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## Identification of QTLs influencing combustion quality in *Miscanthus sinensis* Anderss. II. Chlorine and potassium content

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**Abstract** Chlorine and potassium content are important traits related to combustion quality of *Miscanthus* species. These traits were analysed in a cross between F<sub>1,1</sub> and F<sub>1,7</sub> entries of *Miscanthus sinensis* Anderss, both lines offspring of the cross between MS-90-2 and MS-88-110. Quantitative trait locus (QTL) analyses were performed on a previous linkage map constructed with the offspring cross mapping strategy. The MAPQTL 4.0 package was used to perform QTL analyses. Six potential QTLs were detected with data collected over a 2-year period. Of these, four were associated with chlorine and two with potassium. These results could be used as an initial step to develop a marker-aided selection programme for biomass with low mineral content.

**Keywords** *Miscanthus* · Offspring cross · Biomass · QTL · Combustion quality

### Introduction

The European Union is promoting the use of renewable energies to reduce the CO<sub>2</sub> emitted into the atmosphere. Among the potential energy sources, biomass is expected to highly contribute to a reduction in the greenhouse effect. One genus receiving a great deal of attention is *Miscanthus* Anderss. since it shows a high potential as a biomass resource. This genus consists of C<sub>4</sub> grasses originated from South-east Asia and was introduced from Japan to Denmark in 1935 by Aksel Olsen (Nielsen 1990). In this genus, *Miscanthus* × *giganteus* Greef et Deu is the most investigated species with respect to biomass production. According to Lewandowski et al. (2000), this species can be grown at a low level of fertilizer input, especially nitrogen. Although the potential of *M.* × *giganteus* for biomass production has been demonstrated throughout Europe, with mean yields up to 25 t/ha (dry matter) (Lewandowski et al. 2000), this species is characterized by an extremely limited variability that hampers its breeding. In fact, a single clone is used for biomass production. On the contrary, a high genetic variability is available for breeding purposes in the genus *Miscanthus* (Greef and Deuter 1993). Deuter and Abraham (1998) consider that *M. sinensis* Anderss. and *M. sacchariflorus* (Maxim.) Benth. are the most interesting species to broaden the genetic base of *M.* × *giganteus* for breeding.

In addition, both species are considered to be involved in the origin of *M.* × *giganteus*. In this way, *M.* × *giganteus* is a triploid seed-sterile ( $2n = 3x = 57$ ) originating from a cross between *M. sinensis* ( $2n = 2x = 38$ ) and *M. sacchariflorus* ( $2n = 4x = 76$ ) (Greef and Deuter 1993; Linde-Laursen 1993). One of the two genomes of *M. sacchariflorus* was inherited from *M. sinensis* while the other was given by an unidentified species (Adati and Shiotani 1962). Consequently, of the three genomes of *M.* × *giganteus*, two have a high homology to *M. sinensis*, while the third is a non-homologous genome (Greef and Deuter 1993; Linde-Laursen 1993).

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Biomass with a low mineral content is required for efficient power production. Chlorine (Cl) and potassium (K) play a large role in fouling, ash slagging and corrosion processes during biomass combustion (Miles et al. 1996; Obernberger et al. 1997, Sander 1997). However, both elements are also important plant nutrients. Although the mineral content of *M. × giganteus* has been described as being lower than in other potential crops such as maize (Beale and Long 1997), it is higher than that found in woody crops. In this way, the mineral content of annual crops and the result from annual growth of woody crops is high enough to cause problems during combustion (Miles et al. 1996; Sander 1997). The mineral content of *M. × giganteus* is at its highest at the start of the growing season and gradually decreases during development.

The same tendency has been reported in other species, such as *Bambusa bambos* (Shanmughavel and Francis 1996) and *Quercus leucotrichophora* (Lata and Bisht 1993). Consequently, Miles et al. (1996) proposed special combustion chambers for annual crops since the design of the former has a dipe bearing on avoiding problems during biomass combustion. Other strategies have been proposed to reduce the mineral content in cereals (Sander 1997). First, the use of Cl-free fertilizers should be considered –  $K_2SO_4$  or  $KNO_3$  instead of KCl. The second strategy would be to choose a variety with a low mineral content. The last alternative would be to wash the straw in the field (adopting a prolonged period between harvest and baling) (Sander 1997; Hernández-Allica 2001). However, Sander (1997) proposed that the most realistic method to improve straw quality in cereals would be the application of Cl-free fertilizers since the choice of variety is normally dependant on the grain yield. Hernández Allica et al. (2001) showed that the Cl level increased with increased Cl dose from fertilizer, while the K level was not influenced by the amount of K fertilizer. On the contrary, farmers prefer early cultivation after harvest and, therefore, the maintenance of straw in field would be discarded. With *Miscanthus*, however, all of these alternatives could be applied. *Miscanthus* species have therefore been established as a perennial rhizomatous crop. Consequently, plants may be harvested after winter and before re-sprouting, allowing the leaching of minerals by the rain in the period between ripening and harvesting. Although total yield is lower in the spring than after ripening, due to tops and leaves losses, the combustion quality is highly improved. Therefore it seems to be an interesting strategy for combustion quality improvement. In addition, the loss of leaves would help obtain a higher quality biomass since it is the plant organ with a highest mineral content (Lewandowski and Kicherer 1997). New varieties with a lower mineral content may also be obtained. Jorgensen (1997) found different *M. sinensis* genotypes with a lower mineral content than *M. × giganteus* that obtained the same yields than *M. × giganteus* when grown in Denmark. Clifton-Brown and Lewandowski (2002) also investigated different genotypes of *M. sinensis* and found differences in

chemical content, indicating that at least 2 years of analysis are needed since chemical content can vary from the first to the second year.

As the traits affecting combustion quality characteristics are under complex genetic control and chemical analyses are relatively expensive, the development of molecular markers for marker-assisted selection (MAS) and breeding is an attractive alternative approach. The complete establishment of *Miscanthus* in the field takes at least 3 years (Lewandowski et al. 2000; Clifton-Brown and Lewandowski 2002) and, therefore, the detection of molecular markers associated with traits of interest may be interesting for breeding the species when integrated in a MAS programme.

*M. sinensis* is the preferred species for mapping purposes since it is a diploid species, one of the parental lines of *M. × giganteus* and a great variability is available for breeding purposes. In this way, the first genetic linkage map for this species has been recently developed (Atienza et al. 2002), with the aim of using it for the detection of quantitative trait loci (QTLs) related to combustion quality and agronomic traits.

The objectives of the investigation reported in this paper were to locate QTLs controlling variation in Cl and K content in the map developed by Atienza et al. (2002), which consists of 257 random amplified polymorphic DNA (RAPD) markers and constructed using the offspring cross mapping strategy (Atienza et al. 2002).

## Materials and methods

### Plant material

A population of 89  $F_1$  hybrids from a cross between siblings  $F_{1,1}$  (P1) and  $F_{1,7}$  (P7) originating from a cross between MS-90-2 and MS-88-110 was used for mapping. Both parents are highly heterozygous and the grandparents contrasting for the traits of interest. Plants were maintained in a greenhouse at the Instituto de Agricultura Sostenible (IAS-CSIC), of Córdoba, Spain.

### Field trial and phenotypic measurements

The population was evaluated in a field trial at Córdoba using a randomized block design with two replications. Each field plot consisted of three plants separated by 75 cm. Distance between plots was 100 cm and between rows 150 cm. The phenotypic measurements taken were as follows.

Moisture content was determined by drying the samples at 80 °C for 3 days directly after harvest. For chemical analysis, the samples were milled through a 1-mm sieve and the samples analysed for Cl and K. Biomass was digested with acids [ $HNO_3/HClO_4$ , 85:15 (v:v)] according to the MAFF procedure (MAFF 1981).  $K^+$  was quantified using an atomic absorption spectrometer (VARIAN SpectroAA-250 plus). Cl was quantified by first extracting the samples using a weak (0.1 M) nitric acid, followed by a potentiometric titration using silver nitrate (LaCroix et al. 1970). Standard addition was used at low Cl concentrations in plant dry matter. Field data were collected and analysed for 2 consecutive years.

**Table 1** Model used to test the effect of QTL alleles (Knott et al. 1997) from Sewell et al. (2000, 2002)

|                    |   |
|--------------------|---|
| Parental cross     | $Q_1Q_2 \times Q_3Q_4 = Q_1Q_3, Q_1Q_4, Q_2Q_3, Q_2Q_4$ |
| P1 effect          | $(Q_1Q_3 + Q_1Q_4) - (Q_2Q_3 + Q_2Q_4)$                 |
| P7 effect          | $(Q_1Q_3 + Q_2Q_3) - (Q_1Q_4 + Q_2Q_4)$                 |
| Interaction effect | $(Q_1Q_3 + Q_2Q_4) - (Q_2Q_3 + Q_1Q_4)$                 |

### QTL analyses

QTLs were placed on a previous linkage map (Atienza et al. 2002) based on 257 RAPD markers. This map was developed using the offspring cross mapping strategy (Atienza et al. 2002).

QTL analyses were performed using the MAPQTL 4.0 package (Van Ooijen et al. 2000). This programme allows the detection of QTLs in cross pollinating populations such as the one used in this investigation. A sequential procedure was used for QTL detection. First, the non-parametric test of Kruskal-Wallis (Lehmann 1975) was carried out. This test does not use the map information, thereby enabling the detection of association between markers and traits individually. In a second step, interval mapping (IM) analysis was performed (Lander and Botstein 1989; Van Ooijen 1992). IM was used to select markers significantly associated with the trait. These were used as an initial set of cofactors. The backwards elimination procedure implemented in MAPQTL 4.0 was applied to the initial set of cofactors. Only significant markers at  $P < 0.02$  were used as cofactors in the multiple QTL method (MQM) (Jansen 1993, 1994; Jansen and Stam 1994) analysis for QTL detection. After the selection of cofactors, MQM analysis was performed. A mapping step size of 2 cM was used for both IM and MQM analyses. The all markers mapping approach (Knott and Haley 1992; Maliepaard and Van Ooijen 1994) was used to estimate the genetic information of not completely informative markers with respect to the four possible allelic combinations. Five neighbouring markers at each side of a marker were considered for this purpose.

The significance thresholds accepting the presence of potential QTLs in IM were determined using simulation tables (Van Ooijen 1999). The LOD critical values for MQM analysis were empirically determined using the permutation test (1,000 replications) (Churchill and Doerge 1994). According to Van Ooijen (1999) two different LOD thresholds can be considered: the chromosome wide significance threshold ( $\alpha_c$ ) and the genome wide significance threshold ( $\alpha_g$ ). Both thresholds are established at a significance level of 5%, which means that the probability of finding a LOD above the threshold in a chromosome ( $\alpha_c$ ) or in the whole genome ( $\alpha_g$ ) by chance is just 5%.

QTLs above the  $\alpha_c$  were considered to be potential QTLs in MQM. The significance of QTLs was evaluated using the LOD thresholds, Kruskal-Wallis (KW) analyses, validation test over 2 years and co-localization of QTLs for correlated traits.

The QTL positions were estimated as the position with the maximum LOD score on a linkage group. Uncertainty of the map position was indicated by a two-LOD support interval (Conneally et al. 1985; Van Ooijen 1992).

Since in this population a QTL can segregate for four different alleles, i.e. parental mating type  $Q_1Q_2 \times Q_3Q_4$ , four different genotypes can be obtained. In this way three deviance effects can be calculated (Sewell et al. 2000, 2002) using the model proposed by Knott et al. (1997). Consequently, three effects were calculated: P1 effect (difference in effect of the alleles inherited from P1), P7 effect (difference in effect of the alleles inherited from P7) and interaction effect (i.e. deviation from additivity, where a value of zero indicates complete additivity, when there is genetic information from both parents) (Table 1; Sewell et al. 2000, 2002).

**Table 2** Phenotypic values<sup>a</sup> for chlorine (Cl) and potassium (K)

|          | Cl   |      | K    |      |
|----------|------|------|------|------|
|          | 2000 | 2001 | 2000 | 2001 |
| Average  | 0.13 | 0.12 | 0.16 | 0.08 |
| Maximum  | 0.20 | 0.37 | 0.33 | 0.14 |
| Minimum  | 0.06 | 0.03 | 0.08 | 0.04 |
| St. Dev. | 0.03 | 0.06 | 0.05 | 0.02 |

<sup>a</sup> Values in percentage dry matter

## Results

### Phenotypic values and correlations between traits

The mineral content was only determined in stems, while leaves and tops were discarded. There were two reasons for this. Firstly, leaves and tops can be lost in varying amounts depending on the environmental conditions during the winter, which would then be a source of variation between years related to the higher or lower leaf and top content. In addition, a variation within years would also appear since leaf and top losses would vary from genotype to genotype. Consequently, in considering only stems we have eliminated a source of variation at both intra- and inter-years. Secondly, since the stem is the most important yield component, a reduction in the mineral content of this plant organ would reduce the mineral content of the total plant in a higher proportion than the same relative reduction in leaves or tops.

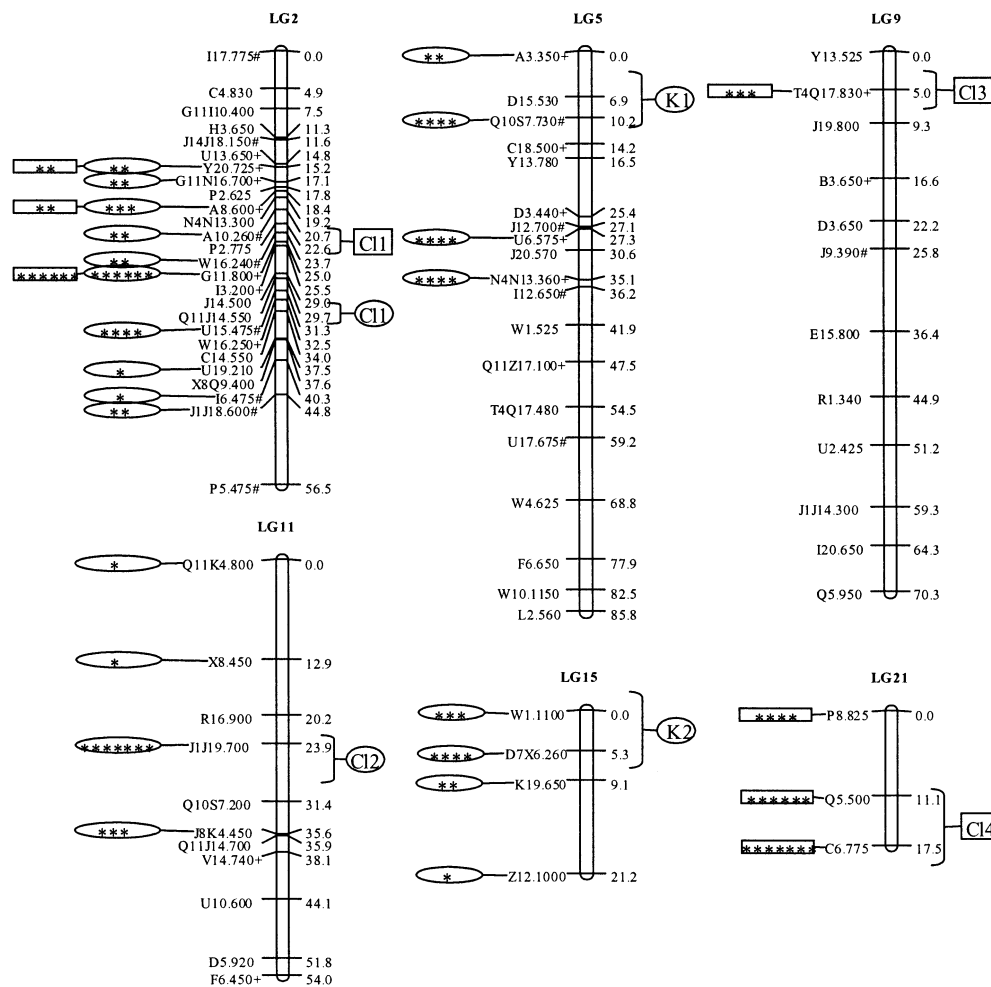
The phenotypic values for Cl and K are shown in Table 2. Both traits were significantly correlated ( $P > 0.05$ ) in both years of analysis. In this way,  $r_{K-Cl} = 0.62$  for the year 2000 and  $r = 0.82$  for 2001. Likewise, data from both years were correlated ( $r_{Cl} = 0.56$  and  $r_K = 0.42$ ). Consequently, it would be possible to obtain QTLs for both traits in the same positions in a year as well as to locate QTLs on the same positions over both years of analysis.

### Detection of QTLs

Six QTLs were detected for Cl and K when both years were considered: four (Cl1, Cl2, Cl3 and Cl4) for Cl and only two (K1 and K2) for K (Table 3). The localization of these QTLs is shown in Fig. 1. Significance LOD thresholds are important parameters for determining the significance of QTLs. Although these thresholds are sometimes arbitrarily fixed, this method leads to false QTLs being considered to be real ones. Consequently, we have determined the  $\alpha_c$  and  $\alpha_g$  LOD thresholds by two different methods. First,  $\alpha_c$  and  $\alpha_g$  were calculated using simulation tables (Van Ooijen 1999) to detect putative QTLs in IM. In our work,  $\alpha_c = 2.9$  and  $\alpha_g = 4.4$  from the simulation tables. Once MQM was completed,  $\alpha_c$  and  $\alpha_g$  were empirically determined for each trait and year (Table 3). Although the permutation test is more accurate than simulation tables for LOD threshold determination,

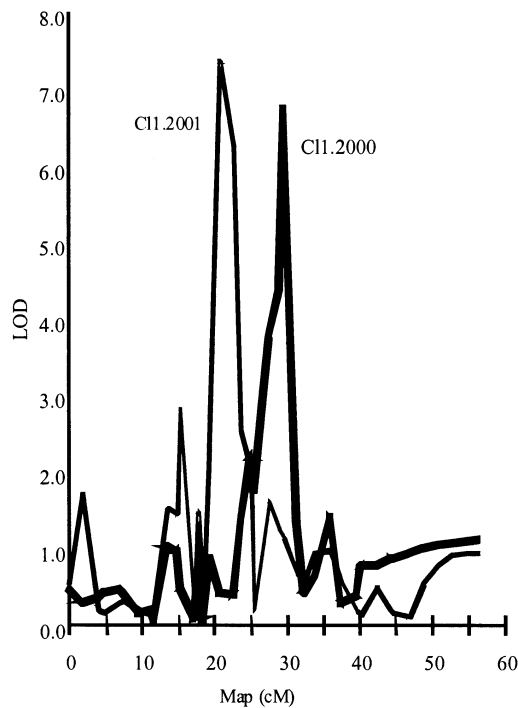
**Table 3** QTL parameters for Cl and K content

| QTL | Year | LG | Confidence interval <sup>a</sup> | cM <sup>b</sup> | LOD <sup>c</sup> | Effect <sup>d</sup> |                |        | Percentage <sup>e</sup> | ac <sup>f</sup> | ag <sup>g</sup> |
|-----|------|----|----------------------------------|-----------------|------------------|---------------------|----------------|--------|-------------------------|-----------------|-----------------|
|     |      |    |                                  |                 |                  | P <sub>1</sub>      | P <sub>7</sub> | I      |                         |                 |                 |
| Cl1 | 2000 | 2  | 29.08←→30.23                     | 29.6            | 6.7              | -0.0957             | -0.0300        | 0.0063 | 35.0                    | 3.1             | 4.4             |
| Cl2 | 2001 | 2  | 20.14←→22.86                     | 20.7            | 7.4              | 0.1659              | 0.2576         | 0.1963 | 28.6                    | 4.6             | 5.5             |
| Cl3 | 2000 | 11 | 22.19←→27.72                     | 23.9            | 4.6              | 0.0433              | 0.0491         | 0.0127 | 18.5                    | 2.7             | 4.4             |
| Cl4 | 2001 | 9  | 2.43←→6.17                       | 5.0             | 5.8              | -0.2301             | -0.1877        | 0.0373 | 31.7                    | 3.8             | 5.5             |
| Cl5 | 2001 | 21 | 11.05←→N                         | 12.1            | 4.2              | -0.0879             | -0.0879        | 0.0429 | 30.2                    | 1.95            | 5.5             |
| K1  | 2000 | 5  | 3.75←→10.57                      | 6.0             | 7.2              | 0.0722              | 0.0113         | 0.0146 | 11.8                    | 3.7             | 5.3             |
| K2  | 2000 | 15 | N←→8.19                          | 0.0             | 4.0              | -0.0742             | -0.0742        | 0.0575 | 34.6                    | 2.6             | 5.3             |

<sup>a</sup> N, End of linkage group (LG)<sup>b</sup> Peak position in centiMorgans<sup>c</sup> Maximum LOD<sup>d</sup> Deviance effects (P<sub>1</sub>, P<sub>7</sub> and interaction) as showed by Sewell et al. (2000, 2002)<sup>e</sup> Percentage of phenotypic variance explained by the QTL<sup>f</sup> Chromosome wide significance threshold determined by permutations<sup>g</sup> Genome wide significance LOD threshold determined by permutations

**Fig. 1** Localization of QTLs for Cl and K in the linkage map of *Miscanthus sinensis*. Distances are in Kosambi (centiMorgan) units, cumulative. QTL names and significance of the Kruskal-Wallis analysis are identified with *ellipses* for the year 2000 and with *boxes* for 2001. The significance of the Kruskal Wallis analysis is: \* $P < 0.1$ , \*\* $P < 0.05$ , \*\*\* $P < 0.01$ , \*\*\*\* $P < 0.005$ , \*\*\*\*\* $P < 0.001$ , \*\*\*\*\* $P < 0.0005$ , \*\*\*\*\* $P < 0.0001$ . Two-LOD support interval is shown by a *bracket* at the *right side* of the linkage groups. Marker nomenclature is as follows. (1) Single primer: markers obtained from single primer reactions were named with the primer designation followed by the molecular weight of the marker. In this way, the marker P8.650 was obtained with primer OP-P8 (OP,

Operon Technologies) and its molecular weight is 650. (2) Pairwise primer combinations: markers obtained from the combination in a single PCR reaction with two primers were designated by a code for the both primers followed by the molecular size of the amplified fragment. Therefore, marker X7X9.200 was amplified from the combination of the primers OP-X7 and OP-X9 and has a molecular weight of 200. (3) Parental origin: a symbol was added to the marker name in order to distinguish mono- and bi-parental markers. Mono-parental markers from P<sub>1</sub> are followed by the symbol +, while those obtained from P<sub>7</sub> were followed by #. The bi-parental markers have no symbol after their name



**Fig. 2** LOD profile of C11 (over both years of analysis)

this test is a time-consuming task. Since it would be possible that no QTL is segregating for one trait it would be desirable to have an overview with simulation tables and IM. Consequently, the permutation test should not be performed when no QTLs are found in IM.

Nevertheless, LOD thresholds should not be the unique parameter for deciding on the significance of QTLs in MQM analysis. On the contrary, there are other characteristics to be considered. First, markers within the confidence interval must be significantly associated with the trait in the KW analysis. In this way, a QTL would probably be false when there is not any significant marker in KW analysis. Other parameters to be taken into account would be validation through different years and the co-localization of QTLs for correlated traits over the same genome positions.

As observed in Fig. 2, C11 was detected on the same chromosome region in both years. Consequently, this QTL may be considered as validated. However, the localization of C11 in Fig. 1 seems to be different from the first to the second year since it is calculated using a two-LOD support interval and the peak LOD does not exactly coincide in both years. The rest of the QTLs for chlorine, C12, C13 and C14, were only detected in a single year. The same happened with K1 and K2, which were only detected in the year 2000; no significant QTL was detected for K in 2001. Co-localization of QTLs for K and Cl was not detected. Most QTLs detected for Cl exceeded the LOD threshold determined using permutations (Table 3). However, C14 only showed a peak LOD of 4.2, which is not surprising since this QTL is on LG21 which only groups three markers. Nevertheless, the high signif-

icance of the association between markers and Cl content found in the KW analysis is strong evidence for considering this QTL to be real. Consequently, all QTLs detected for Cl seem to be real ones. The same happened with K2. The peak LOD of this QTL is 3.99, which is under the LOD threshold determined using permutations (Table 3). However, the low number of linked markers of this group do not allow MQM to show all its potential for QTL detection and, consequently, the peak LOD may be underestimated. Besides, the association shown by markers in the KW analysis seems to show that this QTL is real. In the same way, K1 exceeded the LOD threshold determined using permutations and, therefore, seems to be real. Moreover, the KW significance of the linked markers is satisfactory.

## Discussion

### QTL effects

*M. sinensis* is characterized by its auto-incompatibility. Besides, it is a highly heterozygous species. Both characteristics make *M. sinensis* more similar to forest trees than to herbaceous crops while few QTL analyses have attempted to elucidate the gene action for QTLs in forest trees (Sewell et al. 2000), attempts have been made to investigate QTLs effects. In this way, Sewell et al. (2000, 2002) have applied an outbreed QTL model (Knott et al. 1997) which allows the calculation of an interaction effect plus parental effects (Table 1). As explained in the Materials and methods, an interaction effect of zero would mean additivity with some restrictions. However, all of the QTLs detected in our work showed non-zero interaction values (Table 3), which would suggest epistatic or dominance effects (Sewell et al. 2000; 2002). In addition, the QTL effects of the unique QTL detected through both years (C11) were different from one year to the other (Table 3). Variation in allele effects with plant age have been reported in *Pinus* (Lerceteau et al. 2001). These authors suggested environmental or epistatic interactions to explain this change.

### Potential use of QTLs in MAS

One of the problems in breeding *Miscanthus* is the long establishment period of these species – 3 years are needed in northern European countries before the first relevant data is obtained, which greatly delays the breeding process. Likewise, mineral content has been shown to be related to plant age (Clifton-Brown and Lewandowski 2002). Therefore, the genetic study of these traits would be of interest in *Miscanthus* breeding. Since these traits are complex traits under polygenic control, the detection of QTLs associated with them could be of great importance in the development of a MAS programme. The investigations of QTLs associated with mineral content in other species generally differed with respect the objec-

tives: most have been related with salinity (Tozlu et al. 1999; Monforte et al. 1997). Consequently, the main goal of these studies was to detect QTLs in order to obtain high yield, good quality etc. under conditions of salinity: they did not pay attention to whether minerals were excreted, accumulated or not absorbed by roots. On the contrary, we need plants with low mineral content at harvest and would select for leaching ability, leaf losses (since minerals are in higher proportion in this plant organ) or a lower requirement for minerals. We believe that the present investigation is the first time this type of study has been carried out. Nevertheless, salinity studies have provided some interesting results. Monforte et al. (1997) found two different types of QTLs in *Lycopersicum*: both stable QTLs across environments and others that only appear in some environments. While QTLs stable across different environments have been considered by some (Strauss et al. 1992) as the only ones suitable for MAS development, several authors have described interactions between genotype and environment (Monforte et al. 1997; García et al. 2000). Consequently there are QTLs appearing at some environments but not in others. Although sometimes the lack of expression of QTLs over different environments is considered as an indication of false QTLs, García et al. (2000) considered this as an erroneous interpretation. The inclusion of QTLs expressing in different environments in the same genotype would allow genotypes suitable for different climatic conditions to be obtained. We have found both types of QTLs in our work. First, ClI was found over both years of analysis and, therefore, it may be considered as being stable over time. Conversely, we found QTLs expressing in a single year and, consequently, they could be dependent on environmental conditions. In addition, the different QTLs could be age-dependant. This would be important since mineral content is considered to be dependant on plant age in *Miscanthus* (Clifton-Brown and Lewandowski 2002). First year analyses could identify QTLs expressing in the first stage of development, during field establishment, while the second year may correspond to QTLs expressed with fully established plants. This is possible since although 3 years are needed to obtain relevant data on *Miscanthus* in Northern European countries, the warmer conditions of southern Spain allowed us to get a complete establishment during the first year and to obtain the same yields described for the third year in North of Europe in the second year. Therefore, the first year may be considered as the establishment period while the second would correspond to a fully established crop.

Although the detection of QTLs for Cl and K content may be an important step for the development of a MAS programme, we are in a preliminary stage of the investigation. In this way, the alleles and their effect could differ in other genetic backgrounds and, consequently, the utilization of QTLs detected in this work should be taken with care. In conclusion, this study should be considered as a preliminary work to evaluate the possibilities of developing a MAS programme including

these traits in the *Miscanthus* genus which could shorten the breeding times.

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## References

- Adati S, Shiotani I (1962) The cytotaxonomy of the genus *Miscanthus* and its phylogenetic status. *Bull Fac Agric Mie Univ* 25:1–24
- Atienza SG, Satovic Z, Petersen KK, Dolstra O, Martín A (2002) Preliminary genetic linkage map of *Miscanthus sinensis* with RAPD markers. *Theor Appl Genet* 105:946–952
- Beale CV, Long P (1997) Seasonal dynamics of nutrient accumulation and partitioning in the perennial C<sub>4</sub>-grasses *Miscanthus × giganteus* and *Spartina cynosuroides*. *Biomass Bioenergy* 32:314–331
- Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. *Genetics* 138:963–971
- Clifton-Brown JC, Lewandowski I (2002) Screening *Miscanthus* genotypes in field trials to optimise biomass yield and quality in southern Germany. *Eur J Agron* 16:97–110
- Conneally PM, Edwards JH, Kidd KK, Lalouel J-M, Morton N, Ott J, White R (1985) Report of the committee on methods of linkage analysis and reporting. *Cytogenet Cell Genet* 40:356–359
- Deuter M, Abraham J (1998) Genetic resources of *Miscanthus* and their use in breeding Biomass for energy and industry. In: Kopetz H, Weber T, Palz W, Chartier P, Ferrero GL (eds) *Proc Int Conf. Würzburg*, pp 775–777
- García MR, Asíns MJ, Carbonell EA (2000) QTL analysis of yield and seed number in *Citrus*. *Theor Appl Genet* 101:487–493
- Greef JM, Deuter M (1993) Syntaxonomy of *Miscanthus × giganteus* GREEF et DEU. *Angew Bot* 67:87–90
- Hernández Allica J, Mitre AJ, González Bustamante JA, Itoiz C, Blanco F, Alkorta I, Garbisu G (2001) Straw quality for its combustion in a straw-fired power plant. *Biomass Bioenergy* 21:249–258
- Jansen RC (1993) Interval mapping of multiple quantitative trait loci. *Genetics* 135:205–211
- Jansen RC (1994) Controlling the type I and type II errors in mapping quantitative trait loci. *Genetics* 138:871–881
- Jansen RC, Stam P (1994) High resolution of quantitative traits into multiple loci via interval mapping. *Genetics* 136:1447–1455
- Jorgensen U (1997) Genotypic variation in dry matter accumulation and content of N, K and Cl in *Miscanthus* in Denmark. *Biomass Bioenergy* 12:155–169
- Knott SA, Haley CS (1992) Maximum likelihood mapping of quantitative trait loci using full-sib families. *Genetics* 132:1211–1222
- Knott SA, Neale DB, Sewell MM, Haley CS (1997) Multiple marker mapping of quantitative trait loci in an outbred pedigree of loblolly pine. *Theor Appl Genet* 94:810–820
- LaCroix R, Keeney DR, Walsh LM (1970) Potentiometric titration of chloride in plant tissue extracts using the chloride ion electrode. *Commun Soil Sci Plant Anal* 1:1–6
- Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121:185–199
- Lata K, Bisht NS (1993) Foliar nutrients during growth phases of *Q. leucotrichohora* growing at different aspect in Garhwal Himalaya. *Indian J For* 16:62–66
- Lehmann EL (1975) *Nonparametrics*. McGraw-Hill, New York

- Lerceteau E, Szmidt AE, Andersson B (2001) Detection of quantitative trait loci in *Pinus sylvestris* L. across years. *Euphytica* 121:117–122
- Lewandowski I, Kicherer A (1997) Combustion quality of biomass: practical relevance and experiments to modify the biomass quality of *Miscanthus* × *giganteus*. *Eur J Agron* 6:163–177
- Lewandowski I, Clifton-Brown JC, Scurlock JMO, Huisman W (2000) *Miscanthus*: European experience with a novel energy crop. *Biomass Bioenergy* 19:209–227
- Linde-Laursen I (1993) Cytogenetic analysis of *Miscanthus* 'Giganteus' an interspecific hybrid. *Hereditas* 119:297–300
- MAFF (1981) The analysis of agricultural materials (RB427). Ministry of Agriculture and Fisheries and Food, London, pp 156–157
- Maliepaard C, Van Ooijen JW (1994) QTL mapping in a full-sib family of an outcrossing species. In: Van Ooijen JW, Jansen J (eds) *Biometrics in plant breeding: applications of molecular markers*. Proc 9th Meet EUCARPIA Section Biometrics Plant Breed Plant Research International, Wageningen, pp 140–146
- Miles TR, Miles TR Jr, Baxter LL, Bryers RW, Jenkins BM, Oden LL (1996) Boiler deposits from rifting biomass fuels. *Biomass and Bioenergy* 10:125–138
- Monforte AJ, Asíns MJ, Carbonell (1997) Salt tolerance in *Lycopersicon* species VI. Genotype-by-salinity interaction in quantitative trait loci detection: constitutive and response QTLs. *Theor Appl Genet* 95:706–713
- Nielsen PN (1990) Elefantengrassanbau in Dänemark-Praktikerbericht. *Pflug Spaten* 3:1–4
- Obernberger I, Bidermann F, Widmann W, Riedl R (1997) Concentrations of inorganic elements in biomass fuels and recovery in the different ash fractions. *Biomass Bioenergy* 12:211–224
- Sander B (1997) Properties of Danish biofuels and the requirements for power production. *Biomass Bioenergy* 12:177–183
- Sewell MM, Bassoni DL, Megraw RA, Wheeler NC, Neale DB (2000) Identification of QTLs influencing wood property traits in loblolly pine (*Pinus taeda* L.). I. Physical wood properties. *Theor Appl Genet* 101:1273–1281
- Sewell MM, Davis MF, Tuskan GA, Wheeler NC, Elam CC, Bassoni DL, Neale DB (2002) Identification of QTLs influencing wood property traits in loblolly pine (*Pinus taeda* L.). II. Chemical wood properties. *Theor Appl Genet* 104:214–222
- Shanmughavel P, Francis K (1996) Above ground biomass production and nutrient distribution in growing bamboo (*Bambusa bambos* (L.) Voss. *Biomass Bioenergy* 10:383–391
- Strauss S, Lande R, Namkoong G (1992) Limitations of molecular-marker-aided selection in forest tree breeding. *Can J For Res* 22:1050–1061
- Tozlu I, Guy CL, Moore GA (1999) QTL analysis of Na<sup>+</sup> and Cl<sup>-</sup> accumulation related traits in an intergeneric BC<sub>1</sub> progeny of *Citrus* and *Poncirus* under saline and nonsaline environments. *Genome* 42:692–705
- Van Ooijen JW (1992) Accuracy of mapping quantitative trait loci in autogamous species. *Theor Appl Genet* 84:803–811
- Van Ooijen JW (1999) LOD significance thresholds for QTL analysis in experimental populations of diploid species. *Heredity* 83:613–624
- Van Ooijen JW, Boer MP, Jansen RC, Maliepaard C (2000) MAPQTL version 4.0: software for the calculation of QTL positions on genetic maps. Plant Research International, Wageningen