Theor Appl Genet (2002) 105:946–952
DOI 10.1007/s00122-002-0956-7

S.G. Atienza · Z. Satovic · K.K. Petersen · O. Dolstra
A. Martín

Preliminary genetic linkage map of *Miscanthus sinensis* with RAPD markers

Received: 13 January 2002 / Accepted: 26 February 2002 / Published online: 19 June 2002

Abstract We have used an "offspring cross" mapping strategy in combination with the random amplified polymorphic DNA (RAPD) assay to construct the first genetic map of the species *Miscanthus sinensis* ($2n = 2x = 38$). This map is based on an outbred population of 89 individuals resulting from the cross between two genotypes from a previously designed cross. Consequently, both parents are fullsibs. The same proportion of bi-parental markers (heterozygotic in both parents) and pseudo-testcross markers (heterozygotic in one parent and null in the other), mono-parental markers, have been obtained. A total of 383 RAPD markers were analysed within the 89 F1 plants. Out of these markers, 257 were mapped into 28 linkage groups which spanned a total map length of around 1,074.5 cM with an average density of 4.2 cM per marker. Out of 257 mapped markers, 62 were inherited from F1.1 (P1), 63 from F1.7 (P7) and 132 were bi-parental markers. The contribution to the map was equal from both parents. This map provides a useful tool for genetic analyses of agronomically interesting characters in *M. sinensis* such as flowering, yield, plant height, stem diameter and mineral constitution. The offspring cross mapping strategy is proposed to obtain a higher efficiency in developing integrated maps including both parents.

Keywords *Miscanthus sinensis* · Map · RAPD · Offspring cross · Pseudo-testcross

Communicated by H.F. Linskens

S.G. Atienza (✉) · A. Martín
Departamento de Agronomía y Mejora Genética Vegetal,
Instituto de Agricultura Sostenible,
Consejo Superior de Investigaciones Científicas,
Apdo. 4084, E-14080 Córdoba, Spain
e-mail: es2atpes@uco.es
Tel.: +34-957-499-210, Fax: +34-957-499-252

Z. Satovic
Department of Seed Science and Technology,
Faculty of Agriculture, Svetosimunska 25, 10000 Zagreb, Croatia

K.K. Petersen
Department of Horticulture,
Danish Institute of Agricultural Sciences,
Kirstinebjergvej 10, P.O. Box 102, 5792 Aarslev, Denmark

O. Dolstra
Plant Research International,
P.O. Box 16, 6700 AA Wageningen, The Netherlands

Introduction

The combustion of fossil fuels is contributing significantly to an increase in the CO_2 content of the atmosphere. Due to this fact, the European Union is promoting the use of so-called CO_2-neutral energy, i.e. wind-, sun-, water-power, and combustion of biomass. In fact, the objective of the European Union is that in 2010 12% of the energy consumption comes from renewable energy.

Biomass resources may be divided into four groups: wood, herbaceous, oil and sugar crops (Venendaal et al. 1997). The species within the *Miscanthus* genus are C4 grasses. C4 plants at favourable temperature conditions produce a higher yield than the C3 ones due to higher water-, radiation-, and nitrogen-use efficiencies. However, the spring growth of C4 plants needs warmer conditions than for C3 plants to be initiated (Long 1983). This is a clear disadvantage of *Miscanthus* under most European growing conditions. The genus *Miscanthus* Anderss., which was introduced by Aksel Olsen in 1935 from Japan to Denmark (Nielsen 1990), has a high potential as a biomass resource. This genus is characterized by a high genetic variability and is distributed into four sections (cf. Greef and Deuter 1993): Sect. I. *Triarrhena* HONDA, Sect. II. *Eumiscanthus* HONDA, Sect III. *Kariyasua* OHWI ex HIRAYOSHI, and Sect IV. *Diandra* KENG. Among the genus *Miscanthus*, *Miscanthus* × *giganteus* Greef et Deu is the most-cultivated species for biomass production in the European Union. This species yields up to 25 t/ha (dry matter) from the 3rd year onwards when spring harvested. However, huge differences in biomass yields (from 2 t/ha to 44 t/ha) have been reported (cf. Lewandowski et al. 2000). This species is highly efficient in nutrient acquisition (Himken et al.

1997). Neukirchen et al. (1999) have proposed the great rooting depth and the high root density in the subsoil as factors that overcome periods of low water and nutrient availability, especially when rapid above-ground biomass growth takes place. *M.* × *giganteus* is a triploid ($2n = 3x = 57$) resulting from a cross between *Miscanthus sinensis* ($2n = 2x = 38$) and *Miscanthus sacchariflorus* ($2n = 4x = 76$) (Greef and Deuter 1993; Linde-Laursen 1993). Out of two genomes of *M. sacchariflorus* one was inherited from *M. sinensis* while the other was donated by an unidentified species (Adati and Shiotani 1962). Therefore, the genomic constitution of *M.* × *giganteus* consists of two genomes with high homology to *M. sinensis* and a third non-homologous genome (Greef et Deuter 1993; Linde-Laursen 1993).

The mineral content of biomass causes different problems during combustion in two ways. The emission of contaminant gases such as HCl, NO, SO, and others, is raised with increasing mineral contents in the biomass. In addition, minerals can produce complications in power plants, such as fouling, slagging and corrosion. Therefore, one of the most-important breeding goals in *Miscanthus* is to minimize the content of inorganic elements such as chlorine (Cl), calcium (Ca), potassium (K), phosphorus (P), nitrogen (N), sulphur (S) and silicium (Si). The traits affecting combustion quality characteristics are under complex genetic control. Besides, the high costs of chemical analyses make the development of molecular markers for marker-assisted selection (MAS) and breeding attractive.

Genetic linkage maps are an efficient tool to improve genetic investigations (Paterson et al. 1991; Gebhardt and Salamini 1992; Staub et al. 1996). They also allow the development of more sophisticated breeding strategies like MAS. Molecular markers have facilitated the construction of linkage maps. The polymerase chain reaction (PCR)-based markers have been shown to be of a great importance, including random amplified polymorphic DNA (RAPD)(Welsh and McClelland 1990; Williams et al. 1990) and amplified fragment length polymorphisms (AFLPs) (Vos et al. 1995).

Although the first molecular markers maps were constructed for annual crops (Staub et al. 1996), recently, maps have also been developed for a number of perennial crops such as *Eucalyptus* (Grattapaglia and Sederoff 1994; Verhaegen and Plomion 1996; Marques et al. 1998), *Pinus* (Kubisiak et al. 1995), *Quercus* (Barreneche et al. 1998), *Malus* (Maliepaard et al. 1998), *Rose* (Debener and Mattiesch 1999; Rajapakse et al. 2001) *Hevea* spp. (Lespinasse et al. 2000) and *Populus* (Yin et al. 2001). The outbreeding nature of these species, in combination with high levels of heterozygosity, allow the utilisation of the double "pseudo-testcross" strategy for map construction (Gratapaglia and Sederoff 1994) due to the difficulties in producing mapping populations based on inbred genotypes.

Therefore, the populations for mapping in outbreeding species are produced from the cross between two highly heterozygotic individuals from which no previous genetic information is available. This cross is particularly useful if dominant PCR-markers, like RAPD and AFLP markers, are used (cf. Debener and Mattiesch 1999).

M. × *giganteus* is not suitable for mapping since it is triploid and sterile. The cultivated material has an extremely limited variability. *M. sinensis* and *M. sacchariflorus* are considered the most interesting species of the genus to broaden the genetic base of *M.* × *giganteus* for breeding (Deuter and Abraham 1998). High variability of the chemical content has been reported among entries of *M. sinensis* (Jorgensen 1997). This is the reason why *M. sinensis* is the species of choice for mapping, in addition to its diploid genomic constitution and its contribution with two genomes to *M.* × *giganteus*.

First molecular studies in *Miscanthus* used isozymes (Von Wuhlish et al. 1994) and AFLPs (Greef et al. 1997). Recently, Hernandez et al. (2000) have shown microsatellites to be a cost-effective tool in mapping *Miscanthus*. At present these microsatellites are being studied in our mapping population (work in progress) (Hernández, personal communication).

The objective of this work is to develop the first map of *M. sinensis* as a tool for the ongoing study of the genetic nature of various combustion-related biomass traits.

Materials and methods

Plant material

A population of 89 F1 hybrids from a cross between siblings F1.1 (P1) and F1.7 (P7) originating from a cross between MS-90-2 and MS-88-110 was used for mapping, both parents being highly heterozygous. Plants were maintained in a greenhouse at the Instituto de Agricultura Sostenible (IAS-CSIC) of Córdoba, Spain.

RAPD procedures

DNA was extracted from young leaf and stem tissue using the CTAB method of Murray and Thompson (1980) with the modifications proposed by Hernandez et al. (2000).

Templates for polymerase chain reactions (PCRs) consisted of 20–40 ng of DNA. Amplifications were performed according to Hernández (1998). Single primers or pairwise combinations of primers (hereafter referred to as primers) were used to initiate the amplifications. Primers were obtained from Operon Technologies (Alameda, Calif., USA). A Ampli*Taq* DNA Polymerase Stoffel Fragment from PE Biosystems (Foster City, Calif., USA) was used. Amplifications were performed on a PE biosystems 9600 thermocycler (Foster City, Calif., USA).

PCR amplification products were electrophoresed on gels consisting of 1% (w/v) Seakem agarose: 1% (w/v) NuSieve agarose from FMC (Rockland, Me., USA), and TBE buffer. Amplified fragments were visualized by ethidium bromide fluorescence and photographed with a GDS 5000 system CCD camera from UVP (Cambridge, UK). RAPD markers were scored for presence or absence and the marker segregation types were coded according to JoinMap 3.0 (Van Ooijen and Voorrips 2001), including the following coding classes for CP populations: lm × mm, nn × np, hk × hk for the presence of heterozygosity only in P1, only in P7, and in both parents, respectively.

Table 1 Analyses of RAPD markers using single primers and pairwise primer combinations

	Number of primers			Number of amplicons per primer		Polymorphic markers		
	Tested	Used	Efficiency	Average	Range	Total	Average	Range
Single primers	120	110	92%	7.2	2–14	308	2.8	1–7
Pairwise combinations	35	21	60%	7.2	2–14	75	3.6	1–8

Table 2 Parental origin of the markers used for mapping

	Number of markers	Percentage	Markers mapped at LOD 4.0	Map contribution (%)
Mono-parental markers				
F1-1 (P1)	74	24.4	62	24.1
F1-7 (P7)	73	24.1	63	24.5
Bi-parental markers				
P1+P7	156	51.5	132	51.4
Total	303		257	

Map construction

The map was constructed using the JoinMap 3.0 package (Van Ooijen and Voorrips 2001). Only markers that fit the expected ratios (1:1 for mono-parental markers; 3:1 for bi-parental markers) were considered (chi-square test, $p > 0.05$). A map was constructed with a LOD of 4.0 for the grouping of markers. The order of the markers of each linkage group was determined using a minimum LOD score of 1.0 and a recombination threshold of 0.4 (ripple value = 1, jump threshold = 5, Kosambi mapping function) for all linkage groups except LG1 in which it was necessary to use a LOD of 0.4 for mapping.

Results and discussion

Marker segregation and parental origin

We have used both single primers and pairwise primer combinations for RAPD amplification. The numbers of primers used in this study are shown in Table 1. Both strategies generated on average the same average number of markers per amplification (7.2) and the number of amplified fragments was also in the same range (2–14) (Table 1). However, the average number of polymorphic markers observed per amplification was higher for the pairwise combinations (3.6) than for the single primers (2.8) (Table 1). Nevertheless, the efficiency of the pairwise combinations was lower than that of single primers (Table 1). In this way, out of 35 pairwise combinations tested only 21 were used while the rest were discarded since they did not produce polymorphic markers or else the patterns obtained were non-reproducible. By contrast, the single-primer efficiency was around 90% with the same number of amplifications (data not shown). Therefore, we only used single primers to complete the work. The final efficiency of single primers was 92% (Table 1) but was only 60% for pairwise combinations.

A total of 383 markers were obtained. Out of these, 80 (20.9%) showed distorted segregation and were excluded from further analyses. So, 303 markers remained for map construction. The parental origin of these markers is shown in Table 2. The number of polymorphic markers contributed by the parents to the mapping study was almost equal. This is expected since they are full sibs. By contrast, the pseudo-testcross strategy presents problems on the contribution of parents, especially when interspecific crosses are used. In this way, Yin et al. (2001) reported two maps for the parents used in their interspecific cross in *Populus* employing the pseudo-testcross strategy, one of the maps being much longer than the other. This is a consequence of the low level of heterozygosity of one of the parents. However, using two full sibs, a similar level of heterozygosity is expected for both parents and, consequently, the contribution to the map should be equal for both parents.

Map construction

Out of 303 markers which fit Mendelian inheritance 257 were mapped into 28 linkage groups at a LOD of 4.0 (Fig. 1). These groups were numbered sequentially from the highest to the lowest number of linked markers. A total number of 46 markers were unlinked (12 from P1, 10 from P7 and 24 bi-parental markers). The parental origin of the 257 mapped markers is shown in Table 2. The map is a well-balanced distribution of markers from both parents over the map. Out of 28 groups, there were 21 with at least three markers. Likewise, seven pairs of markers constituted the linkage groups from 22 to 28, denoted as LG22 to LG28. The groups LG24, LG25 and LG26 only contained markers from P1 while LG27 and LG28 contained only markers from P7. The rest of the linkage groups have contributions from both parents. LG1 spanned a total length of 125.8 cM and grouped 35 markers, being the longest, and the linkage group with the higher number of markers.

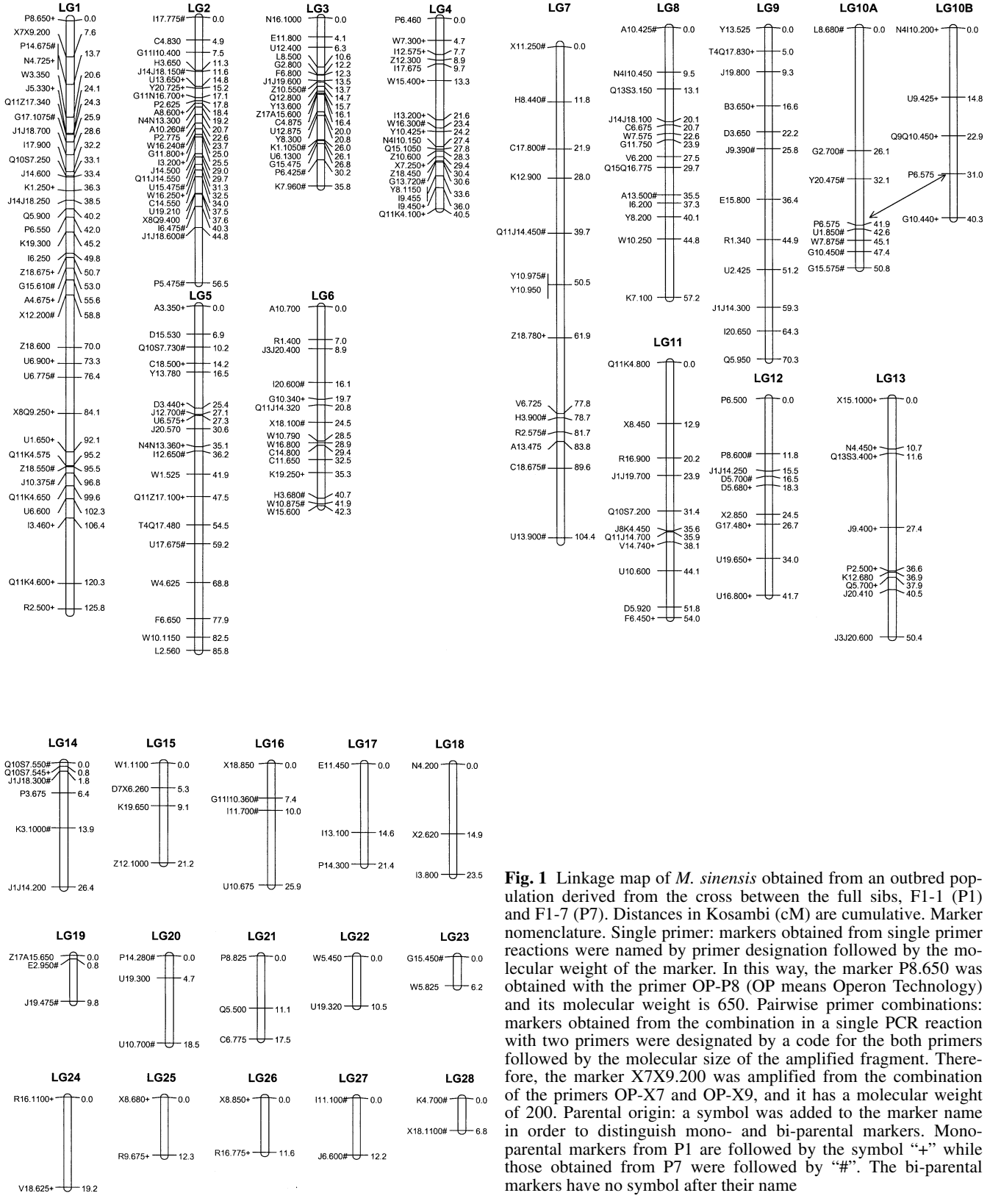

Fig. 1 Linkage map of *M. sinensis* obtained from an outbred population derived from the cross between the full sibs, F1-1 (P1) and F1-7 (P7). Distances in Kosambi (cM) are cumulative. Marker nomenclature. Single primer: markers obtained from single primer reactions were named by primer designation followed by the molecular weight of the marker. In this way, the marker P8.650 was obtained with the primer OP-P8 (OP means Operon Technology) and its molecular weight is 650. 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, the marker X7X9.200 was amplified from the combination of the primers OP-X7 and OP-X9, and it has a molecular weight of 200. Parental origin: a symbol was added to the marker name in order to distinguish mono- and bi-parental markers. Monoparental markers from P1 are followed by the symbol "+" while those obtained from P7 were followed by "#". The bi-parental markers have no symbol after their name

The number of linkage groups reported in this work is higher than the haploid chromosome number of *M. sinensis* (x = 19). However, some groups have only a few markers, and some, i.e. LG24 to LG28, contained only markers from one single parent. More markers are needed to join them to other linkage groups. The distribution of bi-parental markers over most linkage groups have made possible the construction of a single map. The high

number of bi-parental markers mapped, as well as the mono-parental ones, allows the construction of a good single map. Nevertheless, linkage group 10 could not be integrated into a single map since there is only one shared marker (P6.575). Therefore, linkage subgroups, 10A and 10B, are presented (Fig. 1).

Map length

The total map length can not be precisely established since LG10 is divided into two subgroups linked by only one common marker (P6.575), as explained above (Fig. 1). Such a group can only be integrated into a single map when there are at least two common bi-parental markers bridging each linkage group. By contrast, when there are no bi-parental markers, joining groups into a map is impossible. Furthermore, when there is only one common bi-parental marker in two linkage groups, it is possible to establish the relation between both linkage groups; however, the relative order between both linkage groups can not be established and consequently both linkage groups would remain separately as linkage subgroups. This happened to linkage subgroups 10A and 10B which are only joined by P6.575. In this way, the map would cover between 1,052.8 and 1,074.5 cM depending on the relative order between LG10A and LG10B. This size is smaller compared to those reported for species such as rice (1,670 cM) (Nagamura et al. 1993) and *Populus* (2,299.7 cM for the parent *P. alba*; Yin et al. 2001). However, this size is large in comparison to the map size reported for roses (370 cM for the parent 93/1-119; Debener and Mattiesch 1999), sugar beet (508 cM; Nilsson et al. 1997) or *Arabidopsis* (520 cM; Hauge et al. 1993).

The average distance between markers was 4.18 cM and provides a medium-density map compared to the high-density maps reported for other species like tomato (Tanksley et al. 1992), medium-density maps for almond (Joobeur et al. 2000) and rose (Debener and Mattiesch 1999), or low-density maps for *Pinus* (Devey et al. 1996).

The offspring cross and the pseudo-testcross mapping strategies

The mostly used mapping strategy in outbreeding species is the pseudo-testcross strategy (Grattapaglia and Sederoff 1994) in combination with the use of PCR-markers, such as RAPDs. This method often makes use of the high degree of heterozygosity found in interspecific hybrids. This cross yields a high number of markers showing a pseudo-testcross configuration, (i.e. mono-parental markers). By contrast, they produce a very low number of bi-parental markers (heterozygous in both parents), which hampers the construction of a single integrated map.

What we propose is to use two parents of the same offspring. This would generate both a high number of markers showing a pseudo-testcross configuration and a high number of bi-parental markers. In this way, two individuals from an outbred species are crossed. After this, two individuals from the offspring are selected and crossed for mapping purposes. Since at least two bi-parental markers per linkage group are needed to establish the relative order between two homologous groups (one from each parent) a high number of bi-parental markers have to be obtained to develop an integrated map.

For mapping we have used a population derived from the cross between two *M. sinensis* entries: F1-1 (P1) and F1-7(P7). Both parents are offspring of the cross between the two *M. sinensis* genotypes MS-88-110 and MS-90-2. This strategy allowed us to obtain sufficient bi-parental markers (51.5%) to develop a single genetic linkage map using the JoinMap 3.0 package (Van Ooijen and Voorrips 2001). Since both parents used for mapping are part of the same offspring we have decided to name this strategy the “offspring cross”. The number of bi-parental markers employed for this study is much higher than reported in other works. In this way, Debener and Mattiesch (1999) found only 19.67% bi-parental markers. The difference with the reports of Grattapaglia and Sederoff, (1994), (1.97% of bi-parental markers), and Yin et al. (2001), (2%), is striking and is related to the use of the interspecific populations used for mapping.

Advantages of the offspring cross strategy

The offspring cross strategy used in this study has several advantages compared to the pseudo-testcross proposed by Grattapaglia and Sederoff (1994). Firstly, the high genetic relation between the parental lines allows the construction of a single map for both parents since a high number of bi-parental markers are generated, while maintaining advantages of the pseudo-testcross strategy for map construction using dominant markers. The construction of an integrated map is a very important tool for quantitative trait locus (QTL) studies when both parents are expected to contribute to the genetic variation for the traits under study. In this case it is better to use a single map with an all-marker mapping approach (Knott and Haley 1992; Maliepaard and Van Ooijen 1994). Up to now, the construction of single linkage maps for outbreeding species has been possible in several studies due to the use of codominant markers such as RFLPs and microsatellites (Hemmat et al. 1994; Conner et al. 1997; Maliepaard et al. 1998). Dominant bi-parental markers, such as RAPDs and AFLPs, are not commonly used since the recombination frequency estimates with them can be inaccurate (Maliepaard et al. 1997). Nevertheless these types of markers have been used for map integration in rubber tree (Lespinasse et al. 2000), populus (Wu et al. 2000) and chestnut (Casasoli et al. 2001). The offspring cross strategy is also a valuable tool for RFLPs

and SSRs. Essentially, the offspring cross strategy is based on the same principle used up to now for the integration of genetic maps, i.e. the use of bi-parental markers. However, the difference is that the number of bi-parental markers using two full sibs is expected to be higher than using a normal cross. In this way, Maliepaard et al. (1998) mapped 290 markers in two parental maps of apple. They used 127 markers inherited from one parent, 96 derived from the other parent and 67 were multi-allelic markers. Therefore, the integration of 290 markers was based on only 67 bi-parental markers. However, they could have had a higher quantity of bi-parental markers with the offspring cross strategy. In this way, they mapped 37 RFLPs and six isozyme loci in one parent and 30 RFLPs and ten isozyme loci in the other. The offspring of this cross would have all these loci in common, as well as 67 previously detected. Consequently, a cross between two full sibs would generate a much higher number of bi-parental markers and so the integrated map would be better. In addition, the use of bi-parental RAPD markers would facilitate the saturation of this map in a second step. It is obvious that it is not possible to start a cross in a woody perennial and to wait several years to perform a new cross. However, the offspring cross strategy can be applied to woody crops since there are breeding programs for several species and, therefore, the initial cross is already available. Consequently, the starting point for this species would be the choice of adequate individuals from an available progeny. The quality of an integrated map relies on the number of bi-parental markers. Although two homologous groups can be integrated with just two bi-parental markers, the correct integration of the markers mapped in only one parent will depend on the linkage of these mono-parental markers with bi-parental markers since there is no linkage between markers mapped separately in each parent. In this way, the order of the "single markers" in the integrated map would be better as more bi-parental markers are mapped across the genome. As a consequence, any strategy to increase the number of bi-parental markers would be a valuable tool for map integration. We think that the offspring cross strategy is able to do this. On the other hand, although the utilization of codominant markers such as RFLPs or SSRs is desirable for the development of genetic maps, these markers are not available in sufficient numbers in a number of species. Likewise, these markers are much more expensive than RAPDs and AFLPs. Nevertheless, the offspring cross strategy allows a more efficient use of codominant markers since the number of bi-parental markers between both parents is higher and so the resulting integrated map is expected to be better. On the other hand, when no prior breeding work has been developed in a species, it would be better to use two individuals from the same species than an interspecific cross since the genetic similarity between both parents would be higher, and so the possibility to obtain a single map is increased.

The second advantage of the offspring cross strategy compared to the pseudo-testcross is that both parents will contribute equally to the map. Besides, interspecific crosses may produce markers showing distorted segregations. Likewise, different levels of heterozygosity among both parents have been reported (Yin et al. 2001) and so the maps obtained for each parent may differ in length due to partial homozygosity. The integrated maps obtained with the offspring cross strategy can be used for QTL detection using the MapQTL 4.0 package.

Conclusions

We have reported the first linkage map of *Miscanthus sinensis*, a species with high potential as a biomass crop. It is expected that this crop contributes considerably to a sustainable energy supply in the European Union. A single map has been developed by using polymorphic mono- and bi-parental markers in an integrated way. In addition, this map is very well balanced since both parents contributed the same number of markers. The development of genetic linkage maps is the first step towards the detection of genetic factors contributing to the variation for agronomic traits. In this way, this map constitutes a useful starting point in *M. sinensis* to study the genetics of important agronomic traits such us flowering, yield and height or combustion quality traits such us mineral content.

The offspring cross strategy is a valuable tool for the development of integrated maps for outbreeding species since the number of bi-parental markers between both parents is increased. Therefore, although the "offspring cross strategy" requires two generations (a first cross to originate the offspring and a second cross between two individuals of this progeny), we think this strategy is a very valuable tool for the mapping purposes of outbreeding species. Although this work has been developed in a herbaceous crop with a fairly short life cycle, the offspring cross strategy would be useful and readily applicable in woody crops, for instance.

Acknowledgements This research was supported by project FAIR CT98-3571. The population used in this study was developed by K.K.Petersen and K. Kristiansen (Danish Institute of Agricultural Sciences). The first author thanks Junta de Andalucía for a pre-doctoral fellowship and for support for a short period in Zagreb (Croatia).

References

Adati S, Shiotani I (1962) The cytotaxonomy of the genus *Miscanthus* and its phylogenic status. Bull Fac Agr Mie Univ 25:1–24

Barreneche T, Bodenes C, Lexer C, Trontin JF, Fluch S, Streiff R, Plomion C, Roussel G, Steinkellner H, Burg K, Favre JM, Glössl J, Kremer A (1998) A genetic linkage map of *Quercus robur* L. (pedunculate oat) based on RAPD, SCAR, microsatellite, minisatellite, isozyme and 5S rDNA markers. Theor Appl Genet 97:1090–1103

Casasoli M, Mattioni C, Cherubini M, Villani F (2001) A genetic linkage map of European chestnut (*Castanea sativa* Mill.) based on RAPD, ISSR and isozyme markers. Theor Appl Genet 102:1190–1199

Conner PJ, Brown SK, Weeden NF (1997) Randomly amplified polymorphic DNA-based genetic linkage maps of three apple cultivars. J Am Soc Hort Sci 122:350–359

Debener T, Mattiesch L (1999) Construction of a genetic linkage map for roses using RAPD and AFLP markers. Theor Appl Genet 99:891–899

Deuter M, Abraham J (1998) Genetic resources of *Miscanthus* and their use in breeding biomass for energy and industry, Proc Int Conf Würzburg, 8–11 June, pp 775–777

Devey ME, Bell JC, Smith DN, Neale DB, Moran GF (1996) A genetic linkage map for *Pinus radiata* based on RFLP, RAPD and microsatellite markers. Theor Appl Genet 92:673:679

Gebhardt C, Salamini F (1992) Restriction fragment length polymorphism analysis of plant genomes and its application to plant breeding. Int Rev Cytol 135:201–237

Grattapaglia D, Sederoff R (1994) Genetic linkage maps of *Eucalyptus grandis* and *Eucalytus urophylla* using a pseudotestcross mapping strategy and RAPD markers. Genetics 137:1121–1137

Greef JM, Deuter M (1993) Syntaxonomy of *Miscanthus* ×*giganteus* Greef et Deu. Angew Bot 67:87–90

Greef MJ, Deuter M, Jung C, Schondelmaier J (1997) Genetic diversity of European *Miscanthus* species revealed by AFLP fingerprinting. Genet Res Crop Evol 44:185–195

Hauge BM, Hanley SM, Cartinhour S, Cherry JM, Goodmann HM, Korneef M, Stam P, Chang C, Kempin S, Medrano L, Meyerowitz EM (1993) An integrated genetic/RFLP map of the *Arabidopsis thaliana* genome. Plant J 3:745–754

Hemmat M, Weeden NF, Manganaris AG, Lawson DM (1994) Molecular marker linkage map for apple. J Hered 84:4–11

Hernandez P (1998) Utilización de la PCR en el desarrollo de líneas de adición de *Hordeum chilense* en *Triticum aestivum*. PhD thesis University of Córdoba

Hernandez P, Dorado G, Laurie DA, Martín A, Snape JW (2000) Microsatellites and RFLP probes from maize are efficient sources of molecular markers for the biomass energy crop *Miscanthus*. Theor Appl Genet 102:616–622

Himken M, Lammel J, Neukirchen D, Czypionkakrause U, Olfs HW (1997) Cultivation of *Miscanthus* under west european conditions: seasonal changes in dry matter production, nutrient uptake and remobilization. Plant and Soil 189:117–126

Joobeur T, Periam N, de Vicente MC, King GJ, Arus P (2000) Development of a second generation linkage map for almond using RAPD and SSR markers. Genome 43:649–655

Jorgensen U (1997) Genotypic variation in dry matter accumulation and content of N, K and Cl in *Miscanthus* in Denmark. Biomass Bioenergy 12:3 155-169

Knott SA, Haley CS (1992) Maximum likelihood mapping of quantitative trait loci using full-sib families. Genetics 132:1211–1222

Kubisiak TL, Nelson CD, Nance WL, Stine M (1995) RAPD linkage mapping in a longleaf pine ×slash pine F1 family. Theor Appl Genet 90:1119–1127

Lespinasse D, Rodier-Goud M, Grivet L, Leconte A, Legnate H, Seguin M (2000) A saturated genetic linkage map of rubber tree (*Hevea* spp.) based on RFLP, AFLP, microsatellite, and isozyme markers. Theor Appl Genet 100:127–138

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

Long SP (1983) C4 photosynthesis at low temperature. Plant Cell Envir 6:345–363

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 Meeting of the EUCARPIA Section Biometrics in Plant Breeding, 6–8 July 1994, Wageningen, The Netherlands, pp 140–146

Maliepaard C, Jansen J, Van Ooijen JW (1997) Linkage analysis in a full-sib family of an outbreeding plant species: overview and consequences for applications. Genet Res 70:237–250

Maliepaard C, Alston FH, van Arkel G, Brown LM, Chevreau E, Dunemann F, Evans KM, Gardiner S, Guilford P, van Heusden AW, Janse J, Laurens F, Lynn JR, Manganaris AG, den Nijs APM, Periam N, Rikkerink E, Roche P, Ryder C, Sansavini S, Schmidt H, Tartarini S, Verhaegh JJ, Vrielink-van Ginkel M, King GJ (1998) Aligning male and female linkage maps of apple (*Malus pumila* Mill.) using multi-allelic markers. Theor Appl Genet 97:60–73

Marques CM, Araujo JA, Ferreira JG, Whetten R, O'Malley DM, Liu BH, Sederoff R (1998) AFLP genetic maps of *Eucalyptus globulus* and *E. tereticornis*. Theor Appl Genet 96:727–737

Murray YHG, Thompson WF (1980) Rapid isolation of high-molecular-weight plant DNA. Nucleic Acids Res 8:4321–4326

Nagamura Y, Yamamoto K, Harushma Y, Wu J, Antonio BA, Sue N, Shomura A, Lin S-Y, Miyamoto Y, Toyama T, Kirihara T, Shimizu T, Wang Z -X, Tamura Y, Ashikawa I, Yano M, Kurmat N (1993) A high density STS and EST linkage map of rice. Rice Genome 2:11–13

Neukirchen D, Himken M, Lammel J, Czypoinka-Krause U, Olfs H-W (1999) Spatial and temporal distribution of the root system and root nutrient content of an established *Miscanthus* crop. European J Agron 11:301–309

Nielsen PN (1990) Elefantengrassanbau in Dänemark-Praktikerbericht. Pflug und Spaten 3, 1–4

Nilsson NO, Hallden C, Hansen M, Hjerdin A, Säll T (1997) Comparing the distribution of RAPD and RFLP markers in a high density linkage map of sugar beet. Genome 40:644–651

Paterson AH, Tanksley SD, Sorrels ME (1991) DNA markers in plant improvement. Adv Agron 46:39–90

Rajapakse S, Byrne DH, Zhang L, Anderson N, Arumuganathan K, Ballard RE (2001) Two genetic linkage maps of tetraploid roses. Theor Appl Genet 103:575–583

Staub JE, Serquen FC, Gupta M (1996) Genetic markers, map construction, and their application in plant breeding. Hort Sci 31:729–741

Tanksley SD, Ganal MW, Prince JW, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Röder MS, Wing RA, Wu W, Yound ND (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Van Ooijen JW, Voorrips RE (2001) JoinMap Version 3.0, software for the calculation of genetic linkage maps. Plant Research International, Wageningen, The Netherlands

Venendaal R, Jorgensen U, Foster CA (1997) European energy crops: a synthesis. Biomass Bioenergy 13:3 147–185

Verhaegen D, Plomion C (1996) Genetic mapping in *Eucalyptus urophylla* and *Eucalyptus grandis* using RAPD markers. Genome 39:1051–1061

Von Wuhlisch G, Deuter M, Muhs H (1994) Identifizierung verschiedener *Miscanthus*-Sorten mittels Isoenzymen. J Agron Crop Sci 172:247–254

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Fritjers A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new concept for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213–7218

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphism amplified by arbitrary primers are useful as genetic markers Nucleic Acids Res 18:6531–6535

Wu RL, Han YF, Hu JJ, Fang JJ, Li L, Li ML, Zeng Z-B (2000) An integrated genetic map of *Populus deltoids* based on amplified fragment length polymorphisms. Theor Appl Genet 100:1249–1256

Yin T, Huang M, Wang M, Zhu L-H, Zeng Z-B, Wu R (2001) Preliminary interspecific genetic maps of the *Populus* genomes constructed from RAPD markers. Genome 44:602–609