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Abstract

Utilization of exotic germplasm offers an approach to broaden genetic variability in breeding populations. This study was conducted in order to 1) compare germplasm of exotic origin with adapted Swedish barleys with respect to genetic differences and 2) to evaluate first cycles of pre-breeding i.e. agronomic traits in complex exotic x adapted crosses. Allozyme studies showed the following Nei's gene diversities among parents: 0.13 (adapted parents), 0.16 (landraces) and 0.25 (*H. spontaneum*). Cluster analysis based both on allozyme and agronomic data indicated that parental groups were genetically divergent. Earliness, straw length, number of ears per plant and thousand kernel weight (TKW) were studied. The best sources for earliness were adapted parents and landraces. Mean straw length was greatest in *H. spontaneum* lines. Number of ears per plant was quite similar in all groups. The highest TKW was among landraces and adapted parents. Hybrids from the complex crossing programme exceeded parents in earliness and TKW. An index composed from the four traits showed the most favorable frequency distributions for adapted parents and hybrids. Both genetic and agronomic studies indicate that new variation from exotic germplasm may be introduced into barley breeding material. In addition, through recombination, agronomically valuable genotypes can be obtained and they can be utilized in long-term breeding programes.

INTRODUCTION

Genetic variation serves as the basis which plant breeders depend upon to develop improved cultivars. Adequate genetic variation must be available in breeding stocks in order for plant breeders to make further improvements in crops. The most important sources of genetic variation are breeders' own breeding populations. For genotypic diversity not already in the breeding program elite, adapted germplasm from comparable programs within the same ecogeographical region can be chosen to facilitate their ease of incorporation and utilization. Only when sufficient variation is not available from these sources, do breeders turn to gene banks or seek for variation in exotic material (Baenziger & Peterson 1991).

The difficulties involved in introducing new genetic variation into breeding programmes coupled with wariness on the part of breeders have led to concern about the genetic similarity of modern cultivars. As a consequence of this, cultivars of today are genetically vulnerable (Ford-Lloyd & Jackson 1986) i.e. they are incapable to fight against pests, pathogens or environmental conditions due to large number of genetically identical individuals in a cultivar (Wilkes 1989). In addition, genetic similarity may lead to slower gain in breeding. Finally, in the future there could be difficulties meeting the new demands of our changing agricultural environment, if we do not diversify the genetic base of our crops.

One way to solve the problem of genetic erosion or at least reduce the rate of erosion while still producing cultivars that are commercially competitive, is to establish genetically diverse breeding populations. Usually, some cycles of pre-breeding are needed before unimproved germplasm can be introgressed into a breeding population. Pre-breeding involves the transfer of certain characteristics from exotic material into breeding material that is more similar to the improved cultivars currently in use. The end products of pre-breeding are usually deficient in certain desirable characters; however, they are attractive to plant breeders due to their greater potential for direct utilization in a breeding programe than the original unadapted exotic sources (Wynne & Halward 1989).

Utilization of exotic germplasm has been reported earlier, among others, in two temperate cereals, oats (Lawrence & Frey 1973, 1975, Frey et al.1984) and barley (Vega & Frey 1980, Rogers 1982, Lehmann & Bothmer 1988). These studies support the idea that useful genes affecting quantitative as well as qualitative traits can be obtained from exotic germplasm. In their reviews on utilization of exotic germplasm Frey et al. (1984), Bramel-Cox & Cox (1988) and Cox (1991) emphasize the importance of evaluation of exotic germplasm for its utility.

Table 1. Parent lines used to develop the experimental barley population

Name/Origin Accession n:o		2/6-row	Naked/ covered	Seed colour black/light	*Hordeum* ssp. *vulgare/spontaneum*
Aisling	Sweden	2	c	l	ssp. *vulgare*
Alfa	Sweden	2	c	l	ssp. *vulgare*
Ariel	Sweden	2	c	l	ssp. *vulgare*
Ellinor	Sweden	2	c	l	ssp. *vulgare*
Golf	Sweden	2	c	l	ssp. *vulgare*
Hulda	Sweden	2	c	l	ssp. *vulgare*
Ida	Sweden	2	c	l	ssp. *vulgare*
Jet	Ethiopia	2	n	b	ssp. *vulgare*
Kara	Sweden	2	c	l	ssp. *vulgare*
Korinna	Sweden	2	c	l	ssp. *vulgare*
Magda	Sweden	2	c	l	ssp. *vulgare*
Milka	Sweden	2	c	l	ssp. *vulgare*
Nancy	Sweden	2	c	l	ssp. *vulgare*
Shirley	Sweden	2	c	l	ssp. *vulgare*
Sv 87368	Sweden	2	c	l	ssp. *vulgare*
Sv 89412	Sweden	2	c	l	ssp. *vulgare*
Sv 89425	Sweden	2	c	l	ssp. *vulgare*
Sv 87443	Sweden	2	c	l	ssp. *vulgare*
Sv 892052	Sweden	2	c	l	ssp. *vulgare*
Sv 892368	Sweden	2	c	l	ssp. *vulgare*
Sv 892508	Sweden	2	c	l	ssp. *vulgare*
Sv 892596	Sweden	2	c	l	ssp. *vulgare*
Sv 898133	Sweden	2	c	l	ssp. *vulgare*
Sv 898219	Sweden	2	c	l	ssp. *vulgare*
H 4035	Pakistan	6	n	l	ssp. *vulgare*
H 4086	Pakistan	6	n	l	ssp. *vulgare*
H 4166	China	2	c	l	ssp. *vulgare*
H 4175	China	6	c	l	ssp. *vulgare*
H 4241	China	6	c	l	ssp. *vulgare*
H 4246	China	6	c	l	ssp. *vulgare*
H 4248	China	6	n	l	ssp. *vulgare*
H 7405	China	2	c	l	ssp. *vulgare*
H 7614	China	6	c	l	ssp. *vulgare*
H 3038	Syria	2	c	l	ssp. *spontaneum*
H 3042	Syria	2	c	l	ssp. *spontaneum*
H 3057	Jordan	2	c	l	ssp. *spontaneum*
H 3059	Jordan	2	c	l	ssp. *spontaneum*
H 3064	Jordan	2	c	l	ssp. *spontaneum*

In addition, comparisons of divergence between wild and cultivated populations with variation within the cultivated gene pool are needed to make utilization of wild germplasm more efficient. Hence, the objectives of this study were 1) to compare germplasm of exotic origin (unadapted landraces and wild barley) with adapted Swedish barleys with respect to genetic differences and agronomic performance and 2) to evaluate first cycles of pre-breeding, that is to say, agronomic traits in complex exotic x adapted crosses.

MATERIALS AND METHODS

Plant material

The experimental population was developed by intermating 40 barley lines selected for phenotypic diversity and resistance to various barley diseases. The material included 25 spring barley varieties and lines adapted to Swedish conditions and 15 exotic lines. The latter comprised 10 cultivated landraces and 5 accessions of wild barley, *Hordeum vulgare* ssp. *spontaneum* (hereafter called *H. spontaneum*) (Table 1). From 1 to 7 individuals of each accession were used as parents in each crossing generation. The plants within accessions were chosen at random. The parents were intercrossed pairwise so that an exotic parent was always crossed with an adapted one. As a result 20 2-way hybrids were obtained from the first crossing generation. These hybrids were further intercrossed in a half-diallel design and from this 190 double cross hybrids were produced. In the third generation, the hybrids from the previous crossing generation were intercrossed pairwise and 95 hybrid lines were achieved. These highly heterozygous 8-way hybrids, which contained from 25 to 50 % exotic germplasm, were used in the glasshouse experiment.

Allozyme studies

Allozyme variation of 6-phosphogluconate dehydrogenase (6PGD), malate dehydrogenase (MDH), aconitate hydratase (ACO), esterase (EST), NADH dehydrogenase (NDH) and glucosephosphate isomerase (GPI) at 11 loci was assayed to characterize genetic diversity in the parental material. The methods of horizontal starch gel electrophoresis, including details of sample preparations and staining methods have been described in detail earlier by Veteläinen (1994).

Glasshouse experiment

The experiment was conducted in a randomized block design in a glasshouse. Because it was not known whether the hybrids were of spring or winter type, all the hybrids as well as the *H. spontaneum* seeds were vernalized at + 4 °C for 16 days prior to sowing. The vernalization medium was 0.8 % water-agar together with calcium-sulfate (0.01 %) (Ahokas 1982). Ten seeds from each of the parental and hybrid line were sown in separate pots. The experiment was divided into ten blocks and each block was divided into two groups. Group A included all the parental lines and group B the 8-way hybrids. This experimental arrangement was made to minimize interplant competition for light. Nitrogen fertilizer was added when the third plant leaf had emerged. An 18-hour photoperiod was used in the glasshouse with a day/night temperature 18 °/14° C . These light and temperature conditions were designed to imitate Nordic conditions during the growing period.

Traits

Four different agronomic traits were measured from each plant. Heading date was recorded as days from planting to the date when the first head was emerged. Straw length (cm) was measured from the tallest tiller. Number of ears per plant was counted at maturity. Thousand kernel weight (TKW) was measured in grams. An index (scale 4-15) from four components was constructed as follows:

$$INDEX = i_{ear.em} + i_{no/ears} + i_{straw} + i_{TKW}$$

Each trait was divided into four classes (Table 2). The class including the top lines scored 4, while class with the lowest values scored 1 for each index component. Early heading plants with short straw and high TKW were considered most favorable (score 4). However, a moderate number of synchronous emerging ears per plant were considered best in this study. Therefore, the lowest and the highest class were treated similarly in the case of number of ears per plant, when calculating the index. Phenotypic classes were used for cluster analysis (Table 2.).

Statistical analyses

The General Linear Models (GLM) procedure of the Statistical Analysis System (SAS Inst. 1990) was used for the analyses of variance, which was carried out separately for parental material and hybrids. Differences found by the analysis of variance between the means of hybrids and different parental groups were further analysed by Tukey's test. To analyse electrophoretic and agronomic class data, the NTSYS-pc statistical package was used (Rohlf 1993). The statistical method used for electrophoretic data took into account the presence or absence of each allozyme band as differential feature. A total of 28 bands were considered for the statistical comparisons. First, a similarity matrix was formed by calculating Dice's (1945) similarity coefficient for each of the pair of parental lines. Then, the matrix was submitted to average linkage cluster analysis (UPGMA) to produce a dendrogram. Correspondingly, to analyse agronomic class data , simple matching coefficient (SM) (Sokal & Michener 1958) was calculated in order to produce a dendrogram. The formula was SM=m/n, where m is number of matches in class and n total sample size.

To compare different parent groups, Nei's (1975) measure of gene diversity was calculated for each parental group (adapted parents, landraces, *H. spontaneum*). The formula used was $H = 1 - p_{ij}2/m$, where p_{ij} is the frequency of the *ith* allele at the *jth* locus and m is the total number of loci examined.

RESULTS AND DISCUSSION

Genetic diversity and cluster analyses

Of the 11 allozyme loci, 9 (82 %) showed polymorphism among the 40 parental lines. Altogether, 28 alleles were found, of which 9 were found exclusively in exotic parents and 4 only in *H. spontaneum*. The maximum number of alleles at a given locus was four. Gene diversities within parental populations were 0.13 (adapted parents), 0.16 (landraces) and 0.25 (*H. spontaneum*), which indicates that wild barleys were genetically the most variable parent group.

Associations among the parental lines of the experimental population revealed by UPGMA cluster analyses based on electrophoretic and agronomic data are presented in Fig. 1. and 2., respectively. The parental material was divided into four main clusters when using electrophoretic data. The first cluster included Swedish varieties and lines with one exception, which was the Chinese 6-rowed landrace H 7614. The second main cluster included landraces and two Swedish breeding lines Sv 892368 and Sv 89412. The latter line included two different genotypes a and b (Fig. 1). The difference was found in one single EST-locus. The occurrence of these Swedish lines within the cluster of Asiatic landraces may be caused by the primitive landrace, *Hordeum distichum* cv.

Table 2. Phenotypic classes and score values for index

Trait	Phenotypic class	Score for index
Ear emergence, days	30-55	4
	56-80	3
	81-105	2
	106-120	1
Number of ears per plant	1-5	1
	6-10	3
	11-15	2
	15<	1
Straw length, cm	60-80	4
	81-100	3
	101-120	2
	120-135	1
TKW, g	10-28	1
	29-38	2
	39-48	3
	49-65	4

Laevigatum, which has been used as a resistance source to barley mildew in the establishment of these two Swedish lines. The third cluster included all *H. spontaneum* lines, except one from Jordan, which was genetically most distant from all the other parental lines. Overall results from the cluster analysis conformed with the country of origin (Table 1) within the landrace and *H. spontaneum* clusters. The cluster analysis based on agronomic data (Fig. 2) reveals that unadapted material is not only genetically, but also agronomically different from adapted material. The distinction of landraces from *H. spontaneum* lines is not so pronounced in this case, possibly suggesting that these two groups are adapted to similar environmental conditions. Thus, overall results of diversity and cluster analyses revealed that adapted parents, landraces and *H. spontaneum* were genetically divergent and that exotic germplasm could be utilized as a source of new genetic variation.

Agronomic traits in parents and hybrids

All genetic variation is not necessarily useful for breeding purposes and genes to be utilized should either contribute directly, or in combination with other previously evaluated breeding material (Smith & Duvick 1989). Therefore, the next step was to evaluate four easily measurable agronomic traits in order to detect possible additional desirable characters in parental material. Furthermore, the parents were compared with the hybrids in terms of agronomic performance.

The results from analysis of variance are shown in Table 3. Summary statistics with Tukey's test for different parental groups and hybrids are presented in Table 4. The earliest heading parent group was landraces followed by the adapted parents.

The *H. spontaneum* lines were considerably later. Hybrids were remarkably earlier than all the parent groups suggesting that utilization of *H. spontaneum* in this extent (12.5 % of parental material) did not affect earliness negatively.

Straw length had the lowest mean among adapted parents while *H. spontaneum* had the highest. This is in agreement with an earlier study (Jaradat 1989) that showed that one of the most important traits distinguishing *H. vulgare* from *H. spontaneum* is plant height. However, the mean was lower among hybrids than among landraces and *H. spontaneum* lines. Thus, in this respect, exotic germplasm was inferior to adapted one, but affected the performance of the hybrids only moderately.

To acquire an estimate of the yielding capacity of the parental lines, two yield components were measured. Both TKW and number of ears per plant have been shown earlier (Puri et al. 1982, Benbelkacem et al. 1984) to be positively correlated with yield. In an earlier study (Rogers 1982), *H. spontaneum* grain yields were found to be extremely low. Yet, when crossed with adapted cultivars, transgressive high-yielding segregates were found in their progeny. In this study, TKW means were almost similar among landraces and adapted parents, but lower in *H. spontaneum*. The hybrid mean exceeded all the parental means in this trait. The second yield component, number of ears per plant, was quite similar in all the parent groups, although slightly higher in adapted than in exotic parents. Thus, there are some indications that exotic material included genes which would not be seriously detrimental to yielding capacity.

The analysis of agronomic traits in the parents shows that exotic germplasm is not necessarily inferior to adapted, when measuring individual traits. However, agronomic performance is a sum of several traits and

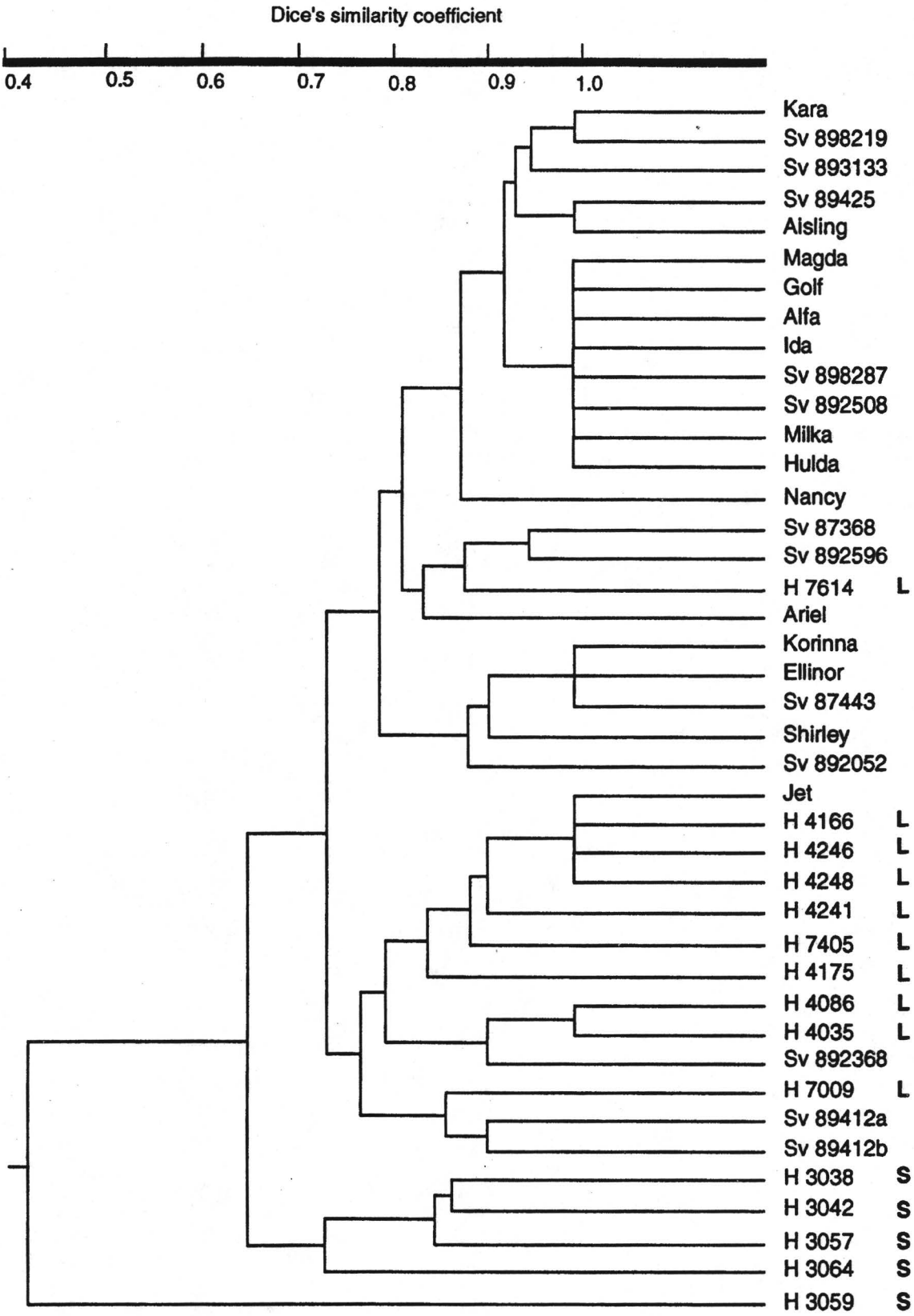

Fig. 1. Dendrogram of 40 parent lines revealed by UPGMA cluster analysis based on electrophoretic data, L = landrace, S = *Hordeum spontaneum*.

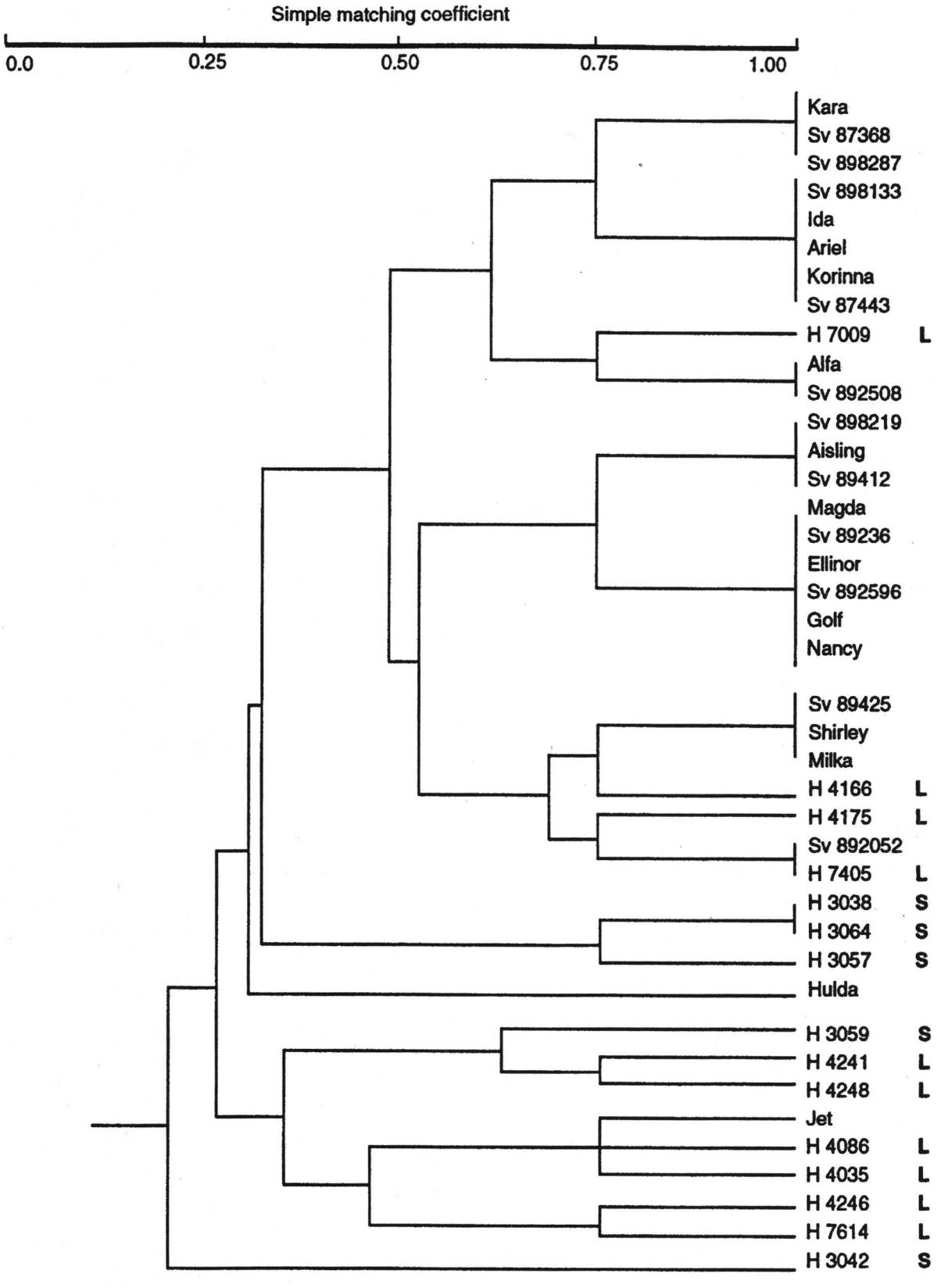

Fig. 2. Dendrogram of 40 parent lines revealed by UPGMA cluster analysis based on agronomic data, L = landraces, S = *Hordeum spontaneum*.

therefore an index was calculated for each line. The frequency distribution (Fig. 3) for the index shows that adapted parents exceed exotic germplasm in overall performance. Yet, it is apparent that landraces may possess a desirable combination of traits, for example, early plants with short straw and high TKW. Twenty per cent of the landraces studied were in the classes with the highest indices. Contrarily, all *H. spontaneum* lines had a very poor combination of agronomic traits. Around 50 % of the hybrids had indices 13 or 14 but only 20 % of the adapted parents fell into the highest classes.

These studies suggest that new genetic variation from exotic sources can be introduced into barley breeding material. In addition, through recombination agronomically valuable genotypes can be achieved and utilized in long-term breeding.

Table 3. Analyses of variance of parent and hybrid lines of four traits

Source of variation	df	MS					
		Ear emergence		No ears/ Plant	Straw length		1000 kernel weight
Blocks							
- A (parent lines)	9	85.1		27.1 **	109.9		150.1 *
- B (8-way hybrids)	9	225.2		20.4 **	566.7 **		184.8 **
Parent lines	39	2150.6 **		48.2 **	3280.8 **		281.4 **
- between groups	2	24669.7 **		398.6 **	40427.5 **		988.6 **
- adapted	24	303.7 **		21.4 **	463.8 **		247.2 **
- landraces	9	1866.8 **		54.5 **	3476.1 **		234.6 **
- spontaneum	4	2610.7 **		19.9	1170.1 **		238.4 **
8-way hybrid lines	94	547.2 **		15.6 **	703.8 **		228.2 **

* significant at the 5 % level
** significant at the 1 % level

Table 4. Summary statistics and Tukey$_F$s test for 4 traits measured on parent and 8-way hybrid lines (C.V= coefficient of variation)

Group	Ear emergence (days)				No ears/plant				Straw length (cm)				1000 kernel weight (g)			
	min	max	C.V	mean=)	min	max	C.V	mean	min	max	C.V	mean	min	max	C.V	mean
Adapted (N=25)	42.1	72.5	10.2	56.3B	9.0	15.6	12.2	12.2A	66.6	96.0	8.7	80.1C	32.0	49.3	12.6	40.3B
Landraces (N=10)	34.8	82.7	26.5	51.5C	4.7	12.9	26.5	8.8C	71.9	130.1	19.2	97.1B	33.0	47.8	11.9	40.5B
Spont. (N=5)	65.1	115.3	19.6	91.8A	8.7	13.0	13.9	11.0B	102.7	134.6	10.2	124.2A	26.6	40.7	16.0	32.5C
Hybrids (N=95)	31.1	70.1	15.6	48.1C	8.2	14.3	11.6	10.8B	73.6	119.2	9.1	93.4B	39.5	62.8	9.9	49.2A

=) Values within the same column followed by the same letter are not significantly different from each other at the 5 % probability level according to Tukey$_F$s test

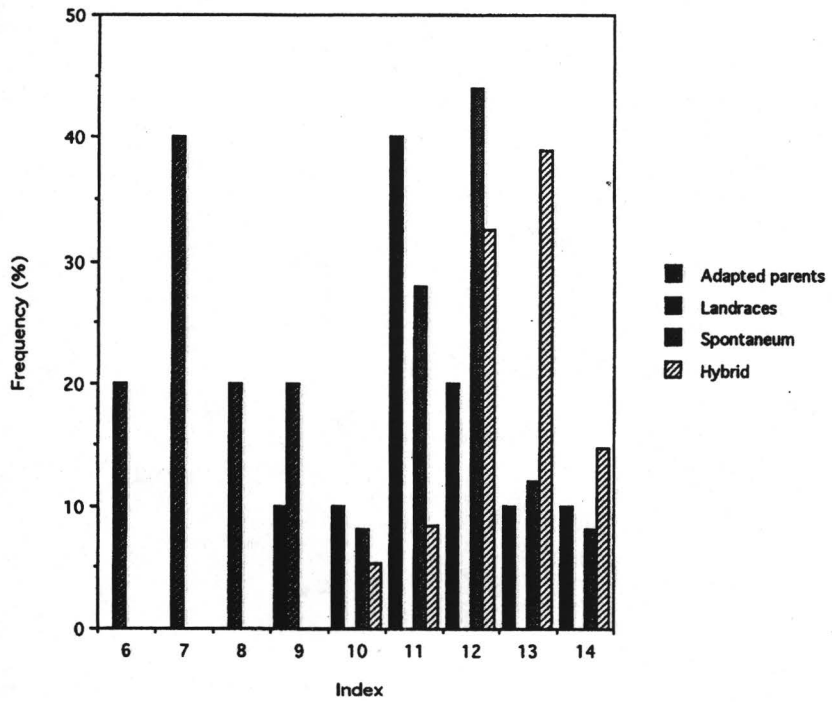

Fig. 3. Frequency distribution for index combined from four traits measured in parents and hybrids.

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