for 7 days and then subjected to alternating temperatures of 20ºC during the day and 3ºC during the night for another 7 days. The solution was sprayed on the seeds every alternate day. Fungicide was sprayed once in 3 days whenever fungal growth was observed. Almost 50% germination was observed counting to 1974 seedlings. The seedlings were planted in bulks of 6 individuals with 1-3 bulks per parent plant. Marker analysis was performed as described before. As Set II of population C1 matured only in October 2006, harvest was too late for integrating Set II in the time schedule of the RS experiment. Hence in what follows population C1 - Set I, is simply called C1.

3.3.3 Third selection cycle

The third selection cycle was conducted between September 2006 and February 2007. Population C2 traces back to 4 accessions with 5 to 18 families per accession, 6 to 144 plants per family, and 1928 plants in total.

The seeds were sown in Jiffy pots which were placed in trays. The trays were planted in growth chamber at 3ºC for 3 days and then transposed in the greenhouse for 2 weeks. Collection of leaf samples, vernalization, and marker analysis were carried out as in the first RS cycle. MegaBACE sequencer enabled to carry out the marker analysis starting from this selection cycle (see section 3.4.5, p.20). One hundred and thirteen plants descending from 4 accessions were selected for the next RS cycle. These plants were arranged in a random manner in the green house for intermating as in the first RS cycle and the temperature was between 1 and 10ºC during flowering. Two plants did not survive to maturity. The harvested spikes were dried under changing temperatures (30ºC for 16h and 15ºC for 8h) for a period of 7 days. The total number of harvested seeds was 4165. Six seeds from each selected plant were preserved for final evaluation and the rest of the seed was advanced to the fourth selection cycle.

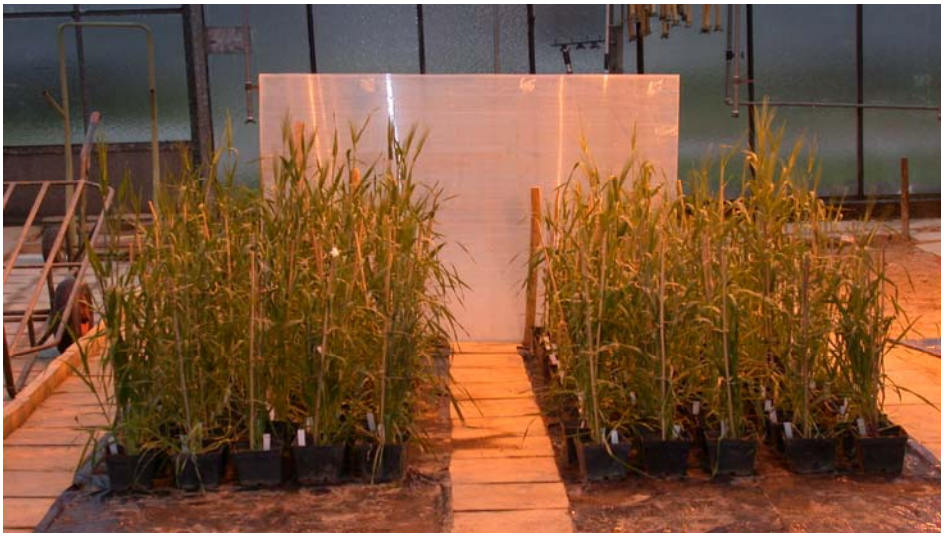

Fig. 3.2 Plants in the greenhouse with a plastic film placed opposite to the blowing device for air circulation.

3.3.4 Fourth selection cycle

The fourth selection cycle was conducted between April 2007 and September 2007. Population C3 consisted of 4 accessions, with 5 to 57 families per accession, 6 to 60 plants per family, and 3505 plants in total.

Seeds were subjected to temperatures alternating between 25°C and 7ºC every 12h for 7 days. A germination test experiment was performed with 3 random seeds from each accession. The seeds were laid in a plastic box on filter paper moistened with water, placed in a vernalisation chamber at 3ºC for 7 days, and watered every second day. The plastic box was then placed in greenhouse for 2 days at 20ºC. Germination rate was 100%. The seedlings were transferred into plastic trays and placed in a greenhouse. Collecting leaf samples, vernalization, and marker analysis were carried out as in the first RS cycle. The selected fraction comprised 149 plants. These plants were grown in a random manner for intermating as in the previous cycles and the temperature was between 15 and 30ºC during flowering. Only 68 plants descending from 23 families derived from 4 accessions survived and seeds were harvested from each of these plants. Six random seeds from each plant were used for final evaluation and the rest was stored at 4ºC for further experiments.

Table 3.2 List of accessions and number of families involved in RS populations C1 to C3.

		Number of families		
List	Accession	C1	C2	C3
1	HOR 7400	1 (1)		
2	HOR 18456	12 (81)		
3	HOR 18468	6 (35)		
4	HOR 19786	8 (83)		
5	HOR 19788	3 (9)		
6	HOR 19803	1 (8)		
7	IG31495	1 (4)		
8	IG31498	5 (31)	^{2}5 (270)	^{5}28 (1010)
9	IG31513	7 (143)	^{3}7 (372)	^{5}21 (690)
10	IG33094	7 (113)		
11	IG36035	1 (6)		
12	IG36041	12 (86)	^{4}5 (104)	^{1}5 (174)
13	IG36046	1 (6)		
14	IG135258	1 (6)		
15	IG138242	5 (60)	^{2}18 (1182)	^{13}57(1631)
16	Mafraq14	1 (5)		

Superscripts refer to the number of families in the previous cycle from which the families in a given cycle were derived. The number in the parenthesis denotes the number of plants per accession.

3.4 Molecular marker analysis

3.4.1 DNA extraction and quantification

The extraction of DNA involved a two-stage procedure. First the DNA was extracted from bulks of 6 plants, then from the individual plants of the selected bulks. One fresh leaf was collected from each seedling two weeks after germination and placed in 96 deep well microtiter plates. A carbide-tungsten bead was placed in each well and 450µl of 3% warm CTAB extraction buffer was added to the leaf material. The

microtiter plates were then shaken for 10 min. Thereafter, the leaf tissue was ground the leaf tissue to enable a complete digestion of the cell walls. Subsequently, genomic DNA was isolated using the protocol of Saghai-Maroof et al. (1984) with minor modifications.

Concentration of DNA were determined on a 0.8% agarose gel (300 ml TBE + 2.4g agarose). A 1:10 dilution was made of all DNA samples using 40 µl H_2O bidest and 5 µl bromophenol blue and 5 µl DNA. Lambda DNA standards at concentrations of 50, 80 and 100ng/µl were prepared from an original concentration of 500µg/ml and were used to estimate the DNA concentrations. The DNA samples and lambda DNA were loaded in the wells of submerged gels in an electrophoresis unit, containing 1 X TBE buffer and an electric current of 80 V was applied for 1 h. The DNA fragments were observed under UV light using a video capture system. The concentration of DNA was estimated according to the band thickness in comparison with the lambda DNA standards accordingly. The samples were then diluted to different concentrations to adjust to the final concentration of 10 ng/µl of DNA.

3.4.2 Optimization of microsatellite markers

Pre-PCR multiplexing was optimized using a set of 17 SSR primer pairs established at the Scottish Crop Research Institute (SCRI 1999, Macaulay et al. 2001) which were reported to be highly polymorphic. The melting temperatures of these primer pairs were determined using the "Oligo Analysis" procedure implemented in the VECTOR NTI ADVANCE™ 10 software. Most of the primers ranged between 50ºC to 60ºC. Ten different temperatures with a difference of 1ºC for each sample were tested in a single run during the annealing step. This enabled to calculate the exact annealing temperature on a temperature gradient thermocycler using 11 wild barley samples (see Appendix I, Table 9.1). After the program was run, the samples were analysed in a gel. The temperature at which the primer performed best amplifications was selected as optimum annealing temperature for that primer. To avoid overlapping of the bands of different markers, the marker combinations were optimized according to their fragment sizes such that they differ by a minimum of 50 base pairs. Two sets of three markers possessing the same annealing temperature were combined for triplex PCR (Table 3.3).

Table 3.3 Charactersitics of two SSR triplets used for genotyping populations C2 to C3 in the course of RS experiment and for the final comparison of all entries in this study.

Triplet	SSR	Chromo--some	Primer (F=Forward, R=Reverse)	[1]Repeat motive	Size (bp)	Annealing Temp. (°C)
I	Bmag0323	5H	F:TTTGTGACATCTCAAGAACAC R:TGACAAACAAATAATCACAGG	(CT)24	158	52
I	GBM1015	4H	F:TTGTTGGAACATACAAACATGC R:GTTCCGTGTGAATTAGCGGT	(CATA)12	228	52
I	GBM1031	3H	F:CAGTTGGCTTCTACCCCAAA R:GCTACGACCCACAACAACAA	(GA)14	294	52
II	Bmag0206	7H	F:TTTTCCCCTATTATAGTGACG R:TAGAACTGGGTATTTCCTTGA	(GT)5(AG)14	239	55
II	Bmag0353	4H	F:ACTAGTACCCACTATGCACGA R:ACGTTCATTAAAATCACAACTG	(AG)21	119	55
II	Bmag0222	5H	F:ATGCTACTCTGGAGTGGAGTA R:GACCTTCAACTTTGCCTTATA	(AC)9(AG)17	179	55

[1]Repeat motive: number of simple sequence repeats in a respective microsatellite marker.

In source population C0, the SSR markers, GBM1015 and GBM1031 were not used, while in C1 population, triplet I was not used. In C2 and C3 populations, both the triplet sets were used. But for analyzing the data in the course of recurrent selection experiment, only triplet II which was common in all the four populations C0 to C3 was used to avoid any discrepancy.

3.4.3. Polymerase Chain Reaction

Polymerase chain reaction (PCR) was performed by means of a PTC-100™ programmable thermal controller (MJ Research, Inc.) in a 20µl reaction mix called the stock solution which was prepared at the concentrations listed in Table 3.4.

Table 3.4 Composition of the PCR stock solution.

Component	Volume (µl)	Concentration of stock solution	Final Concentration
Bidest water	6.6		
$MgCl_2$	1.2	25mM	1.5nM
10 x PCR buffer	2.0	10 x 15mM	1 x 1.5mM
dNTP-Mix	2.0	2mM	0.2mM
Forward primer	1 x 3	5µM	250nM
Reverse primer	1 x 3	5µM	250nM
Taq-Polymerase	0.2	5U/µM	0.5U/10µl
Template-DNA	2.0	10ng/µl	10ng/µl
Total	20		

The samples were heated to 94°C for 1 min to denature the target DNA, as at this high temperature, the kinetic motion of the DNA molecule disrupts the weak hydrogen bonds that join the complementary DNA strands together (Table 3.4). With the lowering of temperature to 52-57°C, the forward and reverse primers were allowed to anneal to their complementary sequences in the template DNA as a primer was needed for the DNA polymerase to start its reaction. When the temperature is risen to 72°C for 1.30 min, *Taq*-polymerase attaches to each priming site, synthesizing the new DNA-strand along with the incorporation of dNTPs (deoxyribo nucleotide triphosphates). After 35 cycles, the reaction was held at 72°C for 10 min to increase the yield of completely elongated products. The reaction conditions were the same for the two sets of primers, except for the annealing temperatures (Table 3.3). Amplification products were cooled down and stored immediately at 4°C after the PCR.

Table 3.5 PCR steps and conditions

Step	Conditions
Hot start	94°C for 1 min
Denaturation of DNA	94°C for 1 min
Annealing of primers	52°C to 57°C respectively for 1 min
Extension	72°C for 1.30 min
Repeat step	For 35 cycles
Final extension	72°C for 10 min
Cooling	4°C for 15 min

3.4.4 Gel Electrophoresis

A 3% metaphor agarose gel was used by dissolving 9g of metaphor agarose in 300 ml of diluted TBE (Tris-borate-EDTA) buffer by permanent mixing with a magnetic stirrer. Agarose solution was boiled in a microwave oven and cooled to 60°C before pouring into gel casting plates containing combs for wells. In this metaphor agarose gel approach, bromophenolblue (2µl) was added as a dye to the samples of PCR product and mixed well before loading into wells of the gel for electrophoresis. The PCR product and 50 bp DNA ladder were loaded onto the gel and an electric current of 120 Voltage was applied for 3h. Amplified SSR markers were separated on a metaphor agarose gel and visualized under ultraviolet light with a video capture system after staining with ethidium bromide. Marker bands were assessed visually. The gel was stained with ethidium bromide (1%), a fluorescent dye, which intercalated into the DNA molecule for 5 min, and consequently the gel was destained in distilled water for 20 min. The DNA in the gel was visualized under UV light and photographed using a video capture system (Flowgen IS 1000).

Plants that are heterozygous, show double bands (denoted by red circles in Fig. 3.3) and those being homozygous, show one single band per microsatellite marker according to the co-dominant nature of the marker type. An example of a typical PCR-triplex result is given in Fig. 3.3.

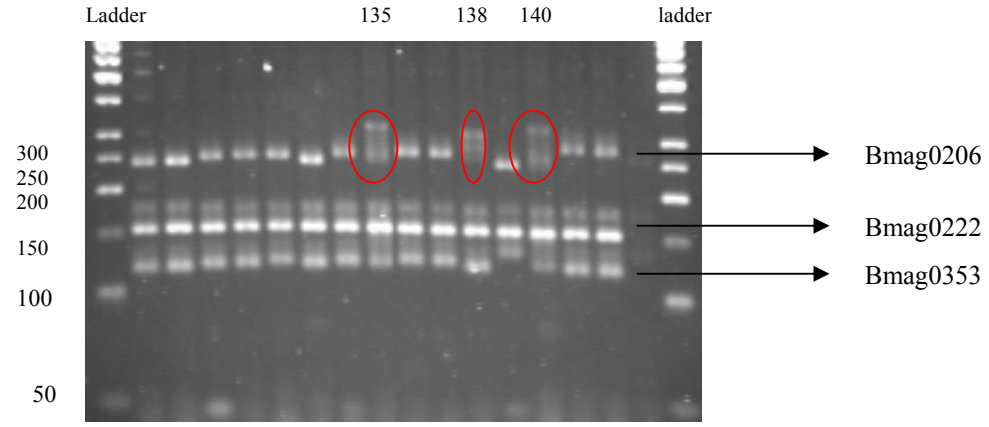

Fig. 3.3 Metaphor agarose gel showing the banding pattern of an SSR triplex among genotypes of 15 barley plants belonging to the same family. The samples flagged by the red circles 135, 138 and 140 are the DNA bulks of C1 Set-I population showing double bands for Bmag0206 indicating heterozygosity.

For efficiency reasons, first the DNA of six-plant bulks was analyzed. Bulks which showed heterozygous banding pattern were reanalyzed i.e. each of the six plants per bulk was genotyped individually to infer outcrossing rates based on the appearance of new alleles which had not been observed in the mother plants. The bands from the reanalyzed samples were assessed using the software program TOTALLAB.

3.4.5 SSR marker analysis on a MegaBACE sequencer

Starting from population C2, a MegaBACE sequencer was used for genotyping. Results were more precise than those obtained from the metaphor agarose gels and the new procedure saved a lot of time.

The MegaBACE genotyping system had four spectral channels to detect the emissions of four dyes per capillary. The forward primer for each of the 6 SSR markers was labeled at the 5' end with fluorescent dyes suited for multiplexing. The dyes were FAM (blue), TET (green), HEX (Yellow), and standard ET-ROX 400 (red). The PCR products from each sample were mixed with 5µl of ET-ROX 400, centrifuged and denaturated at 94ºC for a minute. The samples were then introduced into the 96 capillaries of a capillary array by electro kinetic injection and separated by electrophoresis through a matrix. The samples were run at 10 KV voltage with 45 sec injection time for 60 min at 60ºC. As the DNA fragments passed through the detection cell,

the fluorescent dyes were excited by an argon laser and emission spectra of the various dyes were detected by a charged couple device (CCD) camera and the captured electrons were represented as relative florescence units (rfu). Each run comprised 96 samples and lasted 60 min.

The MegaBACE fragment profiler (Amersham Biosciences) was used for genotyping. The software automates genotyping through a progression of steps which include 1) trace processing 2) fragment sizing, (3) allele calling, and (4) quality scoring.

During trace processing, signal intensity of all four traces of the raw electropherogram are placed at the same level and the unwanted signals from other colors in each data channel are removed. Fragment sizing is done by calculating the length of the DNA fragments identifying the peaks using the internal DNA size standard of ET-ROX dye labeled known fragment sizes. Allele calling on the microsatellite PCR products is performed by selecting the allele peaks from the sized peak list for each trace (Fig. 3.4). The peak selections are based on the size range of the marker and the expected peak pattern for the marker type predicting the best fit of the peaks to allele bins. The peak selections that fall within a given allele bin are considered the same allele. While scoring, the peaks or the alleles are clustered in “bins” that are spaced at the distance of the repeat unit present in the given microsatellite i.e., every subsequent bin is the size of the previous bin plus the repeat size in base pairs (MegaBACE Fragment Profiler v1.0). Sized peaks and alleles were manually reviewed and SSR markers showing stuttered multiple peaks were treated as missing values.

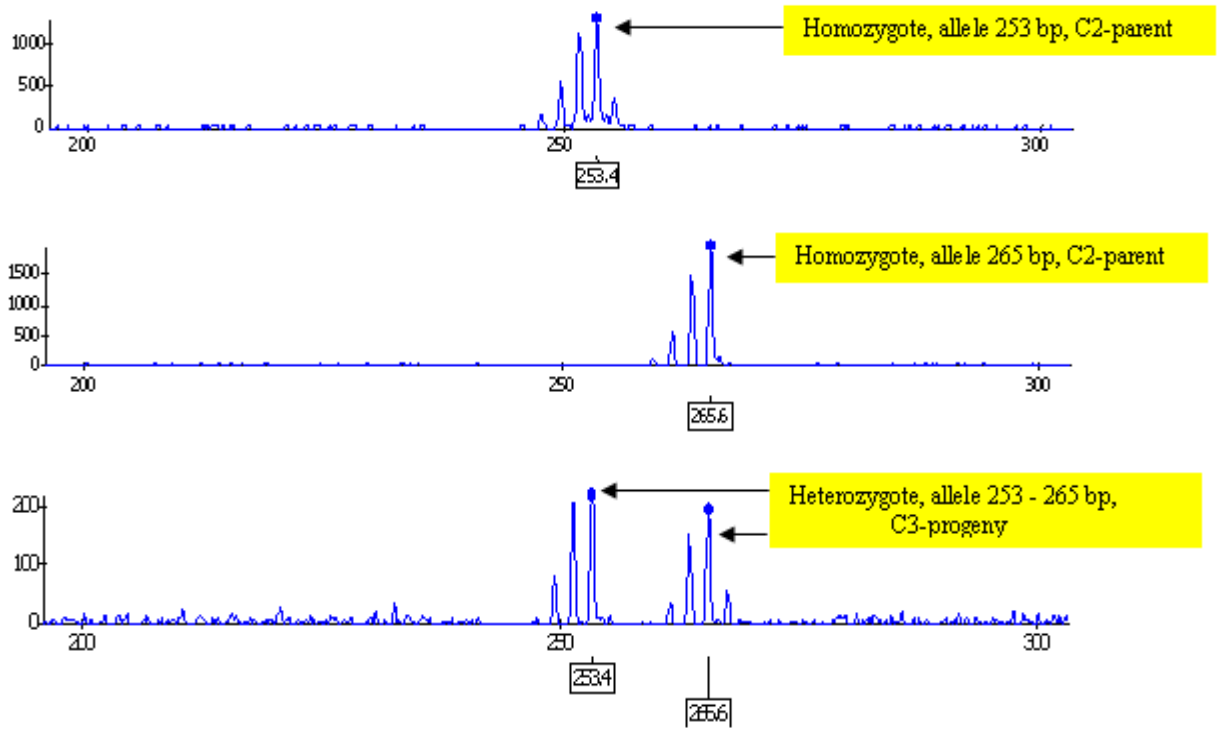

Fig 3.4 Graphs of FAM (blue) labeled microsatellite marker locus Bmag 206 from three DNA samples, two of which are homozygous and one is heterozygous. The heterozygous sample was obtained from a C3 progeny of the first, of the two C2 parent plants.

3.5 Evaluation of selection progress

3.5.1 Phenotyping in final evaluation

Preserved seeds from the four selection cycles were sown in trays in the greenhouse. DNA extraction, PCR and genotyping were performed in the same way as during the preceding two recurrent selection cycles. The list of accessions used in final evaluation is given in Appendix II, Table 9.3.

The seedlings were subjected to vernalization at 4ºC for 6 weeks and then transplanted into pots and grown in the greenhouse for assessing the characters days to ear emergence, anther extrusion, open flowering, row-type and grain weight per plant. The plants were arranged in a randomized manner under the restriction that adjacent plants did not belong to the same accession. The temperature in the greenhouse during flowering was extremely hot reaching to 40ºC which is unfavorable for achieving high outcrossing rates.

Trait assessment

Days to ear emergence

The date of ear emergence was recorded in days from sowing when awn tips were just visible. Only the ear bearing tillers at the time of harvest were included in the statistical analysis. The first emerging spike of a plant was tied with a red colored clip and considered as a primary ear. The spike emerging thereafter was tied with a blue colored clip and considered as a secondary ear. All later emerging ears were considered as tertiary ears.

Number of ear-bearing tillers

The number of ear-bearing tillers was recorded plant-wise at maturity.

Anther extrusion

Anther extrusion was assessed by observing the presence or absence of extruded anthers (Fig. 3.5). Anther extrusion was mainly observed in the middle of the ear. The trait was recorded as percentage of plants extruding anthers relative to the total number of plants in the respective cycle.

Open flowering

Open flowering was estimated by observing the flowers against a dark background to see if any of the flowers opened at anthesis (Fig. 3.5). If a plant had at least one open flower, it was noted as open-flowering plant. Therefore, the percentage of open-flowering was estimated as the number of plants having at least one open flower relative to the number of plants in the respective cycle.

Row-type

Two-row types were distinguished from six-row types. Some plants showing 4 or 5 rows were considered as six-row types. The proportion of two-row and six-row plants was calculated as a percentage the total number of plants in the respective cycle.

Grain yield

At the hard dough stage, when the kernel loses its green color, the grain was harvested from each plant approximately 30 days after flowering. At this stage even the glumes and the

peduncle lose their green color. The harvested grain from each of the plants was kept in paper covers and dried for 72 hours at 30-35ºC in a drying chamber. The dried grain was threshed to separate it from the straw and chaff. Care had to be taken that the grain was not damaged. Grain yield was recorded in grams per plant.

Seed number

The dried cleaned seed from each harvested plant was counted manually.

Hundred-grain weight

It is a measure of average seed weight, expressed in grams of 100 seeds. The seed was counted manually from each plant.

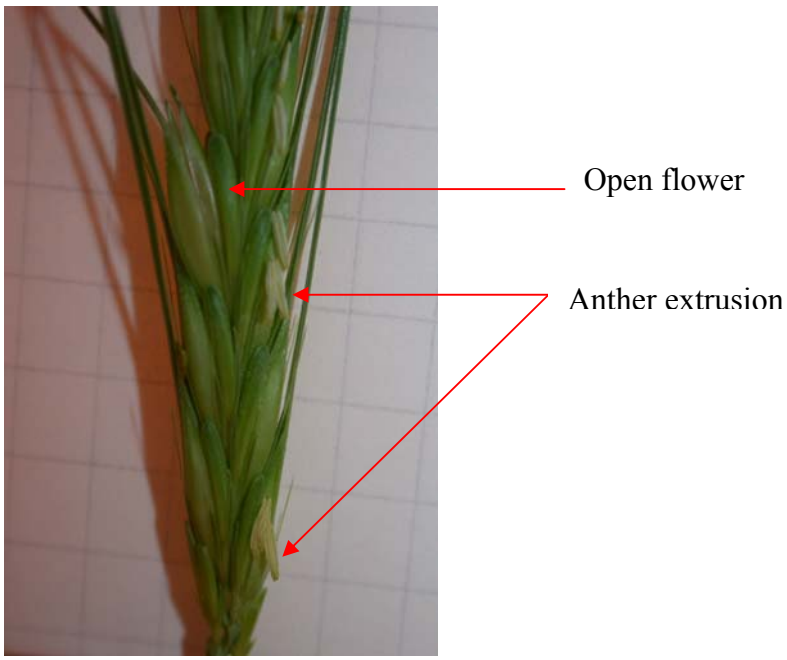

Fig. 3.5 Spike of a barley plant showing anther extrusion and open flowering.

3.5.2 Outcrossing rate

Offspring of the finally evaluated plants from each RS cycle were genotyped for estimating the outcrossing rate. Six seeds from each of 24 random parent plants per cycle were grown to the seedling stage for DNA extraction. Genotyping was conducted as described for the last two RS cycles using the MegaBACE sequencer. Inferred multi-locus outcrossing rates of the parent

plants were estimated using the MLTR software of Ritland (2002); see section 3.6 (Data analysis).

3.6 Data Analysis

3.6.1 Observed heterozygosity

The observed heterozygosity (H_O) statistic was used to quantify the progress from selection. The parameter was calculated using the software POWERMARKER. It measures the proportion of heterozygotes observed in a family or population averaged across all loci analysed (Weir 1996). Observed heterozygosity at a single locus is calculated as

$$H_O = \frac{\text{Number of heteroyzygotes at a locus}}{\text{Total number of individuals in a population}}$$

3.6.2 Polymorphic Information Content

The polymorphic information content (PIC) of a given marker locus, which is closely related to gene diversity, provides an estimate of the discriminatory power of a marker. It was estimated according to Botstein et al. (1980) as

$$PIC = 1 - \sum_{i=1}^{k} p_i^2 - \sum_{i=1}^{k} \sum_{j=i+1}^{k} 2 p_i^2 \; p_j^2$$

where p_i and p_j are the frequencies of marker alleles i and j, respectively.

3.6.3 Multilocus outcrossing rate

Multilocus outcrossing rates (t_m) were estimated in the final comparative evaluation of populations C1 to C4 employing the MLTR software (Ritland and Jain 1981, Ritland 2002 version 3.2). This software is based on a mixed mating model, according to which a certain amount of progeny (t_m) originates from outcrossing and the remaining from selfing ($1 - t_m$). The software applies a likelihood procedure and is an iterative two-step process in which the most likely maternal parent genotype is inferred from the marker genotype of its progeny. Standard errors of the t_m estimates were calculated from the standard deviation of 1000 bootstrap estimates by resampling families within populations.

3.6.4 Floral and other characteristics

The PROC GLM procedure of the software SAS (SAS Institute 2004) was used for the statistical analysis of the phenotypic traits assessed in the final evaluation experiment. For all computations, counts were log transformed and percentage data transformed to the arcsine of their square roots.

3.7. Population parameters

3.7.1 Selection differential

The selection differential (S) is defined as the difference between the means of the selected parents (μ_S) and the unselected parental population (μ). Hence, the selection differential S, is calculated as $S = \mu_S - \mu$ (Falconer and Mackay 1996).

3.7.2 Response to selection

Response to selection (R) is defined as the difference between the mean of the progeny of the selected parents (μ') and the mean of the parental population before selection (μ) (Falconer and Mackay 1996). Therefore, in this study the response is simply calculated as the difference between the means of the succeeding selection cycles: $R = \mu' - \mu$.

3.7.3 Realized heritability

Expressing the selection response as a proportion of the selection differential is referred to as realized heritability (h^2) and is given by equation $h^2 = R/S$ (Falconer and Mackay 1996).

4. Results

4.1 Observed heterozygosities in the course of the RS experiment

a) Population level

The average observed heteroyzgosity (H_O) increased from 0.60% in population C0 to 3.24% in C3. The selected fractions considerably surpassed the population means (Fig. 4.1, Table 4.1).

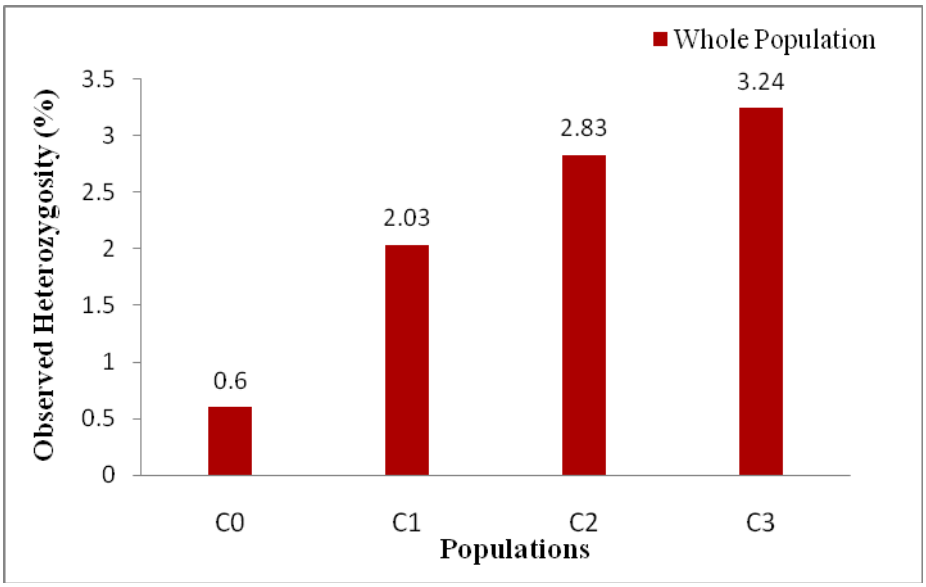

Fig. 4.1 Observed heterozygosities of the populations C0 to C3 in the course of the RS experiment and of their respective selected fractions.

The markers used for the analysis of the data in the populations C0 to C3 were Bmag0353, Bmag0222 and Bmag0206 as the remaining were not used in C0 and C1 populations (see page.17).

Table 4.1 Mean number of alleles per locus and polymorphic information content values of SSR markers in RS populations C0 to C3.

	Whole population		Selected fraction	
Population	*A*	PIC	*A*	PIC
C0	24.6	0.889	15.3	0.837
C1	13.0	0.820	7.7	0.654
C2	9.3	0.588	9.0	0.690
C3	11.7	0.777	10.0	0.747

H_O = percentage observed heterozygosity, *A* = mean number of alleles per locus, PIC = polymorphic information content.

The mean number of alleles per marker locus strongly decreased from population C0 to C3 in the whole populations as well as in the selected fractions. The SSR markers displayed high PIC values throughout the four RS cycles. Polymorphism decreased only little from C0 to C3.

b) Accession level

From a source population of 201 accessions, 16 accessions were selected for the second cycle. Data of all 201 accessions are listed in Appendix I (Table 9.1) and the designation of the 16 accessions advanced to C3, are given in Table 3.2. Observed heterozygosites of all the accessions from C1 to C3 are listed in Appendix II (Table 9.2) and the comparison of 4 accessions maintained through all selection cycles is presented in Fig. 4.2.

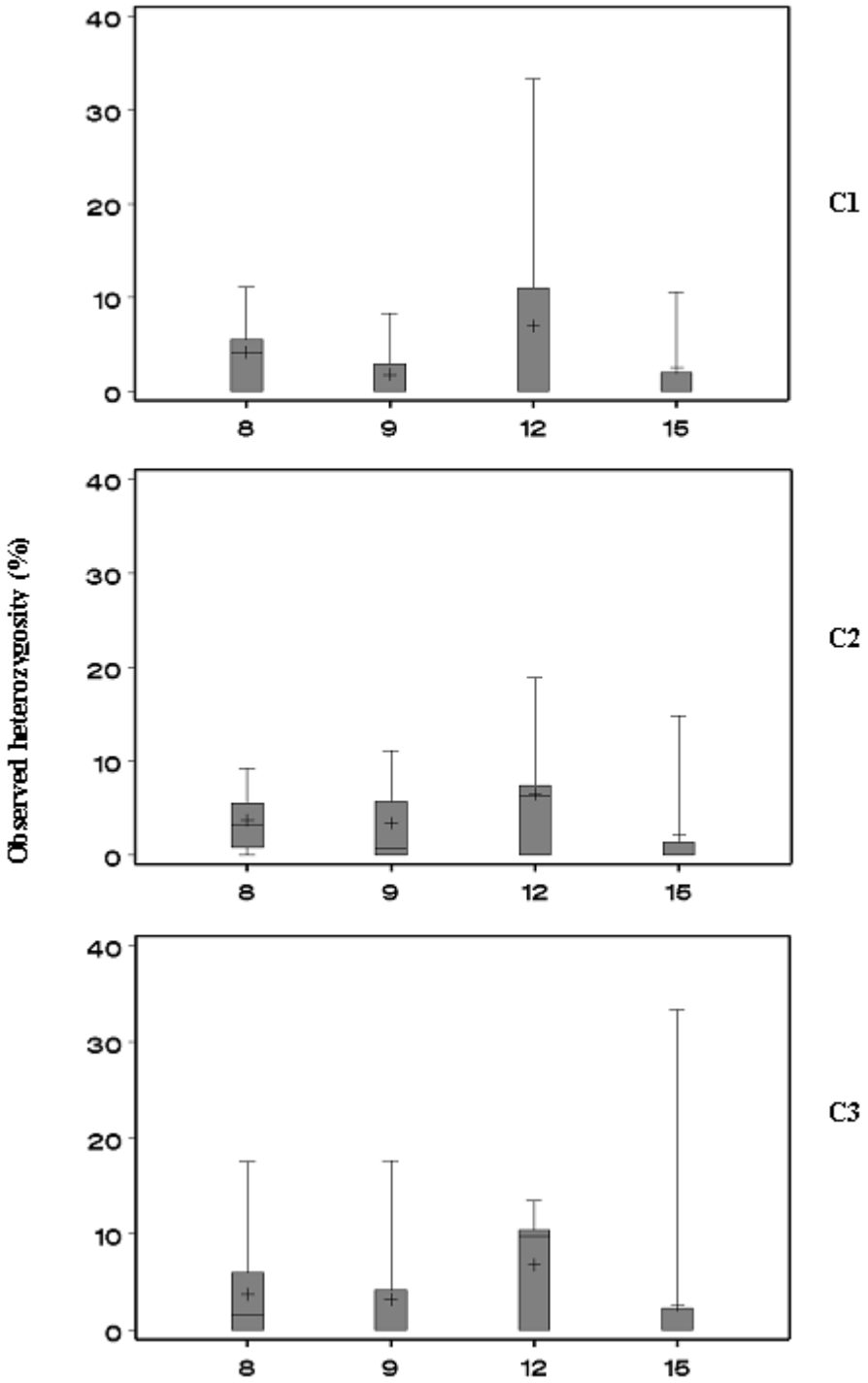

Fig. 4.2 Box-whisker plots of the observed heterozygosities of families within accessions in the course of the RS experiment.

Numbers 8 to 15 on the abscissa refer to 4 accessions listed in Table 4.2. Whiskars indicate the data's smallest and largest values in each particular accession of the respective cycles.

Only accessions 8, 9, 12 and 15 (corresponding to IG31498, IG31513, IG36041 and IG138242, see Table 3.2) were maintained through all 4 RS cycles. By far the highest

heterozygosity was observed in accession IG 36041. The mean of H_O amounted to 6.9 in C1, 6.5 in C2 and 6.7% in C3. Most accessions had families displaying H_O values around 10% or more.

c) Family level

The observed heterozygosities varied considerably among families (single plant progenies). The H_O values ranged from 0 to 33% in C1 and C3 populations while in C2, it ranged from 0 to 19% (Appendix II, Table 9.3). The H_O values surpassed 20% in two of the accessions, IG36041 in C1 and IG138242 in C3 populations.

d) Population parameters

Selection was most effective in populations C0 and C2 (Table 4.2) although the selection differentials were not larger, in C2 even smaller, than in populations C1 and C3. Since the selection differential was several times larger than the selection response in all RS cycles, estimates of the realized heritability were very small throughout. Proportions selected varied between 3.2 and 4.9%.

Table 4.2 Response to selection, selection differential, realized heritability, and selected proportion obtained from the main course of recurrent selection cycles.

Population	S	R	h^2	p
C0	16.95	1.43	0.084	0.032 (112/3420)†
C1	18.80	0.80	0.042	0.047 (32/678)
C2	7.27	1.41	0.056	0.049 (95/1934)
C3	16.22	a	a	0.042 (149/3529)

R = response to selection, S = selection differential, h^2 = realized heritability, p = proportion selected.

a = not applicable, † Number of plants selected/size of the population.

4. 2 Outcrossing rates and observed heterozygosities observed in the final evaluation experiment.

a) Population level

In the final evaluation experiment, the observed heterozygosity values of the progeny obtained from random seed samples preserved after each RS cycle increased from C1 (0.23%) to C4 (1.29%) samples (Fig. 4.3). Accordingly, the average outcrossing rate also increased from C1 (1.4%) to C4 (2.8%) samples when grown in the same environment in greenhouse during the same season (summer 2008) (Fig. 4.4).

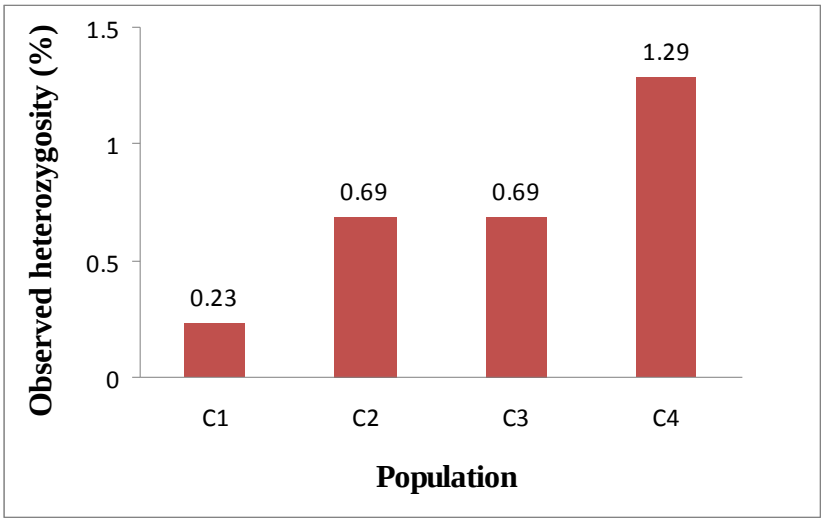

Fig. 4.3 Observed heterozygosity values in populations C1 to C4 assessed in the final evaluation experiment.

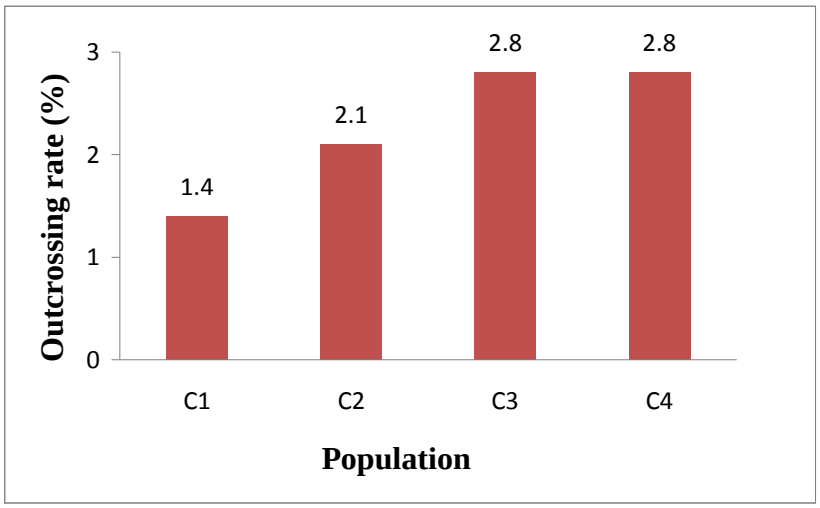

Fig 4.4 Multi-locus outcrossing rates estimated from the marker data of the final evaluation experiment.

b) Family level

The observed heterozygosity estimates displayed a wide range of variation among families from all RS cycles. Family heterozygosity estimates are given in Appendix II, Table 9.3). Observed heterozygosity estimates of families ranged from 0 to 3% in C1 and C2 and 0 to 9% in C3 and C4 populations of final evaluation experiment.

Analysis of variance

Outcrossing rate estimates significantly increased step by step from C1 to C4. Only the increases from C1 to C2 and from C3 to C4 did not reach the 5% significance level (Table 4.3).

Table 4.3 Differences between all possible pairs of RS cycles for multi-locus outcrossing rate (t_m) and observed heteroyzgosity (H_O) estimates.

Difference (progeny samples)	*df*	t_m (%)	H_O (%)
C1-C2	92	1.6	0.4
C1-C3	92	14.9**	0.4
C1-C4	92	15.5**	0.9
C2-C3	92	13.2**	0.0
C2-C4	92	13.8**	0.5
C3-C4	92	0.6	0.5

** Significant at 0.05 probability level.
df = degrees of freedom
.

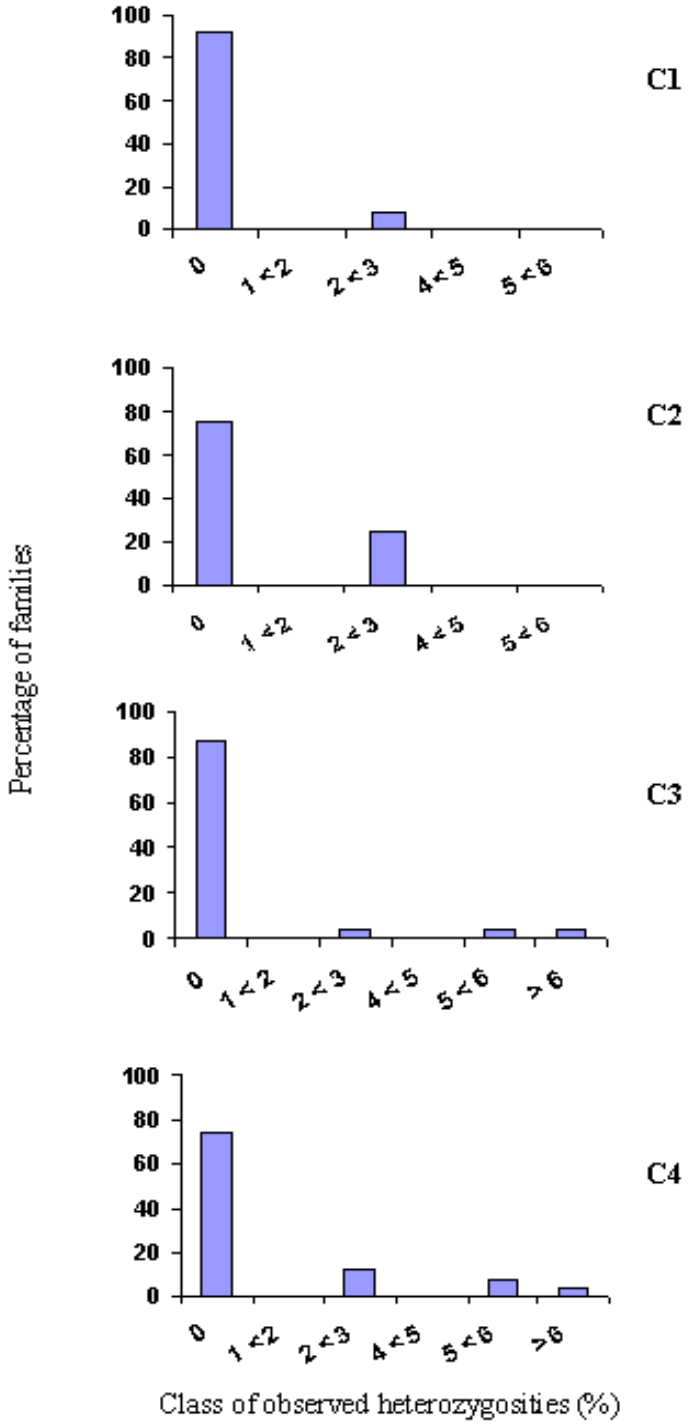

Fig 4.5 Frequency distribution of observed heterozygosities in the final evaluation experiment.

4.3 Evaluation of quantitative traits in the final evaluation experiment

4.3.1 Trait means

Considerable and significant differences between populations C1 to C4 were observed for seed number and ear bearing tillers (Fig 4.6). From C1 to C2, an increase in open flowering and tillering was accompanied by a strong decrease in anther extrusion. No significant trends existed for all other traits. After the third cycle, only a small increase in anther extrusion and open flowering was detected.

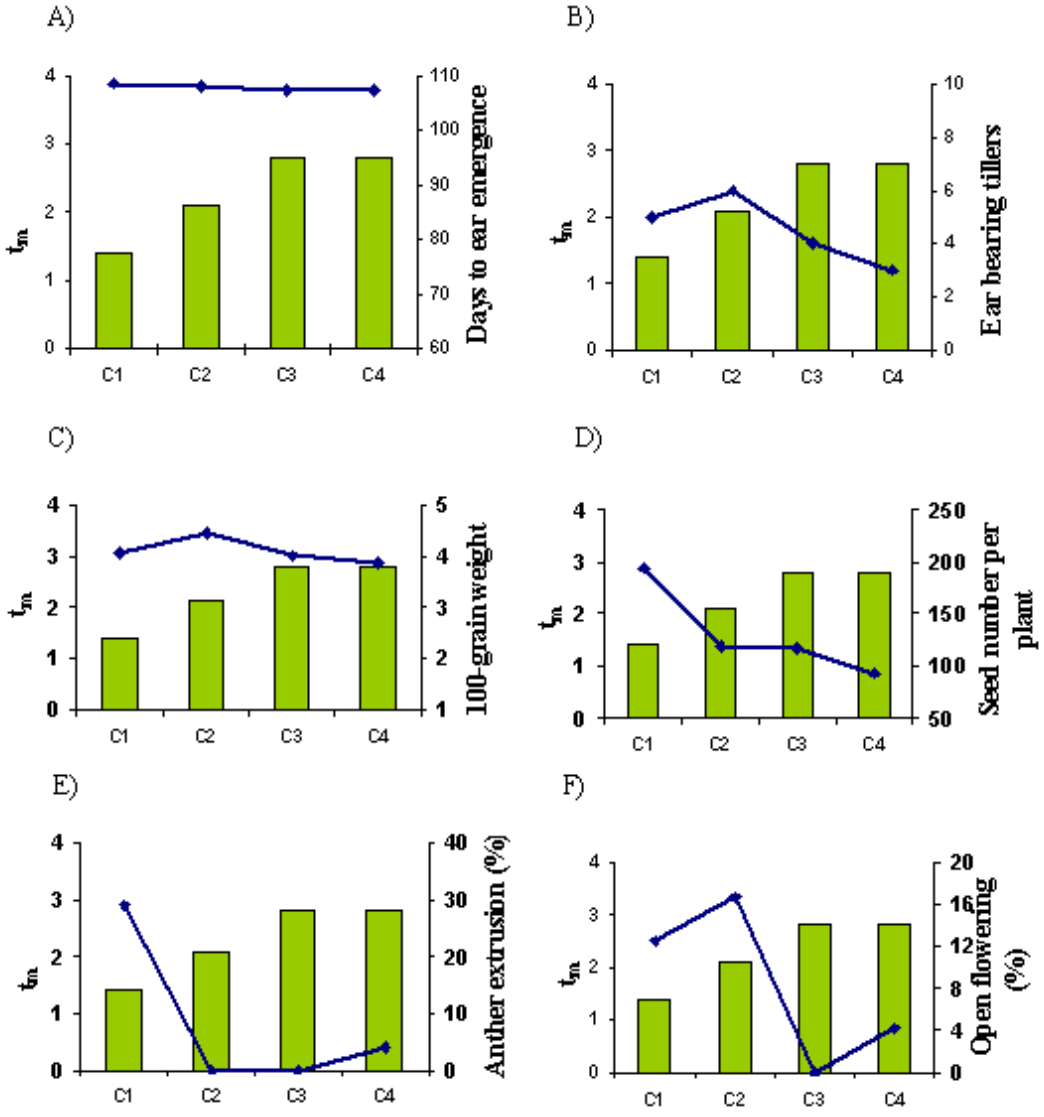

Fig 4.6 Mean values for A) days to ear emergence, B) number of ear bearing tillers, C) seed weight, D) seed number, E) anther extrusion and F) open flowering against multilocus outcrossing rate (t_m) expressed in percentage of the progeny sample size in the final evaluation experiment.

4.3.2 Comparisons between RS cycles

Significant differences among cycles were only observed for total number of ear bearing tillers and seed number (Table 4.4).

Table 4.4 All possible differences between RS populations for tillers per plant and seeds per spike; Standard errors (SE) are given in parenthesis.

Difference (progeny samples)	Tillers	Seed number
C1-C2	-0.83 (1.04)	93.98 (34.28)**
C1-C3	0.77 (1.05)	95.07 (34.28)**
C1-C4	1.87 (1.04)	115.73 (34.64)**
C2-C3	1.05 (1.05)	1.08 (33.53)
C2-C4	2.70 (1.04)*	21.74 (33.89)
C3-C4	1.10 (1.05)	20.66 (33.89)

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

4.3.3 Correlations

No significant correlations existed between outcrossing rate and any of the quantitative traits assessed in the final evaluation experiment (Table 4.5). Small but significant correlations of ear emergence to tillering and ear emergence indicate that early heading genotypes tend to have fewer tillers and less seeds per plant.

Table 4.5 Coefficients of phenotypic correlations among observed heterozygosity and various quantitative traits assessed in the final evaluation experiment.

Trait	DTE	Tillers	SN	GWT
Tillers	-0.34*			
SN	-0.38**	0.40**		
GWT	-0.18	0.22*	0.02	
H_O	-0.08	-0.04	-0.09	0.14

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

DTE = days to ear emergence, Tillers = total number of ear bearing tillers per plant, SN = seed number per plant, GWT = 100 grain-weight, H_O = observed heterozygosity.

5. Discussion

5.1 Limitations of the experiment

From a broad genetic base of 201 accessions, 16 accessions were selected at the end of the first selection cycle. These 16 accessions were supposed to be continued to the succeeding cycles to maintain sufficient genetic variation in the population. But only 4 accessions were successfully advanced to the next cycles because of strong seed dormancy at the beginning of the second selection cycle. So from this cycle onwards the genetic diversity was much lower than intended.

A set of six co-dominant SSR markers was selected for the experiment initially. But because of technical bottlenecks, only four of them could be used in the first cycle and three in the second. All six markers were used in further selection cycles and in the final evaluation after a MegaBACE sequencer became available to substitute the metaphore agarose gel technique. Both technologies yielded precise data. However, it was difficult to assign the alleles detected by the first approach to those of the second. Therefore, critical comparisons among the heterozygosity levels of populations C1 to C4 were only possible from data assessed before or after the technology change.

Estimation of multi-locus outcrossing rates requires populations in inbreeding equilibrium. Since this assumption is not fulfilled in a population under strong selection, no such estimates were computed in the course of the RS experiment. In the final evaluation experiment, less bias was to be expected. Still the estimates obtained need to be interpreted with caution. As all four RS populations contributed to the pollen cloud, less improved plants partly may have pollinated the more improved ones and vice versa. This would cause an overestimation of the outcrossing rate in the early RS populations and an underestimation in the advanced ones.

5.2 Discussion of results obtained in the course of the RS experiment

5.2.1 Observed heterozygosity in the source population

In the source population, the cultivated varieties had H_O values ranging from 0 to 19%, while the landraces had values ranging from 0 to 28%. On average, the H_O values amounted to 0.64% in the varieties and 0.79% in the landraces.

In a study conducted by Allard et al. (1972) in California, USA, on the effect of natural selection over 25 generations in barley Composite Cross V, composed by intercrossing 30 North American varieties, the percentage of observed heterozygosity decreased rapidly in early generations to 6.7% in F_4 and 1.5% in F_6. Thereafter, no further consistent change was observed. In studies of Nevo et al. (1979, 1986 a, b), the mean observed heterozygosity was 0.3% in 28 populations from Israel and 0.0% in 11 wild barley populations from Iran and Turkey. As compared to these latter studies, the H_O values obtained in our study reached much higher levels corroborating the efficiency of recurrent selection.

In wheat, literature reports on H_O values are very scarce. In a study with 43 accessions of U.S wheat cultivars and breeding lines representing seven U.S market classes, the average level of H_O was 0.9% (Chao et al. 2007). Similarly, a mean H_O value of 0.6% was found in 39 Italian emmer wheat accessions (Pagnotta et al. 2005). In the partially allogamous species sorghum, Rabbi et al. (2009) reported H_O values ranging between 2 and 8% in seven cultivars sampled from Kenya and Sudan. Muraya et al. (2009) obtained H_O values between 1 and 49% in 62 wild sorghum populations from Kenya. In predominantly cross pollinated rapeseed, an H_O value ranging from 42% to 51% was observed in a genetic diversity study of eight cultivars collected from Europe and China (Yong-guo et al. 2009). In allogamous maize, a mean H_O value of 69% was observed in 54 landraces of south-west China (Yao et al. 2007).

5.2.2 Observed heterozygosity in the course of the RS experiment

Among the accessions selected at the end of the first selection cycle, four of them (Numbers 4, 9, 12 and 16, see Table 3.2), were six-rowed varieties showing values of H_O ranging from 0.0 to 28%, compared to two-rowed (0.0 to 19%) indicating that the six-rowed varieties are more heterozygous than the two-rowed varieties. The 12 landraces (including the six-rowed variety, RUM) from Jordan and 11 wild barley populations did not contain any heterozygous plant while they displayed outcrossing rates (t_m) of 0.0 to 0.6% and 0.0 to 1.8 %, respectively, in the study of Abdel-Ghani et al. (2004). This could be attributable to the influence of environment as the plants were raised under greenhouse conditions in this study, while they were grown in the field in Jordan in the latter.

In the present experiment, significant differences in heterozygosity were only obtained between the source population and the rest of the populations (C1, C2 and C3), while no significance was observed between C1, C2 and C3 populations. This might have been due to the broader genetic base present in the source population than in the later generations. Furthermore, the H_O estimates had rather large standard errors due to sampling effects caused by variation between and within families.

5.2.3 Observed heterozygosities of individual accessions

Among the four accessions advanced to C3, only IG31513 displayed increased heterozygosity from C0 to C3 whereas the other three accessions showed a reduction from C0 to C1 and remained constant at that level until C3. This indicates that the dormancy problem at the onset of the second RS cycle may have affected seeds resulting from outcrossing more than those resulting from selfing. This would not only have decreased heterozygosity but also variation for outcrossing related traits within accessions.

5.2.4 Observed heterozygosity in selected fractions

In the selected fractions, the level of observed heterozygosity varied considerably between selection cycles (Fig 4.1). In view of the extremely low heritability of the H_O values, this

should largely be due to the different environmental conditions in the individual greenhouse seasons.

5.2.5 Response to selection

A significant positive response to selection was obtained for the mean observed heterozygosities in the populations over the four cycles of selection. The response was highest in the first cycle which may be attributable to the strong selection and the great genetic diversity in population C0. The low response obtained in populations C1 and C2 probably was caused by a loss of genetic variation due to deep dormancy at the onset of the second cycle. These findings are in agreement with recurrent selection studies in maize which indicated that genetic drift reduced selection response (Helms et al. 1989, Eyherabide and Hallauer 1991).

5.2.6 Marker polymorphism

The SSR markers used in this study proved to be highly polymorphic (Table 4.1). The PIC values ranged from 0.58 to 0.88 giving evidence for high informativeness. However, the average number of alleles per marker dramatically decreased from C0 to C1 confirming the reduction of genetic diversity mainly caused by the dormancy problem. Observed heterozygosity values increased from populations C0 to C3 for all the common markers except Bmag222 which showed a slight decrease from C2 to C3. Chen et al. (2010) reported a mean number of alleles of 4.1 and PIC values ranging from 0.23 to 0.88 in 116 barley accessions from China using 21 SSR markers. In a study of genetic diversity of wild barley in 16 populations of Jordan, 18 microsatellite markers revealed a total of 249 alleles with an average of 13.8 alleles per locus (Baek et al. 2003). Malysheva-Otto et al. (2006) found a mean number of 16.7 alleles per locus with PIC values ranging from 0.64 to 0.78 in 953 cultivated barley accessions from all continents except Australia, profiled with 48 SSR markers.

The number of alleles per locus and the PIC values estimated in this study are higher than in the previous reports for barley. This could be due to the diverse genetic origins of the barley accessions chosen for this study.

5.3 Discussion of the results obtained in the final evaluation experiment

5.3.1 Observed heterozygosity and outcrossing rate

The level of heterozygosity increased from 0.23% in C1 to 1.29 in C4 (Fig 4.3) when the preserved seed from all RS cycles was grown in a common greenhouse experiment. This confirms the results obtained in the course of the RS experiment. The multi-locus outcrossing estimates in this study ranged from 1.4 % in C1 to 2.8 % in C4 (Fig.4.4). Parzies et al. (2008) found season specific outcrossing rates ranging from 0.0 to 3.4% for all genotypes when comparing anther-extruding and non-extruding barley genotypes. Abdel-Ghani et al. (2004) found low outcrossing rates of 0.0 to 1.8% in wild and cultivated barley accessions from Jordan. Brown et al. (1978) reported a mean outcrossing rate of 1.7 % in 28 populations of wild barley from Israel with a range of 0.0 to 9.6% using isozyme markers.

5.3.2 Variation of outcrossing among families

All the families in populations C1 and C2 had outcrossing rates below 50% while in C3 and C4 three families considerably surpassed this threshold. Outcrossing rate estimates ranged from 0.0 to 91% in these last cycles. There were 3 out of 24 families in C3 and C4 which exceeded 50% outcrossing rate of which 2 families belong to IG31498, a landrace from Syria. Such high values of outcrossing rates in individual families have never been reported in barley. High t_m estimates were reached inspite of hot and dry conditions during the flowering period in the greenhouse. To some extent the values may be overestimated because of small family sizes.

Barnaud et al. (2008) showed a similar range of variation, from 0.0 to 73 % among families of sorghum landraces in Cameroon while the mean outcrossing rate recorded was 18 %. The authors concluded that the high outcrossing rate observed in one particular family was due to the loosely arranged panicles. In common bean, the mean outcrossing rate was found to be 6.9 % in Southern California while the outcrossing rate of individual families ranged between 0.0 and 78 % (Wells et al. 1988, Ibarra-Perez F.J et al. 1997, Ferreira et al. 2000). This variation can be explained by genetic variation occurring not only among, but also within families. These literature reports and the present results clearly demonstrate that the outcrossing of autogamous and partially autogamous plants is a heritable trait and thus can be improved by selection.

5.3.3 Correlations between outcrossing rate and presumably outcrossing related traits

No significant correlations were obtained between outcrossing rate and presumably outcrossing related traits. Abdel-Ghani et al. (2005) found that cold and humid conditions favour outcrossing in barley. In contrast, the climatic conditions were very hot and dry during flowering in May 2008, recorded temperatures being 30-40°C in the greenhouse. This might be one of the reasons for the relatively low outcrossing rates obtained in the present study.

5.3.4 Impact of flowering characteristics on the outcrossing rate

The outcrossing rates increased from C1 to C4 populations while the heading date was almost the same for all populations indicating that there is no impact of flowering on the outcrossing rates. Flowering time is an adaptative charactersitic in the subtropical climate of West Asia and North Africa. Late flowering avoids frost damage to reproductive tissues. In spring cultivars, late flowering stimulates vegetative growth which is a disadvantage in drought stress environments as it depletes the soil water reserve leaving too little water for anthesis and grain filling (Einfeldt et al. 2005). Indeed, wild barley accessions from Israel typically carry the early flowering *Ppd-H1* gene (which is a major determinant of long day response) whereas cultivated barley carries the recessive late flowering allele of that gene (Wang et al. 2010). High outcrossing rates could be advantageous in both late and early flowering populations because increased heterozygosity improves vigor and phenotypic stability. However, under severe drought or heat stress, assuring reproduction by self-pollination should be equally important. Therefore, it is not surprising that no significant correlation was observed between outcrossing rate and flowering date in our material.

In self pollinated species the ears usually show up before anthers extrude and the flowers open. This was also the case in our study. Even though the anthers extrude after ear emergence, a plant's pollen may already have shed on its own stigma before the flower opens and outside pollen can get access to the stigma. In contrast, the chasmogamous plants open their flowers before pollen shedding and generally keep it open for about 30 min (Honda et al. 2005). In this study, anther extrusion was observed in 30% of the plants in C1 and 12 to 16% in C2 populations. Interestingly, this did not lead to high oucrossing rates in those populations which could probably be due to the hot and dry conditions present in the greenhouse.

Anther dehiscence in barley was initially thought to be a consequence of desiccation (Schmid 1976). However, in cleistogamous two-rowed barley, it has been reported that the rapid pollen swelling is associated with anther dehiscence (Matsui et al. 1999 a, b, 2000 a, b, Rehman et al. 2004). The latter authors concluded that the swelling of pollen occurs inside the anther absorbing the moisture from interpollen space, exerting pressure to bulge theca, rupture septa and unfold the locules by bending the locule wall outward causing anther dehiscence.

Matsui et al. (1999) reported that anther dehiscence in rice is a moisture requiring process and increases with increase in relative air humidity. This is in accordance with findings of Abdel-Ghani et al. (2004) that under severe drought and heat stress, the spikes remain in the flag sheath favouring self pollination while the spike is still enclosed and that under high annual precipitation and cool temperatures during flowering time enhanced outcrossing rates can be observed. Later Rehman et al. (2006) found that presence of potassium (K) flux during maturation of anthers, pollen and stigma is the driving force behind the synchronization of the stigma becoming receptive thus favouring successful pollination. In view of the above studies, it can be concluded that pollen maturation and anther dehiscence depend on the air humidity in barley.

Honda et al. (2005) observed that barley flowers normally open synchronously with anther dehiscence and filament extension, and pollen is subsequently scattered from extruded anthers. As the pollen remains viable for at least 48h (Parzies et al. 2005), while the stigma are short-lived, selection for genotypes favoring protandry, long stamen filaments and open flowering might favor anther dehiscence and crosspollination.

Cleistogamy of barley was reported to be under the control of two tightly linked genes (*cly1* and *Cly2*) with epistatic interaction between the two (Turuspekov et al. 2004). In contrast, Abdel-Ghani found quantitative variation for anther extrusion in F3-popualtions in Jordan, and obtained intermediate to high heritability estimates indicating polygenic inheritance. However, no significant difference in outcrossing rates was detected between anther-extruding and non-extruding barley genotypes indicating that high anther extrusion is not a sufficient condition to cause increased outcrossing rates (Parzies et al. 2008). As variation in anther extrusion can only influence the outcrossing rate of chasmogamous plants, no correlation between the two traits can be expected in predominantly cliestogamous populations. This is in accordance with the present results. Gene *Cly*1 has pleiotropic effects on lodicule size and auxin response (Honda et

al. 2005). Recently, Nair et al. (2010) isolated this gene and found that its expression was strongest in the lodicule primordial confirming its impact on flowering behaviour.

5.4 General conclusions and outlook

The level of observed heterozygosity increased in the course of recurrent selection cycles in this study. This was confirmed in the evaluation experiment conducted in a greenhouse under common environmental conditions. Selection for heterozygosity led to a doubling (from 1.4 to 2.8%) of the outcrossing rate. Although the achieved outcrossing rate is still too low to create measurable progress in agronomic productivity, results are important as they demonstrate for the first time that enhancing the heterozygosity level of an "autogamous" cereal crop is feasible by recurrent selection. The marker-based approach employed in the present study, proved to be excellently suited for this purpose. The improved population deserves to be further improved in breeding programs in the WANA barley growing areas.

6. Summary

Improving the grain yield in drought stress environments such as the semi-arid areas of the West Asia North Africa (WANA) region has been a persistent problem since many years. Although barley (*Hordeum vulgare* L.) is widely grown in this region, the possibility of a crop failure is high. Being an autogamous crop, barley cultivars display almost complete homozygosity. Population genetic studies have shown that heterozygous barley genotypes possess a significantly increased stress tolerance, thus, being superior in both the level and stability of yielding performance. Therefore, increasing the level of heterozygosity in barley was the general aim of this study. For this purpose, a new marker-assisted recurrent selection (RS) approach was developed and applied to a genetically broad based world collection of barley germplasm.

The specific objectives of this study were: (1) to investigate the efficacy of the above approach, (2) to determine the gain in heterozygosity over four RS cycles and to evaluate the selection results in a final experiment under common environmental conditions, (3) to estimate the selection differential, response to selection and realized heritability and (4) to provide barley materials with increased heterozygosity to plant breeding programs in the WANA region.

Applying the RS approach, only plants showing superior levels of heterozygosity at co-dominant molecular marker (SSR) loci were advanced to successive selection cycles. These heterozygous plants were expected to carry a combination of advantageous alleles a) for open flowering from the female parent, and b) for pollen shedding from the male parent. For marker assessment, bulking of the plants and multiplexing of the SSR markers was practised in each selection cycle to save time and labour. The most polymorphic bulks were genotyped plant-wise and seed of the most heterozygous plants was advanced to the subsequent RS cycles.

In the **course of the RS experiment**, a base population was compiled from 201 gene bank accessions held by the “International Center for Agricultural Research in Dry Areas” (ICARDA) and the “Institute of Plant Genetics and Crop Plant Research” (IPK) in Germany. Selection led to a stepwise increase in the heterozygosity from 0.60% in the base population to 3.24% after four cycles of selection. In the base population, the six-rowed landraces showed higher heterozygosity than the two-rowed.

Selection response was highest in the first RS cycle which may be attributable to a major decline of the genetic variance from cycle to cycle and to a severe reduction of the population size due to strong dormancy among the entries selected in the first RS cycle. Very low realized heritabilities for observed heterozygosity were obtained in each RS cycle. Nevertheless, significant selection response was obtained.

In order to compare the results of the individual RS cycles under common environmental conditions, preserved seed from each of the selected parent plants was grown in a **final greenhouse experiment**. Beside heterozygosity, various development, flowering and performance traits (days to ear emergence, anther extrusion, open flowering, number of ear bearing tillers, 100-grain weight and seed number) were additionally assessed in this experiment. The observed heterozygosity increased from 0.23% in population C1 via 0.69% in C2 and C3 to 1.29% in C4.

The marker genotypes assessed in the final experiment were used to estimate multi-locus outcrossing rates. Values increased from 1.4% in C1 via 2.1% in C2 to 2.8% in C3 and C4. Generally, the increase from cycle to cycle was significant. Only the progress from C1 to C2 and from C3 to C4 did not reach the 5% significance level. All estimates were probably downward biased due to extremely high temperatures in the greenhouse during flowering. Great differences existed between the outcrossing rates of individual families within populations. Only non-significant weak to negligible correlations were obtained between floral traits and the outcrossing rate.

The observed positive response to recurrent selection substantiates the efficacy of the present approach for enhancing the level of heterozygosity in barley, offering good perspectives for improving the productivity of the crop in the stress prone WANA region. The new selection approach, in principle, is applicable to other autogamous or partially autogamous crop plants as well.

7. **Zusammenfassung**

Die Verbesserung des Kornertrags unter Trockenstressbedingungen, wie sie beispielsweise in den semi-ariden Gebieten der sogenannten WANA-Region (West-Asien und Nord-Afrika) auftreten, stellt seit vielen Jahren ein hartnäckiges Problem dar. Obgleich Gerste (*Hordeum vulgare* L.) in dieser Region weitverbreitet angebaut wird, besteht eine hohe Gefahr von Ernteausfällen. Aufgrund ihrer autogamen Blühbiologie weisen Gerstensorten eine fast vollständige Homozygotie auf. Populationsgenetische Studien zeigten, dass heterozygote Gerstengenotypen eine signifikant höhere Stresstoleranz besitzen und dementsprechend sowohl im Ertragsniveau als auch in der Ertragsstabilität überlegen sind. Die Erhöhung des Heterozygotiegrades der Gerste war daher das Hauptziel dieser Arbeit. Zu diesem Zweck wurde ein neuer Ansatz der marker-gestützten rekurrenten Selektion (RS) auf hohen Heterozygotiegrad entwickelt und auf eine weltweite, genetisch diverse Gerstenkollektion angewendet.

Die Ziele dieser Arbeit waren insbesondere (1) die Überprüfung der Wirksamkeit des oben genannten Ansatzes, (2) die Bestimmung des Heterozygotiegewinns über vier RS-Zyklen sowie die Evaluierung der einzelnen Selektionszyklen in einem vergleichenden Abschlußexperiment unter gemeinsamen Umweltbedingungen, (3) die Schätzung des des Selektionsdifferentials, Selektionsgewinns sowie der realisierten Heritabilität, und (4) die Bereitstellung von Gerstenmaterial mit erhöhtem Heterozygotiegrad für Pflanzenzüchtungs-programme in der WANA-Region.

Der Heterozygotiegrad wurde mittels kodominanter Mikrosatellitenmarker bestimmt. Hierbei wurden nur solche Pflanzen selektiert, die Heterozygotie an möglichst vielen Markerloci aufwiesen. Es konnte dann angenommen werden, dass diese Pflanzen aus der Kreuzung zwischen einer Mutterpflanze mit genetischer Veranlagung für Offenblütigkeit und einer Bestäuberpflanze mit erhöhter Pollenschüttung hervorgegangen war. Für die Marker-auswertung wurden zunächst jeweils sechs Pflanzen in einer Mischprobe analysiert. Proben mit hohem Polymorphiegrad wurden anschließend einzelpflanzenweise genotypisiert. Um den Arbeits- und Zeitaufwand gering zu halten, kam ab dem zweiten Selecktionszyklus eine Multiplex-Analyse zum Einsatz (zwei Kombinationen zu je drei Markern).

Im **Verlauf des RS-Experimentes** wurde eine Ausgangspopulation aus 201 Gersten-Akzessionen zusammengestellt, die aus den Sammlungen der Genbanken am International Center for Agricultural Research in Dry Areas (ICARDA) und am Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK) in Deutschland stammen. Es konnte ein schrittweiser Anstieg des Heterozygotiegrades von 0,60% in der Ausgangspopulation auf 3,24% nach vier Selektionszyklen festgestellt werden. In der Ausgangspopulation zeigten die sechsreihigen Landrassen einen höheren Heterozygotiegrad als die zweireihigen.

Der Selektionsgewinn war im ersten RS-Zyklus am höchsten, was auf eine starke Abnahme der genetischen Varianz im Verlauf des Experimentes sowie eine erhebliche Dezimierung des Populationsumfanges durch viele hochgradig dormante Prüfglieder nach dem ersten RS-Zyklus zurückführbar ist. Für den Heterozygotiegrad ergaben sich in den einzelnen RS-Zyklen sehr niedrige Schätzwerte der realisierten Heritabilität. Trotzdem wurde ein insgesamt signifikanter Selektionsgewinn erzielt.

Zum Vergleich der in den einzelnen RS-Zyklen selektierten Fraktionen unter gemeinsamen Umweltbedingungen wurde konserviertes Saatgut in einem **abschließenden Gewächshausexperiment** angebaut. Neben der Heterozygotie wurden verschiedene Entwicklungs-, Blüh- und Leistungsmerkmale (Tage bis zum Ährenschieben, Antherenextrusion, offenes Abblühen, Anzahl ährentragender Halme, Hundertkorngewicht und Kornzahl je Ähre) erhoben. Die beobachtete Heterozygotie stieg von 0,30% in der C1-Population über 0,69% in C2 und C3 auf 1,29% in C4. Die unterschiede zwischen den zyklen en ueberwiegen war statistisch signifikant.

Die im Abschlussexperiment festgestellten Markergenotypen wurden auch zur Schätzung der Multilocus-Auskreuzungsraten herangezogen. Die Werte stiegen von 1,4% in C1 über 2,1% in C2 auf 2,8% in C3 und C4. Der Anstieg von C1 zu C3 und C4 war signifikant. Alle Schätzwerte sind wahrscheinlich nach unten verzerrt, da während der Blüte im Gewächshaus extrem hohe Temperaturen herrschten. Große Unterschiede existierten zwischen den Auskreuzungsraten einzelner Familien innerhalb der Populationen. Es bestanden keine signifikanten Korrelationen zwischen der Auskreuzungsrate und den Blühmerkmalen.

Der beobachtete positive Selektionsgewinn bekräftigt die Effektivität des hier beschriebenen Ansatzes zur Steigerung des Heterozygotiegrades beim „Selbstbefrucher“ Gerste. Hiermit

eröffnen sich gute Perspektiven zur Verbesserung der Produktivität dieser Kulturart in der stressanfälligen WANA-Region. Der praktizierte Ansatz ist grundsätzlich auch auf andere autogame oder partiell autogame Nutzpflanzenarten anwendbar.

8. References

Allard RW, Hansche PE. 1964. Some parameters of population variability and their implications in plant breeding. Advances in Agronomy 16:281-325.

Allard RW, Kahler AL, Weir BS. 1972. The effect of selection on esterase allozymes in a barley population. Genetics 72:489-503.

Abdel-Ghani AH, Parzies HK, Ceccarelli S, Grando S, Geiger HH. 2005. Estimation of quantitative genetic parameters for outcrossing-related traits in barley. Crop Science 45:98-105.

Abdel-Ghani AH, Parzies HK, Ceccarelli S, Grando S, Geiger HH. 2003. Evaluation of floral characteristics of barley in the semi-arid climate of Syria. Plant Breeding 122:273-5.

Abdel-Ghani AH, Parzies HK, Omary A, Geiger HH. 2004. Estimating the outcrossign rate of barley landraces and wild barley populations collected from ecologically different regions of Jordan. Theoretical Applied Genetics 109:588-95.

Botstein D, White RL, Skolnick M, Davis RW. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. The American Journal of Human Genetics 32:314-31.

Baek HJ, Beharav A, Nevo E. 2003. Ecological genomic diversity of microsatellites in wild barley, *Hordeum spontaneum*, populations in Jordan. Theoretical Applied Genetics 106:397-410.

Becker HC, Damgaard C, Karlsson B. 1992. Environmental variation for outcrossing rate in rapeseed (*Brassica napus*). Theoretical Applied Genetics 84:303-6.

Bernardo R, Moreau L, Charcosset A. 2006. Number and fitness of selected individuals in marker-assisted and phenotypic recurrent selection. Crop Science 46:1972-80.

Bernaud A, Trigueros G, McKey D, Joly HI. 2008. High outcrossing rates in fields with mixed sorghum landraces: How are landraces maintained? Heredity 101:445-52.

Brantestam KA, von Bothmer R, Dayteg C, Rashal I, Tuvesson S, Weibull J. 2007. Genetic diverisy changes and relationships in spring barley (*Hordeum vulgare* L.) germplasm of nordic and baltic areas as shown by SSR markers. Genetic Resources and Crop Evolution 54:749-58.

Brown AHD, Nevo E, Zohary D, Dagan O. 1978. Genetic variation in natural populations of wild barley (*Hordeum spontaneum*). Genetica 49:97-108.

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

Brun R, Peterson CJ, Byrne I. 1997. Yield and yield stability factors associated with hybrid wheat. In Proc. of the 5th Int. Wheat Symp. Ankara, Turkey. CIMMYT, Mexico.

Byrne I, Rasmusson DC. 1974. Recurrent selection for mineral content in wheat and barley. Euphytica 23:241-9.

Ceccarelli S. 1978. Single-gene inheritance of anther extrusion in barley. Journal of Heredity 69:210-1.

Chao S, Zhang W, Dubcovsky J, Sorrells M. 2007. Evaluation of genetic diversity and genome-wide linkage disequilibrium among US wheat (*Triticum aestivum* L.) germplasm representing different market classes. Crop Science 47:1018-30.

Chen A, Reinheimer J, Brũlé-Babel A, Baumann U, Pallotta M, Fincher GF, Collins NC. 2009. Genes and traits associated with chromosome 2H and 5H regions controlling sensitivity of reproductive tissues to frost in barley. Theoretical Applied Genetics 118:1465-76.

Chen F, Chen D, Vallés MP, Gao Z, Chen X. 2010. Analysis of diversity in chinese cultivated barley with simple sequence repeats: Differences between eco-geographic populations. Biochemical Genetics 48:44-56.

Delogu G, Lorenzoni C, Maracco A, Martiniello P, Odoardi M, Stanca AM. 1988. A recurrent selection programme for grain yield in winter barley. Euphytica 37:105-10.

Díaz-Lago JE, Stuthman DD, Abadie TE. 2002. Recurrent selection for partial resistance to crown rust in oat. Crop Science 42:1475-82.

Dizkirici A, Guren HE, Onde S, Temel F, Akar T, Budak H, Kaya Z. 2008. Microsatellite (SSR) variation in barley germplasm and its potential use for marker assisted selection in scald resistance breeding. International Journal of Integrative Biology 4:9-15.

Djé Y, Forcioli D, Ater M, Lefebvre C, Vekemans X. 1999. Assessing population genetic structure of sorghum landraces from north-western Morocco using allozyme and microsatellite markers. Theoretical Applied Genetics 99:157-63.

Djé Y, Heuertz M, Ater M, Lefebvre C, Vekemans X. 2004. In *situ* estimation of outcrossing rate in sorghum landraces using microsatellite markers. Euphytica 138:205-12.

Djé Y, Heuertz M, Lefebvre C, Vekemans X. 2000. Assessment of genetic diversity within and among germplasm accessions in cultivated sorghum using microsatellite markers. Theoretical Applied Genetics 100:918-25.

Doggett H. 1972. Recurrent selection in sorghum populations. Heredity 28:9-29.

Doll H. 1987. Outcrossing rates in autumn and spring-sown barley. Plant Breeding 98:339-41.

Duvick DN, editor. 1999. Heterosis: Feeding people and protecting natural resources. In *The genetics and exploitation of heterosis in crops* (*eds*. Coors JG, Pandey S), pp.19-30. Crop science society of America, Madison, WI.

Einfeldt CHP, Ceccarelli S, Grando S, Gland-Zwerger A and Geiger HH. 2005. Heterosis and mixing effects in barley under drought stress. Plant Breeding 124:350-5.

Enjalbert J, David JL. 2000. Inferring recent outcrossing rates using multilocus individual heterozygosity: Application to evolving wheat populations. Genetics 156:1973-82.

Eyherabide GH, Hallauer AR. 1991. Reciprocal full-sib recurrent selection in maize: II. contribution of additive, dominance and genetic drift effects. Crop Science 31:1442-8.

FAO. 2007. Statistical year book. http://www.fao.org.

Falconer DS, Mackay TFC, 1996. Introduction to quantitative genetics. *fourth edition*. Pearson Education Ltd., Essex, England.

Ferreira JJ, Alvarez E, Fueyo MA, Roca A, Giraldez R. 2000. Determination of the outcrossing rate of *Phaseolus vulgaris* L. using seed protein markers. Euphytica 113:259-63.

Giles RJ, McConnell G, Fyfe JL. 1974. The frequency of natural cross-fertilization in a composite cross of barley grown in Scotland. The Journal of Agricultural Science 83:447-50.

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

Haussmann BIG. 1995. Effects of heterozygosity and heterogeneity on the adaptedness of sorghum to a semi-arid area of Kenya. Ph.D. Diss. University of Hohenheim, Stuttgart, Germany.

Haussmann BIG, Obilana AB, Blum A, Ayiecho PO, Schipprack W, Geiger HH. 1998. Hybrid performance of sorghum and its relationship to morphological and physiological traits under variable drought stress in Kenya. Plant Breeding 117:223-9.

Haussmann BIG, Obilana AB, Ayiecho PO, Blum A, Schipprack W and Geiger HH. 2000. Yield and yield stability of four population types of grain sorghum in a semi-arid area of Kenya. Crop Science 40:319-29.

Helms TC, Hallauer AR, Smith OS. 1989. Genetic drift and selection evaluated from recurrent selection programs in maize. Crop Science 29:602-7.

Hockett EA, Aastveit K, Glibertson KM. 1989. Outcrossing on genetic male sterile barley in Norway and the United States. Heriditas 111:167-9.

Honda I, Turuspekov Y, komatsuda T and Watanabe Y. 2005. Morphological and physiological analysis of cleistogamy in barley (*Hordeum vulgare*). Physiologia Plantarum 124:524-31.

Ibarra-Perez FJ, Ehdaie B, Waines JG. 1997. Estimation of outcrossing rate in common bean. Crop Science 37:60-5.

ISTA. 1999. International Rules for seed testing. International seed testing association, Seed Science and Technology 27: Supplement, p.178. Zurich, Switzerland.

Ivandic V, Hackett CA, Nevo E, Keith R, Thomas WTB, Forster BP. 2002. Analysis of simple sequence repeats (SSRs) in wild barley from the fertile crescent: Associations with ecology, geography and flowering time. Plant Molecular Biology 48:511-27.

Jilal A, Grando S, Henry RJ, Lee LS, Rice N, Hill H, Baum M, ceccarelli S. 2008. Genetic diversity of ICARDA's worldwide barley landrace collection. Genetic Resources and Crop Evolution 55:1221-30.

Johansen B, von Bothmer R. 1994. Pollen size in *Hordeum* L.: Correlation between size, ploidy level, and breeding system. Sexual Plant Reproduction 7:259-63.

Jordaan JP, Engelbrecht SA, Malan JH and Knobel HA, editor. 1999. Wheat anad heterosis. In *The genetics and exploitation of heterosis in crops* (*eds.* Coors JG, Pandey S), p.411-420. Crop science society of America, Madison, WI. .

Kahler AL, Clegg MT and Allard RW. 1975. Evolutionary changes in the mating system of an experimental population of barley (*Hordeum vulgare* L.). Proceedings of the National Academy of Sciences 72:943-6.

Lawrie RJ, Matus-Cádiz MA, Hucl P. 2006. Estimating out-crossing rates in spring wheat cultivars using the contact method. Crop Science 46:247-9.

Lee EA, Doerksen TK, Kannenberg LW. 2003. Genetic components of yield stability in maize breeding populations. Crop Science 43:2018-27.

Leon. 1991. Heterosis and mixing effects in winter oilseed rape. Crop Science 31:281-4.

Lord E. 1981. Cleistogamy: A tool for the study of floral morphogenesis, function and evolution. The Botanical Review 47:421-49.

Macaulay M, Ramsay L, Powell W. 2001. A representative 'highly informative' genotyping set of barley SSRs. Theoretical Applied Genetics 102:801-9.

Maestri E, Malcevschi A, Massari A, Marmiroli N. 2002. Genomic analysis of cultivated barley (*Hordeum vulgare*) using sequence-tagged molecular markers. Estimates of divergence based on RFLP and PCR markers derived from stress-responsive genes and simple-sequence repeats (SSRs). Molecular Genetics and Genomics 267:186-201.

Malysheva-Otto LV, Ganal MW, Roeder MS. 2006. Analysis of molecular diversity, population structure and linkage disequilibrium in a worldwide survey of cultivated barley germplasm (*Hordeum vulgare* L.). BMC Genetics 7:6.

Matsui T, Omasa A, Horie T. 1999a. Rapid swelling of pollen grains in response to floret opening unfolds locule in rice. Plant Production Science 2:196-9.

Matsui T, Omasa K, Horie T. 1999b. Mechanism of anther dehiscence in rice (*Oryza sativa* L.). Annals of Botany 84:501-6.

Matsui T, Omasa A, Horie T. 2000a. Rapid swelling of pollen grains in the dehiscing anther of two-rowed barley (*Hordeum distichum* L. emend. LAM). Annals of Botany 85:345-50.

Matsui T, Omasa A, Horie T. 2000b. Mechanism of septum opening in anthers of two-rowed barley (*Hordeum vulgare* L.). Annals of Botany 86:47-51.

Matsui T OK. 2002. Rice (*Oryza sativa* L.) cultivars tolerant to high temperature at flowering: Anther characteristics. Annals of Botany 89:683-7.

Mayer M, Gland A, Ceccarelli S and Geiger HH. 1995. Comparison of doubled haploid lines and F2 bulks for the improvement of barley in the dry areas of north Syria. Plant Breeding 114:45-9.

Muraya MM. 2009. Gentic diversity and mating system of wild sorghum in Kenya and adaptive value of wild x cultivated sorghum hybrids. Ph.D. Diss.University of Hohenheim, Stuttgart, Germany.

Nair SK, Wang N, Turuspekov Y, Pourkheirandish M, Sinsuwongwat S, Chen G, Sameri M, Tagiri A, Honda I, Watanabe Y, Kanamori H, Wicker T, Stein N, Nagamura Y, Matsumoto T and Komatsuda T. 2010. Cleistogamous flowering in barley arises from the suppression of microRNA-guided HvAP2 mRNA cleavage. Proceedings of the National Academy of Sciences 107:490-5.

Nevo E, Beiles A, Kaplan D, Storch N, Zohary D. 1986a. Genetic diversity and environmental associations of wild barley, *Hordeum spontaneum* (*Poaceae*) in Iran. Plant Systematics and Evolution 153:141-64.

Nevo E, Brown AHD, Zohary D. 1979. Genetic diversity in the wild progenitor of barley in Israel. Experentia 35.

Nevo E, Zohary D, Beiles A, Kaplan D, Storch N. 1986b. Genetic diversity and environmental associations of wild barley, *Hordeum spontaneum*, in Turkey. Genetica 68:203-13.

Olmedo-Arcega OB, Elias EM, Cantrell RG. 1995. Recurrent selection for grain yield in durum wheat. Crop Science 35:714-9.

Pagnotta MA, Mondini L, Atallah MF. 2005. Morphological and molecular characterization of Italian emmer wheat accessions. Euphytica 146:29-37.

Parlevliet JE, van Ommeren A. 1988. Recurrent selection for grain yield in early generations of two barley populations. Euphytica 38:175-84.

Parzies HK, Fosung Nke C, Abdel-Ghani AH, Geiger HH. 2008. Outcrossing rate of barley genotypes with different floral characteristics in drought stressed environments in Jordan. Plant Breeding 127:536-8.

Parzies HK, Schnaithmann F, Geiger HH. 2005. Pollen viability of *Hordeum spp*. genotypes with different flowering characteristics. Euphytica 145:229-35.

Parzies HK, Spoor W, Ennos RA. 2000. Outcrossing rates of barley landraces from Syria. Plant Breeding 119:520-2.

Pederson JF, Toy JJ, Johnson B. 1998. Natural outcrossing of sorghum and sudangrass in the central great plains. Crop Science 38:937-9.

Powell W, Machray GC, Povan J. 1996. Polymorphism revealed by simple sequence repeats. Trends in Plant Science 1:215-22.

Rabbi IY. 2009. Population dynamics of crop sorghum: Genetic structure, pollen dispersal and outcrossing rates. Ph.D. Diss.University of Hohenheim, Stuttgart, Germany.

Rehman S, Rha ES, Ashraf M, Lee KJ, Yun SJ, Kwak YG, Yoo NH, Kim J-K. 2004. Does barley (*Hordeum vulgare* L.) pollen swell in fractions of a second? Plant Science 167:137-42.

Rehman S, Yun SJ. 2006. Developmental regulation of K accumulation in pollen, anthers, and papillae: Are anther dehiscence, papillae hydration, and pollen swelling leading to pollination and fertilization in barley (*Hordeum vulgare* L.) regulated by changes in K concentration? Journal of Experimental Botany 57:1315-21.

Reich, VH and Atkins, RE. 1970. Yield stability of four population types of grain sorghum, *Sorghum bicolor* (L.) Moench, in different environments. Crop Science 10:511-7.

Ritland K. 2002. Extension of models for the estimation of mating systems using *n* independent loci. Heredity 88:221-8.

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

Rodriguez OA, Hallauer AR. 1988. Effects of recurrent selections in corn populations. Crop Science 28:796-800.

Roux F, Touzet P, Cuguen J, Le Corre V. 2006. How to be early flowering: An evolutionary perspective. Trends in Plant Science 11:375-81.

Saghai Maroof MA, Soliman K, Jorgensen R, Allard RW. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proceedings of the National Academy of Sciences 81:8014-8.

Satake T, Yoshida S. 1978. High-temperature induced sterility in indica rice at flowering. Japanese Journal of Crop Science 47:6-10.

Schimd R. 1976. Filament histology and anther dehiscence. Botanical Journal of the Linnean Society 73:303-15.

Schnell, FW and Becker HC. 1986. Yield and yield stability in a balanced system of widely differing population structures in *Zea mays* L. Plant Breeding 97:30-8.

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

Shorter R, Gibson P, Frey KJ. 1978. Outcrossing rates in oat species crosses (*Avena sativa* L.) X (*Avena sterilis* L.). Crop Science 18:877-8.

Singh SP, Terán H, Germán Muñoz C, Carlos Takegami J. 1999. Two cycles of recurrent selection for seed yield in common bean. Crop Science 39:391-7.

Springer NM, Stupar RM. 2007. Allelic variation and heterosis in maize: How do two halves make more than a whole? Genome Research 17:264-75.

Stelling D, Ebmeyer E, Link W. 1994a. Yield stability in faba bean, *Vicia faba* L. 2.Effects of heterozygosity and heterogeneity. Plant Breeding 112:30-9.

Stracke S, Haseneyer G, Veyrieras JB, Geiger HH, Sauer S, Graner A, Piepho HP. 2009. Association mapping reveals gene action and interactions in the determination of flowering time in barley. Theoretical Applied Genetics 118:259-73.

Struss D, Plieske J. 1998. The use of microsatellite markers for detection of genetic diversity in barley populations. Theoretical Applied Genetics 97:308-15.

Tautz D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Research 17:6463-71.

Turuspekov Y, Mano Y, Honda I, Kawada N, Watanabe Y, Komatsuda T. 2004. Identification and mapping of cleistogamy genes in barley. Theoretical Applied Genetics 109:480-7.

Virmani SS and Athwal DS. 1973. Genetic variability in floral characteristics influencing outcrossing in *Oryza sativa* L. Crop Science 13:66-7.

Wahle G GH. 1978. Vergleich der phaenotypischen stabilitaet von inzucht linen, einfachhybriden und populationen bei winterroggen. Zeitschrift Fur Pflanzenzuchtung 80:211-22.

Wells WC, Isom WH, Waines JG. 1988. Outcrossing rates of six common bean lines. Crop Science 28:177-8.

Weir BS. 1996. Genetic data analysis II: Methods for discrete population genetic data. Sinauer Associates, Inc., Sunderland, MA, USA.

Weyhrich RA, Lamkey KR, Hallauer AR. 1998. Responses to seven methods of recurrent selection in the BS11 maize population. Crop Science 38:308-21.

Wang G, Schmalenbach I, von Korff M, Leon J, Kilian B, Rode J, Pillen K. 2010. Association of barley photoperiod and vernalization genes with QTLs for flowering time and agronomic traits in a BC_2DH population and a set of wild barley introgression lines. Theoretical Applied Genetics 120:1559-74.

Yao Q, Yang K, Pan G, Rong T. 2007. Genetic diversity of maize (*Zea mays* L.) landraces from southwest China based on SSR data. Journal of Genetics and Genomics 34:851-60.

Yong-guo Z, Atta O, Chang-ming LU. 2009. Genetic diversity of European and Chinese oilseed *Brassica rapa* cultivars from different breeding periods. Agricultural Sciences in China 8:931-8.

9. Appendices

9.1 Appendix I

Table 9.1 Details of the genetic material used in the present study.

GENETIC MATERIAL FROM ICARDA								
IDENTITY		**POP_TYPE**	**ORIGIN**	**PROVINCE**	**LONGITUDE**	**LATTITUDE**	**ALTITUDE**	
29049		LA	SYR	Aleppo	37,55	36,18	360	
29059		LA	SYR	Aleppo	37,33	36,25	447	
29061		LA	SYR	Aleppo	37,75	36,48	510	
29075		LA	JOR	Irbid	35,92	32,68	430	
29080		LA	JOR	Irbid	35,60	32,53	-220	
29083		LA	JOR	Zarqa	36,25	32,08	613	
29089		LA	JOR	Amman	35,80	31,72	798	
29095		LA	JOR	Tafila	35,90	30,70	913	
29097		LA	JOR	Ma'an	35,42	30,33	1114	
29102		LA	JOR	Ma'an	35,43	30,15	1553	
29103		LA	JOR	Ma'an	35,48	30,00	1369	
31406	9 Sp-p	LA	SYR	Dar'a	36,23	32,63	601	
31411	12 E	LA	SYR	Homs	37,10	34,60	672	
31416	14 Sp-p	LA	SYR	Raqqa	39,21	36,41	333	
31419	16 E	LA	SYR	Raqqa	39,28	36,40	357	
31429	21 E	LA	SYR	Al Hasakah	40,78	36,09	268	
31436	24 Sp-p	LA	SYR	Aleppo	37,53	36,37	458	
31440	26 Sp-p	LA	SYR	Aleppo	38,02	36,79	342	
31443	28 E	LA	SYR	Raqqa	38,96	36,69	332	
31446	29 Sp-p	LA	SYR	Raqqa	39,00	36,17	270	
31460	36 Sp-p	LA	JOR	Karak	35,63	31,30	479	
31484	48 Sp-p	LA	SYR	Al Hasakah	40,77	36,50	304	
31495	54 E	LA	SYR	Aleppo	37,97	36,67	425	

31498	55 Sp-p	LA	SYR	Aleppo	38,37	36,89	495	
31513	63 E	LA	SYR	Damascus	36,54	33,84	1395	
31518	65 Sp-p	LA	SYR	Aleppo	36,82	36,15	293	
31526	70 E	LA	JOR	Amman	35,78	31,72	772	
31529	J-3	LA	JOR	Amman	35,95	31,70	717	
31531	J-5-1	LA	JOR	Ma'an	35,50	30,50	959	
31537	J-7-2	LA	JOR	Tafila	35,67	30,78	1245	
31538	J-8-1	LA	JOR	Karak	35,77	30,97	786	
31545	J-11-3	LA	JOR	Irbid	35,78	32,53	613	
31548	J-13-3	LA	JOR	Irbid	36,07	32,53	587	
31555	J-23	LA	JOR	Amman	36,07	31,60	778	
32573	Arabi Abiad	LA	SYR	Dar'a	36,34	32,63	698	
32575	Arabi Aswad	LA	SYR	Dar'a	36,34	33,11	608	
32584	Arabi Aswad	LA	SYR	Al Hasakah	40,68	36,59	327	
32585	Arabi Aswad	LA	SYR	Al Hasakah	40,61	36,66	351	
32586	Arabi Aswad	LA	SYR	Al Hasakah	40,58	36,74	370	
32587	Arabi Aswad	LA	SYR	Al Hasakah	40,62	36,97	414	
32588	Arabi Aswad	LA	SYR	Al Hasakah	40,80	36,70	328	
32595	Arabi Abiad	LA	SYR	Hama	36,87	35,02	349	
32597	Arabi Baladi	LA	SYR	Hama	37,02	35,10	486	
32598	Halabi	LA	SYR	Hama	36,96	35,38	392	
32742	BMW 46-1	LA	SYR	Lattakia	35,83	35,62	64	
32801	Baladi	LA	SYR	Damascus	36,51	33,82	1451	
32804	LR 122	LA	SYR	Homs	36,42	34,67	460	
33094	Arabi Aswad	LA	SYR	Dayr Az Zawr	40,57	34,92	158	
35240	SY 22171	LA	SYR	Sweida	36,59	32,83	988	
35996	91JOR-8	LA	JOR	Irbid	35,85	32,55	593	
36000	91JOR-27	LA	JOR	Balqa	35,58	32,17	-253	
36002	91JOR-34	LA	JOR	Irbid	35,65	32,48	359	
36003	91JOR-36	LA	JOR	Irbid	35,65	32,48	359	
36006	91JOR-44	LA	JOR	Irbid	35,67	32,41	500	

36009	91JOR-62	LA	JOR	Amman	35,71	31,94	487	
36012	91JOR-69	LA	JOR	Amman	35,65	31,80	-156	
36020	91JOR-93	LA	JOR	Amman	36,37	32,05	626	
36028	91JOR-115	LA	JOR	Karak	35,63	31,12	1158	
36029	91JOR-118	LA	JOR	Karak	35,63	31,12	1158	
36033	91JOR-125	LA	JOR	Karak	35,93	31,15	884	
36035	91JOR-127	LA	JOR	Karak	35,83	31,15	973	
36037	91JOR-130	LA	JOR	Karak	35,83	31,28	870	
36041	91JOR-142	LA	JOR	Amman	36,80	31,78	513	
36042	91JOR-143	LA	JOR	Amman	36,80	31,78	513	
36046	91JOR-162	LA	JOR	Ma'an	35,47	30,42	1459	
128173	BM 4 sel.	LA	SYR	Homs	38,36	34,57	388	
128195	91JOR-27 sel.	LA	JOR	Balqa	35,58	32,17	-253	
128199	91JOR-89 sel.	LA	JOR	Zarqa	36,27	32,12	595	
135258	A-23	LA	JOR	Mafraq	36,50	32,25	766	
138214		UM	---					
138215	Arta	CV	SYR					
138216	Tadmor	CV	SYR					
138217	ER/Apm	CV	---					
138218	WI 2291	CV	---					
138219	Zanbaka	CV	SYR					
138220	SLB 05-96	CV	---					
138221	Moroc 9-75	CV	---					
138222	SLB 34-40	CV	SYR	Raqqa	38,76	35,63	284	
138223	Zanbakian	CV	---					
138224	Harmal	CV	---					
138225	Sara	CV	SYR					
138226	Rihane-03	CV	SYR					
138227	Alanda-01	CV	---					
138228	Martin	CV	TUN					
138229	Saida	CV	DZA					

138230	Tichedrett	CV	DZA					
138231	Manel	CV	TUN					
138232	Badia	CV	---					
138233	Express	CV	FRA					
138234	IPA7	CV	IRQ					
138235	Arig8	CV	MAR					
138236	Rum	CV	JOR					
138240	Matnan-01	CV	---					
138241	Assala-04	CV	---					
138242	Salmas	CV	---					
138243	CalM	CV	EGY					
138244	Mari/Aths*2	CV	CYP					
138245	Aths	CV	GRC					
138246	Giza 125	CV	EGY					
138247	Giza 126	CV	EGY					
138248	Furat 1	CV	SYR					
138249	Furat 2	CV	SYR					
138250	Sadik-1	CV	---					
138251	Sadik-2	CV	---					
138252	Pamir 9	CV	---					
138253	Katara	CV	LBY					
138254	Barjouj	CV	LBY					
138255	Momtaz	UM	LBY					
138256	Batal-1	CV	---					
138257	Litani	CV	LBN					
138258	Birlik	CV	---					
138259	Furat3	CV	SYR					
138260	Keel	CV	AUS					
138261	Barque	CV	AUS					
138262	Shege	CV	ETH					
138263	Demhay	CV	ERI					

138264	Atsa	CV	ERI					
138265	Tarida	CV	LBY					
138266		UM	---					
138267		UM	---					
138268		UM	---					
138269		UM	---					
138270		UM	---					
138271		UM	---					
138272		UM	---					
138273		UM	---					
138274		UM	---					
138275		UM	---					
138276		UM	---					
138277		UM	---					
LAND RACES FROM JORDAN								
Karak-Mutah		LA	Jordan					
Jloun		LA	Jordan					
Mafraq		LA	Jordan					
Mafraq		LA	Jordan					
Amman		LA	Jordan					
Salt		LA	Jordan					
Faqo		LA	Jordan					
Tafila		LA	Jordan					
Tafila		LA	Jordan					
Irbid-Sama Al-Rosan		LA	Jordan					
RUM		LA	Jordan					
ACSAD		LA	Jordan					

wild barley populations								
Sahab 58								
Sahab 53								
Sahab 48								
Ma´daba - Mleh 68								
Ma´daba - Mleh 33								
Ma´daba - Mleh 21								
Ma´daba - Balawneh 64								
Ma´daba - Balawneh 26								
Ma´daba - Balawneh 53								
Irbid - Sal 52								
Mafraq - Bwadah 37								
Mazeh - Thahabiah 12								

GENETIC MATERIAL from IPK

IDENTITY	**Land**	**Bot. Name**	**s_name**	**SOURCE**		**Origin**		
HOR 4083	Syrien	H. vulgare		Leipzig				
HOR 4469	Syrien	H. vulgare		Leningrad, UdSSR				
HOR 7400	Syrien	H. vulgare		Leningrad, UdSSR				
HOR 12826	Syrien	H. vulgare		IPK				
HOR 12827	Syrien	H. vulgare		IPK	Nord-Syrien, in der Nähe von Dadat am Euphrat			
HOR 12828	Syrien	H. vulgare		IPK	Nord-Syrien, in der Nähe von Dadat am Euphrat			
HOR 12829	Syrien	H. vulgare		IPK	Nord-Syrien, in der Nähe von Dadat am Euphrat			
HOR 12830	Syrien	H. vulgare		IPK	Nord-Syrien, bei Qal'at Saman			
HOR 13792	Jordanien	H. vulgare	ICARDA 70 SP, P	Braunschweig	Nord-Syrien, Feld bei Tale Ahmar am Euphrat			
HOR 13795	Jordanien	H. vulgare	ICARDA 6 SP, P	Braunschweig	Madaba; Lat: 3141N; Lon: 03550E; Alt: 650			
HOR 13796	Syrien	H. vulgare	ICARDA 52 E	Braunschweig	Madaba; Lat: 3141N; Lon: 03550E; Alt: 650			
HOR 13808	Syrien	H. vulgare	ICARDA 4 SP, P	Braunschweig	Aleppo, Suran; Lat: 3617N; Lon: 03723E; Alt: 450			
HOR 13809	Syrien	H. vulgare	ICARDA 2 E	Braunschweig	Homs, Qudair; Lat: 3510N; Lon: 03851E; Alt: 460			
HOR 14095	Syrien	H. vulgare	ICARDA 23 E	Braunschweig	Raqqa, Rusafa; Lat: 3553N; Lon: 03847E; Alt: 250			
HOR 14097	Syrien	H. vulgare	ICARDA 30 SP, P	Braunschweig	Raqqa, Qalat Jaber; Lat: 3555N; Lon: 03830E; Alt: 300			

HOR 14098	Syrien	H. vulgare	ICARDA 30 E	Braunschweig	Homs, Homs; Lat: 3441N; Lon: 03653E; Alt: 550
HOR 14099	Syrien	H. vulgare	ICARDA 14 E	Braunschweig	Homs, Homs; Lat: 3441N; Lon: 03653E; Alt: 550
HOR 14100	Syrien	H. vulgare	ICARDA 19 E	Braunschweig	Raqqa, Dachan; Lat: 3609N; Lon: 03913E; Alt: 320
HOR 14101	Syrien	H. vulgare	ICARDA 13 E	Braunschweig	Hassake, Tel Tamr; Lat: 3624N; Lon: 04033E; Alt: 325
HOR 14107	Syrien	H. vulgare	ICARDA 16 P	Braunschweig	Raqqa, Raqqa; Lat: 3604N; Lon: 03909E; Alt: 280
HOR 14108	Syrien	H. vulgare	ICARDA 18 E	Braunschweig	Raqqa, Kantoni; Lat: 3626N; Lon: 03928E; Alt: 360
HOR 14112	Syrien	H. vulgare	ICARDA 28 E	Braunschweig	Hassake, Rasel Ain; Lat: 3650N; Lon: 04005E; Alt: 350
HOR 14113	Syrien	H. vulgare	ICARDA 29 SP, P	Braunschweig	Raqqa, Tel Abiad; Lat: 3634N; Lon: 03501E; Alt: 325
HOR 14121	Syrien	H. vulgare	ICARDA 15 E	Braunschweig	Raqqa, Suluq; Lat: 3610N; Lon: 03900E; Alt: 280
HOR 16631	Syrien	H. vulgare	A.DAMASKUS	Braunschweig	Raqqa, Kantari; Lat: 3624N; Lon: 03917E; Alt: 350
HOR 18034	Syrien	H. vulgare	A.DAMASKUS	Braunschweig	
HOR 18453	Jordanien	H. vulgare	ICARDA 69 E	Braunschweig	
HOR 18455	Syrien	H. vulgare	ICARDA 5 SP, P	Braunschweig	Tel Er Rumeith; Lat: 3225N; Lon: 03602E; Alt: 500
HOR 18456	Syrien	H. vulgare	ICARDA 2 SP, P	Braunschweig	Raqqa, Rusafa; Lat: 3553N; Lon: 03847E; Alt: 250
HOR 18457	Jordanien	H. vulgare	ICARDA 69 SP, P	Braunschweig	Raqqa, Rusafa; Lat: 3553N; Lon: 03847E; Alt: 250
HOR 18465	Syrien	H. vulgare	ICARDA 4 E	Braunschweig	Tel Er Rumeith; Lat: 3225N; Lon: 03602E; Alt: 500
HOR 18466	Jordanien	H. vulgare	ICARDA 36 E	Braunschweig	Homs, Qudair; Lat: 3510N; Lon: 03851E; Alt: 460
HOR 18468	Syrien	H. vulgare	ICARDA 1 E	Braunschweig	Kerak, Qasr; Lat: 3118N; Lon: 03538E; Alt: 900
HOR 18472	Syrien	H. vulgare	ICARDA 5 E	Braunschweig	Raqqa, Rusafa; Lat: 3553N; Lon: 03847E; Alt: 250
HOR 18476	Jordanien	H. vulgare	ICARDA 68 E	Braunschweig	Raqqa, Rusafa; Lat: 3553N; Lon: 03847E; Alt: 250
HOR 18480	Jordanien	H. vulgare	ICARDA 7 E	Braunschweig	Ramtha; Lat: 3130N; Lon: 03600E; Alt: 500
HOR 18484	Jordanien	H. vulgare	ICARDA 70 E	Braunschweig	Kerak, Qasr; Lat: 3118N; Lon: 03538E; Alt: 900
HOR 19782	Syrien	H. vulgare	ICARDA 9 E	Braunschweig	Madaba; Lat: 3141N; Lon: 03550E; Alt: 650
HOR 19786	Syrien	H. vulgare	ICARDA 51 SP, P	Braunschweig	Dera'a Sayaa; Lat: 3238N; Lon: 03614E; Alt: 600
HOR 19788	Syrien	H. vulgare	ICARDA 14 SP, P	Braunschweig	Raqqa, Sabkhe; Lat: 3548N; Lon: 03924E; Alt: 200
HOR 19789	Syrien	H. vulgare	ICARDA 44 E	Braunschweig	Raqqa, Duchan; Lat: 3609N; Lon: 03913E; Alt: 320
HOR 19799	Syrien	H. vulgare	ICARDA 17 E	Braunschweig	Raqqa, Kantari; Lat: 3624N; Lon: 03917E; Alt: 350
HOR 19802	Syrien	H. vulgare	ICARDA 29 E	Braunschweig	Hassake, Tel Hanish; Lat: 3640N; Lon: 03950E; Alt: 410
HOR 19803	Syrien	H. vulgare	ICARDA 12 SP, P	Braunschweig	Raqqa, Suluq; Lat: 3610N; Lon: 03900E; Alt: 280
HOR 19814	Syrien	H. vulgare	ICARDA 31 E	Braunschweig	Homs, Furqlos; Lat: 3435N; Lon: 03710E; Alt: 680

HOR 19818	Syrien	H. vulgare	ICARDA 44 SP, P	Braunschweig	Homs, Jdeide; Lat: 3518N; Lon: 03710E; Alt: 410
HOR 19819	Syrien	H. vulgare	ICARDA 33 SP, P	Braunschweig	Raqqa, Kantari; Lat: 3624N; Lon: 03917E; Alt: 350
HOR 19820	Syrien	H. vulgare	ICARDA 32 SP, P	Braunschweig	Damascus, Sidnaya; Lat: 3345N; Lon: 03628E; Alt: 1400

9.2 Appendix II

Table 9.2 Observed heterozygosity (H_O) and size (number of plants) of individual families included included in populations C1 to C3 in the course of the experiment along the observed heterozygosity of the accessions selected in C0.

The same colour with in each accession in C1, C2 and C3 indicate that the families in C3 descend from C2 which of these descend from C1.

C0		C1			C2			C3		
Accession	**HO**	**Family**	**size**	**HO**	**Family**	**size**	**HO**	**Family**	**size**	**HO**
HOR 7400	2.777778	1	1	0						
HOR 18456	18.98148	1	12	0.0000						
		2	8	0.0000						
		3	7	0.0000						
		4	7	0.0000						
		5	4	0.0000						
		6	7	0.0476						
		7	7	0.0476						
		8	3	0.0000						
		9	8	0.0000						
		10	4	0.0000						
		11	5	0.0000						
		12	9	0.0000						
HOR 18468	5.555556	1	7	0.0000						
		2	3	0.0000						
		3	1	0.0000						
		4	6	0.0000						
		5	8	0.0417						
		6	10	0.0000						
HOR 19786	11.11111	1	25	0.0000						
		2	9	0.0370						

C0		C1			C2			C3		
Accession	**HO**	**Family**	**size**	**HO**	**Family**	**size**	**HO**	**Family**	**size**	**HO**
		3	11	0.0000						
		4	7	0.0000						
		5	12	0.0000						
		6	4	0.0000						
		7	14	0.0000						
		8	1	0.0000						
HOR 19788	7.407407	1	1	0.0000						
		2	3	0.0000						
		3	5	0.0000						
HOR 19803	0	1	8	0.0000						
IG31495	4.166667	1	4	0.0000						
IG31498	11.11111	1	8	0.0417	1	18	0.0556	1	48	0.0069
		2	6	0.0556				2	27	0.0370
		3	5	0.0000				3	42	0.0000
		4	9	0.1111	2	36	0.0370	4	48	0.0000
		5	3	0.0000				5	35	0.0095
								6	42	0.0000
								7	42	0.0000
								8	18	0.0000
								9	48	0.0213
								10	42	0.0952
								11	48	0.0417
								12	24	0.0000
								13	42	0.0714
								14	6	0.0000
								15	18	0.0000
								16	48	0.1752
					3	126	0.0080	17	48	0.0417

C0		C1			C2			C3		
Accession	**HO**	**Family**	**size**	**HO**	**Family**	**size**	**HO**	**Family**	**size**	**HO**
								18	48	0.1250
								19	48	0.0564
								20	6	0.0000
								21	42	0.0000
								22	36	0.0093
								23	42	0.0556
								24	6	0.0000
								25	30	0.0333
					4	48	0.0278	26	48	0.1334
								27	48	0.0625
					5	42	0.0926	28	30	0.0667
IG31513	1.851852	1	12	0.0000						
		2	24	0.0290	1	6	0.0556			
					2	6	0.0000			
					3	36	0.0571	1	48	0.0000
								2	42	0.1759
								3	36	0.0556
		3	32	0.0104	4	144	0.0069	4	24	0.1703
		4	8	0.0000				5	24	0.0139
		5	24	0.0833				6	24	0.0700
		6	12	0.0000				7	24	0.0000
								8	48	0.0000
								9	30	0.0556
								10	24	0.0417
					6	36	0.0000	11	48	0.0388
								12	30	0.0000
		7	31	0.0000	5	138	0.0051	13	42	0.0000
								14	18	0.0000
								15	24	0.0000
								16	24	0.0278
								17	36	0.0000

C0		C1			C2			C3		
Accession	**HO**	**Family**	**size**	**HO**	**Family**	**size**	**HO**	**Family**	**size**	**HO**
								18	24	0.0000
								19	48	0.0000
								20	48	0.0000
					7	6	0.1111	21	24	0.0145
IG33094	18.51852	1	24	0.0000						
		2	17	0.0000						
		3	24	0.0370						
		4	17	0.0392						
		5	9	0.0000						
		6	21	0.0000						
		7	1	0.0000						
IG36035	0	1	6	0.0000						
IG36041	28.54031	4	14	0.0952	1	12	0.0000			
		1	18	0.0556	2	18	0.0741			
		2	2	0.0000						
		3	12	0.0000						
		5	7	0.0000	4	12	0.0000			
		6	2	0.3333	3	30	0.1889			
		7	8	0.1250	5	32	0.0625	1	48	0.1042
		8	4	0.0000				2	48	0.1349
		9	6	0.0000				3	48	0.0980
		10	3	0.0000				4	6	0.0000
		11	9	0.2222				5	24	0.0000
		12	1	0.0000						
IG36046	0	1		0.0000						
IG135258	0	1		0.0000						

C0		C1			C2			C3		
Accession	**HO**	**Family**	**size**	**HO**	**Family**	**size**	**HO**	**Family**	**size**	**HO**
IG138242	8.333333	1	22	0.1061	1	24	0.0000	1	41	0.0325
					2	24	0.0000	2	24	0.0000
		3	12	0.0000				3	24	0.1250
		4	3	0.0000	3	108	0.0000	4	23	0.0588
		5	6	0.0000	4	6	0.0000	5	24	0.0864
					5	30	0.0000	6	18	0.0000
								7	24	0.0000
								8	24	0.0000
								9	60	0.0167
					6	96	0.0140	10	42	0.0000
								11	12	0.0000
								12	30	0.0000
								13	48	0.0069
								14	18	0.0000
								15	30	0.0000
								16	24	0.0139
								17	24	0.0000
								18	30	0.0444
					7	102	0.0033	19	36	0.0000
								20	24	0.0139
								21	24	0.0000
								22	48	0.0000
								23	24	0.0139
								24	24	0.0000
								25	30	0.0000
								26	42	0.0000
								27	24	0.0000
					8	18	0.0000	28	36	0.0370
								29	24	0.0000
					9	78	0.0000	30	24	0.0000
					10	96	0.0139	31	30	0.0333

C0		C1			C2			C3		
Accession	**HO**	**Family**	**size**	**HO**	**Family**	**size**	**HO**	**Family**	**size**	**HO**
					11	6	0.0000	32	24	0.0000
								33	48	0.0069
								34	24	0.0000
					12	108	0.0432	35	48	0.0903
								36	24	0.0139
					13	54	0.0617	37	18	0.0556
					14	72	0.1111	38	43	0.0433
								39	30	0.0000
								40	36	0.0000
								41	18	0.0185
								42	24	0.0000
								43	48	0.0000
								44	12	0.0000
					15	108	0.0093	45	30	0.0222
								46	6	0.0000
								47	24	0.0000
								48	12	0.1667
					16	102	0.0000	49	48	0.0139
					17	132	0.0051	50	24	0.0000
								51	24	0.0000
								52	24	0.0278
								53	24	0.3333
								54	42	0.1905
								55	6	0.0000
		2	17	0.0196	19	18	0.1481	56	30	0.0000
								57	30	0.0222
Mafraq14	0	1	5	0						

Table 9.3 Comparison of average values of observed heterozygosity in the final evaluation experiment.

C1			C2		
Accession	**Fam**	**H_O(%)**	**Accession**	**Fam**	**H_O(%)**
IG33094	1	0.00	IG138242	1	0.00
IG33094	2	0.00	IG36041	2	2.78
IG31495	3	0.00	IG31513	3	0.00
IG31495	4	0.00	IG31498	4	2.78
IG31513	5	0.00	IG31513	5	0.00
IG138242	6	0.00	IG138242	6	2.78
IG36041	7	0.00	IG138242	7	0.00
IG36041	8	0.00	IG138242	8	2.78
IG31498	9	0.00	IG138242	9	0.00
IG135258	10	0.00	IG138242	10	0.00
IG36046	11	0.00	IG138242	11	0.00
HOR19788	12	0.00	IG138242	12	2.78
HOR19786	13	2.78	IG138242	13	0.00
HOR18456	14	0.00	IG31498	14	0.00
HOR18456	15	0.00	IG31498	15	0.00
HOR19803	16	2.78	IG138242	16	0.00
HOR18468	17	0.00	IG31513	17	0.00
HOR18468	18	0.00	IG31513	18	0.00
HOR7400	19	0.00	IG138242	19	0.00
Mafraq14	20	0.00	IG31513	20	0.00
IG31495	21	0.00	IG31498	21	0.00
IG36035	22	0.00	IG31513	22	2.78
IG138242	23	0.00	IG138242	23	0.00
IG36041	24	0.00	IG138242	24	0.00

……continued.

C3			C4		
Accession	**Fam**	**H_O(%)**	**Accession**	**Fam**	**H_O(%)**
IG31498	1	8.33	IG31498	1	0.00
IG31498	2	0.00	IG31498	2	5.56
IG31498	3	0.00	IG31498	3	0.00
IG31498	4	0.00	IG31498	4	0.00
IG31498	5	5.56	IG31498	5	0.00
IG31498	6	2.78	IG31498	6	0.00
IG31498	7	0.00	IG31498	7	0.00
IG31513	8	0.00	IG31498	8	0.00
IG31513	9	0.00	IG31498	9	0.00
IG31513	10	0.00	IG31498	10	2.78
IG31513	11	0.00	IG31498	11	0.00
IG138242	12	0.00	IG31498	12	2.78
IG138242	13	0.00	IG31498	13	0.00
IG138242	14	0.00	IG31498	14	0.00
IG138242	15	0.00	IG31498	15	0.00
IG138242	16	0.00	IG31513	16	0.00
IG138242	17	0.00	IG31513	17	8.33
IG138242	18	0.00	IG31513	18	0.00
IG138242	19	0.00	IG31513	19	0.00
IG138242	20	0.00	IG31513	20	2.78
HOR19786	21	0.00	IG138242	21	0.00
HOR19786	22	0.00	IG138242	22	5.56
HOR18456	23	0.00	IG138242	23	0.00
IG36041	24	0.00	IG36041	24	0.00

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11. Curriculum vitae

Personal Details

Name : Aruna Nandety

Date of birth : 01-07-1978

Place of birth : Guntur, India

Nationality : Indian

Sex : Female

Email : anandety@gmail.com

School Education

1982-1993: Secondary School Certificate, Board of Secondary Education

1993-1995: Board of Intermediate Education

University Education

1996-2000: Bachelor of Science (Agriculture), Acharya N. G. Agricultural University, Bapatla, India.

2003-2005: Master of Science (M.Sc.) Environmental Protection and Agricultural Food Production, University of Hohenheim, Stuttgart, Germany. MSc. thesis title: Genetic diversity of sorghum (*Sorghum bicolor* L.) landraces from Central Sudan.

Professional Experience

2001- 2002: Agricultural Officer under state government of Andhra Pradesh, India.

Aruna Nandety

Stuttgart 22.10.2010

12. Erklärung

Hiermit erkläre ich Eides statt, dass die vorliegende Arbeit von mir selbst verfasst und lediglich unter Zuhilfenahme der angegebenen Quellen und Hilfsmittel angefertigt wurde. Wörtlich oder inhaltlich übernommene Stellen wurden als solche gekennzeichnet.

Die vorliegende Arbeit wurde in gleicher oder ähnlicher Form noch keiner anderen Institution oder Prüfungsbehörde vorgelegt.

Aruna Nandety
Stuttgart, den 22.10.2010