Contribution of Biotechnological Methods to Cereal Breeding

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One of the chief aims of Hungarian cereal breeding is to increase the efficiency of breeding, to select cereal varieties capable of adapting to the ecological conditions of Cereal Europe and to develop genotypes with the potential to give the best quality in this environment.

The advantages of haploid production has been widely utilized in the breeding programme. A method combining early selection, the SSD technique and anther culture was developed leading to the selection of two registered wheat varieties. *In vivo* and in *vitro* selection in combination with anther culture has been applied to increase the aluminium tolerance of wheat. In addition, doubled haploid lines represent the basic material of genetic analyses at the DNA level and in molecular gene mapping.

Winter hardiness, and its components are determined by a number of independently inherited genes, and the existence of epistatic effects within the gene groups complicates the selection of favourable alleles. Application of molecular markers and QTL analyses could be a useful tool for dissecting complex phenotypes to develop barley genotypes with superior winter habit. In cooperation with the North American Barley Genome Mapping Initiative the role and dynamics of *Ppd-H1* locus of photoperiod sensitivity and *Sh2* locus of growth habit have been determined on plant developmental and agronomic traits in a winter x spring barley mapping reference population. Comparative QTL mapping has been initiated in a winter x winter barley population. A new QTL peak for photoperiod sensitivity was located on chromosome 4 in this population with a complex effect on several agronomic traits.

Research is carried out on wheat quality to determine the exact HMW glutenin composition of old Hungarian varieties, which are made up of various genotypes and have good breadmaking quality. It appears that the HMW glutenin subunits present in the old Hungarian wheat variety Bánkúti 1201 posseses a specific DNA fragment measuring approx. 1300 bp. Technological analyses have shown that these genotypes have better quality.

The agroecological conditions in Hungary are well suited to the production of all cereal species with the exception of rice. There is thus a long tradition of research on wheat, barley and maize, the importance of which is enhanced by the unique genetic background of the old landraces found in Central Europe. With the spread of tissue culture and molecular genetic methods the main objectives of cereal researchers can be summarized as follows:

1. an improvement in the efficiency of breeding and the shortening of the breeding time;
2. the molecular genetic analysis of traits with a decisive influence on the production of cereals in this region;
3. the genetic analysis of the valuable traits of endemic genotypes.

1. During the last decade plant breeders have been interested in the development of haploids and the formation of homozygous lines in a single generation. The main advantages of haploid breeding are

- the shortening of the breeding time and the increase in selection efficiency;

- recessive gene effects and possible gametoclonal variations important for classical breeding are manifested in the phenotypes unmasked;

- the efficiency of haploid breeding compared to diploid breeding is higher when the genes concerned are large and the frequencies of useful alleles are low;

- doubled haploid lines represent the basic material of genetic analyses at the DNA level and in molecular gene mapping, where homozygotic genotypes are required in the population and exact reproducibility is an important criterion.

All these advantages of haploid production were utilized in the current programme. A method combining early selection and the SSD technique was developed [2], leading to the selection of two registered wheat varieties. The use of doubled haploids for the improvement of technological quality was studied in order to examine the extent to which *in vitro* androgenesis influences breadmaking quality properties [1].

In vitro selection methods, including selection in microspore and pollen populations, offer the chance to broaden genetic variation, especially to defend the plants from the negative effects of stress factors in cases where very few resistance sources are available. In our experiments [8] a combination of *in vivo* and *in vitro* selection was used to increase aluminium tolerance, which is an important limiting factor for the cultivation of wheat on low pH soils world-wide. It was concluded that the application of *in vivo* selection at the seedling stage greatly enhanced the probability of fixing aluminium tolerance in the DH lines, and this ratio could be further increased when selection pressure was maintained during the *in vitro* phase. On the other hand, anther culture itself did not represent an appropriate screening method because of the enhancing effect of aluminium on plant regeneration and the moderate correlation existing between the reaction to aluminium applied *in vitro* and *in vivo*.

2. The adaptation of cereal species in the early phases of development which has a decisive influence on the success of production is influenced by traits connected with winterhardiness: frost resistance, vernalisation requirement and photoperiod sensitivity. There are complex interactions among the genes determining winterhardiness-related traits, so different combinations of allele phases at these loci may result in the same phenotype. These traits differ considerably in varieties of different origins. In association analysis carried out in a range of cultivated barley germplasm [13] a strong positive correlation was found between frost resistance and vernalisation requirement, and also between vernalisation requirement and the heading date recorded at a daylength of 18 hours.

A knowledge of genotypes which break this correlation is important for breeders. Among the winter barley varieties, for example, Dicktoo and Scio have good frost resistance, but have no vernalisation requirement. However, under short days these genotypes behave like those with a strong vernalisation requirement. Under short photoperiod regimes, the strong photoperiod sensitivity slows down the plant development of these varieties, thus replacing vernalisation. Rodnik represents a different type, since it is frost-sensitive and has a very long vernalisation requirement, but is daylight-insensitive. The 17 winter barley varieties examined could be divided into 7 groups on the basis of traits determining early adaptation.

The presence of weak alleles or the absence of frost tolerance can be masked by vernalisation or even, to some extent, by photoperiod sensitivity. In the association study a weak correlation was observed between photoperiod sensitivity and frost resistance. It was found that spring barley varieties exhibited a certain level of frost tolerance, which was a consequence of their photoperiod sensitivity. The variability between spring barley varieties exceeded that of winter varieties. A number of genotypes carrying the different combination of traits determining early adaptation could again be demonstrated in this group. For instance, spring barley varieties were found which were photoperiod-sensitive, and/or had a vernalisation requirement.

No correlation was found between vernalisation requirement and photoperiod sensitivity. This can be attributed to the fact that the loci of these two latter traits are located independently of each other on different chro-

Table 1. Effects of loci on chromosome 2 (the *Ppd-H1* locus) and chromosome 7 (the *Sh2* locus) on the development and photoperiod sensitivity of 19 selected DH lines derived from the cross of *Dicktoo* x *Morex* at an 18 h light/6 h dark photoperiod regime.

Trait	Marker genotypes on chromosome 2[1] and 7[2]				Multilocus R2 (%)	Dicktoo	Morex
	DDDD	DDMM	MMMM	MMDD			
DEV31 uv (days)	20.3b[3]	16.7c	20.8b	38.2a	92.4	25.0	21.0
DEV31 v (days)	17.8b	16.6b	18.3b	25.2a	89.2	18.0	16.5
DEV49 uv (days)	30.3c	24.5d	38.3b	55.0a	96.8	33.0	39.0
DEV49 v (days)	27.0c	26.0c	36.5b	41.7a	91.5	31.0	37.0
Tillers uv (no.)	6.3b	4.0b	5.3b	11.9a	82.1	13.0	6.0
Tillers v (no.)	2.3b	2.4b	3.0ab	5.2a	54.9	5.5	4.0
Plant height uv (cm)	42.2b	42.2b	49.8ab	53.7a	49.7	38.7	50.7
Plant height v (cm)	42.3b	41.0b	53.9a	53.9a	64.8	44.0	56.5

1 for markers *CDO64-ABC454*
2 for markers *Dhn1-BCD265b*
3 values with a different letter within a row are significantly different at P<0.05 level, by F-protected LSD tests.

mosomes [10, 15, 19].

Analyses carried out on barley varieties with different geographical origins indicate that adaptational traits related to early development are determined by several independently inherited genes, while the various phenotype combinations suggest that certain genes are linked. Although winter hardiness is a complex character, Fowler and Gusta [5] consider that the possibilities for improving it have been exhausted. Due to the many interacting components, including low temperature tolerance, growth habit, photoperiod response, etc., a better understanding of the genetic mechanism determining winter hardiness could be obtained by dissecting the genetic basis of each component, and QTL analysis could be a useful tool for developing barley genotypes with superior winter habit.

Within the framework of the North American Barley Genome Mapping Initiative a population of 100 DH lines was developed from a winter spring barley cross using the varieties Dicktoo and Morex to determine the genes controlling winter hardiness and other components of early phase adaptation. Regardless of the fact that winter survival is influenced by several traits, chromosome 7 was the only important region where significant QTL effects were detected. QTLs for heading date under 16 and 24 hours light were mapped to the same region and were attributed to the segregation of alleles at the *Sh2* locus [15]. Heading date QTLs were mapped on chromosomes 1, 2, 3, 5 and 7 under field conditions, which means that all the QTLs identified in controlled environments were present in field trials [15]. This study reported, among other things, another QTL with a considerable effect on heading date on the short arm of chromosome 2 under long photoperiod regimes. Laurie et al. [10, 11] identified this locus as *Ppd-H1*.

On the basis of earlier comparative mapping studies and the synteny established among the *Triticeae* [20], the objective of the present work was to study the effects of alleles at the *Ppd-H1* and *Sh2* loci on developmental phases using 19 Dicktoo Morex DH lines representing the four possible marker genotype classes on chromosomes 2 and 7 [7]. The controlled environmental tests give a better insight into the dynamics of these two regions under changing environmental factors. Based on the detailed photoperiod regime experiments it is hypothesized that the *Ppd-H1* and *Sh2* loci contain coding regions of regulatory elements controlling the activation of overlapping sets of structural genes determining plant development. While *Ppd-H1* acts as a daylength receptor activated by long days (14 hours or more in this population), *Sh2* acts as a temperature receptor, activated by low tem-

Table 2. Phenotypic correlations among traits at an 18 h ligh/6 h dark photoperiod regime without vernalization (above the diagonal) and with vernalization (below the diagonal) in 19 DH lines derived from the cross of *Dicktoo x Morex*. Traits and the photoperiod sensitivity index are defined in the text.

Trait	Plant height	Tillers	DEV31	DEV49	Photoperiod sensitivity (b value)
Plant height		0.61**	0.58**	0.61*	- 0.64**
Tillering	0.14		0.92***	0.89***	- 0.77***
DEV31	0.41	0.83***		0.97***	- 0.84***
DEV49	0.60**	0.74***	0.90***		- 0.90***
Photoperiod		- 0.67***	- 0.85***	- 0.92***	
sensitivity (b value)	- 0.66***				

significant at * P<0.05, ** P<0.01, and ***P<0.001 level, respectively

peratures. It seems that these two loci are different not only in their activating environmental factors, but also in that they determine different plant developmental phases. *Sh2* basically determines the turning point from the vegetative growth stage to the generative growth stage. *Ppd-H1* has a strong influence on the speed of node formation in the stem and spike and on the formation of the terminal spikelet.

When studying the effect of an 18-hour photoperiod in both the vernalised and non-vernalised treatments, genotypes with Dicktoo alleles at chromosome 2 marker loci had significantly earlier heading dates than genotypes with Morex alleles at these loci (Table 1). Without vernalisation the DDMM genotypes were significantly earlier than the DDDD genotypes. Pan et al. [15] described this type of interaction between the chromosome 2 and the chromosome 7 heading date QTLs as a two-locus epistasis. The basis of this epistatic interaction is the independent assortment of alleles at the *Ppd-H1* and *Sh2* loci that occurred in this winter spring cross.

With an 18-hour photoperiod the Dicktoo allele at the *Ppd-H1* locus had a significant effect in reducing plant height in both the vernalised and non-vernalised experiments. The phenotypic correlations confirm the multiple effects of the *Ppd-H1* locus on plant growth, because the b values representing photoperiod responsiveness were negatively correlated with plant height, tillering, the appearance of the first main stem node and the

days to heading, both with and without vernalisation (Table 2).

At photoperiods of less than 12 hours the *Ppd-H1* locus was not a significant determinant of trait expression; the *Ppd-H1* locus on chromosome 2 was only activated at longer photoperiod regimes. The majority of phenotypic variation was detected by the *CDO64* marker locus and this chromosome region represents a homoeologue of the *Ppd* locus of wheat [12, 10]. The sensitivity of genotypes to longer photoperiod in this study is attributed to the Dicktoo allele.

The *Sh2* locus on chromosome 7 did not play role in determining photoperiod response (Table 1), as illustrated by the proportion of phenotypic variance (R2) for the growth and development traits. Based on comparative RFLP mapping, it is likely that the *Sh2* locus is homoeologous to the *Vrn1* locus on chromosome 5 of wheat [11, 6]. As Dicktoo does not have a vernalisation requirement, Takahashi and Yasuda [19] consider that it possesses one of the possible alleles in the multiple allelic series at the *Sh2* locus.

Based on the results of the detailed photoperiod regime study several interesting aspects became evident: (1) Although neither Dicktoo nor Morex is vernalisation responsive they may carry different alleles at the *Sh2* locus, assuming that the appearance of a heading date QTL in that region is an effect of that locus. (2) The heading date QTL on chromosome 7 is in the same region as

the low temperature tolerance and crown fructan content QTLs. These coincident QTLs are probably the results of a multi-locus cluster. (3) Dicktoo represents a specific combination in the multi-locus cluster on chromosome 7, as it does not carry the winter allele at *Sh2*, but carries a strong allele at the locus for frost tolerance. (4) Though low temperature tolerance showed the phenotypic distribution characteristic of quantitative traits, only one large QTL was identified. (5) It is probable that there are other regions containing major or minor genes that cannot be mapped in the Dicktoo x Morex population because of the similar allele composition between these two varieties and/or the overshadowing effect of the three major loci *Ppd-H1*, *Sh2* and frost tolerance.

To examine the latter possibility, the comparative mapping of a DH population with winter barley genetic background has been initiated. In this population the two winter barley parents - Dicktoo and Kompolti korai represent different combinations of winterhardiness-related traits. Dicktoo has no vernalisation response and is photoperiod-sensitive, while Kompolti korai is vernalisation-responsive and photoperiod-insensitive. In addition, the latter variety possesses a lower level of low temperature tolerance than Dicktoo. Based on phenotypic measurements, their population shows segregation for all traits with transgressive segregants. The segregation was especially apparent for heading date and its two components vernalisation response and photoperiod sensitivity.

Of the major genes identified in Dicktoo × Morex, neither the *Ppd-H1* nor the *Sh2* loci were important in the winter × winter barley population. Although Dicktoo and Kompolti korai show polymorphism at the DNA level for markers around both the *Ppd-H1* and *Sh2* loci, in spite of this difference these loci have no significant effects on winterhardiness-related traits. A QTL peak for photoperiod sensitivity was located on chromosome 4 at *BMAC310*. This locus had a significant effect on the heading date of vernalised plant material under long photoperiod regimes (16 and 24 h). Lines with the photoperiod sensitivity allele in this region headed earlier. In addition to heading date, this locus had an effect on several agronomic traits. Lines with the photoperiod-sensitive allele at this locus were shorter, had fewer tillers, and lower total and 1000 kernel weights. There were also differences between the two varieties in the region around *ABG366*. This marker is close to the *mHs* morphological marker in the reference map of Dicktoo × Morex, the pu-

tative position of the *sh* locus [19]. QTLs for vernalisation response, photoperiod sensitivity, heading date under long daylength, frost tolerance and several yield components mapped to this region in Dicktoo × Kompolti korai, suggesting that the two varieties carry different alleles at the *sh* locus.

In the Dicktoo × Kompolti korai population, although significant QTLs were identified, neither determined as large a percentage of phenotypic variation as in the winter × spring barley cross. In Dicktoo × Morex the single low temperature QTLs on chromosome 7 accounted for about 70 % of the phenotypic variation, while in Dicktoo × Kompolti korai the largest low temperature QTL on chromosome 4 explained only 18 % of the variation. Possible explanations are that: (1) there are unmapped loci in other regions of the genome, (2) there is a basic similarity in the major loci determining these traits in this accession and they segregate only for certain minor genes, and (3) there are epistatic interactions modifying the effects of the individual alleles. The completion of the map in the Dicktoo × Kompolti korai population will facilitate more extensive comparative mapping.

3. Endemic varieties, or landraces, played a significant role in the history of Central European wheat production. Landraces from the Tisza region were outstanding populations for breadmaking quality. The main disadvantage of these populations was their susceptibility to stem rust. In order to improve this trait, a progeny of the Tisza landrace, Bánkúti 5, was crossed with the Canadian variety Marquis, which is also partially of Central European origin, since one of its parents, Red Fife, originates from the same region as the Tisza landraces.

Biochemical markers have been employed for the selection of wheat breadmaking quality for the last twenty years using allelic variants of the *Glu-1* gene [, 14, 17, 18]. According to Payne et al. [16] these allelic variants represent 55-67 % of the variation. A study of old Hungarian wheat varieties showed that one of them, Bánkúti 1201, which had a Tisza landrace and Marquis in its parentage, contained HMW glutenin subunits 3+12 [9] or 2+12 [3] on chromosome 1D. In the course of SDS-PAGE analysis different genotypes were observed as regards the *Glu-1A* and *Glu-1B* loci, proving the heterogeneity of the old variety populations.

To explain the background of good breadmaking quality combined with the presence of 2+12 or 3+12 HMW glutenin subunit composition, generally responsible for poor quality properties, further investigations were carried out using a primer pair specific for the upstream region of the 1Dx5 gene and another primer pair specific for a similar region of the 1Dx2 gene.

The 53 Bánkúti 1201 lines selected consisted of 5 different groups on the basis of their HMW glutenin patterns. Subunits 2* and 1 were observed at the Glu-1A locus, subunits 7+9 and 7+8 on the 1B chromosome, and subunits 2+12 at the Glu-1D locus. In the course of PCR analysis using the primer pair specific for 1Dx5 an extra fragment of 1300 bp was present in 80 % of the Bánkúti genotypes. The lines were divided into three groups according to the presence or absence of this fragment. On the basis of the SDS test, genotypes with the 1300 bp fragment had better quality compared to genotypes whose fragment composition was similar to that of Chinese Spring, the check variety with 2+12 HMW glutenin subunit composition.

When primer pair 1Dx2 was used in the investigations, the uppermost fragment of the Bánkúti 1201 lines was observed at the same height as that of Chinese Spring, while the middle fragment of the Bánkúti lines was found in a similar position to the middle fragment of Cheyenne, the check variety for 5+10 HMW glutenin subunit composition.

From these results it can be concluded that the HMW glutenin composition of the old endemic variety Bánkúti 1201, which has outstanding breadmaking quality, is not identical to the 2+12 subunits responsible for poor quality. It is suggested that its subunit is a variant of gene 1Dx5. Further experiments will be required for a more detailed study of the specific fragments and to analyse the LMW glutenin composition.

This study confirmed the importance of investigations on populations of endemic varieties which show heterogeneity for different traits, thus providing useful sources for plant breeding research. The present experiment is a good example of the application of molecular markers, which are of use over a wider range in marker-aided selection than biochemical markers.

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