MARKER-ASSISTED SELECTION IN IMPROVEMENT OF QUANTITATIVE TRAITS OF FORAGE CROPS

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Summary
Quantitative trait loci (QTLs) for seven component traits of nitrogen-use efficiency (NUE) found in a mapping study in perennial ryegrass have been substantiated in a marker selection experiment, being a divergent mass selection programme in an F_2 population. The criterion used for plant selection was a summation index based on the number of positive QTL alleles, in fact markers for five chromosomal regions having 1-5 NUE-related QTLs. The evaluation studies showed a strong indirect response of marker selection on NUE.

Keywords
perennial ryegrass, *Lolium perenne*, marker selection, nitrogen-use efficiency

Introduction
Most agronomical characteristics of forage crops have a quantitative, polygenic, and mostly complex nature. Genetic improvement of such traits is for these reasons laborious and also time-consuming. The improvement of the nitrogen-use efficiency (NUE) in perennial ryegrass (*Lolium perenne, 2n = 14*), the major grass species in northern Europe, is in this respect a good example. The high inputs of nitrogen in animal husbandry needed to realize high forage yields do namely cause severe water pollution (Loo et al., 2003). Lowering of the nitrogen inputs through breeding efforts is therefore of utmost importance.
Selection for NUE, however, is not easily implemented in conventional grass breeding based on field evaluations. Adequate testing requires separate and long-term trials with good control of the N-stress; such experiments tend to be rather inaccurate. To circumvent the disadvantages of field-testing we used in our studies a hydroponics system in which the crop situation is simulated with growth-dependent N application (Van Loo et al, 1992). The aim is to grow plants having an equal suboptimal N content. A test usually requires several cuts. The trait is vigour-related and complex, and is extremely important in relation to the regrowth after cutting.
The afore-mentioned aspects of NUE make it a trait very attractive for marker-assisted selection. To this end, an analysis of the genetic variation present in an F_1 plant was made. This plant originated from a cross between two genotypes contrasting in NUE. The F_1 was crossed with a doubled haploid and the resulting test cross progeny was used for the analysis of variation for NUE. This approach was chosen to avoid inbreeding effects and to be able to use dominant molecular markers. The performance of the mapping population for NUE-related traits was studied on hydroponics set at a moderately low nitrogen deficiency (3.6 % N of leaf dry weight). The outcome of the mapping study was a genetic map with seven linkage groups. Putative genes (quantitative trait loci (QTLs)) for the components of NUE were found on four linkage groups. The location of the selection markers for QTLs is depicted in Fig. 1. The map shows five genomic sites with 1-5 QTLs, in total 13 QTLs for seven NUE-related traits. Three sites contain more than one QTL.
The findings of the current study are typical for genetic analyses of quantitative traits in forage crops and also indicative of the problems associated with exploitation of QTL
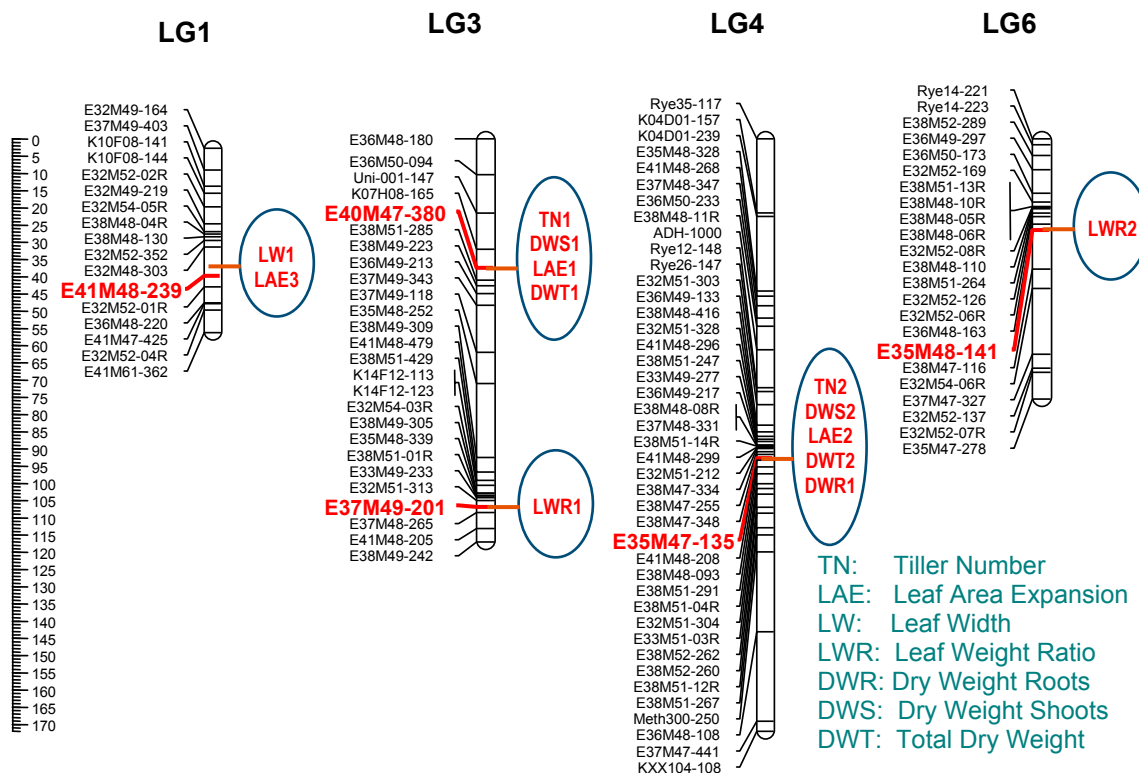
LG1 **LG3** **LG4** **LG6**

Fig. 1. Map position of the selection markers (in red) for QTLs for seven components of nitrogen-use efficiency

information through marker-assisted breeding. Exploitation problems encountered were uncertainties with respect to effect and location of QTLs, the fairly large number of QTLs often found, the cosegregation of QTLs, and the weighing of the different component traits of NUE and NUE-QTLs. This presentation will show how we have solved or circumvented these breeding problems in a divergent marker selection study to validate the QTLs found in the mapping study.

Materials and Methods

The plant materials used in the validation study was an F_2 generation obtained by selfing of the heterozygous F_1 genotype used to generate the mapping population mentioned above (Loo et al, 2003). In total, about 200 genotypes were genotyped for five AFLP-selection markers with help of the fluorescent AFLP^{TM}-technique of Applied BioSystems. The markers were codominantly scored using the heights of the fluorescence peaks relative to those of homozygous fragments as criterion.

The genotyping data were subsequently used as basis for a divergent mass selection programme. The selection strategy is outlined in Fig.2. The selection criterion was a genotype-specific selection index, being the summation of all positive QTL-alleles (or chromosome segments) over the five QTL sites considered (Figs.1 and 2). The selections were than multiplied using a polycross scheme (after vegetative propagation) to get sufficient seeds for evaluation on hydroponics and under various field conditions.

The marker selections were evaluated for NUE in a replicated trial with two cuts on hydroponics at two N-levels, being 2.5% and 5% N in leaves (Van Loo et al., 2003). The
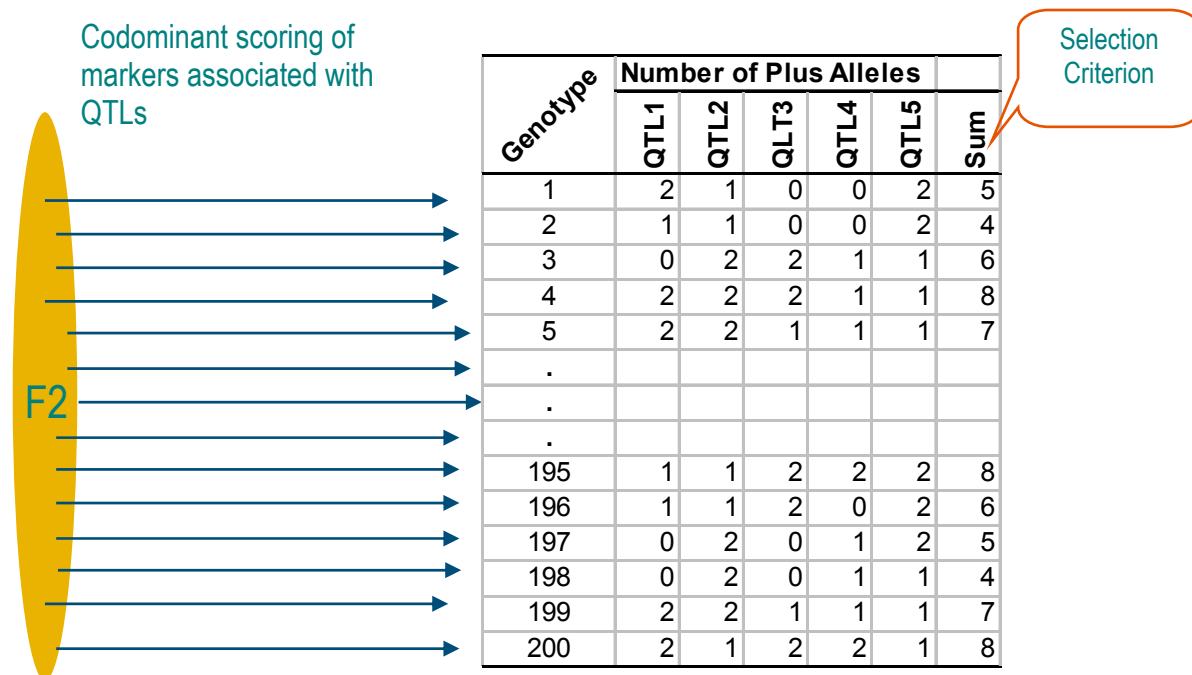
Codominant scoring of markers associated with QTLs

Selection Criterion

F2

Genotype	Number of Plus Alleles					
	QTL1	QTL2	QLT3	QTL4	QTL5	Sum
1	2	1	0	0	2	5
2	1	1	0	0	2	4
3	0	2	2	1	1	6
4	2	2	2	1	1	8
5	2	2	1	1	1	7
.						
.						
.						
195	1	1	2	2	2	8
196	1	1	2	0	2	6
197	0	2	0	1	2	5
198	0	2	0	1	1	4
199	2	2	1	1	1	7
200	2	1	2	2	1	8

Fig. 2 Strategy applied for marker selection

same set of plant characteristics as in the original mapping studies was monitored after each cut. Leaf-area expansion rate, leaf length and width, as well as tiller number were determined one week after cutting. The determination of shoot and root dry weight followed three weeks later.

Results and Discussion
The AFLP technique is usually not the marker technology of choice for selection purposes, because of its dominant nature and high costs per selection marker. Codominant scoring of the five selection markers, however, was well feasible. The trimodal frequency distributions namely allowed proper classification of plants, although some misclassification can not be fully excluded. The advantages of codominant AFLP scoring from a selection point of view are so large, that a small number of genotyping errors are not seen as a serious handicap.
The choice to use a summation index as criterion for selection was primarily made because of the difficulty to weigh the individual NUE-related traits and the co-localization of QTLs. The designation of the positive QTL-alleles (chromosome fragments) turned out to be straightforward. Fig.3 shows the F_2 frequency distribution for the number of 'Plus Alleles'. The population mean is somewhat below the expected number of 5 due to the fact that the AFLP marker on LG1 showed a skew segregation. This is likely due to gametophytic selection in favour of the negative QTL-allele due to linkage with an incompatibility locus.
The intensities of selection were set at about 25%, representing about 50 genotypes per selections (Fig. 3). The selection pressure was kept fairly low because of logistic reasons, being the need to have sufficient seeds for measuring selection responses. An advantage was that influence of genetic drift accompanying marker selection was minimized in this way. The cut-off point for the top selection was 6 positive alleles and that for the opposite selection 3
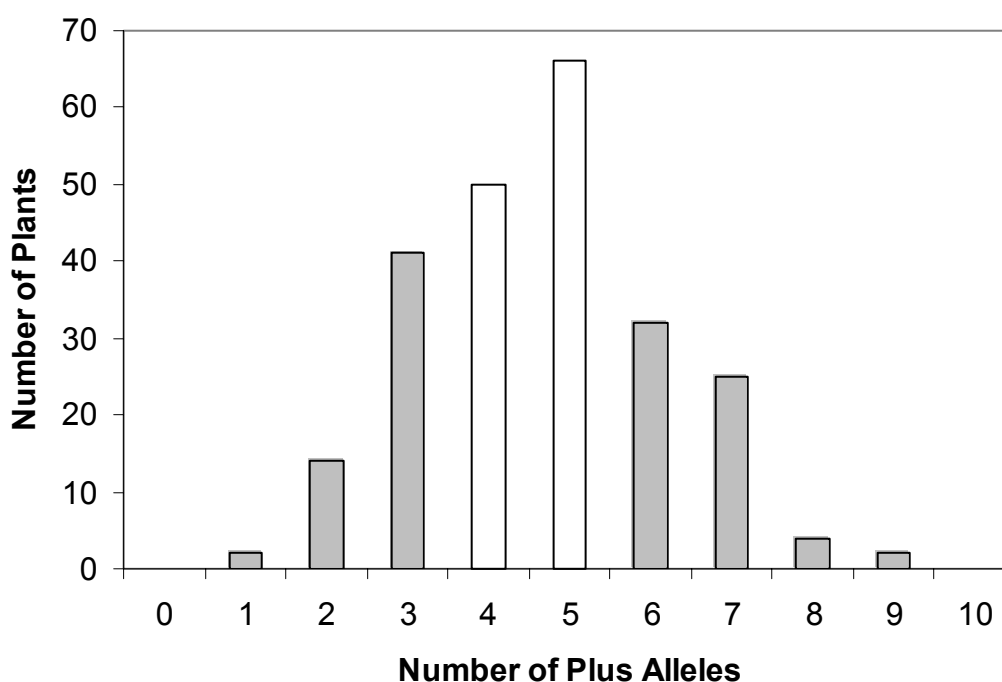
Fig 3 Frequency distribution for number of plus alleles found for five QTLs. The two marker selections, i.e. NUEplus and NUEmin, were marked

(Fig.3). The frequency of the plus alleles was on average 0.66 and 0.27, respectively. Selection showed for all NUE-loci a positive selection response. However, he between-selection difference in allele frequency of the loci ranged from 0.18 to 0.77, showing that index selection did not affected all NUE-loci to the same degree. The differences were probably mainly due to chance.

The indirect responses to marker selection as determined on hydroponics are summarized in Fig. 4. At low N-supply, the NUEplus selection showed a remarkable 40% higher tillering rate and dry matter production than the NUEmin selection. The 40% higher tillering rate is associated with a 40% higher leaf area increase after defoliation (data not shown). Relative root growth (expressed as ratio of root to total growth) and leaf length were hardly changed through marker selection. At high N-supply, the performance of NUEplus and NUEmin was fairly similar (Fig.4).

In conclusion, divergent mass selection has shown that marker selection using a summation index can be very effective for difficult and complex quantitative traits such as NUE. A collateral advantage of such an approach is that it offers a true validation of the putative genes (QTLs) for the traits of interest. The associated response to marker selection namely indicates the presence of true NUE-related genes; in particular in the vicinity of markers strongly affected by the selection imposed. The results also indicate that recurrent mass selection to increase the number of positive alleles is worthwhile.
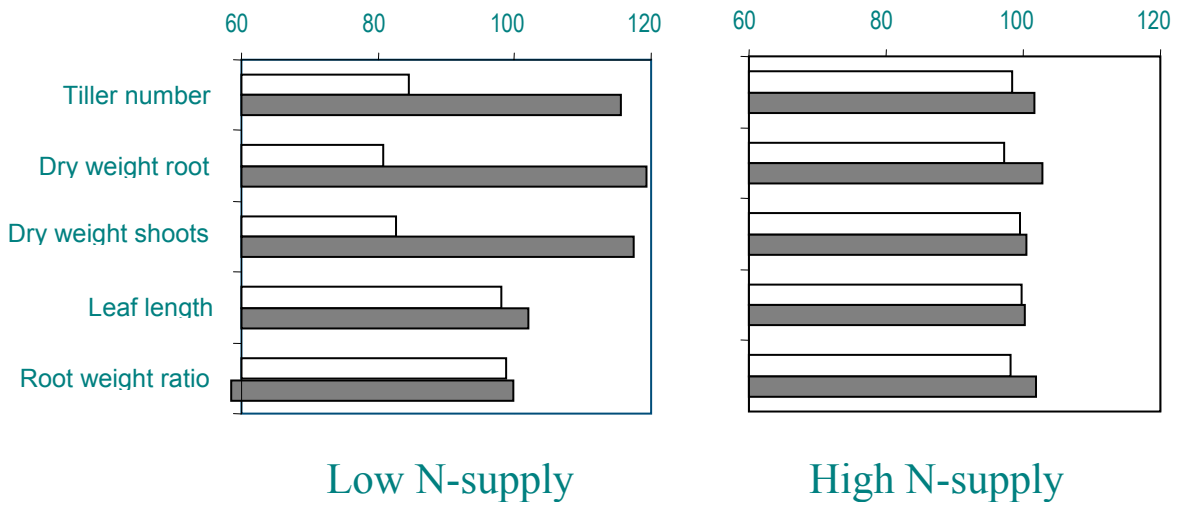
Fig. 4. Performance of the marker selections, NUEmin (white) and NUEplus (dark) on hydroponics at low N-supply (2.5 %N in leaves) and high N-supply (5% N in leaves). The performance is expressed as percentage of mean of the two selections

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