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Deviations from Mendelian segregation, attributable to genetic factors and in response to exogenous selective agents, have been reported in a number of plant species. These deviations can be caused by unintentional gametophytic selection, such as pollen lethals or differential response of gametophytes to tissue culture procedures. Unintentional gametophytic selection is in general undesirable, because it can seriously affect genetic analysis.

Directed gametophytic selection as a result of a controlled selective pressure during sexual reproduction can be exploited for breeding purposes. Assuming an overlap of gametophytic and sporophytic gene expression, the frequency of alleles conferring sporophytic resistance to a selective agent can be increased by directed gametophytic selection.

The objectives of this research were 1) to test if unintentional gametophytic selection lead to a shift in gene frequencies in the progeny of a relatively wide (winter x spring) cross, and 2) to test if directed gametophytic selection for cold tolerance was effective when

the F_1 of a winter x spring cross was subjected to low temperature during pollination.

Segregation of isozyme, storage protein, DNA, and morphological markers was compared in three populations derived from the same winter x spring cross: a control F_2 (F_{2C}), a doubled haploid (DH) population, and an F_2 derived from F_1 plants self pollinated at 10°C (F_{2T}).

When goodness-of-fit to expected ratios was tested, no aberrant segregation was found in the F_{2C}. The DH population showed significant deviations from hypothesized genotype frequencies at two loci. The comparison of gene frequencies between the DH and the F_{2C} population showed that the DH population differed significantly from the F_{2C} only at one of the loci tested. Alleles of Dicktoo, the winter habit parent, were significantly overrepresented in the DH population. Unintentional gametophytic selection was operative during production of the DH population, but not in the F_{2C}.

In the F_{2T} a significant excess of one parent (Dicktoo) was observed at two loci (pTA71 and ACO1). However, only for pTA71 was the heterogeneity test between the F_{2T} and F_{2C} significant. Segregation of the other markers was not significantly affected by the cold treatment. When data were pooled over all loci, except pTA71, no significant differences in gene frequency were detected between the two F_2 populations. Gametophytic selection as a consequence of the cold treatment was limited to a small portion of the genome.

When linkage data from the F_2 and the DH population were compared, estimates of recombination frequencies were in close agreement. Despite aberrant segregation, the DH population should be suitable for linkage analyses.

GAMETOPHYTIC SELECTION IN BARLEY (*HORDEUM VULGARE* L.)

by

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INTRODUCTION

The alternation of generations is a well known phenomenon in plants. The diploid sporophyte produces haploid spores that give rise to the gametophytes, which in turn produce gametes that complete the life cycle by developing into new sporophytes. In Angiosperms the male and female gametophytes are reduced to the pollen grain and the embryo sac. Despite their limited size and lifespan the gametophytes are subjected to selection.

Gametophytic selection can occur unintentionally, due to qualitative factors causing death of gametes carrying certain alleles. In wheat for example, Loegering and Sears (1963) found a pollen killer gene that resulted in severely distorted segregation ratios of a linked gene regulating resistance to stem rust.

Zamir and Tadmor (1986) reported distorted segregation ratios of marker genes in F_2 and backcross progeny of intraspecific and interspecific crosses of *Lens*, *Capiscum*, and *Lycopersicum*. The segregation distortion was most pronounced in F_2 progeny of interspecific crosses. Because wide crosses are needed to broaden the genetic basis of many crops, unintentional gametophytic selection is of concern.

Among female gametophytes, selection has been shown for species like *Oenothera muricata* and *Lycopersicum esculentum* (Burnham, 1962; Rick, 1966). Female gametophytic selection has also to be taken into account, when tissue culture systems are used for the production of

gynogenetic doubled haploids (DH). Genes regulating tissue culture response may cause segregation distortion at linked loci. The use of such populations in linkage analyses could bias estimates of recombination frequencies and make them inappropriate for quantitative trait locus (QTL) mapping.

Selection for quantitative traits has been demonstrated to be operative on the male gametophytes, mainly due to their large numbers and direct exposure to the environment (Mulcahy, 1986). Genetic variability and vigor of a population can be manipulated by controlling the number of pollen grains (Ter-Avanesian, 1978) and the length of the styles (Mulcahy and Mulcahy, 1975). When stress tolerant and stress susceptible genotypes are crossed and the resulting gametophytic generation is exposed to environmental stresses such as temperature (Zamir et al., 1982; Mulinix and Iezzoni, 1988) or heavy metals (Searcy and Mulcahy, 1985), selection favors the stress tolerant gametophytes, leading to a shift in mean performance of progeny.

In order to utilize gametophytic selection in breeding certain requirements have to be met. Genes have to be expressed in the pollen and there needs to be a substantial overlap of gene expression in the gametophyte and the sporophyte. Isozyme studies in tomato and barley demonstrated the presence of postmeiotic gene expression in pollen grains and that approximately 60 percent of sporophytic genes were also expressed in the gametophytes (Tanksley et al., 1981; Pedersen et al., 1987).

In this study the trait of interest was cold tolerance. Cold tolerance is extremely difficult to evaluate in the field and selecting

cold tolerant genotypes is in general hampered by pronounced genotype x environment interactions. If the gametophytic response to cold is correlated with sporophytic cold tolerance, selection of pollen grains under low temperature may result in sporophytes with increased cold tolerance (Zamir and Gadish, 1987). Because the gametophyte is haploid, recessive genes could be selected for and the vast number of pollen grains allows for many genotypes to be screened.

The objective of this study was to test for the presence of gametophytic selection in a winter x spring barley cross. Unintentional selection was tested for in an F_2 and a Hordeum bulbosum-derived DH population. Directed gametophytic selection for cold tolerance was attempted by selfing an F_1 under low temperature.

GAMETOPHYTIC SELECTION
IN A WINTER X SPRING BARLEY CROSS

Abstract

Unintentional and directed gametophytic selection were tested in a winter x spring barley cross by comparing segregation of isozyme, storage protein, DNA, and morphological markers in three populations derived from the same cross: a control F_2 (F_{2C}), a doubled haploid (DH) population and an F_2 derived from F_1 plants self pollinated at 10°C (F_{2T}). When goodness-of-fit to expected ratios was tested, no aberrant segregation was found in the F_{2C}. The DH population showed significant deviations from hypothesized genotype frequencies at two loci, but only at one of the loci was the gene frequency significantly different from the F_{2C}. Alleles of Dicktoo, the winter habit parent, were significantly overrepresented in the DH population. Unintentional gametophytic selection was operative during production of the DH population, but not in the F_{2C}. In the F_{2T} a significant excess of one parent (Dicktoo) was observed at two loci (pTA71 and ACO1). However, only for pTA71 was the heterogeneity test between the F_{2T} and F_{2C} significant. Segregation of the other markers was not significantly affected by the cold treatment. When data were pooled over all loci, except pTA71, no significant differences in gene frequency were detected between the two F_2 populations. Gametophytic selection as a consequence of the cold treatment was limited to a small portion of the genome. When linkage data from the F_2 and the DH population were compared, estimates of recombination frequencies were in close agreement. Despite aberrant segregation, the DH population should be suitable for linkage analyses.

Introduction

Both genetic and environmental factors responsible for selection at the gametophytic level can significantly affect the following sporophytic generation (Rick, 1966; Zamir et al., 1982; Mulinix and Iezzoni, 1988). We studied unintentional and directed gametophytic selection in barley by comparing segregation of Mendelian markers in three populations derived from a winter x spring cross: a control F_2, a Hordeum bulbosum-derived doubled haploid (DH) population, and an F_2 population derived from F_1 plants that were self pollinated at 10°C.

Unintentional shifts in gene frequency are undesirable when progeny are used for breeding or linkage analyses. The problem was recognized early in the study of plant genetics. Jones (1928) reviewed the phenomenon of distorted Mendelian segregation for a number of loci in several species. Preferential transmission of alleles from one parent can occur, especially in the progeny of wide crosses (Zamir and Tadmor, 1986). Preferential transmission of alleles from one parent is also a concern when populations, such as Hordeum bulbosum-derived DH lines in barley, are obtained through tissue culture. The female gametes may respond differentially to culture methods, causing segregation distortion of marker genes.

Directed selection at the gametophytic level could potentially enhance efficiencies in breeding. Substantial overlap between sporophytic and gametophytic gene expression has been demonstrated for a number of species, including barley (reviewed by Mascarenhas, 1989). Zamir et al. (1982) reported preferential transmission of Lycopersicum

hirsutum alleles in backcrosses of L. esculentum x (L. esculentum x L. hirsutum) under low temperature and concluded that due to gametophytic gene expression, pollen grains carrying alleles from the cold tolerant L. hirsutum parent were more successful in fertilization. Such a relationship of sporophytic and gametophytic gene expression could be exploited for the production of F_2 progenies with increased frequencies of desired alleles.

Zamir et al. (1981) were able to demonstrate differential in vitro pollen germination and pollen tube growth under low temperature for two tomato species (L. hirsutum and L. esculentum). We could not duplicate this experiment in barley, probably due to the very short viability of barley pollen. Kison (1979) estimated the average viability of barley pollen to be only five to ten minutes. In vitro studies of barley pollen germination and pollen tube growth under cold stress were therefore precluded.

Based on these considerations this study had two objectives: 1) to determine if there was an unintentional shift in gene frequencies in F_2 and Hordeum bulbosum-derived DH progeny of a relatively wide (winter x spring) barley cross. 2) to test if gametophytic selection for cold tolerance was operative since the parents of our cross, the spring cultivar Morex and the winter cultivar Dicktoo, differ in their sporophytic expression of cold tolerance.

Materials and Methods

Crosses were made between cultivars Morex, a six-row spring malting barley, and Dicktoo, a winter habit six-row feed cultivar that has consistently ranked among the most cold tolerant entries in repeated tests throughout the United States.

Twenty F_1 plants were grown in the greenhouse at 20/16°C (day/night) with a 16 h photoperiod. Three days before anthesis of the main culm, five of the 20 F_1 plants were moved to a growth chamber at a constant 10°C, with a 16 h photoperiod. Below 10°C pollen tube growth is in general inhibited (Richards, 1986). Spikes were checked daily for protrusion of anthers and pollen shed. After an average of 14 days the first and second tiller inflorescences had gone through fertilization and plants were returned to the greenhouse. Ten of the 20 F_1 plants were used for the extraction of 130 Hordeum bulbosum-mediated DH lines using in vitro floret culture as described by Chen and Hayes (1989). All DHs were vernalized for 6 weeks at 8°C with a 8 h photoperiod. Five of the 20 F_1 plants constituted the parents of the control F_2 population.

Seed was harvested from the first and second tiller of each cold treated and control F_1 plant. Percent seed set was calculated as number of seeds/number of florets for each spike. Twenty six seeds were randomly chosen from each plant and planted in the greenhouse at two planting dates. After three weeks plants were transferred to a vernalization chamber at 8°C with a 8 h photoperiod for six weeks. Plants were transplanted to soil after vernalization and grown to maturity in the greenhouse.

Segregation of each of ten Mendelian markers: five isozymes, three hordeins, and two morphological markers, awn roughness (R/r) and rachilla hair length (S/s), was followed in 130 F_2 control plants (F_{2C}), 130 F_2 plants derived from cold treated F_1s (F_{2T}) and 130 DH lines. In addition, one polymerase chain reaction (PCR) marker and one restriction fragment length polymorphism (RFLP) marker were assayed in both F_2 populations. Markers were located on five of the seven barley chromosomes (Table 1).

Aconitate hydratase 1 (ACO1), esterases 1 and 4 (EST1, EST4), glucosephosphate isomerase 1 (GPI1), and phosphogluconate dehydrogenase 2 (PGD2) were evaluated in horizontal starch gel systems as described by Nielsen and Johansen (1986). Hordeins B,C, and D (HORB, HORC, HORD) were separated in 12% SDS-polyacrylamide gels as described by Blake et al. (1982). When plants were four weeks old, DNA was extracted from 1 g fresh leaf tissue (Dellaporta et al., 1983) for RFLP and PCR analysis. 15 μg aliquots were digested with Bam HI, electrophoresed in 0.8% agarose gels and transferred to Zeta-probe nylon membranes (Reed and Mann, 1985). Filters were hybridized according to Sambrook et al. (1989) with 5x Denhardt's reagent, 6x SSC, 0.5% SDS and 100 μg/ml herring sperm DNA. The probe, pTA71, a clone from the ribosomal barley and wheat gene cluster (Gerlach and Bedrook, 1979) was labeled using primer extension (Feinberg and Vogelstein, 1984). Primers for the PCR reaction were generated based on the sequence of clone pMSU21 (Shin, 1988). The amplification reaction consisted of 30 cycles with a cycling protocol of 1 minute at 94°C, 2 minutes at 37°C and 4 minutes at 72°C. Total reaction volumes were 30 μl. Reaction products were electrophoresed in 1.4%

agarose gels. Awn roughness and rachilla hair length were evaluated under a stereomicroscope and phenotypes were classified into smooth or rough and short or long, respectively.

All markers were assumed to follow monohybrid patterns. Goodness-of-fit to hypothesized ratios was tested at each locus in all three populations with the log likelihood ratio test (G-test). Fit to a phenotypic ratio of 3:1 was tested for markers with dominant inheritance; pTA71 was scored for the homozygous recessive Dicktoo phenotype, while at the other dominant loci the Morex phenotype was homozygous recessive. For codominant markers, a fit to expected gene frequencies (1:1) was tested to allow for a comparison between the F_{2C} and the DH population. The G-test was preferred over the Chi-square test, because of its additivity (Sokal and Rohlf, 1981). Heterogeneity was tested for single loci between populations and for pooled data between populations. When testing pooled segregation within populations, markers with dominant inheritance were tested separately from codominant markers. The marker pTA71 was excluded from the pooled tests within populations, since it was the only marker with a homozygous recessive Dicktoo phenotype. G-tests were computed using GOODFT (BIOM, Rohlf, 1986).

The multipoint linkage map for chromosome 5 and two-point recombination frequencies for the remaining markers were calculated using Mapmaker (Lander et al., 1987). The standard error of the recombination frequency estimates from the F_2 and the DH populations was calculated as described by Allard (1956).

Results and Discussion

Segregation data and heterogeneity tests are summarized in Tables 2 and 3 for the DHs and in Tables 4 and 5 for the F_2s. Linkage data are presented in Table 6.

Unintentional shifts in gene frequency

Since the cross under study will be utilized in mapping of cold tolerance genes, the parents, Dicktoo and Morex, were chosen in order to maximize genetic variation for cold tolerance in the progeny and polymorphism at marker loci. This resulted in a relatively wide (winter x spring) cross and we were concerned that alleles from one parent might be preferentially transmitted to the progeny and that estimates of recombination frequencies would be biased by aberrant segregation at more than one locus (Bailey, 1961). The F_{2C} and DH population were used to test for unintentional shifts in gene frequency of the progeny of this winter x spring cross.

In the F_{2C}, EST1 showed a significant deviation from the expected 3:1 ratio (Table 5). EST1 and EST4 are tightly linked on chromosome 3 (Kahler and Allard, 1970). In the F_{2C}, EST4 exhibited the same reduction of the homozygous recessive Morex genotype as EST1. However, deviations from the expected gene frequency (p=q=0.5) were not significant at the EST4 locus (Table 4). Because no recombinant genotype between EST1 and EST4 was found in the F_{2C}, we assumed that the gene frequency at EST1 would not grossly differ from estimates obtained for EST4, and that there was thus no evidence for unintentional selection in the F2C.

For all ten markers in the DH population goodness-of-fit to a 1:1 ratio was tested. Significant deviations from expected genotype frequencies were detected at two loci, PGD2 and GPI1, in favor of the Dicktoo genotype. The two loci are not linked, indicating that independent factors likely caused skewness in segregation. Genotype frequencies summed over all loci in the DH population were significantly different from the expected 1:1 ratio, with 54 percent Dicktoo genotypes and 46 percent Morex genotypes.

A comparison between the DH population and the F_{2C} was based on gene frequencies of markers with codominant inheritance in the F_2 (Table 3). Heterogeneity tests between DHs and the F_{2C} were only significant at the GPI1 locus. Gene frequency at the other loci did not significantly differ from the F_{2C}. The highly significant heterogeneity test for data pooled over the seven loci used in the comparison between DHs and the F_{2C} indicated significant preferential transmission of the Dicktoo alleles during the DH production process.

These results did not agree with data presented by Powell et al. (1986a,b) and Schön et al. (1990), who demonstrated, based on segregation of Mendelian markers and distributions of quantitative traits, that Hordeum bulbosum-derived DH lines were derived from a random sample of gametes. Generalization of results may not be appropriate, especially when relatively wide crosses are concerned. An analysis of marker segregation at additional RFLP loci is underway and will show the extent of unintentional gametophytic selection throughout the genome of this winter x spring cross.

Markers showing aberrant segregation are likely linked to genes responsible for overrepresentation of parts of the Dicktoo genome in the DH progeny. The significant excess of Dicktoo alleles in the DH population may be attributable to differential survival rates during the colchicine treatment required to double the genome of haploid plants generated by Hordeum bulbosum-mediated chromosome elimination. Plants with more tillers, in general plants with winter growth habit, have a greater chance to survive the colchicine treatment. However, the overall doubling efficiency in our program is greater than 90% (unpublished data) and excessive mortality during the colchicine doubling phase was not observed with the Dicktoo x Morex population. In addition, neither genes for growth habit nor genes for tillering have been mapped to barley chromosome 5, where distortion was most pronounced.

Success rates in DH production are known to be genotype dependent (Powell, 1988; Hayes and Chen, 1989). Alleles present in Dicktoo that confer a selective advantage during culture may provide an alternative explanation for aberrant segregation. Megaspore competition based on partial incompatibility with Hordeum bulbosum pollen must also be considered as a cause for deviations from expected ratios.

Effectiveness of directed selection

Since cold tolerance is extremely difficult to evaluate in the field and is complicated by hardening requirements (Fowler and Carles, 1979), it would be desirable to increase the frequency of cold tolerance alleles in breeding populations through gametophytic selection. In barley, some of the requirements for the use of gametophytic selection

are met. Postmeiotic gene expression and overlap of gene expression in the sporophyte and the gametophyte have been demonstrated (Pedersen et al., 1987). Clegg et al. (1978) showed that in Composite Cross V selection was operative at the gametophytic level. Furthermore, there is evidence for gametophytic expression of cold tolerance. In tomato, Zamir et al. (1982) reported an increase of the alleles of the cold tolerant parent at three out of nine loci. Qian et al. (1986) found differential genotypic response to low temperatures (15/10°C and 11/6°C) for ten wheat cultivars as measured by pollen maturation and seed set.

We tested gametophytic selection for cold tolerance by comparing the F_{2C} and F_{2T} population. The cold treatment significantly reduced seed set from 91.8% in the F_{2C} to 65.7% in the F_{2T} ($p < 0.01$).

Segregation of pTA71 deviated significantly from the expected 3:1 ratio in the F_{2T} ($p < 0.01$) in favor of the homozygous recessive Dicktoo phenotype. The heterogeneity test showed a significant difference in phenotype frequency between the F_{2C} and the F_{2T} for pTA71 (Table 5). As noted previously there was a significant reduction of homozygous recessive Morex phenotypes at EST1 in the F_{2C}. At the same locus the F_{2T} segregated according to expectations. A heterogeneity test indicated a significant difference between the two F_2 populations at the EST1 locus, but again this was not reflected in segregation at EST4. The two remaining dominant markers S/s and R/r did not deviate from expectations in either F_2 population.

Goodness-of-fit to the hypothesized gene frequency ($p=q=0.5$) was tested at the eight codominant marker loci (Table 4). No deviations were detected in the F_{2C}. There was a significant excess of alleles from one

parent (Dicktoo) at the ACO1 locus in the F_{2T}. However, the heterogeneity test identified the excess of Dicktoo alleles at locus ACO1 to be not significantly different from the F_{2C} (Table 4). For the remaining codominant markers, goodness-of-fit tests were not significant.

A strong effect of the cold treatment was detected in a portion of the genome linked to pTA71, which raises the question if pTA71 and/or linked genes are involved in the genetic control of cold tolerance in barley. In a study on the geographic distribution of different alleles at this marker locus, it was found that certain alleles were favored depending on environmental conditions (Saghai-Maroof, pers. communication).

pTA71 is known to hybridize to two independent loci (Rrn1 and Rrn2) located on chromosomes 6 and 7 (Saghai-Maroof et al., 1984). Independence tests between pTA71 and ACO1, S/s and R/r in the F_{2C} indicated that none of these markers were linked to pTA71. The polymorphism identified with pTA71 could therefore not be mapped to either chromosome.

Since segregation at ACO1 was also significantly skewed in favor of Dicktoo, the two markers might both be linked to a gene responsible for the aberrant segregation on chromosome 6. Gametophytes or zygotes carrying the Dicktoo allele at this locus may have been favored under cold temperature.

Cold tolerance in barley is considered to be a quantitatively inherited trait (Nilan, 1964), but there is some evidence that cold tolerance genes may be located on chromosomes 2 and 5 (Rhode and

Pulham, 1960). However, no effects of the cold treatment were detected with markers located on chromosome 5, nor with the PCR marker on chromosome 2. Sutka and Snape (1989) mapped a cold tolerance gene on chromosome 5A in wheat. Barley chromosome 7 is considered to be homoeologous to the group 5 chromosomes in wheat (Islam and Shepherd, 1981). The effect observed at the locus marked by pTA71 might have also been related to a gene homologous to the one in wheat and located on barley chromosome 7.

To test if deviations favored the alleles of one parent, segregation data were pooled over all codominant markers in both F_2 populations (Table 4). While the goodness-of-fit test to a hypothesized 1:1 ratio was not significant in the F_{2C}, the F_{2T} showed a highly significant excess of Dicktoo alleles over all loci. Although Dicktoo alleles were significantly overrepresented in the F_{2T}, no significant overall change in gene frequency as a consequence of the cold treatment could be detected at loci with codominant inheritance, since the heterogeneity test between F_{2C} and F_{2T} was not significant.

Other portions of the genome, not covered by the twelve markers used in our study, may have been affected by the cold treatment. It remains to be shown, however, if the frequency increase of the Dicktoo phenotype at the pTA71 locus is correlated with an increase of sporophytic cold tolerance in the F_{2T}.

Suitability of DH lines for linkage analysis

If DH lines are to be used for breeding and mapping, F_1-derived DH lines should represent a random sample of gametes. Since genotype

frequencies in the DH population did not meet expectations at two loci, we tested the possible consequences of these results on linkage by comparing linkage data obtained from the F_2 and DH population. For all markers, chromosome locations and estimates of recombination frequencies are available in the literature. GPI1, PGD2, HORB, HORC, and HORD have all been mapped to chromosome 5 and ACO1 to chromosome 6 (Brown et al., 1989). The PCR marker on chromosome 2 was mapped by Shin (1988). EST1 and EST4 are known to be situated on chromosome 3 in tight linkage (Kahler and Allard, 1970). The two morphological markers form a linkage group on chromosome 7 (Nilan, 1964). Polymorphism detected with pTA71 can be located on either chromosome 6 or 7 (Saghai-Maroof et al., 1984).

For the comparison of estimated recombination fractions between DHs and F_2s, calculations were based on all 260 F_2 individuals, since the non-significant heterogeneity tests between F_2 populations for loci included in the linkage analysis allowed data to be pooled. All estimates of recombination values were in close agreement with values previously reported in the literature. Estimates of recombination frequencies based on DH data matched the F_2 data very well (Table 6). Linkage analysis in the DH population was not significantly affected by the shift in gene frequency in favor of Dicktoo.

Table 1. Phenotype, expected F_2 phenotypic ratios, and chromosome location of twelve marker loci used to follow segregation in progeny of a Dicktoo x Morex cross.

Marker	Phenotype	Expected ratio	Chromosome
PCR	PCR polymorphism	1:2:1	2
EST1	esterase 1	3:1	3
EST4	esterase 4	1:2:1	3
GPI1	glucosephosphate isomerase 1	1:2:1	5
PGD2	phosphogluconate dehydrogenase 2	1:2:1	5
HORB	hordein B	1:2:1	5
HORC	hordein C	1:2:1	5
HORD	hordein D	1:2:1	5
ACO1	aconitate hydratase 1	1:2:1	6
pTA71	RFLP (rDNA)	3:1	6 or 7
S/s	rachilla hair length	3:1	7
R/r	awn roughness	3:1	7

Table 2. Single locus and pooled genotype frequencies and G-statistics testing fit to a 1:1 ratio in the doubled haploid population.

Marker	MM : DD †	G-statistic
EST4	35 : 53	3.708
EST1	24 : 35	2.063
GPI1	49 : 75	5.492 *
PGD2	49 : 73	4.752 *
HORB	44 : 48	0.174
HORC	42 : 50	0.697
HORD	38 : 54	2.797
ACO1	52 : 48	0.160
S/s	56 : 46	0.982
R/r	60 : 45	2.150
Pooled	449 : 527	6.240 *

† MM = number of Morex genotypes, DD = number of Dicktoo genotypes

* significant at the 0.05 level

Table 3. Goodness-of-fit tests to hypothesized allele frequencies (1:1) and heterogeneity tests (HET_B) between the control F_2 (F_{2C}) and the doubled haploid (DH) population for seven codominant markers.

Marker	G-statistic		
	F_{2C}	DH	HET_B
EST4	2.184	7.416 **	1.117
GPI1	0.018	10.985 **	5.673 *
PGD2	0.443	9.504 **	2.771
HORB	0.510	0.348	0.005
HORC	0.143	1.393	1.307
HORD	0.186	5.594 *	1.953
ACO1	0.209	0.320	0.002
Pooled	0.779	23.910**	8.308 **

*,** significant at the 0.05 and 0.01 level

Table 4. Codominant markers, their single locus and pooled allele frequencies, G-statistics testing fit to a 1:1 ratio, and heterogeneity tests (HET_B) between the control F_2 (F_{2C}) and the treated F_2 (F_{2T}).

Marker	F_{2C}		F_{2T}		HET_B
	MM : DD †	G-statistic	MM : DD †	G-statistic	G-statistic
PCR	52 : 62	0.878	65 : 61	0.127	0.855
EST4	100 : 122	2.184	122 : 120	0.017	1.338
GPI1	112 : 110	0.018	112 : 130	1.340	0.806
PGD2	108 : 118	0.443	100 : 106	0.175	0.025
HORB	93 : 103	0.510	104 : 120	1.144	0.044
HORC	129 : 123	0.143	112 : 136	2.326	1.821
HORD	94 : 100	0.186	98 : 122	2.623	0.633
ACO1	89 : 83	0.209	62 : 88	4.529 *	3.496
Pooled	777 : 821	1.212	775 : 883	7.040 **	1.153

† MM = number of Morex alleles, DD = number of Dicktoo alleles
*,** significant at the 0.05 and 0.01 level

Table 5. Dominant markers, their single locus and pooled phenotypic frequencies, G-statistics testing fit to a 3:1 ratio, and heterogeneity tests (HET_B) between the control F_2 (F_{2C}) and the treated F_2 (F_{2T}).

Marker	F_{2C}		F_{2T}		HET_B
	D_:M_ †	G-statistic	D_:M_ †	G-statistic	G-statistic
pTA71	13:52	0.190	23:29	9.062 **	7.989 **
EST1	92:19	3.995 *	84:36	1.536	5.355 *
S/s	77:26	0.003	65:20	0.099	0.074
R/r	72:31	1.369	62:23	0.189	0.210
Pooled +	241:76	0.179	211:79	0.762	0.849

+ pTA71 not included

† D_ = number of Dicktoo phenotypes, M_ = number of Morex phenotypes

*,** significant at the 0.05 and 0.01 level, respectively

Table 6. Comparison of recombination frequencies (r) and associated standard errors (SE) between the F_2 and the doubled haploid (DH) population.

Locus pair	F_2		DH	
	r	SE	r	SE
EST1 - EST4	0.005	0.003	0.017	0.016
HORB - HORC	0.108	0.016	0.152	0.037
HORC - GPI1	0.076	0.012	0.071	0.028
GPI1 - HORD	0.349	0.043	0.338	0.051
HORD - PGD2	0.279	0.038	0.267	0.048
S/s - R/r	0.242	0.026	0.242	0.043

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CONCLUSION

Unintentional and directed gametophytic selection was investigated in this research.

In the F_2 population from a winter x spring barley cross, no preferential transmission of alleles from either parent was detected, indicating that in order to maximize genetic variability and marker polymorphism, relatively wide crosses may be useful for breeding and genetic analyses.

A doubled haploid population derived from the F_1 of the same cross using the Hordeum bulbosum method exhibited distorted segregation at two loci. However, a comparison of linkage data obtained from the doubled haploid and F_2 population showed close agreement of estimates of recombination frequencies between the two populations. Doubled haploid lines proved suitable for linkage analyses, despite distorted segregation at two loci.

As a consequence of the cold treatment during pollination, aberrant segregation of one molecular marker was detected in a second F_2 population from the same cross. The affected portion of the genome may play a role in cold tolerance of the gametophytes. Provided there is an overlap of gene expression between the gametophytic and sporophytic generations, it may also be responsible for sporophytic expression of cold tolerance.

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