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Colinearity and gene density in grass genomes

Beat Keller and Catherine Feuillet

Grasses are the single most important plant family in agriculture. In the past years, comparative genetic mapping has revealed conserved gene order (colinearity) among many grass species. Recently, the first studies at gene level have demonstrated that microcolinearity of genes is less conserved: small scale rearrangements and deletions complicate the microcolinearity between closely related species, such as sorghum and maize, but also between rice and other crop plants. In spite of these problems, rice remains the model plant for grasses as there is limited useful colinearity between *Arabidopsis* and grasses. However, studies in rice have to be complemented by more intensive genetic work on grass species with large genomes (maize, Triticeae). Gene-rich chromosomal regions in species with large genomes, such as wheat, have a high gene density and are ideal targets for partial genome sequencing.

The botanical family of the grasses (Poaceae) comprises >10 000 species. Their reproductive mechanism, plant anatomy and genetic variability results in a high level of adaptability enabling grass species to grow in most terrestrial habitats. In the past few thousand years, humans have taken advantage of these natural resources by domesticating and breeding a small subset of the grass species. These efforts have resulted in many important crop plants, such as wheat, rice, maize and sorghum. Many species, including wheat, are grown in different climate zones and environmental conditions, demonstrating the diversity in the gene pool of a single species. Wheat and rice each contribute ~20% of the calories ingested by the world's population (FAOSTAT home page; http://apps.fao.org/). In total, ~60% of the world's food production is obtained from grasses, which makes them economically by far the most important plant family.

In terms of genome organization, grasses represent a highly diverse family. Their chromosome number varies from 2n = 4 for the two species *Zingeria biebersteiniana* and *Colpodium versicola*[1], to 2n = 266 for the polyploid grass *Poa litorosa*[2]. Their genome sizes also vary greatly; for example, the genomes of the two crop species, rice (4.3×10^8 bp) and bread wheat (1.7×10^{10} bp), differ by a factor of 40 (Ref. 3). Comparative genetics enables us to analyse the genome structure in these different species. If gene organization and order are conserved between species, a smaller reference genome can be used as a model for gene isolation from large genomes. In addition, comparative genetics provides the basis for understanding genome evolution.

1360 - 1385/00/$ – see front matter PII: S1360-1385(00)01629-0

In this review, we address the question of comparative gene organization and gene density in the grass family. We also evaluate the use of model genomes for gene isolation in species with large genomes.

Comparative mapping in grasses: genetics reveals colinearity

At the beginning of the 1990s, cross-RFLP mapping analysis of genomes of closely related grass species, such as wheat, barley and rye or sorghum and maize, revealed a good conservation of markers within large chromosomal segments of the grass genomes[4]. These conserved regions are assumed to have derived from a common ancestor and plant geneticists have frequently used the term synteny to refer to this conservation across species. However, this use corresponds to an extension of the original definition of synteny (Box 1), and has recently been contested[5]. We will refer to orthology when describing the conservation of chromosomal segments or gene loci of common ancestry in different species and to homoeology when large chromosomal fragments or entire chromosomes are concerned (Box 1). Orthology and colinearity (conservation of the gene order) between grass genomes (Fig. 1) was also found in the first comparative maps between the taxonomically more distant genomes of maize, rice and wheat (reviewed in Ref. 4). It was remarkable that the conservation of markers and of marker order was independent of the chromosome number and the haploid DNA content of the species studied.

These observations prompted exciting possibilities, such as:

- Comparing the gene organization in grass genomes with different sizes.
- Studying the evolution of the grass genomes.
- Considering grasses as a single genetic system and defining a model grass genome.

The rice genome was proposed to consist of 19 linkage blocks that, assembled in different ways, form the basis of the Triticeae, maize, foxtail millet (*Setaria italica*), sugar cane or sorghum genomes[6]. Moreover, taken as a single unit, these blocks could represent the ancestral grass genome, which was proposed to have had a single chromosome pair[7]. In the past five years, several studies including those on many different members of the Poaceae family have confirmed the significance of these first observations and enabled a more detailed view of the length of the conserved regions and the possible rearrangements between the homoeologous chromosomes (reviewed in Ref. 8). A consensus grass map has now been established based on 25 rice linkage blocks and includes the genomes of oats, Triticeae, maize, sorghum, sugar cane and foxtail millet[4].

The development of such comparative analysis was helped greatly by the tremendous improvement in the linkage maps of different grass species. Combined comparative mapping efforts have helped to define 'anchor' probes from wheat, barley, oat, maize and rice[9] (M. Gale, pers. commun.) that correspond to RFLP probes that give a good hybridization signal on a majority of grass species. These probes can be used to evaluate the conservation of linkage groups and the degree of colinearity among the different grass species.

Box 1. Glossary of terms

Colinearity: conservation of the gene order within a chromosomal segment between different species.
Homoeologous: chromosomes that are located in different species or in different genomes in polyploid species and that originate from a common ancestral chromosome.
Orthologous: gene loci that arose from a common ancestor and that are conserved in different species. By extension, a region containing orthologous gene loci will be referred to as an orthologous region and not as a syntenic region.
Paralogous: gene loci that have arisen from a common ancestor and have evolved side by side within one species.
Synteny: originally defined for physical mapping as gene loci located on the same chromosome (without genetic linkage assumption). Since 1993, this term has been used in comparative genetic analysis to refer to chromosomal segments or to gene loci in different organisms located on a chromosomal region originating from a common ancestor. This use is currently contested[5].

Many RFLP probes that were found at orthologous locations correspond to anonymous markers. However, major genes and also quantitative trait loci (QTL) for important traits, such as vernalization, flowering time, plant height, dwarfism and shattering, also show orthologous relationships in barley, wheat, maize and rice[10,11] (reviewed in Ref. 4). Such results are particularly valuable for QTL analysis because the conservation of genes contributing to a specific trait possibly reflects the relevance of these genes in the evolution of this particular trait[12].

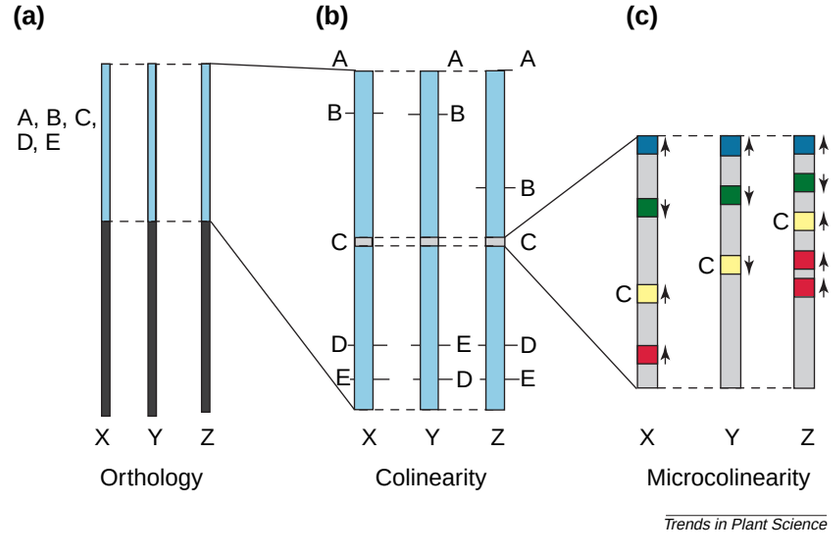

Fig. 1. Different levels of conservation between the grass genomes. (a) Orthology: refers to the conservation of loci of common evolutionary ancestry between different species. The A, B, C, D and E markers, which can originate from different species, have been localized in the chromosomal regions, highlighted in blue in three different grass species (X, Y, Z). If these regions have arisen from a common ancestor and have been conserved during the evolution of species X, Y and Z, then the markers A, B, C, D and E are likely to be orthologous. (b) Colinearity: corresponds to the conservation of the linear arrangement of the markers within a chromosomal segment. Small rearrangements (between E and D for example) can be detected at this level within a colinear region. (c) Microcolinearity: corresponds to the conservation of the order of coding regions on DNA fragments that are >50 kb. Microrearrangements such as deletions, inversions and duplications are often detected at this level. The colored boxes and the arrows represent the coding regions and the orientation of transcription, respectively.

Table 1. Publicly available BAC libraries of grass genomes

Plant	Number of clones	Genome coverage	Insert size (kb)	Ref.
Triticum monococcum	276 480	5.6	115	34
Triticum tauschii	144 000	3.7	119	35
Hordeum vulgare	313 344	6.3	106	BBACL
Oryza sativa[a]	11 000	3.5	125	36
	18 432	3.3	107	37
	14 208	4.4	130	38
Sorghum bicolor	13 440	2.8	157	39
Saccharum sp.	103 296	4.5	130	40
Zea mays	131 712	3.5	80	CUGI
	142 848	5.0	110	GS

[a]Examples selected from more than a dozen rice BAC libraries.
Abbreviations: BBACL, Barley Bacterial Artificial Chromosome Library (http://wheat.pw.usda.gov/ggpages/BarleyNewsletter/42/oral37.html); CUGI, Clemson University Genomic Institute (http://www.genome.clemson.edu); GS, Genome Systems (http://www.genomesystems.com).

Recent mapping work has revealed some exceptions to orthology and colinearity. Several disease resistance gene analogs (RGAs) and resistance genes are not well conserved among the grass genomes. Mapping of RGAs isolated from rice and barley in rice, barley and foxtail millet showed limited orthology[13]. A lack of colinearity was also found between wheat and rice at the wheat-leaf rust-resistance gene locus *Lr1* on chromosome 5DL (Ref. 14). These data suggest that comparative analysis might be more difficult for genes that evolve rapidly. Comparison of the chromosomal organization at the genetic map level has also revealed many rearrangements (inversions, translocations and insertions) and in some cases was indicative of evolutionary mechanisms leading to the modern grass species. For example, extensive comparative analysis between the genomes of maize, domesticated panicoids and rice has revealed rearrangements that occurred specifically during the evolution of the Panicoideae and Oryzoideae subfamilies[15].

Table 2. Expected versus observed gene density at different loci in grass genomes

Plant	Loci	Length[a]	Number of genes[b]	Observed gene density[c]	Expected gene density[c]	Ref.
Rice	*Adh1*	350	33	10	20	41
	Sh2/a1	28	3	8	20	42
Rice (12 PACs)	*chr1/6*	150	20–30	5.3	20	RGP
Maize	*Adh1*	225	9	25	50	17
	Zein[d]	78	10	6	50	43
Sorghum	*Adh1*	78	14	5	30	17
Barley	*mlo*	60	3	20	100–200	44
	HvLrk	160	11	15	200	24
	Mla[d]	204	11	18	200	45
Wheat	*Lrk10*	16	3	5	200–250	24
Triticum tauschii	*Lrk10*	75	4	15	200–250	CF
	Cre3	100	6	15	200–250	ML
	SBE-I[d]	16	3	5	200–250	46
Triticum monococcum	*Lrk10*	150	>6	<25	200–250	CF

[a]Length in kb of the analysed fragments.
[b]Number of genes that were detected or predicted in the analysed fragments.
[c]Gene density expressed as one gene per x kb.
[d]Correspond to duplicated genes.
Abbreviations: RGP, Rice Genome Project (http://www.staff.or.jp/GenomeSeq.html); CF, Catherine Feuillet *et al.*, unpublished; ML, Odile Moullet and Evans S. Lagudah, pers. commun.

(Micro)colinearity among the grass genomes and its many exceptions

Most of the comparative data that have been published to date has been based on linkage analysis. The recent development of large insert libraries of grass genomes in yeast artificial chromosomes (YAC) and particularly in bacterial artificial chromosomes (BAC; Table 1) has allowed the isolation and the sequencing of large genomic fragments (100–500 kb). This gave new insights about the conservation of gene order between the different grass genomes at the sub-megabase level (microcolinearity; Fig. 1) and more generally about the gene organization in small and large grass genomes. In 1996, Jeffrey Bennetzen and coworkers analysed and sequenced genomic fragments at two orthologous loci of the genomes of rice, maize and sorghum. At the *Sh2/a1* locus, good conservation of the gene content and order was found between the three grass species although the distance between the genes varied[16]. More recently, by sequencing genomic DNA fragments of 78 kb and 218 kb from sorghum and maize, respectively, three additional genes have been identified in the *Adh1* locus of sorghum compared with maize[17]. This region was previously defined as 'microsyntenic' based on cross-hybridization[18]. A lack of colinearity that could only be detected at the microstructural level has also been reported in a detailed study of the *Rpg1* barley resistance locus and the orthologous region in rice[19]. Many probes were successfully derived from rice BACs and YACs to saturate the barley *Rpg1* region. However, three probes originating from the end of a rice BAC clone containing markers flanking the *Rpg1* region, mapped 2.5 cM proximal to the *Rpg1* gene in barley, in a chromosomal region that was previously shown to be non-orthologous in rice. This demonstrated that a region of 10–15 kb had moved to a non-orthologous location. These data suggest that even in regions where colinearity has been found with high-resolution mapping, microrearrangements (deletions, duplications) can occur. This shows the limitations of using a single model genome to isolate orthologous genes in grasses and demonstrates the necessity of further molecular work on grass species other than rice.

Genome organization and gene density in small and large grass genomes

One of the key questions in studying the genome organization and evolution in large and mostly repetitive genomes is whether the genes are randomly distributed along the chromosomes or if there are gene islands interspersed by repetitive sequences. Moreover, is there a difference in gene density between grasses such as rice and wheat given that these grasses differ in genome size and in the amount of repetitive sequences? DNA fractionation and gene localization experiments have suggested that plant genomes are organized in long clusters of genes and transposable elements (forming together the gene space) occupying 12–24% of the genome, separated by long stretches of gene-empty regions that consists mainly of repetitive sequences[20]. The difference in genome size between small and large genome species would be mainly because of the difference in the length of the 'gene-empty' regions. Cytogenetic studies in wheat and barley[21,22] have shown an unequal distribution of the physical and genetic distances, suggesting the presence of gene-rich regions in these genomes. With the development of efficient sequencing technologies and large insert libraries it is now possible to sequence large fragments of genomic DNA and to obtain information about >100 kb of sequence. Based on the sequence, gene prediction can be performed with adequate computer programs (e.g. GeneMark.hmm at http://dixie.biology.gatech.edu/GeneMark/eukhmm.cgi) and the gene distribution can be studied. This complements the earlier studies, which were only based on partial sequencing and hybridization analysis and have often underestimated the number of genes present in large fragments. To date, few data on the gene organization in rice, maize, sorghum, barley and wheat have been published (Table 2). The expected gene density (based on randomly distributed genes) is variable in the different species because of the difference in genome size. However, the observed gene density is similar between the genomes. Most of the available information concerns rice, with ~1.8 Mb of DNA sequenced from chromosome 1 and 6 (Genome sequencing status quo; http://www.staff.or.jp/GenomeSeq.html). On 12 PACs (P1 artificial chromosomes) ~150 kb in length, 20–30 genes were predicted, which would give a gene density of one gene per 5–10 kb – twofold higher than would be expected from the genome size. The same result was observed in maize at the *Adh1* locus (Table 2). In the larger genomes of barley and wheat the observed gene density is one gene per 5–20 kb, much higher than the expected density of one gene per 200–250 kb. Hence, in spite of a 14-fold difference in genome size, the difference in gene density between rice and diploid wheat does not exceed two (Table 2). These data strongly support the idea that there are gene-rich regions and that in large genomes the genes are more densely packed than expected. In the gene-rich regions, it is possible that the gene density and the gene organization are not significantly different in large and small plant genomes. To date, only a few sequences are available from large and repetitive genomes, and the regions analysed where targeted based on the presence of coding regions. Therefore, it is likely that these studies concentrated mainly on the gene-rich space. With the new sequencing projects and the development of large insert libraries, 'gene-empty' regions will certainly be analysed in the near future. It will be interesting to see whether genes, and if so which kind of genes, can be found within the repetitive sequences.

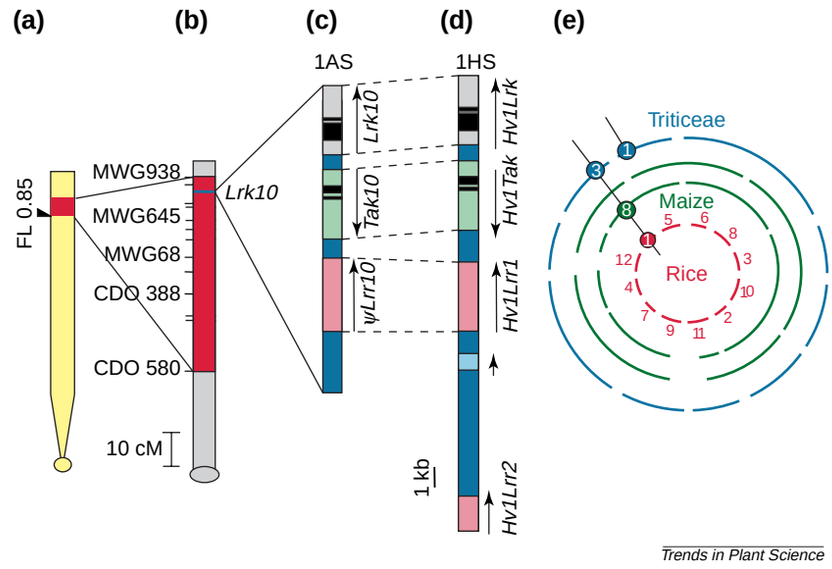

Fig. 2. Microcolinearity at the telomeric region of chromosome 1S in the Triticeae (wheat, barley) and orthologous relationships with rice and maize. (a) Physical map of chromosome group 1 of wheat based on cytogenetic studies[21] with deletion lines (FL, fraction length of the retained chromosomal arm). (b) Consensus genetic linkage map of wheat homoeologous group 1. Barley (MWG) and oat (CDO) RFLP markers, as well as the receptor-like kinase gene *Lrk10* (Ref. 24) are indicated. (c) Physical map of a 16 kb genomic fragment of wheat chromosome 1AS at the *Lrk10* locus containing three coding regions[24]. (d) Physical map of a 25 kb genomic fragment of barley chromosome 1HS (homoeologous to wheat chromosome group 1) containing five coding regions. The type of genes and the gene order are perfectly conserved compared with the orthologous region in wheat, demonstrating a good microcolinearity between barley and wheat at this locus. This region shows a gene density of one gene per 5 kb (Ref. 24). (e) Comparative mapping of the receptor-like kinase *Lrk* and *Tak* genes in wheat, barley, rice and maize shows a duplication from chromosome 3 to 1 that is specific for the Triticeae. The genomes are arranged relative to rice chromosomes, and the chromosome numbers of the orthologous regions in maize and in the Triticeae are indicated by the colored dots[4]. The maize genome is represented as two circles corresponding to the two ancestral genomes before allotetraploidization.

Microcolinearity at chromosome 1S of Triticeae: a case study

In the telomeric region of chromosome 1HS in barley[22] and group 1S in wheat[21], cytogenetic analyses have indicated a fairly high recombination rate and a high density of markers (Fig. 2). In wheat, several genes of agronomic importance have been located in the same genetic region, suggesting the presence of a gene-rich region on this part of the chromosome[21]. Moreover, major resistance genes against obligate biotrophs and genes encoding seed storage proteins have also been mapped in the homoeologous regions of barley, maize and oat[23]. Microcolinearity in this region of wheat and barley has been studied recently[24]. Good conservation of the gene order in both species was found on short genomic fragments of this region isolated from wheat and barley, and a high gene density (five genes in 23 kb; Fig. 2). Based on the analysis of a 160 kb YAC clone of barley, the gene density in this

region was estimated to be at least one gene every 15 kb. These results confirmed the presence of gene-rich regions in Triticeae and showed a perfect microcolinearity between wheat and barley in the region studied. The conservation of receptor-like kinase genes (*Lrk* and *Tak*) present in the high gene-density region of Triticeae group 1 in rice and maize has also been investigated. No conserved regions on rice chromosome 5 have been found, which has been shown to be homoeologous to the Triticeae chromosome group 1 (Ref. 4). By contrast, we found a gene family of at least ten members of *Lrk* and *Tak* receptor-like kinases on rice chromosome 1. Interestingly, this chromosome is homoeologous to the Triticeae chromosome group 3 where *Lrk*- and *Tak*-related genes have also been found in wheat and barley[24]. In maize, the two types of receptor-like kinase were found on chromosome 8 at a position that is orthologous to rice chromosome 1 and Triticeae group 3 (Fig. 2). Thus, *Lrk/Tak* gene clusters were found on homoeologous chromosomes of the Triticeae group 3 in the four grass species examined. The locus on chromosome group 1S is a duplication only present in barley and wheat and therefore seems to be specific for Triticeae. These results indicate that rice does not represent a good model for the cross-genome isolation of genes located on the Triticeae chromosome group 1S. Together with the data obtained from the analysis at the microlevel of large genomic regions in maize, rice and sorghum, these results argue in favor of using a model species as closely related as possible to the species of interest.

Can *Arabidopsis* be used as a model genome for the grasses?

One of the key questions is whether comparative genetics between *Arabidopsis* and grasses can be used to isolate agronomically important genes from cereal crop plants. A unified genetic map of higher plants was proposed[25], and it was predicted that within a distance of <3 cM, ~50% of the genes should remain colinear between monocots and dicots. Recently, a detailed study of the colinearity between rice and *Arabidopsis* in two regions of *Arabidopsis* chromosome 1 spanning <3 cM was performed[26]. Several rice ESTs have been identified by sequence similarities with the *Arabidopsis* genes, which were predicted in these regions. Little evidence for gene conservation was found (two markers spanning 1.2 cM in *Arabidopsis* were found to be associated within 0.3 cM in rice) suggesting that within 3 cM there is probably <50% gene colinearity between rice and *Arabidopsis*. It is thought[26] that conserved regions that are not detectable using the current comparative mapping tools will only be revealed at the DNA sequence level when more rice sequences are available. This greatly limits the interest in using comparative data between *Arabidopsis* and rice for grass genome analysis. Studies on the microcolinearity between maize and sorghum at the *Adh1* locus have shown that two adjacent sorghum genes were also found next to each other in *Arabidopsis*[17]. However, this conservation did not extend to the neighboring genes, suggesting that colinearity of two genes can be found between dicots and monocots but that long conserved segments are rare. In addition, the conservation of five genes on contiguous sequences in *Arabidopsis* and rice has been shown recently[27]. But the finding that these genes are also interspersed by 19 non-orthologous genes in *Arabidopsis*, demonstrates that many rearrangements have occurred since monocots and dicots diverged. These data suggest that in some cases the conservation with *Arabidopsis* will probably be useful for the identification of additional closely linked genes. However, the use of rice and more generally grass genomic data are necessary to support the isolation of genes of agronomic interest from grasses.

Conclusions and outlook

The comparative study of grass genomes has demonstrated that grasses form a single genetic system[28], of which rice provides the model genome[29]. Five years ago it was thought that the use of rice as a model genome would allow genes of agronomic importance to be isolated from other cereal crop plants without the labor intensive map-based cloning in large genomes. The surprising level of rearrangements and deletions at the microlevel between rice and other genomes, and also between closely related species such as sorghum and maize, means that improved strategies are required. These strategies must also take into account the fact that large grass genomes (e.g. wheat and barley) have gene-rich regions where gene density is not significantly different from model species. A modified approach, whereby the rice genome is used for positional cloning of genes in species with larger genomes might work as follows: mapping data and sequence information generated in the rice genome project would be highly useful for the characterization of larger genomes when the gene order is conserved between rice and the grass species of interest. Rice should also be a source of markers to saturate the region of interest when the orthologous genes are not present in rice but the overall orthology is conserved at the molecular map level. However, in addition to analysis of the orthologous region in the rice genome, the same chromosomal segment must also be analysed in the target species to discover rearrangements. For polyploid species, closely related diploid species can be used as models. For the tetraploid and hexaploid wheats, the diploid species *Triticum monococcum* and *Triticum tauschii*, and also barley can provide model genomes. Indeed, in polyploid wheat, cytogenetic stocks and comparative genetics can be skilfully exploited for targeted mapping of gene-rich regions and specific genes of interest that lie in those chromosomal regions[30]. It is also essential that gene-rich regions are sequenced in representative members of the supertribes or tribes with relevant crop species. Large insert libraries exist for undertaking such an approach and their genome coverage of 5–6*x* can be increased if necessary. These sequencing data should facilitate the cloning of agronomically important genes and result in a better understanding of gene organization, gene density and genome evolution. Specific sequencing of coding regions should also be greatly facilitated by libraries enriched in undermethylated, gene-rich DNA (Ref. 31). Relevant information about genome evolution in grasses could come from the analysis of genomes in families closely related to the Poaceae. The sister family Joinvilleaceae[32] provides the closest relatives outside the Poaceae, but little is known about genome organization in this family. Nevertheless, members of the Joinvilleaceae, as well as from other families closely related to the grasses, should help to define genome rearrangements that are specific for the grasses.

The molecular isolation of agronomically relevant genes and the understanding of their action should contribute to breed the cultivars needed in the agriculture of tomorrow. A limited gene transfer between grasses using sexual crosses and embryo rescue has added some important traits to modern cultivars[33]. However, the vast majority of genes and traits in single grass species has never been used in other species. These genes and alleles should increase the range of possibilities available to breeders to improve crop plants. Optimal strategies based on present and future resources of grass genome information should allow the isolation of such genes in the next few years.

Acknowledgements

We would like to thank Robert Dudler and Christoph Ringli for critical reading of the manuscript and Evans Lagudah for disclosing unpublished results. Grants from the SPP Biotechnology (5002-45033) and BBW/EU-Biotech program are gratefully acknowledged.

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